university of groningen

umcg

University of Groningen

New options for evaluation and intervention of cancer therapy induced oral mucositis

Stokman, Monique Astrid

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Stokman, M. A. (2005). *New options for evaluation and intervention of cancer therapy induced oral mucositis*. [Thesis fully internal (DIV), University of Groningen]. [S.n.].

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

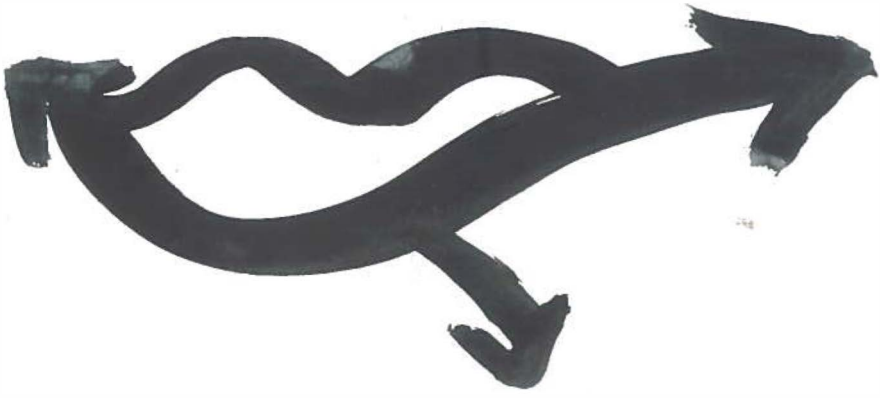
The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 06-10-2025

New options | for evaluation and intervention of cancer therapy induced oral mucositis



M. A. Stokman

New Options for Evaluation and Intervention of Cancer Therapy induced Oral Mucositis

M.A. Stokman

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Stokman, M.A.
New options for evaluation and intervention of cancer therapy induced oral mucositis.

Proefschrift Groningen. - Met lit. opg. - Met samenvatting in het Nederlands.

ISBN 90-75092-99-7

© Copyright 2005 M.A. Stokman
All rights are reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, mechanically, by photocopying, recording, or otherwise, without the written permission of the author.

Design cover and illustration: Monique Vogelsang, Heerenveen NL
Layout: Kader Desktop Publishing, Zuidlaren NL
Printed by: Drukkerij Van Ark, Haren NL

Stellingen

behorende bij het proefschrift

New Options for Evaluation and Intervention of Cancer Therapy induced Oral Mucositis

Groningen, 14 december 2005

M.A. Stokman

1. Mucositis kan niet worden voorkomen door een enkelvoudige interventie.

2. De betrouwbaarheid van het scoren van mucositis wordt verbeterd door het trainen van de onderzoeker.

3. Het bepalen van het percentage vitale epitheelcellen van het mondslijmvlies is een objectieve parameter voor het ontstaan van bestralingsmucositis.

4. De rol van Gram-negatieve bacteriën bij het ontstaan van mucositis is modulerend en niet causaal.

5. Het gezegde "voorkomen is beter dan genezen" is voor mucositis vooralsnog een utopie.

6. Een multidisciplinaire werkgroep hoofdhals tumoren dient een mondhygiënist als teamlid te hebben.

7. Een inzichtelijke complicatie registratie van een ziekenhuis-afdeling is een kenmerk van kwaliteit.

8. Gezien de positionering binnen de mondzorg, zal de mond-hygiënist in staat moeten zijn om alle visueel waarneembare afwijkingen in het aandachtsgebied als zodanig te herkennen.

9. De rol van de mondhygiënist in de gezondheidszorg dient gericht te zijn op alle facetten van preventieve mondzorg.

10. Door de mondzorg uitgevoerd door de mondhygiënist niet op te nemen in de nieuwe Zorgverzekeringswet wordt voorbij gegaan aan het proces van taakherschikking in de gezondheidszorg.

11. Het getuigt van visie om het schrijven van wetenschappelijke publicaties op te nemen in de criteria van het Kwaliteitsregister Paramedici.

12. Je moet datgene accepteren in het leven wat niet te veranderen is en datgene veranderen wat niet te accepteren is.
Mieke Bijenhof (1966-1991)

13. Failure is the opportunity to begin again more intelligently.
Henry Ford

14. Door het verbod voor alle pleziervaartuigen om zogenaamd zwart afvalwater te lozen op het oppervlaktewater, kan het onderwater toilet overboord worden gezet.

15. "Het zal me een zorg zijn" krijgt een heel andere betekenis na de invoering van het nieuwe zorgstelsel.

RIJKS*UNIVERSITEIT* GRONINGEN

New Options for Evaluation and Intervention of Cancer Therapy induced Oral Mucositis

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
woensdag 14 december 2005
om 14.45 uur

door

Monique Astrid Stokman

geboren op 14 december 1963

te Amsterdam

Promotores:	Prof. dr. J.L.N. Roodenburg Prof. dr. E.G.E. de Vries
Copromotor:	Dr. F.K.L. Spijkervet
Beoordelingscommissie:	Prof. dr. J.B. Epstein Prof. dr. J.A. Langendijk Prof. dr. I. van der Waal

Paranimfen: H. van der Lei-Groenewegen
Drs. B. van Minnen

Financial support for the publication of this thesis, received from the following companies or institutions, is gratefully acknowledged:

KerrHawe, Philips Sonicare

Nederlandse Vereniging van Mondhygiënisten, Amgen B.V., Sense Medical B.V., Dental Union B.V., Dentaid BeNeLux B.V., Straumann B.V.

CONTENTS

1 Introduction

Oral mucositis induced by radiotherapy or chemotherapy is a frequently occurring side-effect in patients with cancer. It is painful and restricts oral function, such as speech, swallowing and chewing. Oral mucositis is associated with an increase in the number of systemic infections, days in hospital and overall costs.[1,2] These aspects can limit the cancer therapy and have a negative impact on patients health-related quality of life (HRQOL).[3] Mucositis was reported to be the most troubling side-effect of cancer therapy by 38% of patients treated with head and neck radiation and 42% of the patients treated with high-dose chemotherapy.[4,5] (Figure 1 and 2)

The incidence of mucositis is dependent on the cancer treatment regimen. The current head and neck radiotherapy protocols have a mucositis incidence of 85-100%.[6] The incidence of mucositis can approach 90-100% in patients receiving aggressive myeloablative chemotherapy[7], and is present in 40% of patients with a solid tumor, who have chemotherapy induced myelosuppression.[1]

Secondary infection of mucosal ulcers, are seen in severe mucositis, and can provide a port of entry for microorganisms into the circulation, which can lead to life-threatening septicemia, especially in myelosuppressed patients.[8]

These data illustrate the impact of oral mucositis on cancer treatment. Understanding and knowledge of the development of oral mucositis and the preventive possibilities are becoming increasingly important since the actual regimens for mucositis prevention are, until now, mainly symptomatic or palliative.

The aim of this thesis is to investigate new approaches for evaluation and new options for management of cancer therapy induced oral mucositis.

Many studies have been published concerning prevention of oral mucositis, but most studies had small samples sizes or used different scoring methods which make comparison of the results difficult. Insufficient power and the lack of sensitivity of the outcomes measures make it hard to draw definitive conclusions regarding the use of these interventions for the prevention of oral mucositis. **Chapter 2** reviews this literature on the effectiveness of interventions for the prevention for oral mucositis in cancer patients treated with head and neck radiotherapy, chemotherapy, or chemoradiation. It focuses, whenever possible, on meta-analyses of randomized clinical trials of interventions for the prevention of oral mucositis.

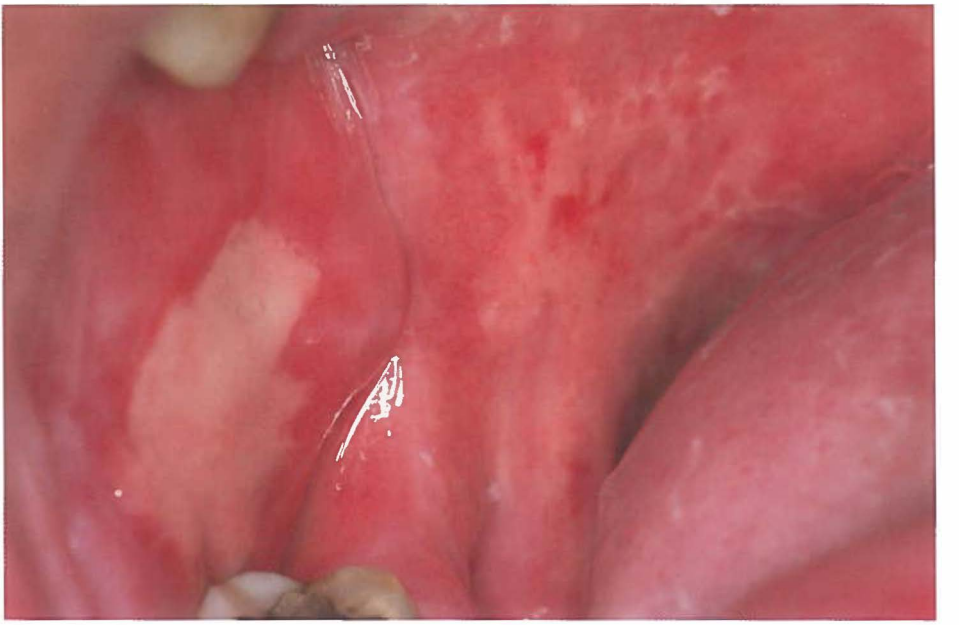


Figure 1. Radiation induced pseudomembranous mucositis on the right cheek and soft palate region of the mouth, after 32 Gy cumulative radiation dose (conventional fractionation schedule, 2 Gy per day, 5 times a week)

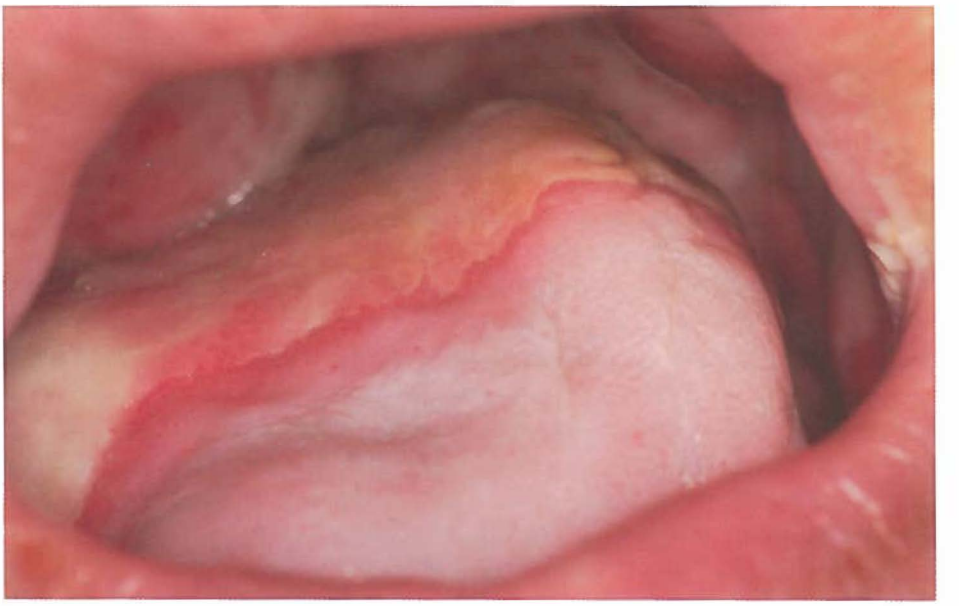


Figure 2. Chemoradiation induced pseudomembranous mucositis on the dorsum of the tongue and pharyngeal region of the mouth, after 52 Gy cumulative radiation dose and two chemotherapy cycles with carboplatin and 5-fluorouracil. A demarcation is noticed between the radiated and non radiated area of the tongue

Accurate evaluation of the effect of new preventive modalities on mucositis is a prerequisite, at which in multicenter trials, establishment of adequate inter-evaluator reliability is an important concern, with mucositis as outcome variable. Training of evaluators in scoring oral mucositis is thought to be important to increase inter-evaluator reliability.[9] In **chapter 3** the effect of training of evaluators on scoring of oral mucositis is studied.

Mostly clinical observational oral mucositis scores are based on a combination of mucosal parameters (signs) together with general complaints (symptoms). Differences in definition and operationalization of these general complaints hamper proper comparison of the outcomes using these scoring systems.[10] With respect to the mechanisms of mucositis, it seems to be important to investigate it more objectively on a cellular level. **Chapter 4** describes an assay based on cells retrieved from an oral mouthwash to quantify oral mucositis in head and neck cancer patients who received radiotherapy and to compare the results with the WHO scoring system.

In neutropenic cancer patients, oral mucositis is a potential portal of entry for the indigenous oral flora leading to bacteremia or sepsis. Fever is one of the first clinical signs of bacterial infection in these patients. The standard therapy for patients with fever and chemotherapy induced neutropenia is hospitalization and intravenous administration of broad-spectrum antibiotics. A risk assessment model has been developed, using objective clinical parameters and the plasma IL-8, to select patients with febrile neutropenia at low risk for bacterial infection.[11] Plasma IL-8 is considered to be a systemic inflammatory response parameter to foci of infection. **Chapter 5** describes the relationship between oral mucositis and systemic IL-8 levels in neutropenic cancer patients with fever, without a local bacterial infection or a clinical sepsis.

Changed oral flora, colonizing the oral mucosa, may aggravate the mucosa reaction following radiation. The carriage and colonization of aerobic Gram-negative bacilli are thought to play a role in the pathogenesis of irradiation mucositis.[12] In **chapter 6** the results are presented of a randomized, double blind, placebo-controlled study evaluating the effects of selective oral flora elimination with an antibiotic lozenge on the development of irradiation induced oral mucositis, feeding, weight loss and colonization of aerobic Gram-negative bacilli and yeast.

Radiation injury to the epithelium, causing mucositis, is associated with production of active oxygen and cytokines. During this process cyclooxy-

genase 2 (COX-2) is induced and has been found to be responsible for the synthesis of prostaglandins which cause the separation of tight cellular junctions and increase in vascular permeability.[13] Flurbipofen is an efficient inhibitor of COX-2, and might therefore delay or prevent mucositis.[14] In addition flurbiprofen has anti-inflammatory and anti-proliferative properties which probably can also delay oral mucositis and/or alleviate the severity of this side-effect. **Chapter 7** describes the outcomes of a pilot study evaluating the effects of flurbiprofen in a tooth patch on the development, severity and duration of pseudomembranous oral mucositis in patients treated with curative head and neck radiotherapy.

Cytoprotective agents, such as amifostine (Ethyol®, WR-2721), may reduce the toxicity induced by radiotherapy or chemotherapy. Amifostine is a prodrug, which is active as a protective agent when dephosphorylated by alkaline phosphatase to its active metabolite WR-1065. Once inside the cell, WR-1065 protects against chemotherapy and radiotherapy induced damage by scavenging free radicals, donating hydrogen ions to free radicals, depleting oxygen, and direct binding and inactivating cytotoxic drugs.[15] **Chapter 8** presents the results of a phase II trial evaluating the effect of local application of WR-1065, the active compound of amifostine, on oral mucositis in non-small cell lung cancer patients treated with epirubicin and gemcitabine. The effect of WR-1065 on mucositis was the primary end point of this study. The secondary end point was to determine the WR-1065 concentration in oral epithelial mucosa cells in this patient population.

Chapter 9 describes the summary of the thesis, considerations on the outcomes and potential future perspectives in mucositis research.

REFERENCES

1. Elting LS, Cooksley C, Chambers M, Cantor SB. The burdens of cancer therapy - Clinical and economic outcomes of chemotherapy-induced mucositis. *Cancer* 2003;98:1531-1539.
2. Sonis ST, Oster G, Fuchs H, Bellm LA, Bradford WZ, Edelsberg J, Hayden V, Eilers J, Epstein JB, LeVeque FG, Miller C, Peterson DE, Schubert MM, Spijkervet FKL, Horowitz M. Oral mucositis and the clinical and economic outcomes of hematopoietic stem-cell transplantation. *J Clin Oncol* 2001;19:2201-2205.
3. Ohrn KE, Sjoden PO, Wahlin YB, Elf M. Oral health and quality of life among patients with head and neck cancer or haematological malignancies. *Support Care Cancer* 2001;9:528-538.
4. Bellm LA, Epstein JB, Rose-Ped A, Martin P, Fuchs HJ. Patient reports of complications of bone marrow transplantation. *Support Care Cancer* 2000;8:33-39.

5. Rose-Ped AM, Bellm LA, Epstein JB, Trotti A, Gwede C, Fuchs HJ. Complications of radiation therapy for head and neck cancers - The patient's perspective. *Cancer Nurs* 2002;25:461-467.

6. Trotti A, Bellm LA, Epstein JB, Frame D, Fuchs HJ, Gwede CK, Komaroff E, Nalysnyk L, Zilberberg MD. Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: a systematic literature review. *Radiother Oncol* 2003;66:253-262.

7. Filicko J, Lazarus HM, Flomenberg N. Mucosal injury in patients undergoing hematopoietic progenitor cell transplantation: new approaches to prophylaxis and treatment. *Bone Marrow Transplant* 2003;31:1-10.

8. Ruescher TJ, Sodeifi A, Scrivani SJ, Kaban LB, Sonis ST. The impact of mucositis on alpha-hemolytic streptococcal infection in patients undergoing autologous bone marrow transplantation for hematologic malignancies. *Cancer* 1998;82:2275-2281.

9. Kobak KA, Engelhardt N, Williams JBW, Lipsitz JD. Rater training in multicenter clinical trials: Issues and recommendations. *J Clin Psychopharmacol* 2004;24:113-117.

10. Parulekar W, Mackenzie R, Bjarnason G, Jordan RCK. Scoring oral mucositis. *Oral Oncol* 1998;34:63-71.

11. Oude Nijhuis CS. Fever and neutropenia in cancer patients; a risk-adapted approach (dissertation). Groningen, The Netherlands: University Medical Center Groningen; 2004.

12. Spijkervet FKL, van Saene JJM, Panders AK, Vermey A, Mehta DM. Mucositis prevention by selective elimination of oral flora in irradiated head and neck cancer patients. *J Oral Pathol Med* 1990;19:486-489.

13. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, van de Putte LBA, Lipsky PE. Cyclooxygenase in biology and disease. *FASEB J* 1998;12:1063-1073.

14. Vane JR, Botting RM. Anti-inflammatory drugs and their mechanism of action. *Inflamm Res* 1998;47:S78-S87.

15. Culy CR, Spencer CM. Amifostine: an update on its clinical status as a cytoprotectant in patients with cancer receiving chemotherapy or radiotherapy and its potential therapeutic application in myelodysplastic syndrome. *Drugs* 2001;61:641-684.

2 Preventive intervention possibilities in radiotherapy and chemotherapy induced oral mucositis: results of meta-analyses

M.A. Stokman
F.K.L. Spijkervet
H.M. Boezen
J.P. Schouten
J.L.N. Roodenburg
E.G.E. de Vries

Submitted

ABSTRACT

Aim This meta-analyses aims to evaluate the effectiveness of interventions for the prevention of oral mucositis in cancer patients, treated with head and neck radiotherapy and/or chemotherapy; with a focus on randomized clinical trials.

Methods A literature search was performed for randomized controlled clinical studies between 1966-2004 aiming at prevention of mucositis, in cancer patients, undergoing head and neck radiation, chemotherapy or chemoradiation. The control group consisted of a placebo, no intervention or another intervention group. Mucositis was scored either by the WHO, or the NCI-CTC score, or the absence or presence of ulcerations, or the presence or absence of grade 3 and 4 mucositis.

Results The meta-analyses included 45 studies fulfilling the inclusion criteria, in which 8 different interventions were evaluated; i.e. local application of chlorhexidine; iseganan; PTA (polymyxin E, tobramycine and amphotericin B); GM-CSF/G-CSF; oral cooling; sucralfate and glutamine; and systemic administration of amifostine and GM-CSF/G-CSF. Four interventions showed a significant preventive effect on the development or severity of oral mucositis; PTA with an odds ratio (OR) = 0.61 (95% confidence interval (CI) 0.39-0.96); GM-CSF OR= 0.53 (CI: 0.33-0.87); oral cooling OR=0.3 (CI: 0.16-0.56); amifostine OR=0.37 (CI: 0.15-0.89).

Conclusion To date, no single intervention completely prevents oral mucositis, so combined preventive therapy strategies seems to be required to ensure more successful outcomes.

INTRODUCTION

Oral mucositis is defined as an injury of the oral mucosa in cancer patients, either induced by radiation of patients who have head and neck cancer, or due to chemotherapy. This has debilitating and painful side-effects and adversely affects the nutritional status of the patient. Mucositis is associated with an increase in the number of systemic infections, days in hospital and overall costs, and these aspects have a negative impact on health-related quality of life (HRQOL).[1-5] Mucositis was reported to be the most troubling side-effect of cancer therapy by 38% of patients treated with head and neck radiation and 42% of the patients treated with high-dose chemotherapy.[6,7]

Many studies have been published about interventions for the prevention of mucositis, but most studies had small samples sizes, or used different scoring methods, which make comparison of the results difficult. Insufficient sample power, the lack of sensitivity of the outcomes measures, and study design flaws, make it hard to draw definitive conclusions regarding the use of these interventions or to provide evidence-based guidelines for the prevention of oral mucositis.[8]

This review aims to evaluate the effectiveness of interventions for the prevention of oral mucositis in cancer patients treated with head and neck radiotherapy, chemotherapy, or chemoradiation. It focuses, whenever possible, on meta-analyses of randomized clinical trials of interventions for the prevention of oral mucositis.

CAUSES OF MUCOSITIS

In a conventional radiotherapy scheme of fractionation, a first mucosal reaction in the form of a white mucosal hyperkeratinization can be observed after a cumulative radiation dose of 10-20 Gy. Clinically, erythema is considered to be the first sign which is usually visible after 20 Gy cumulative dosage. Thereafter, ulcerations can occur which are often covered with a pseudomembranous layer. This more severe stage of mucositis will develop after about 30 Gy, mostly after 3 weeks of radiotherapy.[9] After completion of the radiotherapy the mucositis will decline between 2 to 6 weeks later.

The oral mucositis induced by chemotherapy is more acute than that due to radiotherapy.[10] Around 5-8 days following chemotherapy erythema occurs which usually is followed, within 2 days by edema and ulceration. Mucositis due to chemotherapy lasts for approximately 7 to 10 days.

Mucositis lesions, both in radiotherapy and chemotherapy, are localized in the non-keratinized mucosa such as the buccal and labial mucosa, ventral and lateral surface of the tongue, floor of the mouth and soft palate.

The incidence of mucositis is dependent on the cancer treatment regimen. The current head and neck radiotherapy protocols have a mucositis incidence of 85-100%. For altered fractionated radiation the incidence is 100%, for chemoradiation 89% and for conventional radiation 97%.[11] The incidence of mucositis can approach 90-100% in patient receiving aggressive myeloablative chemotherapy.[12] In solid tumor patients, who have chemotherapy induced myelosuppression, mucositis occurred during 37% of 1,236 cycles of chemotherapy.[1]

The severity of mucositis depends on different factors; e.g. anti-cancer treatment protocol, age and diagnosis of the patient, level of oral hygiene during therapy and genetic factors.[13-15]

Historically, mucositis was thought to arise as a consequence of direct and indirect toxic effects on epithelial cells. The colonization of the damaged mucosa by bacteria, fungi and viruses can superimpose secondary infections.[16-18] Furthermore, it was thought that the development of mucositis was facilitated by trauma, i.e. due to the effects of dentures on the oral mucosa or oral hygiene habits.[13,19]

Nowadays mucositis is recognized as an epithelial and sub-epithelial injury and is thought to develop in a five-stage model, namely: 1) initiation; 2) upregulation with generation of messengers; 3) signaling and amplification; 4) ulceration with inflammation; and 5) healing.[20] In figure 1 possible pathways of interventions for mucositis prevention are depicted in relation to this model. The development of mucositis according this five-stage model is an immediate and simultaneously process in all the different tissue compartments and cellular levels.

1) Initiation

Radiotherapy and chemotherapy induce cell damage. Mucosal cell death is induced by DNA strand breaks and found in both the epithelial and sub-epithelial cells. In this stage most cell destruction is found in the submucosa, whereas the effects on the basal membrane and stratum spinosum are more selective.[21] Simultaneously, non-DNA related cell damage starts by the production of reactive oxygen species by both radiotherapy and chemotherapy.[22] These reactive oxygen species directly damage cells, tissues and blood vessels.

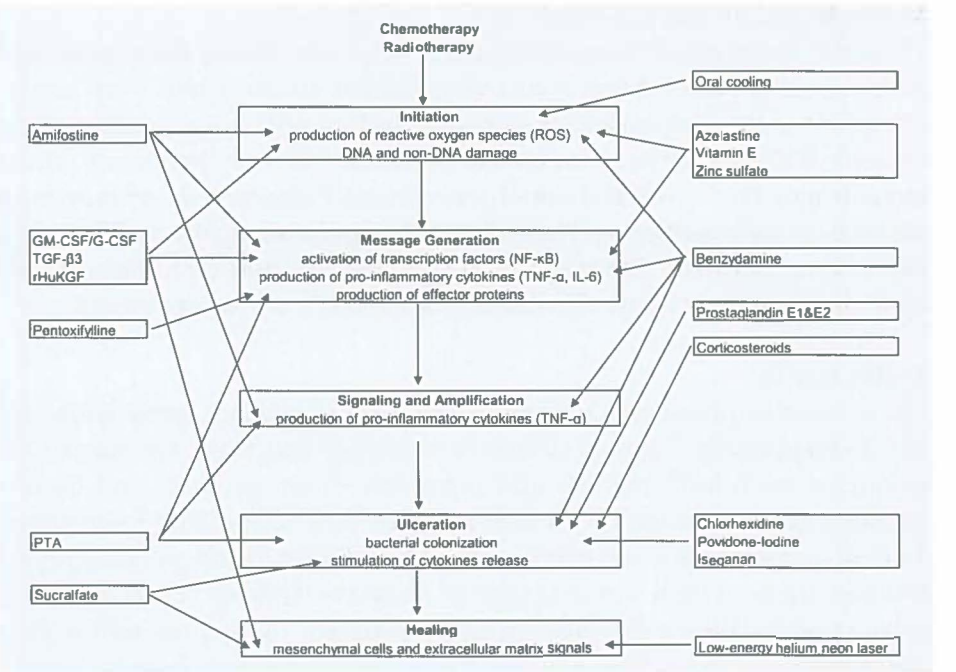


Figure 1. The development of mucositis in a 5 stage model (adapted from Sonis (2004)) and pathways of interventions for mucositis prevention

2) Message generation

Radiotherapy and chemotherapy, together with the induced reactive oxygen species, effectively activate biological control mechanisms, including a select group of transcription factors, of which NF-κB is probably one of the most significant.[23,24] Activated NF-κB can upregulate up to 200 genes, including those that code for pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and adhesion molecules which produce effector proteins that initiate tissue injury. At the same time, radiotherapy, chemotherapy and reactive oxygen species activate both neutral and acidic sphinogmyelinases and ceramide synthase, which mediate death of submucosal endothelial cells and fibroblasts.[25] Moreover, fibroblast destruction is associated with fibronectin and metalloproteinases production, which results in increased apoptosis. This rapid cascade of changes occurs in a clinically normal mucosal lining.

3) Signaling and amplification

The radiotherapy and chemotherapy related production in the mucosa and submucosal of proteins has two distinguishable effects. Apart from target-cell injury these proteins affect signaling and amplification functions. For example TNF-α is not only an efficient mediator of cellular and tissue injury, but also activates NF-κB and sphinogmyelinase. This potentiates the activity of these control mechanisms following the initial damage by radiotherapy and chemotherapy.[24] During this phase, the apoptotic changes in the epithelium result in clinically observable altered mucosal appearance.

4) Ulceration

The ulcerative phase of mucositis is the most clinically significant event and is responsible for pain and loss of barrier function. The ulcers are colonized with both aerobic and anaerobic Gram-positive and Gram-negative microorganisms. Cell wall products from colonizing bacteria are likely to penetrate into the submucosa, where they can activate infiltrating macrophages, which are thought to increase TNF-α, IL-1β and IL-6 production.[26] These cytokines probably promote the expression of pro-apoptotic genes and potentiate tissue injury. Inflammatory cells then migrate by chemotaxis to the base of the lesion, where they produce damaging enzymes.

5) Healing

Mucosal ulcers induced by radiotherapy and chemotherapy heal spontaneously after cessation of therapy by the dynamic mechanisms of epithelial wound healing. It is thought that submucosal extracellular matrix and mesenchyme regulate epithelial-cell migration and proliferation. An intact mucosal surface is created by epithelial cell migration, over the denuded connective tissue.

LITERATURE SEARCH STRATEGY AND STATISTICAL ANALYSES

The Medline, Embase and CINAHL databases were searched for articles published from January 1966 to December 2004, using the following search strategy: [neoplasms] AND [(mucositis OR stomatitis)] AND [limit to (clinical trial OR randomized-controlled trial)]. Citation lists were examined and all interventions identified were listed and were classified according to their possible mechanism of action. The search was repeated for each

intervention using the strategy described above. All articles found with this search were retrieved and selected on the basis of the inclusion criteria. Included in this review were randomized controlled clinical studies aiming prevention of mucositis, of patients undergoing head and neck radiation, chemotherapy or chemoradiation, and written in English. The control group consisted of a placebo, no intervention, or another intervention group. The outcome of mucositis was scored by the WHO score or the NCI-CTC score, the absence or presence of ulcerations or the presence or absence of grade 3 and 4 mucositis. Only studies in which the data on these outcome variables were available were included in the meta-analysis. Studies were excluded if inadequate data were available on the outcome variable of mucositis.

The literature search revealed 109 publications, but in five of these, prevention of oral mucositis was not the study objective. Of the remaining 104 studies, 13 were non-randomized and 29 studies did not contain data in a comprehensive form. Seventeen articles each stood alone as far as their intervention was concerned. Therefore, a total of 45 articles were included in the meta-analyses (Table 1).

A meta-analysis was performed to estimate the effect of the different interventions on the outcome variable of mucositis defined by presence of mucositis, ulceration and grade 3 and 4 mucositis for the several combined studies. When the included studies showed heterogeneity regarding the effect estimates, the results of the meta-analyses are based on the random effects models, otherwise the results are based on the fixed effects models. Random effects (DerSimonian-Laird) meta-analysis computes the odds ratios (OR) of the individual studies, the summary, the random effects variance, and Woolf's test for heterogeneity. The fixed effect (Mantel-Haenszel) meta-analysis computes the Mantel-Haenszel summary. Studies with zero or infinite ORs were omitted, as their variance cannot be calculated with accuracy. ORs were considered to be significant if the 95% confidence interval (95% CI) did not include the value 1.

The 'plot' method presented in the figures shows standard meta-analysis plots. The 95% confidence interval for each study is depicted by a horizontal line, and the point estimate is depicted by a square whose height is inversely proportional to the standard error of the estimate. The summary OR is drawn as a diamond with horizontal limits at the confidence limits and width inversely proportional to its standard error. Meta-analyses were performed using the Rmeta package of the R Project for Statistical Computing (Build 2.0.1).[27]

Table 1. Studies included in the meta-analyses

Reference	No. of study patients (intervention/control)	Cause	Controlled/ Double-blind	Study characteristics	Result-impact on oral mucositis
Antiseptic and antimicrobial agents					
Chloorhexidine					
Cheng, 2003 [31]	34 (17/17)	CTX[a]	Yes/No	Chlorhexidine versus benzydamine	Reduction
Dodd, 1996 [32]	222 (110/112)	CTX	Yes/Yes	Chlorhexidine versus placebo	No difference
Ferretti, 1988 [35]	49 (24/25)	CTX + BMT[n]	Yes/Yes	Chlorhexidine versus placebo	Significant reduction
Ferretti, 1990 [36]	36 (18/18)	CTX	Yes/Yes	Chlorhexidine versus placebo	Significant reduction
Ferretti, 1990 [36]	28 (15/13)	RTX[c]	Yes/Yes	Chlorhexidine versus placebo	No difference
Foote, 1994 [37]	52 (25/27)	RTX	Yes/Yes	Chlorhexidine versus placebo	No difference
Pitten, 2003 [39]	47 (24/23)	CTX	Yes/Yes	Chlorhexidine versus fluoride solution	No difference
Spijkervet, 1989 [40]	30 (15/15)	RTX	Yes/Yes	Chlorhexidine versus placebo	No difference
Iseganan					
Giles, 2004 [44]	502 (251/251)	CTX	Yes/Yes	Iseganan versus placebo	No difference
Trotti, 2004 [45]	424 (253/171)	RTX/CTX + RTX	Yes/Yes	Iseganan versus placebo	No difference
PTA					
El Sayed, 2002 [47]	136 (69/67)	RTX	Yes/Yes	Antimicrobial lozenge versus placebo	No difference
Okuno, 1997 [48]	103 (49/54)	RTX	Yes/Yes	PTA lozenges versus placebo	No difference
Stokman, 2003 [49]	58 (28/30)	RTX	Yes/Yes	PTA lozenges versus placebo	No difference
Symonds, 1996 [50]	221 (112/109)	RTX	Yes/Yes	PTA lozenges versus placebo	No differences
Wijers, 2001 [51]	77 (39/38)	RTX	Yes/Yes	PTA oral paste versus placebo	No difference
Cytokines and/or growth factors					
GM-CSF/G-CSF					
Cartee, 1995 [62]	45 (36/9)	CTX	Yes/Yes	GM-CSF versus placebo	No difference
Chi, 1995 [63]	20 (9/11)	CTX	Yes/No	GM-CSF versus no intervention	Significant reduction
Crawford, 1999 [64]	195 (93/102)	CTX	Yes/Yes	r-metHuG-CSF versus placebo	Significant reduction
Dazzi, 2003 [65]	90 (46/44)	CTX + BMT	Yes/Yes	GM-CSF versus placebo	No differences
Katano, 1995 [68]	14 (7/7)	CTX	Yes/No	G-CSF versus no intervention	Significant reduction
Makkonen, 2000 [69]	40 (20/20)	RTX	Yes/No	GM-CSF versus no intervention	No difference
Nemunaitis, 1995 [70]	109 (53/56)	CTX + BMT	Yes/Yes	rhGM-CSF versus placebo	Significant reduction
Schneider, 1999 [72]	14 (8/6)	RTX	Yes/Yes	r-metHuG-CSF versus placebo	Reduction
Valcarcel, 2002 [73]	35 (16/19)	CTX + BMT	Yes/Yes	rhGM-CSF versus placebo	No differences
van der Lelie, 2001 [74]	36 (18/18)	CTX + BMT	Yes/Yes	GM-CSF versus placebo	No difference

Locally applied non-pharmacological methods					
Oral cooling					
Cascinu, 1994 [77]	84 (44/40)	5-FU[d]	Yes/No	Oral cooling versus no intervention	Significant reduction
Mahood, 1991 [78]	93 (50/43)	5-FU	Yes/No	Oral cooling versus no intervention	Significant reduction
Mouth coating agents					
Sucralfate					
Carter, 1999 [82]	102 (52/50)	RTX	Yes/Yes	Sucralfate versus placebo	No difference
Castagna, 2001 [83]	102 (51/51)	CTX	Yes/Yes	Sucralfate versus placebo	Reduction of mucositis
Cengız, 1999 [84]	28 (18/10)	RTX	Yes/Yes	Sucralfate versus placebo	Significant reduction
Chiara, 2001 [85]	40 (20/20)	CTX	Yes/Yes	Sucralfate versus placebo	No difference
Dodd, 2003 [86]	30 (14/16)	RTX	Yes/Yes	Sucralfate versus salt & soda mouthwash	No difference
Makkonen, 1994 [91]	40 (20/20)	RTX	Yes/Yes	Sucralfate versus placebo	No difference
Nottage, 2003 [92]	80 (41/39)	CTX	Yes/Yes	Sucralfate versus placebo	No difference
Pfeiffer, 1990 [93]	23 (23/23)	CTX	Yes/Yes	Sucralfate versus placebo	Significant reduction
Shenep, 1988 [94]	48 (24/24)	CTX	Yes/Yes	Sucralfate versus placebo	No difference
Radical scavengers					
Amifostine					
Antonadou, 2002 [97]	45 (22/23)	RTX+CTX	Yes/No	Amifostine versus no intervention	Significant reduction
Bourhis, 2000 [98]	24 (12/12)	RTX	Yes/No	Amifostine versus no intervention	Reduction
Brizel, 2000 [99]	303 (153/150)	RTX	Yes/No	Amifostine versus no intervention	No difference
Buntzel, 1998 [100]	39 (25/14)	RTX+CTX	Yes/No	Amifostine versus no intervention	Significant reduction
Hartmann, 2001 [101]	40 (20/20)	CTX+BMT	Yes/No	Amifostine versus no intervention	Significant reduction
Koukourakis, 2000 [102]	39 (19/20)	RTX	Yes/No	Amifostine versus no intervention	Significant reduction
Lorusso, 2003 [103]	187 (93/94)	CTX	Yes/No	Amifostine versus no intervention	Significant reduction
Amino Acid					
Glutamine					
Huang, 2000 [109]	17 (8/9)	RTX	Yes/No	Oral glutamine versus placebo	Reduction
Okuno, 1999 [111]	134 (66/68)	5-FU	Yes/Yes	Oral glutamine versus placebo	No difference

[a]CTX=chemotherapy; [b]BMT=bone marrow transplantation; [c]RTX=radiotherapy; [d]5-FU=5-fluorouracil

INTERVENTION STRATEGIES

For the various intervention strategies, results of the meta-analyses are presented when more than one study of an intervention is available that fulfilled the inclusion criteria.

Basic Oral Care

The NIH consensus (1989) states that patients who receive head and neck radiation or chemotherapy must be evaluated, before the start of cancer treatment for potential risk factors for oral complications, by a thorough oral and dental evaluation, including a radiographic examination.[28]

In two randomized clinical trials various different oral care protocols were tested.[29,30] In both studies an intensive oral care protocol was compared with a standard oral care protocol. The intensive oral care protocol varied in the different studies. In one study, intensive oral care included treatment of dental lesions before the chemotherapy, and tooth and gum brushing during aplasia (granulocytes < 0.5×10^9/l and/or platelet count < 20×10^9/l).[29] In another study radiotherapy patients, were divided in three groups. In two groups the protocol consisted of oral care instructions, tooth brushing and rinsing with sterile water. One group started on day 1 of radiotherapy and one group started 1 week before radiotherapy. The control group received no instructions.[30] Both studies showed that additional oral care is important and has a positive attenuation effect in the development of oral mucositis. No meta-analysis was performed because in one study the data were not presented according to the inclusion criteria on the outcome variable and therefore only one study was available for analysis.

Topical antiseptic and antimicrobial agents

Studies with topical antiseptic and antimicrobial agents attempted to determine whether oral mucositis, due to colonization of the mouth with aerobic and anaerobic Gram-positive and Gram-negative microorganisms or yeast, could be prevented.

Chlorhexidine, as an oral rinse, with concentrations of at least 0.12% is an anti-plaque agent with potential antimicrobial activity. It has been evaluated in ten randomized clinical trials for preventing oral mucositis but the outcomes were different.[31-40] Of these studies only seven fulfilled the inclusion criteria on the outcome variable for the meta-analysis (Table 1).

The study by Ferretti and colleagues was included in the database as two different studies, one for radiotherapy, and one for chemotherapy.[36] Three studies used "presence of ulceration" as the outcome, one study used "presence of mucositis" and four studies used both. The meta-analysis however showed no effect of chlorhexidine in the prevention of mucositis in chemotherapy and radiotherapy patients, OR=0.7 (CI: 0.43-1.12).

Povidone-iodine is an antiseptic agent that is effective against oral bacteria. In the single randomized clinical trial in which povidone-iodine was used as a mouth rinse (Betaisosona® Mund-Antiseptikum, diluted 1:8), radiochemotherapy induced mucositis was significantly decreased.[41]

Iseganan is a structural analogue of naturally occurring protegin-1. Protegins, initially isolated from porcine neutrophils, are antimicrobial peptides involved in local and systemic host defense. Iseganan has rapid microbicidal activity in saliva. It is a microbicidal against a broad spectrum of endogenous oral flora including Gram-positive and Gram-negative bacteria and yeast, and it can be applied topically to the oral mucosa with no detectable systemic absorption.[42] In the three published studies, there was no significant effect of iseganan on the prevention of oral mucositis induced by radiotherapy, chemotherapy or chemoradiation.[43-45] The first study of Giles (2003) is not included in this meta-analysis because of a major randomization flaw.[43] The two included studies used the "presence of ulceration" as the outcome and evaluated both chemotherapy and radiotherapy patients. The meta-analysis showed no effect on prevention of ulcerations, OR=0.75 (CI: 0.55-1.02).

Other studies investigated the effects of topical application of antimicrobials by a combination of polymyxin E, tobramycine and amphotericin B (PTA) in a lozenge or paste for selective elimination of the oral flora in radiotherapy patients. The goal of this combination was to eliminate aerobic Gram-negative bacteria and yeast.[46] Several randomized placebo controlled trials, employing PTA, have been conducted and the results were not in total agreement.[47-51] All five studies, all in radiotherapy patients, were included in the meta-analysis. All these studies used "presence of mucositis" and "presence of ulcerations" as the outcome. The meta-analysis found a preventive effect of PTA on the development of ulcerations with an OR of 0.61 (CI: 0.39-0.96) (Figure 2). However, no effect on the prevention of mucositis was found in radiotherapy patients.

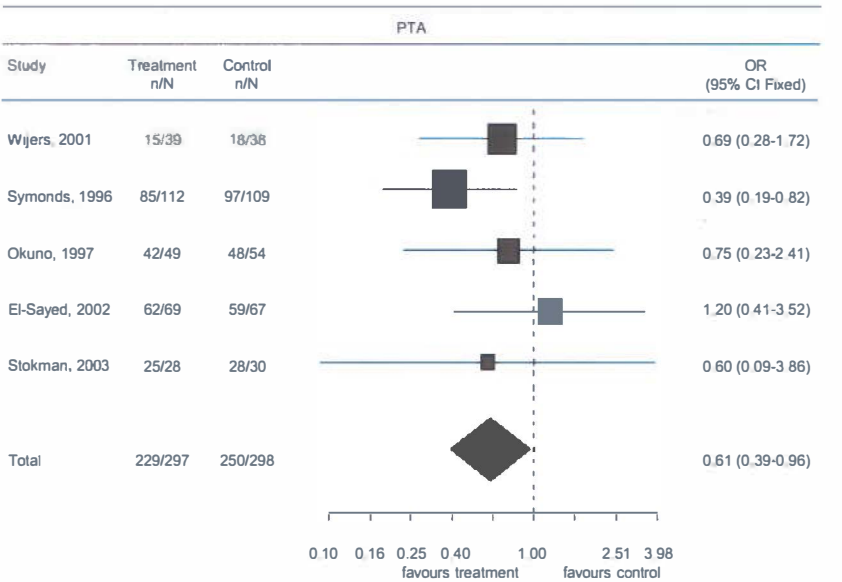


Figure 2. Standard meta-analysis plot result of PTA for the outcome "presence of ulceration"

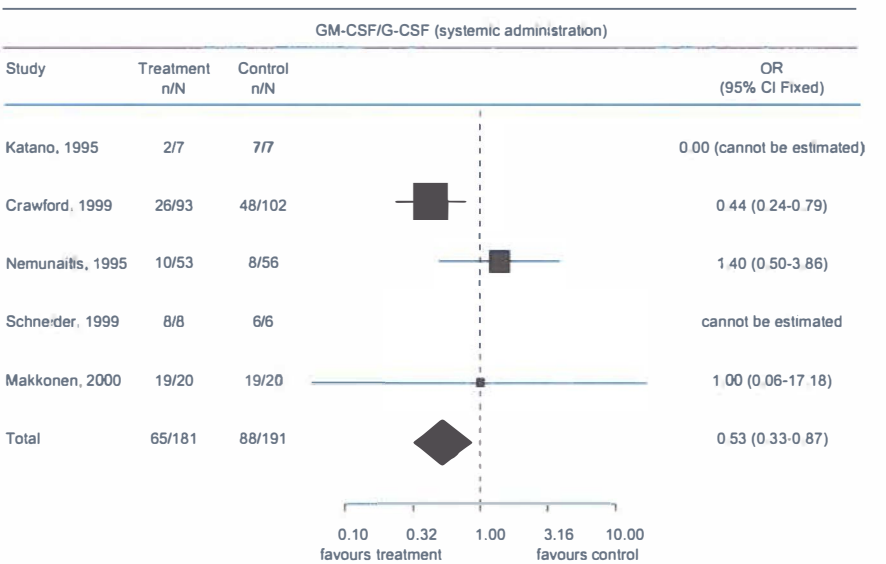


Figure 3. Standard meta-analysis plot result of GM-CSF/G-CSF (systemic administration) for the outcome "presence of mucositis"

Anti-inflammatory agents

Benzydamine is a non-steroidal drug with analgesic, anesthetic, anti-inflammatory, and antimicrobial properties. Benzydamine inhibits the production and effects of inflammatory cytokines, particularly TNF-α. It has especially been evaluated for the prevention and reduction of radiation induced oral mucositis with different outcomes.[52-56] In a large, multicenter, double-blinded randomized trial, benzydamine improved the ulcer-free rate and diminished the incidence of ulceration and erythema.[57] However, no study contained data that fulfilled the inclusion criteria on the outcome variable for the meta-analysis.

Topical prostaglandins are agents believed to possess anti-inflammatory and cytoprotective properties. Both prostaglandin E_1 (misoprostol) and prostaglandin E_2 have been evaluated in a small series of radiotherapy or chemotherapy patients and there were conflicting outcomes.[58-60] The manner in which the data was presented did not permit a meta-analysis.

In one study, corticosteroids showed no reduction in the intensity or duration of radiotherapy induced mucositis.[61]

Cytokines and/or growth factors

The hematopoietic growth factors, granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) can promote the accumulation of activated neutrophils in the mucosa, and can directly induce proliferation of endothelial cells and keratinocytes. The mucosal protective effects of GM-CSF and G-CSF were tested in 13 randomized placebo controlled trials, with systemic administration or local application by mouthwashes, in various chemotherapy, radiotherapy, or chemoradiation regimens.[62-74] Ten out of 13 studies could be included in the meta-analysis (Table 1). Six studies used GM-CSF/G-CSF as a systemic administration [63,64,68-70,72] and 4 studies used GM-CSF/G-CSF as a mouthwash.[62,65,73,74] The "presence of mucositis" was used in 5 out of 6 studies (systemic intervention) and in 3 out of 4 studies (mouthwash) as the outcome. The meta-analysis found a significant effect of GM-CSF and G-CSF in preventing mucositis in the systemic intervention group with an OR of 0.53 (CI: 0.33-0.87) (Figure 3). No preventive effect was found for the topical administration of GM-CSF and G-CSF, OR=0.32 (CI: 0.06-1.67).

Transforming growth factor-β3 (TGF- β3) is a cytokine that stimulates or inhibits cell proliferation depending on cell type. The interim analysis of a randomized clinical trial showed no preventive effect on chemotherapy induced oral mucositis.[75]

Probably, the most promising growth factor is the recombinant human keratinocyte growth factor (rHuKGF; palifermin). In a phase III, randomized, double-blind, placebo-controlled study palifermin, intravenously, significantly reduced the incidence (35%) and duration (3 days) of severe mucositis in patients with hematologic malignancies undergoing autologous peripheral blood progenitor cell transplantation after fractionated total-body irradiation.[76] Palifermin seems promising for the chemotherapy patient but data on radiotherapy patients are not yet available.

Locally applied non-pharmacological methods

Oral cooling of the mouth by sucking on ice cubes causes local vasodilatation and reduces blood flow to the epithelium. Thus, it was thought that this approach would reduce the chemotherapeutic drug delivery to oral mucosa cells. In two randomized trials in patients receiving a 5-fluorouracil bolus a 50% reduction in mucositis was observed by sucking on ice.[77,78] These two studies were included in the meta-analysis. Both studies used "presence of mucositis" as the outcome for the prevention of 5-fluorouracil induced mucositis. The meta-analysis for this outcome revealed a significant preventive effect with an OR of 0.3 (0.16-0.56) (Figure 4).

Low-energy helium-neon laser has been reported to promote wound healing and to reduce pain and inflammation. In three double-blind controlled studies, the severity and duration of oral mucositis in radiation and chemotherapy patients, treated with low-energy helium-neon laser was reduced [79-81]. Unfortunately, the manner in which the data was presented did not permit a meta-analysis.

Mouth coating agents

Sucralfate is a basic aluminium salt of sucrose octasulfate which in the past has been used in the treatment of gastric and duodenal ulcers. Nowadays, it is sometimes used in the treatment of radiation-esophagitis. Sucralfate acts as a protective coating by forming an ionic bond to proteins in the ulcer site. Furthermore, sucralfate stimulates prostaglandin production and mucosal cell renewal. To date, there have been 13 randomized trials with sucralfate, as an oral suspension, performed in various treatment protocols.[82-94] Nine out of 13 studies could be included in the meta-analysis (Table 1). Only two [84,88] reported a significant reduction of radiation induced mucositis Five studies used "presence of mucositis" as outcome, one study used "presence of ulceration" and three studies used both. The meta-analysis

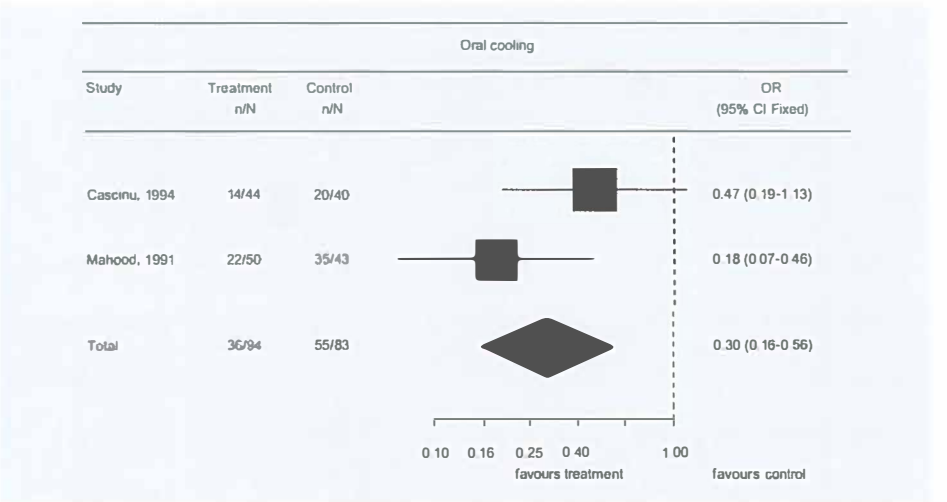


Figure 4. Standard meta analysis plot result of oral cooling for the outcome "presence of mucositis"

found no effect of sucralfate on prevention of mucositis or ulcerations in chemotherapy and radiotherapy patients, OR=0.82 (CI: 0.05-1.33).

Radical scavenger

Amifostine (WR 2721, Ethyol) is an organic thiophosphate compound which selectively protects normal cells from treatment-related toxicity. It is a pro-drug, which is active as a protective agent when dephosphorylated by alkaline phosphatase to its active metabolite WR-1065. WR-1065 is preferentially taken up by normal rather than neoplastic cells because of the higher alkaline phosphatase activity, better vascularization and higher pH of normal tissue. Once inside the cell, WR-1065 protects against chemotherapy and radiation induced damage by scavenging free radicals, donating hydrogen ions to free radicals, depleting oxygen, and by direct binding to and inactivating cytotoxic drugs.[95,96] Nine randomized clinical trials have used amifostine intravenously or subcutaneously administration for the prevention of mucositis in various treatment regimens with different outcomes.[97-105] Seven studies could be included in the meta-analysis (Table 1). All studies used "presence of grade 3 and 4 mucositis" as the outcome and were performed in chemotherapy, radiotherapy or chemoradiation patients. The meta-analysis found a significant effect of amifostine in the prevention of grade 3 and 4 mucositis in chemotherapy and radiotherapy patients with an OR of 0.37 (0.15-0.89) (Figure 5).

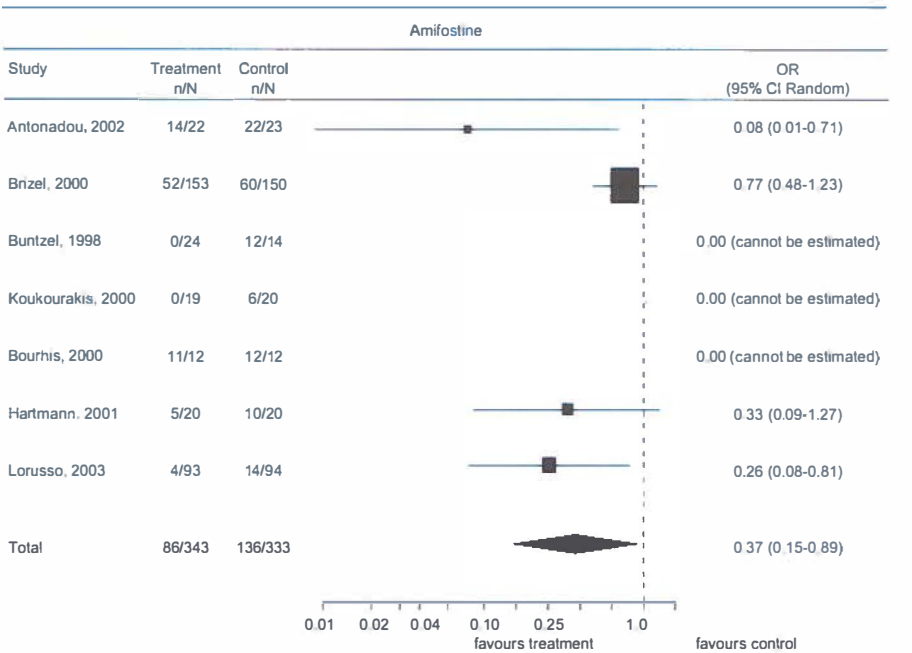


Figure 5. Standard meta analysis plot result of amifostine for the outcome "presence of grade 3 and grade 4 mucositis"

Amino acids

Glutamine is an amino acid, required for the support and maintenance of intestinal growth and function. During episodes of catabolic stress there is a marked intracellular depletion of glutamine.[106] Oral glutamine was tested in five studies with different outcomes.[107-111] Two studies could be used for the meta-analysis using the "presence of mucositis" as outcome variable.[109,111] The meta-analysis found no effect of glutamine on prevention of mucositis in chemotherapy and radiotherapy patients, OR=1.25 (CI: 0.61-2.59).

Anti-oxidants

The use of anti-oxidants such as azelastine hydrochloride [112], vitamin E [113] and zinc sulfate [114] for the prevention of oral mucositis in radiotherapy or chemoradiation seems to be interesting but needs further research.

Antineoplastic agents antagonists

Allopurinol is a structural isomer of hypoxanthine, which is used for the

treatment of gout. Some pilot studies have shown that allopurinol mouthwash decreased 5-fluorouracil induced mucositis. However, in the single randomized, placebo-controlled, double blind study no effect of allopurinol mouthwash on 5-fluorouracil induced mucositis was observed.[115]

Immunomodulatory drugs

Pentoxifylline is a xanthine derivate, capable of downregulating TNF-α production and stimulating vascular endothelial production of prostaglandins I_2 and E_2. The two randomized clinical trials studying the effect of oral administration of pentoxifylline for the prevention of chemotherapy induced mucositis failed to show any benefit.[116,117] The manner in which the data was presented did not permit a meta-analysis.

Anti-cholinergic agents

Oral administration of pilocarpine hydrochloride is approved for treatment of radiation induced xerostomia in several countries. It is a naturally occurring, cholinergic, parasympathomimetic alkaloid that has a broad range of pharmacologic effects, including increasing secretion from the exocrine glands (sweat, salivary, lachrymal, gastric, pancreatic and intestinal glands). Only one randomized, double-blind, placebo-controlled cross-over study has been published, showing a significant decrease in the development of chemotherapy induced oral mucositis.[118]

Miscellaneous

Traumeel S® is a homeopathic medication containing 12 botanical substances and 2 mineral substances. It has been sold over the counter in pharmacies for over 50 years and it is available worldwide for use as an anti-inflammatory, analgesic, anti-edematous and anti-exudative drug. In a randomized, placebo-controlled study, Traumeel S®, as a moutwash, significantly reduced the severity and duration of chemotherapy induced mucositis.[119]

The topical application of *honey* was investigated in a randomized, non-blinded study in radiotherapy patients.[120] In this study, a significant reduction of mucositis grade 3 and 4 was detected.

In a double-blind, randomized study the efficacy of a *calcium phosphate* mouth rinse (Caphosol®) with fluoride treatments was tested for the prevention of mucositis in patients undergoing hematopoietic stem cell transplantation.[121] The use of the mouthwash Caphosol® had a significant effect in decreasing the duration and severity of mucositis.

Aloe vera contains numerous vitamins and minerals, enzymes, amino acids, natural sugars and agents which may be anti-inflammatory and anti-microbial. The aloe vera gel has been used for topical treatment of wounds, minor burns, and skin irritations. In a double-blind, randomized clinical trial for prevention of radiation induced mucositis topical aloe vera did not show any benefit.[122]

Chamomile mouthwash is a solution prepared from the flower of the chamomile plant. This plant contains many different substances which are suggested to have anti-inflammatory, antibacterial and antifungal properties. The use of chamomile mouthwash in a double-blind, randomized clinical trial for the prevention of 5-fluorouracil induced mucositis did not show any benefit either.[123]

CONCLUSION AND PERSPECTIVES

A search of the literature revealed a total of 27 different interventions for the prevention of oral mucositis. Based on our criteria, a meta-analysis could be performed on eight of these interventions. Four interventions, namely PTA lozenges or paste, systemic administration of GM-CSF or G-CSF, oral cooling and amifostine showed a preventive effect on the development or severity of oral mucositis.

Our meta-analyses show that GM-CSF and G-CSF is the only intervention with some beneficial effect on the development of mucositis. PTA and amifostine decreased the severity of mucositis. Oral cooling prevented mucositis in 5-fluorouracil induced mucositis. Perhaps a multiple agent therapy that targets different biological events is necessary for the prevention of oral mucositis.

From the 14 single-published studies nine interventions showed some positive results in prevention of mucositis. Most studies consisted of small samples sizes, were not double-blinded or did not use a placebo-controlled design. Further research with well designed studies is necessary for providing evidence if any of the interventions is effective in prevention of mucositis. Palifermin, the recombinant human keratinocyte growth factor, intravenously, seems to be the most promising intervention for the prevention of mucositis in patients with hematologic malignancies undergoing autologous peripheral blood progenitor cell transplantation after fractionated total-body irradiation.

To date, it can be concluded that no single intervention is capable of completely preventing oral mucositis. Future studies should evaluate combination of interventions for the prevention of oral mucositis.

REFERENCES

1. Elting LS, Cooksley C, Chambers M, Cantor SB. The burdens of cancer therapy - Clinical and economic outcomes of chemotherapy-induced mucositis. *Cancer* 2003;98:1531-1539.
2. Ruescher TJ, Sodeifi A, Scrivani SJ, Kaban LB, Sonis ST. The impact of mucositis on alpha-hemolytic streptococcal infection in patients undergoing autologous bone marrow transplantation for hematologic malignancies. *Cancer* 1998;82:2275-2281.
3. Sonis ST, Oster G, Fuchs H, Bellm LA, Bradford WZ, Edelsberg J, Hayden V, Eilers J, Epstein JB, LeVeque FG, Miller C, Peterson DE, Schubert MM, Spijkervet FKL, Horowitz M. Oral mucositis and the clinical and economic outcomes of hematopoietic stem-cell transplantation. *J Clin Oncol* 2001;19:2201-2205.
4. Stiff P. Mucositis associated with stem cell transplantation: current status and innovative approaches to management. *Bone Marrow Transplant* 2001;27:S3-S11.
5. Ohrn KE, Sjoden PO, Wahlin YB, Elf M. Oral health and quality of life among patients with head and neck cancer or haematological malignancies. *Support Care Cancer* 2001;9:528-538.
6. Bellm LA, Epstein JB, Rose-Ped A, Martin P, Fuchs HJ. Patient reports of complications of bone marrow transplantation. *Support Care Cancer* 2000;8:33-39.
7. Rose-Ped AM, Bellm LA, Epstein JB, Trotti A, Gwede C, Fuchs HJ. Complications of radiation therapy for head and neck cancers - The patient's perspective. *Cancer Nurs* 2002;25:461-467.
8. Rubenstein EB, Peterson DE, Schubert M, Keefe D, McGuire D, Epstein J, Elting LS, Fox PC, Cooksley C, Sonis ST. Clinical practice guidelines for the prevention and treatment of cancer therapy-induced oral and gastrointestinal mucositis. *Cancer* 2004;100:2026-2046.
9. Spijkervet FKL, van Saene HKF, Panders AK, Vermey A, Mehta DM. Scoring irradiation mucositis in head and neck cancer patients. *J Oral Pathol Med* 1989;18:167-171.
10. Dreizen S, Bodey GP, Rodriguez V. Oral complications of cancer chemotherapy. *Postgrad Med* 1975;58:75-82.
11. Trotti A, Bellm LA, Epstein JB, Frame D, Fuchs HJ, Gwede CK, Komaroff E, Nalysnyk L, Zilberberg MD. Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: a systematic literature review. *Radiother Oncol* 2003;66:253-262.
12. Filicko J, Lazarus HM, Flomenberg N. Mucosal injury in patients undergoing hematopoietic progenitor cell transplantation: new approaches to prophylaxis and treatment. *Bone Marrow Transplant* 2003;31:1-10.
13. Dodd MJ, Miaskowski C, Shiba GH, Dibble SL, Greenspan D, MacPhail L, Paul SM, Larson P. Risk factors for chemotherapy-induced oral mucositis: dental appliances, oral hygiene, previous oral lesions, and history of smoking. *Cancer Invest* 1999;17:278-284.
14. Sonis ST, Fey EG. Oral complications of cancer therapy. *Oncology (Huntingt)* 2002;16:680-686.

15. Barasch A, Peterson DE. Risk factors for ulcerative oral mucositis in cancer patients: unanswered questions. *Oral Oncol* 2003;39:91-100.

16. Spijkervet FKL, van Saene HKF, Panders AK, Vermey A. Colonisation index of the oral cavity: a novel technique for monitoring colonisation defence. *Microb Ecol Health Dis* 1989;2:145-151.

17. Makkonen TA, Borthen L, Heimdahl A, Joensuu H, Lehtonen OP, Nord CE. Oropharyngeal colonisation with fungi and gram-negative rods in patients treated with radiotherapy of the head and neck. *Br J Oral Maxillofac Surg* 1989;27:334-340.

18. Rand KH, Kramer B, Johnson AC. Cancer chemotherapy associated symptomatic stomatitis: role of Herpes simplex virus (HSV). *Cancer* 1982;50:1262-1265.

19. Berger AM, Kilroy TJ. Oral complications. In: DeVita VT, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology*. Philadelphia: Lippincott-Raven, 1997;2714-2725.

20. Sonis ST. The pathobiology of mucositis. *Nature Reviews Cancer* 2004;4:277-284.

21. Denham JW, Hauer-Jensen M. The radiotherapeutic injury - a complex 'wound'. *Radiother Oncol* 2002;63:129-145.

22. Gate L, Paul J, Nguyen Ba G, Tew KD, Tapiero H. Oxidative stress induced in pathologies: the role of antioxidants. *Biomed Pharmacother* 1999;53:169-180.

23. Criswell T, Leskov K, Miyamoto S, Luo GB, Boothman DA. Transcription factors activated in mammalian cells after clinically relevant doses of ionizing radiation. *Oncogene* 2003;22:5813-5827.

24. Sonis ST. The biologic role for nuclear factor-kappaB in disease and its potential involvement in mucosal injury associated with anti-neoplastic therapy. *Crit Rev Oral Biol Med* 2002;13:380-389.

25. Paris F, Fuks Z, Kang A, Capodieci P, Juan G, Ehleiter D, Haimovitz-Friedman A, Cordon-Cardo C, Kolesnick R. Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. *Science* 2001;293:293-297.

26. Perfetto B, Donnarumma G, Criscuolo D, Paoletti I, Grimaldi E, Tufano MA, Baroni A. Bacterial components induce cytokine and intercellular adhesion molecules-1 and activate transcription factors in dermal fibroblasts. *Res Microbiol* 2003;154:337-344.

27. R development core team. R: A language and environment for statistical computing. R Foundation for statistical computing 2004. Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

28. Anonymous. Oral complications of cancer therapies: diagnosis, prevention, and treatment. National Institutes of Health. *Conn Med* 1989;53:595-601.

29. Borowski B, Benhamou E, Pico JL, Laplanche A, Margainaud JP, Hayat M. Prevention of oral mucositis in patients treated with high-dose chemotherapy and bone-marrow transplantation - A randomized controlled trial comparing 2 protocols of dental-care. *Oral Oncol, Eur J Cancer* 1994;30B:93-97.

30. Shieh SH, Wang ST, Tsai ST, Tseng CC. Mouth care for nasopharyngeal cancer patients undergoing radiotherapy. *Oral Oncol* 1997;33:36-41.

31. Cheng KKF, Chang AM. Palliation of oral mucositis symptoms in pediatric patients treated with cancer chemotherapy. *Cancer Nurs* 2003;26:476-484.

32. Dodd MJ, Larson PJ, Dibble SL, Miaskowski C, Greenspan D, MacPhail L, Hauck WW, Paul SM, Ignoffo R, Shiba G. Randomized clinical trial of chlorhexidine versus placebo for prevention of oral mucositis in patients receiving chemotherapy. *Oncol Nurs Forum* 1996;23:921-927.

33. Dodd MJ, Dibble SL, Miaskowski C, MacPhail L, Greenspan D, Paul SM, Shiba G, Larson P. Randomized clinical trial of the effectiveness of 3 commonly used mouthwashes to treat chemotherapy-induced mucositis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000;90:39-47.

34. Epstein JB, Vickars L, Spinelli J, Reece D. Efficacy of chlorhexidine and nystatin rinses in prevention of oral complications in leukemia and bone-marrow transplantation. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1992;73:682-689.

35. Ferretti GA, Ash RC, Brown AT, Parr MD, Romond EH, Lillich TT. Control of oral mucositis and candidiasis in marrow transplantation - A prospective, double-blind trial of chlorhexidine digluconate oral rinse. *Bone Marrow Transplant* 1988;3:483-493.

36. Ferretti GA, Raybould TP, Brown AT, Macdonald JS, Greenwood M, Maruyama Y, Geil J, Lillich TT, Ash RC. Chlorhexidine prophylaxis for chemotherapy- and radiotherapy-induced stomatitis: a randomized double-blind trial. *Oral Surg Oral Med Oral Pathol* 1990;69:331-338.

37. Foote RL, Loprinzi CL, Frank AR, O'Fallon JR, Gulavita S, Tewfik HH, Ryan MA, Earle JM, Novotny P. Randomized trial of a chlorhexidine mouthwash for alleviation of radiation-induced mucositis. *J Clin Oncol* 1994;12:2630-2633.

38. Mcgaw WT, Belch A. Oral complications of acute-leukemia - prophylactic impact of a chlorhexidine mouth rinse regimen. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1985;60:275-280.

39. Pitten FA, Kiefer T, Buth C, Doelken G, Kramer A. Do cancer patients with chemotherapy-induced leukopenia benefit from an antiseptic chlorhexidine-based oral rinse? A double-blind, block-randomized, controlled study. *J Hosp Infect* 2003;53:283-291.

40. Spijkervet FKL, Panders AK, Vermey A, van Saene JJM, Mehta DM, Fidler V. Effect of chlorhexidine rinsing on the oropharyngeal ecology in patients with head and neck cancer who have irradiation mucositis. *Oral Surg Oral Med Oral Pathol* 1989;67:154-161.

41. Rahn R, Adamietz IA, Boettcher HD, Schaefer V, Reimer K, Fleischer W. Povidone-iodine to prevent mucositis in patients during antineoplastic radiochemotherapy. *Dermatology* 1997;195 Suppl 2:57-61.

42. Giles FJ, Redman R, Yazi S, Bellm L. Iseganan HCL: A novel antimicrobial agent. *Expert Opin Invest Drugs* 2002;11:1161-1170.

43. Giles FJ, Miller CB, Hurd DD, Wingard JR, Fleming TR, Sonis ST, Bradford WZ, Pulliam JG, Anaissie EJ, Beveridge RA, Brunvand MM, Martin PJ. A phase III, randomized, double-blind, placebo-controlled, multinational trial of iseganan for the prevention of oral mucositis in patients receiving stomatotoxic chemotherapy (PROMPT-CT trial). *Leuk Lymphoma* 2003;44:1165-1172.

44. Giles FJ, Rodriguez R, Weisdorf D, Wingard JR, Martin PJ, Fleming TR, Goldberg SL, Anaissie EJ, Bolwell BJ, Chao NJ, Shea TC, Brunvand MM, Vaughan W, Petersen F, Schubert M, Lazarus HM, Maziarz RT, Silverman M, Beveridge RA, Redman R, Pulliam JG, Devitt-Risse P, Fuchs HJ, Hurd DD. A phase III, randomized, double-blind, placebo-controlled, study of iseganan for the reduction of stomatitis in patients receiving stomatotoxic chemotherapy. *Leuk Res* 2004;28:559-565.

45. Trotti A, Garden A, Warde P, Symonds P, Langer C, Redman R, Pajak TF, Fleming TR, Henke M, Bourhis J, Rosenthal DI, Junor E, Cmelak A, Sheehan F, Pulliam J, Devitt-Risse P, Fuchs H, Chambers M, O'Sullivan B, Ang KK. A multinational, randomized phase III trial of iseganan HCl oral solution for reducing the severity of oral mucositis in patients receiving radiotherapy for head-and-neck malignancy. *Int J Radiat Oncol Biol Phys* 2004;58:674-681.

46. Spijkervet FKL, van Saene JJM, Panders AK, Vermey A, Mehta DM. Mucositis prevention by selective elimination of oral flora in irradiated head and neck cancer patients. *J Oral Pathol Med* 1990;19:486-489.

47. El Sayed S, Nabid A, Shelley W, Hay J, Balogh J, Gelinas M, Mackenzie R, Read N, Berthelet E, Lau H, Epstein J, Delvecchio P, Ganguly PK, Wong F, Burns P, Tu D, Pater J. Prophylaxis of radiation-associated mucositis in conventionally treated patients with head and neck cancer: A double-blind, phase III, randomized, controlled trial evaluating the clinical efficacy of an antimicrobial lozenge using a validated mucositis scoring system. *J Clin Oncol* 2002;20:3956-3963.

48. Okuno SH, Foote RL, Loprinzi CL, Gulavita S, Sloan JA, Earle J, Novotny PJ, Burk M, Frank AR. A randomized trial of a nonabsorbable antibiotic lozenge given to alleviate radiation-induced mucositis. *Cancer* 1997;79:2193-2199.

49. Stokman MA, Spijkervet FKL, Burlage FR, Dijkstra PU, Manson WL, de Vries EGE, Roodenburg JLN. Oral mucositis and selective elimination of oral flora in head and neck cancer patients receiving radiotherapy: a double-blind randomised clinical trial. *Br J Cancer* 2003;88:1012-1016.

50. Symonds RP, McIlroy P, Khorrami J, Paul J, Pyper E, Alcock SR, McCallum I, Speekenbrink ABJ, McMurray A, Lindemann E, Thomas M. The reduction of radiation mucositis by selective decontamination antibiotic pastilles: a placebo-controlled double-blind trial. *Br J Cancer* 1996;74:312-317.

51. Wijers OB, Levendag PC, Harms ER, Gan-Teng AM, Schmitz PI, Hendriks WD, Wilms EB, van der Est H, Visch LL. Mucositis reduction by selective elimination of oral flora in irradiated cancers of the head and neck: a placebo-controlled double-blind randomized study. *Int J Radiat Oncol Biol Phys* 2001;50:343-352.

52. Cheng KKF, Chang AM, Yuen MP. Prevention of oral mucositis in paediatric patients treated with chemotherapy: a randomised crossover trial comparing two protocols of oral care. *Eur J Cancer* 2004;40:1208-1216.

53. Epstein JB, Stevenson-Moore P, Jackson S, Mohamed JH, Spinelli JJ. Prevention of oral mucositis in radiation therapy: a controlled study with benzydamine hydrochloride rinse. *Int J Radiat Oncol Biol Phys* 1989;16:1571-1575.

54. Kim JH, Chu FCH, Lakshmi V, Houde R. Benzydamine HCl, A new agent for the treatment of radiation mucositis of the oropharynx. *Am J Clin Oncol (CCT)* 1986;9:132-134.

55. Prada A, Chiesa F. Effects of benzydamine on the oral mucositis during antineoplastic radiotherapy and/or intra-arterial chemotherapy. *Int J Tissue React* 1987;9:115-119.

56. Samaranayake LP, Robertson AG, Macfarlane TW, Hunter IP, MacFarlane G, Soutar DS, Ferguson MM. The effect of chlorhexidine and benzydamine mouthwashes on mucositis induced by therapeutic irradiation. *Clin Radiol* 1988;39:291-294.

57. Epstein JB, Silverman S Jr, Paggiarino DA, Crockett S, Schubert MM, Senzer NN, Lockhart PB, Gallagher MJ, Peterson DE, LeVeque FG. Benzydamine HCl for prophylaxis of radiation-induced oral mucositis: results from a multicenter, randomized, double-blind, placebo- controlled clinical trial. *Cancer* 2001;92:875-885.

58. Hanson WR, Marks JE, Reddy SP, Simon S, Mihalo WE, Tova Y. Protection from radiation-induced oral mucositis by a mouth rinse containing the prostaglandin E1 analog, misoprostol: a placebo controlled double blind clinical trial. *Adv Exp Med Biol* 1997;400B:811-818.

59. Labar B, Mrsic M, Pavletic Z, Bogdanic V, Nemet D, Aurer I, Radman I, Filipovicgrcic N, Sertic D, Kalenic S, Presecki V. Prostaglandin-E(2) for prophylaxis of oral mucositis following BMT. *Bone Marrow Transplant* 1993;11:379-382.

60. Pillsbury HC, Webster WP, Rosenman J. Prostaglandin inhibitor and radiotherapy in advanced head and neck cancers. *Arch Otolaryngol Head Neck Surg* 1986;112:552-553.

61. Leborgne JH, Leborgne F, Zubizarreta E, Ortega B, Mezzera J. Corticosteroids and radiation mucositis in head and neck cancer. A double-blind placebo-controlled randomized trial. *Radiother Oncol* 1998;47:145-148.

62. Cartee L, Petros WP, Rosner GL, Gilbert C, Moore S, Affronti ML, Hoke JA, Hussein AM, Ross M, Rubin P, Vredenburgh JJ, Peters WP. Evaluation of GM-CSF mouthwash for prevention of chemotherapy-induced mucositis - A randomized, double-blind, dose-ranging study. *Cytokine* 1995;7:471-477.

63. Chi KH, Chen CH, Chan WK, Chow KC, Chen SY, Yen SH, Chao JY, Chang CY, Chen KY. Effect of granulocyte-macrophage colony-stimulating factor on oral mucositis in head and neck-cancer patients after cisplatin, fluorouracil, and leucovorin chemotherapy. *J Clin Oncol* 1995;13:2620-2628.

64. Crawford J, Tomita DK, Mazanet R, Glaspy J, Ozer H. Reduction of oral mucositis by filgrastim (r-metHuG-CSF) in patients receiving chemotherapy. *Cytokines Cell Mol Ther* 1999;5:187-193.

65. Dazzi C, Cariello A, Giovanis P, Monti M, Vertogen B, Leoni M, Tienghi A, Turci D, Rosti G, Nanni O, Rondoni C, Marangolo M. Prophylaxis with GM-CSF mouthwashes does not reduce frequency and duration of severe oral mucositis in patients with solid tumors undergoing high-dose chemotherapy with autologous peripheral blood stem cell transplantation rescue: a double blind, randomized, placebo-controlled study. *Ann Oncol* 2003;14:559-563.

66. Hejna M, Kostler WJ, Raderer M, Steger GG, Brodowicz T, Scheithauer W, Wiltschke C, Zielinski CC. Decrease of duration and symptoms in chemotherapy-induced oral mucositis by topical GM-CSF: results of a prospective randomised trial. *Eur J Cancer* 2001;37:1994-2002.

67. Karthaus M, Rosenthal C, Huebner G, Paul H, Elser C, Hertenstein B, Krauter J, Scharmann T, Geissler RG, Heil G, Ganser A. Effect of topical oral G-CSF on oral mucositis: a randomised placebo-controlled trial. *Bone Marrow Transplant* 1998;22:781-785.

68. Katano M, Nakamura M, Matsuo T, Iyama A, Hisatsugu T. Effect of granulocyte-colony-stimulating factor (G-CSF) on chemotherapy-induced oral mucositis. *Surg Today-Jpn J Surg* 1995;25:202-206.

69. Makkonen TA, Minn H, Jekunen A, Vilja P, Tuominen J, Joensuu H. Granulocyte macrophage-colony stimulating factor (GM-CSF) and sucralfate in prevention of radiation-induced mucositis: A prospective randomized study. *Int J Radiat Oncol Biol Phys* 2000;46:525-534.

70. Nemunaitis J, Rosenfeld CS, Ash R, Freedman MH, Deeg HJ, Appelbaum F, Singer JW, Flomenberg N, Dalton W, Elfenbein GJ, Rifkin R, Rubin A, Agosti J, Hayes FA, Holcenberg J, Shadduck RK. Phase-III randomized, double-blind placebo-controlled trial of rhgm-csf following allogeneic bone-marrow transplantation. *Bone Marrow Transplant* 1995;15:949-954.

71. Saarilahti K, Kajanti M, Joensuu T, Kouri M, Joensuu H. Comparison of granulocyte-macrophage colony-stimulating factor and sucralfate mouthwashes in the prevention of radiation-induced mucositis: A double-blind prospective randomized phase III study. *Int J Radiat Oncol Biol Phys* 2002;54:479-485.

72. Schneider SB, Nishimura RD, Zimmerman RP, Tran L, Shiplacoff J, Tormey M, Contreras R, Juillard GF. Filgrastim (r-metHuG-CSF) and its potential use in the reduction of radiation-induced oropharyngeal mucositis: An interim look at a randomized, double-blind, placebo-controlled trial. *Cytokines Cell Mol Ther* 1999;5:175-180.

73. Valcarcel D, Sanz MA, Sureda A, Sala M, Munoz L, Subira M, Laborda R, Clopes A, Sierra J. Mouth-washings with recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) do not improve grade III-IV oropharyngeal mucositis (OM) in patients with hematological malignancies undergoing stem cell transplantation. Results of a randomized double-blind placebo-controlled study. *Bone Marrow Transplant* 2002;29:783-787.

74. van der Lelie H, Thomas BLM, van Oers RHJ, Ek-Post M, Sjamsoedin SAS, Dijk-Overtoom ML, Timmer JG, dem Borne AEGK. Effect of locally applied GM-CSF on oral mucositis after stem cell transplantation: a prospective placebo-controlled double-blind study. *Ann Hematol* 2001;80:150-154.

75. Foncuberta MC, Cagnoni PJ, Brandts CH, Mandanas R, Fields K, Derigs HG, Reed E, Sonis ST, Fay J, LeVeque F, Pouillart P, Schrezenmeier H, Emmons R, Thiel E. Topical transforming growth factor-beta 3 in the prevention or alleviation of chemotherapy-induced oral mucositis in patients with lymphomas or solid tumors. *J Immunother* 2001;24:384-388.

76. Spielberger R, Stiff P, Bensinger W, Gentile T, Weisdorf D, Kewalramani T, Shea T, Yanovich S, Hansen K, Noga S, McCarty J, LeMaistre CF, Sung EC, Blazar BR, Elhardt D, Chen MG, Emmanouilides C. Palifermin for oral mucositis after intensive therapy for hematologic cancers. *N Engl J Med* 2004;351:2590-2598.

77. Cascinu S, Fedeli A, Fedeli SL, Catalano G. Oral cooling (cryotherapy), an effective treatment for the prevention of 5-fluorouracil-induced stomatitis. *Oral Oncol, Eur J Cancer* 1994;30B:234-236.

78. Mahood DJ, Dose AM, Loprinzi CL, Veeder MH, Athmann LM, Therneau TM, Sorensen JM, Gainey DK, Mailliard JA, Gusa NL, Finck GK, Johnson C, Goldberg RM. Inhibition of fluorouracil-induced stomatitis by oral cryotherapy. *J Clin Oncol* 1991;9:449-452.

79. Barasch A, Peterson DE, Tanzer JM, Dambrosio JA, Nuki K, Schubert MM, Franquin JC, Clive J, Tutschka P. Helium-neon laser effects on conditioning-induced oral mucositis in bone-marrow transplantation patients. *Cancer* 1995;76:2550-2556.

80. Bensadoun RJ, Franquin JC, Ciais G, Darcourt V, Schubert MM, Viot M, Dejou J, Tardieu C, Benezery K, Nguyen TD, Laudoyer Y, Dassonville O, Poissonnet G, Vallicioni J, Thyss A, Hamdi M, Chauvel P, Demard F. Low-energy He/Ne laser in the prevention of radiation-induced mucositis. A multicenter phase III randomized study in patients with head and neck cancer. *Support Care Cancer* 1999;7:244-252.

81. Cowen D, Tardieu C, Schubert M, Peterson D, Resbeut M, Faucher C, Franquin JC. Low energy Helium-Neon laser in the prevention of oral mucositis in patients undergoing bone marrow transplant: results of a double blind randomized trial. *Int J Radiat Oncol Biol Phys* 1997;38:697-703.

82. Carter DL, Hebert ME, Smink K, Leopold KA, Clough RL, Brizel DM. Double blind randomized trial of sucralfate vs placebo during radical radiotherapy for head and neck cancers. *Head Neck* 1999;21:760-766.

83. Castagna L, Benhamou E, Pedraza E, Luboinski M, Forni M, Brandes I, Pico JL, Dietrich PY. Prevention of mucositis in bone marrow transplantation: A double blind randomised controlled trial of sucralfate. *Ann Oncol* 2001;12:953-955.

84. Cengiz M, Ozyar E, Ozturk D, Akyol F, Atahan IL, Hayran M. Sucralfate in the prevention of radiation-induced oral mucositis. *J Clin Gastroenterol* 1999;28:40-43.

85. Chiara S, Nobile MT, Vincenti M, Gozza A, Pastrone I, Ross M, Rosso R. Sucralfate in the treatment of chemotherapy-induced stomatitis: A double-blind, placebo-controlled pilot study. *Anticancer Res* 2001;21:3707-3710.

86. Dodd MJ, Miaskowski C, Greenspan D, MacPhail L, Shih AS, Shiba G, Facione N, Paul SM. Radiation-induced mucositis: A randomized clinical trial of micronized sucralfate versus salt & soda mouthwashes. *Cancer Invest* 2003;21:21-33.

87. Epstein JB, Wong FLW. The efficacy of sucralfate suspension in the prevention of oral mucositis due to radiation-therapy. *Int J Radiat Oncol Biol Phys* 1994;28:693-698.

88. Etiz D, Erkal HS, Serin M, Kucuk B, Hepari A, Elhan AH, Tulunay O, Cakmak A. Clinical and histopathological evaluation of sucralfate in prevention of oral mucositis induced by radiation therapy in patients with head and neck malignancies. *Oral Oncol* 2000;36:116-120.

89. Franzen L, Henriksson R, Littbrand B, Zackrisson B. Effects of sucralfate on mucositis during and following radiotherapy of malignancies in the head and neck region - A double-blind placebo-controlled study. *Acta Oncol* 1995;34:219-223.

90. Lievens Y, Haustermans K, van den Weyngaert D, van den Bogaert W, Scalliet P, Hutsebaut L, Fowler J, Lambin P. Does sucralfate reduce the acute side-effects in head and neck cancer treated with radiotherapy? A double-blind randomized trial. *Radiother Oncol* 1998;47:149-153.

91. Makkonen TA, Bostrom P, Vilja P, Joensuu H. Sucralfate mouth washing in the prevention of radiation-induced mucositis: a placebo-controlled double-blind randomized study. *Int J Radiat Oncol Biol Phys* 1994;30:177-182.

92. Nottage M, McLachlan SA, Brittain MA, Oza A, Hedley D, Feld R, Sin LL, Pond G, Moore MJ. Sucralfate mouthwash for prevention and treatment of 5-fluorouracil-induced mucositis: a randomized, placebo-controlled trial. *Support Care Cancer* 2003;11:41-47.

93. Pfeiffer P, Madsen EL, Hansen O, May O. Effect of prophylactic sucralfate suspension on stomatitis induced by cancer-chemotherapy - A randomized, double-blind cross-over study. *Acta Oncol* 1990;29:171-173.

94. Shenep JL, Kalwinsky DK, Hutson PR, George SL, Dodge RK, Blankenship KR, Thornton D. Efficacy of oral sucralfate suspension in prevention and treatment of chemotherapy-induced mucositis. *J Pediatr* 1988;113:758-763.

95. Culy CR, Spencer CM. Amifostine: an update on its clinical status as a cytoprotectant in patients with cancer receiving chemotherapy or radiotherapy and its potential therapeutic application in myelodysplastic syndrome. *Drugs* 2001;61:641-684.

96. Hospers GAP, Eisenhauer EA. The sulfhydryl containing compounds WR-2721 and glutathione as radio- and chemoprotective agents. A review, indications for use and prospects. *Br J Cancer* 1999;80:629-638.

97. Antonadou D, Pepelassi M, Synodinou M, Puglisi M, Throuvalas N. Prophylactic use of amifostine to prevent radiochemotherapy-induced mucositis and xerostomia in head-and-neck cancer. *Int J Radiat Oncol Biol Phys* 2002;52:739-747.

98. Bourhis J, De Crevoisier R, Abdulkarim B, Deutsch E, Lusinchi A, Luboinski B, Wibault P, Eschwege F. A randomized study of very accelerated radiotherapy with and without amifostine in head and neck squamous cell carcinoma. *Int J Radiat Oncol Biol Phys* 2000;46:1105-1108.

99. Brizel DM, Wasserman TH, Henke M, Strnad V, Rudat V, Monnier A, Eschwege F, Zhang J, Russell L, Oster W, Sauer R. Phase III randomized trial of amifostine as a radioprotector in head and neck cancer. *J Clin Oncol* 2000;18:3339-3345.

100. Buntzel J, Schuth J, Kuttner K, Glatzel M. Radiochemotherapy with amifostine cytoprotection for head and neck cancer. *Support Care Cancer* 1998;6:155-160.

101. Hartmann JT, von Vangerow A, Fels LM, Knop S, Stolte H, Kanz L, Bokemeyer C. A randomized trial of amifostine in patients with high-dose VIC chemotherapy plus autologous blood stem cell transplanation. *Br J Cancer* 2001;84:313-320.

102. Koukourakis MI, Kyrias G, Kakolyris S, Kouroussis C, Frangiadaki C, Giatromanolaki A, Retalis G, Georgoulias V. Subcutaneous administration of amifostine during fractionated radiotherapy: a randomized phase II study. *J Clin Oncol* 2000;18:2226-2233.

103. Lorusso D, Ferrandina G, Greggi S, Gadducci A, Pignata S, Tateo S, Biamonte R, Manzione L, Di Vagno G, Ferrau F, Scambia G. Phase III multicenter randomized trial of amifostine as cytoprotectant in first-line chemotherapy in ovarian cancer patients. *Ann Oncol* 2003;14:1086-1093.

104. Thieblemont C, Dumontet C, Saad H, Roch N, Bouafia F, Arnaud P, Hequet O, Espinouse D, Salles G, Roy P, Eljaafari-Corbin A, du Manoir-Baumgarten C, Coiffier B. Amifostine reduces mucosal damage after high-dose melphalan conditioning and autologous peripheral blood progenitor cell transplantation for patients with multiple myeloma. *Bone Marrow Transplant* 2002;30:769-775.

105. Vacha P, Fehlauer F, Mahlmann B, Marx M, Hinke A, Sommer K, Richter E, Feyerabend T. Randomized phase III trial of postoperative radiochemotherapy +/- amifostine in head and neck cancer - Is there evidence for radioprotection? *Strahlenther Onkol* 2003;179:385-389.

106. Savarese DMF, Savy G, Vahdat L, Wischmeyer PE, Corey B. Prevention of chemotherapy and radiation toxicity with glutamine. *Cancer Treat Rev* 2003;29:501-513.

107. Anderson PM, Schroeder G, Skubitz KM. Oral glutamine reduces the duration and severity of stomatitis after cytotoxic cancer chemotherapy. *Cancer* 1998;83:1433-1439.

108. Anderson PM, Ramsay NKC, Shu XO, Rydholm N, Rogosheske J, Nicklow R, Weisdorf DJ, Skubitz KM. Effect of low-dose oral glutamine on painful stomatitis during bone marrow transplantation. *Bone Marrow Transplant* 1998;22:339-344.

109. Huang EY, Leung SW, Wang CJ, Chen HC, Sun LM, Fang FM, Yeh SA, Hsu HC, Hsiung CY. Oral glutamine to alleviate radiation-induced oral mucositis: a pilot randomized trial. *Int J Radiat Oncol Biol Phys* 2000;46:535-539.

110. Jebb SA, Osborne RJ, Maughan TS, Mohideen N, Mack P, Mort D, Shelley MD, Elia M. 5-Fluorouracil and folinic acid-induced mucositis - No effect of oral glutamine supplementation. *Br J Cancer* 1994;70:732-735.

111. Okuno SH, Woodhouse CO, Loprinzi CL, Sloan JA, LaVasseur BI, Clemens-Schutjer D, Swan D, Axvig C, Ebbert LP, Tirona MRBT, Michalak JC, Pierson N. Phase III controlled evaluation of glutamine for decreasing stomatitis in patients receiving fluorouracil (5-FU)-based chemotherapy. *Am J Clin Oncol (CCT)* 1999;22:258-261.

112. Osaki T, Ueta E, Yoneda K, Hirota J, Yamamoto T. Prophylaxis of oral mucositis associated with chemoradiotherapy for oral carcinoma by azelastine hydrochloride (azelastine) with other antioxidants. *Head Neck* 1994;16:331-339.

113. Ferreira PR, Fleck JF, Diehl A, Barletta D, Braga A, Barletta A, Iiha L. Protective effect of alpha-tocopherol in head and neck cancer radiation-induced mucositis: A double-blind randomized trial. *Head Neck* 2004;26:313-321.

114. Ertekin MV, Koc M, Karslioglu I, Sezen O. Zinc sulfate in the prevention of radiation-induced oropharyngeal mucositis: A prospective, placebo-controlled, randomized study. *Int J Radiat Oncol Biol Phys* 2004;58:167-174.

115. Loprinzi CL, Cianflone SG, Dose AM, Etzell PS, Burnham NL, Therneau TM, Hagen L, Gainey DK, Cross M, Athmann LM, Fischer T, Oconnell MJ. A controlled evaluation of an allopurinol mouthwash as prophylaxis against 5-fluorouracil induced stomatitis. *Cancer* 1990;65:1879-1882.

116. Attal M, Huguet F, Rubie H, Charlet JP, Schlaifer D, Huynh A, Laurent G, Pris J. Prevention of regimen-related toxicities after bone-marrow transplantation by pentoxifylline - A prospective, randomized trial. *Blood* 1993;82:732-736.

117. Verdi CJ, Garewal HS, Koenig LM, Vaughn B, Burkhead T. A double-blind, randomized, placebo-controlled, crossover trial of pentoxifylline for the prevention of chemotherapy-induced oral mucositis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995;80:36-42.

118. Awidi A, Homsi U, Kakail RI, Mubarak A, Hassan A, Kelta M, Martinez P, Sulaiti S, Al Qady A, Jamhoury A, Daniel M, Charles C, Ambrose A, El Aloosy AS. Double-blind, placebo-controlled cross-over study of oral pilocarpine for the prevention of chemotherapy-induced oral mucositis in adult patients with cancer. *Eur J Cancer* 2001;37:2010-2014.

119. Oberbaum M, Yaniv I, Ben Gal Y, Stein J, Ben Zvi N, Freedman LS, Branski D. A randomized, controlled clinical trial of the homeopathic medication TRAUMEEL S (R) in the treatment of chemotherapy-induced stomatitis in children undergoing stem cell transplantation. *Cancer* 2001;92:684-690.

120. Biswal BM, Zakaria A, Ahmad NM. Topical application of honey in the management of radiation mucositis. A Preliminary study. *Support Care Cancer* 2003;11:242-248.

121. Papas AS, Clark RE, Martuscelli G, O'Loughlin KT, Johansen E, Miller KB. A prospective, randomized trial for the prevention of mucositis in patients undergoing hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2003;31:705-712.

122. Su CK, Mehta V, Ravikumar L, Shah R, Pinto H, Halpern J, Koong A, Goffinet D, Le QT. Phase II double-blind randomized study comparing oral aloe vera versus placebo to prevent radiation-related mucositis in patients with head-and-neck neoplasms. *Int J Radiat Oncol Biol Phys* 2004;60:171-177.

123. Fidler P, Loprinzi CL, O'Fallon JR, Leitch JM, Lee JK, Hayes DL, Novotny P, Bartel J, Michalak JC. Prospective evaluation of a chamomile mouthwash for prevention of 5-FU-induced oral mucositis. *Cancer* 1996;77:522-525.

3

Assessment of oral mucositis in clinical trials: impact of training on evaluators in a multicenter trial

M.A. Stokman
S.T. Sonis
P.U. Dijkstra
J.G.M. Burgerhof
F.K.L. Spijkervet

European Journal of Cancer 2005;41:1735-1738

SUMMARY

Background In the assessment of mucositis the inter-evaluator variability needs to be minimized and would likely to be best accomplished by training. The aim of this study was to evaluate the effect of training on concordance of evaluators in scoring oral mucositis.
Methods The evaluators were informed about the pathobiology and clinical appearance of mucositis and were trained in scoring mucositis according the Oral Mucositis Assessment Scale (OMAS). The effect of the training was evaluated by a pre- and post-training test. Each test consisted of 15 slides depicting oral mucositis. The pre- and post-training scores were compared to the reference standard.
Results During 8 months at 6 meetings 65 evaluators were trained. The mean percentage correctly scored slides according the OMAS increased significantly between the pre- and post-training test ($P<0.001$).
Conclusion Training evaluators in scoring oral mucositis has a significant improvement on the outcome of mucositis assessment.

INTRODUCTION

Mucositis of the oral mucosa is a frequent cause of morbidity in cancer therapy with a serious burden on patients. Severe mucositis causes considerable pain and discomfort, leading to a higher need of pain medication, parenteral nutrition and length of hospitalization.[1,2]

Many studies in which agents are tested with potential useful outcomes on mucositis have their shortcomings. Studies were underpowered, lacked an adequate control arm, were not investigator or patient blinded or had other major design flaws.[3] To determine the value of new agents aimed at prevention of mucositis, well designed, sufficiently powered and appropriately executed studies are needed. Due to the lack of sufficient patient numbers at single study sites, often a multicenter design is necessary to obtain data within an acceptable time frame. Moreover, time frames for preventive studies on sequelae of cancer therapy are very tight because of new developments or changes in ablative therapies like changes in fractionation schedules in radiotherapy or new combinations of cancer cytotoxic therapies.[4]

One of the major concerns in controlled multicenter trials with mucositis is the establishment of adequate inter-evaluator reliability to reduce outcome variability. In the implementation of the evaluation method in a multicenter trial, standardization between the different study sites and evaluators is essential for decreasing error variance and reducing type II error, i.e. failing to detect true differences between active drug and placebo. Furthermore a poor inter-evaluator reliability decreases statistical power, resulting in necessity for larger sample sizes to be able to detect significant differences between drug and placebo.[5]

To increase the inter-evaluator reliability in multicenter trials start up training meetings are necessary to standardize evaluators to the same method of scoring and baseline knowledge.

According to the regulations of the United States Food and Drug Administration (FDA) selected investigators and evaluators should be qualified by training and experienced to investigate the device (21Code of Federal Regulations (CFR) 812). Industry-sponsored trials, with the intention for FDA approval, will need to have start up training meetings to conform these regulations.

In testing efficacy of training of evaluators there should be pre- and post-testing conducted to examine on empirical basis whether the training

was effective. This testing should evaluate the improvement in: 1) conceptual understanding of the scoring method; 2) accuracy, i.e. how scores by the evaluator agree with the reference standard; 3) inter-evaluator reliability. Between pre- and post-testing phase, a didactic training should be provided.[6]

One of the most important issues in research is a well defined endpoint. Regarding mucositis studies, in most instances mucositis will be used as primary endpoint defined as ulcerative or pseudomembranous mucositis. Several mucositis rating scales are clinical observational scores, based on a combination of local mucositis parameters (signs) together with general complaints such as pain and effects on eating.[7] Differences in definition and operationalization of these general complaints hamper proper comparison of the outcomes using these scoring systems.

For assessment of the mucosal changes related to anti-cancer therapies the Oral Mucositis Assessment Scale (OMAS) has been developed.[8] The OMAS is a simple, quantitative and accurate mucositis score especially validated for research application in multicenter clinical trials. In this score, a clear definition of mucositis symptoms, erythema and ulceration, are established. The OMAS has been shown to be highly reproducible between observers ($r > 0.8$), responsive over time ($r > 0.9$) and accurately records the anatomic elements considered being associated with mucositis.[8]

The aim of this study was to evaluate the effect of training on concordance of evaluators in scoring oral mucositis according the OMAS score.

MATERIALS AND METHODS

Training meetings were organized as start up of a phase III multicenter clinical trial. During these meetings evaluators were trained in scoring mucositis according the OMAS and informed about the intention of the study, the pathobiology and clinical appearance of mucositis. All meetings were conducted by the same trainer (F.S.).

The training meeting consisted of a pre-testing phase, didactic training and post-testing phase, all of which was performed on the same day. The pre-testing phase consisted of a slideshow of 15 slides with clinical pictures of different regions of the mouth with or without different stages of mucositis. For each slide the evaluator had to fill in on a form the mucositis score, according the OMAS of the depicted region, based on visible ulceration and/or erythema, and the size of the lesion (ulceration) or intention

(erythema) (Table 1). The score forms were collected after the slideshow. The didactic training consisted of a thorough review of the pathobiology of mucositis, the scoring method of mucositis according to OMAS, and an explanation of the different clinical aspects of mucositis. The post-testing phase consisted of a retest of the same 15 mucositis slides of which the evaluator again needed to fill in the score forms.

Table 1. Scoring mucositis according the Oral Mucositis Assessment Scale (OMAS)

Location	Erythema*	Ulcerations/Pseudomembranes**
Upper lip	0 1 2	0 1 2 3
Lower lip	0 1 2	0 1 2 3
Right cheek	0 1 2	0 1 2 3
Left cheek	0 1 2	0 1 2 3
Right ventral and lateral tongue	0 1 2	0 1 2 3
Left ventral and lateral tongue	0 1 2	0 1 2 3
Floor of the mouth	0 1 2	0 1 2 3
Soft palate	0 1 2	0 1 2 3
Hard palate	0 1 2	0 1 2 3

** Erythema: 0 = none; 1 = not severe; 2 = severe*
*** Ulcerations/Pseudomembranes: 0 = no lesion; 1 = < 1 cm^2; 2 = 1 cm^2 – 3 cm^2; 3 = > 3 cm^2*

The scores of the evaluators were compared to a reference standard score. The reference standard was developed as follows. All 15 slides were scored for ulceration and erythema according the OMAS and rated for visible ulceration and/or erythema, independently by three experienced evaluators (S.S., F.S., M.S.) in the field of mucositis scoring prior to the start up meetings. A consensus meeting was held to discuss discrepancies in their scores. Consensus was reached by means of discussion. The outcomes of these scores were used as the reference standard score for each slide.

Statistical analysis

The scores of the evaluators according the OMAS and presence or absence of ulcerations and/or erythema, were dichotomized in either correct or

incorrect comparing to the reference standard. The Student's t-test for dependent samples was used to analyze the mean performance (expressed as the percentages of correct scores) of the evaluators in two ways, one ignoring the missing and secondly considering the missing as incorrect scores. McNemar's test was used to analyze the percentages correct scores of the different slides separately.

A *P* value of <0.05 was considered significant.

RESULTS

In the course of 8 months, 6 start-up training meetings were organized and a total of 65 evaluators were trained. The average group size was 11 evaluators (range 8-15). The professional background of the evaluators varied from dentists, physicians, nurses and research assistants. The results of the dichotomized scores on the 15 slides of the pre- and post-test training are summarized in table 2.

Table 2. Summary of the correct, incorrect and missing scores of the 65 evaluators for the 15 slides according OMAS: T1 is pre-test training scores; T2 is post-test training scores

		T1		T2	
		%	n	%	n
Ulceration	Correct	45	435	62	608
	Incorrect	50	491	28	269
	Missing	5	49	10	98
Erythema	Correct	59	567	69	669
	Incorrect	35	344	21	207
	Missing	6	55	10	99

Comparing the mean performance of the evaluators, ignoring the missing, the mean evaluator's percentage correctly scored slides according to the OMAS increased significantly between pre- and post-test training for both ulceration from 47% to 69% (95% CI: 17 to 26) and erythema from 63% to 77% (95% CI: 11 to 17). Considering the missing as incorrect scores, the mean percentages increased less pronounced but still significant at the 0.001 level. The mean percentage correctly scored ulceration increased between

Table 3. Proportion of scoring improvement of each slide after training of the evaluators

Slide	Mucositis	Proportion of improvement	95% CI
1	Ulceration	0.00	(-0.05 to 0.05)
	Erythema	0.12	(-0.01 to 0.25)
2	Ulceration	0.37*	(0.24 to 0.50)
	Erythema	0.25*	(0.14 to 0.37)
3	Ulceration	0.03	(-0.08 to 0.15)
	Erythema	-0.03	(-0.21 to 0.14)
4	Ulceration	-0.04	(-0.13 to 0.05)
	Erythema	0.06	(-0.02 to 0.14)
5	Ulceration	0.40*	(0.28 to 0.52)
	Erythema	0.02	(-0.06 to 0.09)
6	Ulceration	0.63*	(0.50 to 0.75)
	Erythema	0.20*	(0.07 to 0.33)
7	Ulceration	0.22*	(0.09 to 0.34)
	Erythema	0.31*	(0.17 to 0.45)
8	Ulceration	0.33*	(0.19 to 0.47)
	Erythema	0.42*	(0.27 to 0.57)
9	Ulceration	0.11*	(0.03 to 0.19)
	Erythema	0.29*	(1.13 to 0.45)
10	Ulceration	0.30*	(0.12 to 0.48)
	Erythema	0.02	(-0.02 to 0.06)
11	Ulceration	0.18*	(0.06 to 0.30)
	Erythema	0.27*	(0.10 to 0.44)
12	Ulceration	0.25*	(0.09 to 0.41)
	Erythema	0.07	(-0.08 to 0.22)
13	Ulceration	0.00	(-0.12 to 0.12)
	Erythema	0.08*	(0.00 to 0.15)
14	Ulceration	0.52*	(0.38 to 0.66)
	Erythema	0.14	(-0.02 to 0.30)
15	Ulceration	0.19*	(0.04 to 0.35)
	Erythema	0.07	(-0.01 to 0.15)

** represents a significant improvement ($P < 0.05$)*

pre- and post-test training from 45% to 62% (95% CI: 12 to 23) and for erythema from 59% to 69% (95% CI: 5 to 14)

Comparing the results of the evaluators of scoring the absence or presence of ulceration and/or erythema, the correctly scored slides increased significantly between pre- and post-test training for both ulceration from 83% to 92% (95% CI: 2 to 16) and erythema from 79% to 85% (95% CI: 1 to 12).

The proportion improvements for the different slides are shown in table 3. Analyzing the scores for each of the 15 slides separately, 14 improvements were found for assessment of ulceration and of these improvements, 11 were significant. Also on erythema, on 14 slides improvements were found and 11 were significant.

DISCUSSION

This study has shown that training of evaluators has a positive significant influence on scoring oral mucositis.

Analyzing the slides separately, three slides were poorly scored. More than 87% of the evaluators scored these three slides wrong in the pre-test and post-test after training according to OMAS score. These slides were not easy to evaluate with respect to the size of the lesion. A possible explanation might be a poor slide exposure or poor depiction of the anatomic site. In the future, it would probably be better to keep these three slides out of the training meeting. In a post hoc analysis, leaving these three slides out of the training meeting, the mean evaluator's percentage correctly scored slides according the OMAS increased for ulceration from 52% to 81% and for erythema from 62% to 78%. However, scoring oral mucositis in the clinical situation should be easier because it allows inspection of the whole mouth and all anatomic sites are visible and the evaluator can change the position of the patient in obtaining a better view.

Scoring only the absence or presence of mucositis signs erythema and/or ulceration gave a higher mean percentage of correctly scored slides than when the size or the intention of the lesion had to be taken into account both at pre- and post-testing. Scoring of dimensions on a slide is very difficult and can only be done if a reference, like a tooth, is visible on the slide. Some of the slides did not have such a reference. This could be the explanation of the difference in mean percentages correctly scored slides between both

scoring methods. These outcomes can be an argument for the use of a scoring method, which only evaluates the absence or presence of the mucositis signs erythema and/or ulceration. Clinically, the ulcerative stage of mucositis is the most considerable stage and responsible for the pain and loss of function. In several studies aimed at prevention of mucositis, the primary endpoint is prevention of ulcerations. The World Health Organization Oral Toxicity Scale (WHO) score measures anatomic, symptomatic, and functional components of oral mucositis.[9] The WHO score is easy to use, to learn and measures no lesion dimensions. In contrast, the OMAS is focused on the anatomic compound of mucositis alone, and is more precisely related to the mucosal changes and dimensions of the lesions due to cancer therapies. In clinical research with mucositis as primary endpoint, it would probable be best to use both, a method measuring mucositis in a subjective way and a method measuring only in an objective way.

In this present study, the professional background of the evaluators varied and unfortunately the distribution was unknown. This drawback could be interpreted as flaw of this study, however it is known from others studies that the type of medical professional background does not influence the scoring outcome.[8]

Post-training monitoring of the evaluators and calibration during a (multicenter) study is necessary to determine reliability and to prevent evaluator drift.[6] An evaluator can have high levels of competence and reliability before the study starts but this can fade away in time. It is necessary to involve a positive feedback loop post-training, which could measure the degree of the evaluators' comprehension. The phase III study, for which these trainings where accomplished, was stopped prematurely due to the instability of the intervention agent. Therefore, no information is available from this study about the evaluators reliability and drift with time.

Training is essential to gain standardization of the evaluators and to increase intra- and inter-evaluator reliability. Not only for standardizing of scoring procedures but also to provide every study site and evaluator similar information. Moreover in multicenter trials, concordance between the evaluators at different study sites is a prerequisite.

Based on this study and previous research future multicenter trails on mucositis prevention the following training prerequisites are recommended.[6,10] Training needs to consist of: 1) training of the scoring method used as endpoint of the study; 2) didactic training comprising a review of

pathobiology of mucositis and of the scoring method; 3) training in scoring and processing of data; 4) testing efficacy of training intervention by pre- and post-testing and 5) post-training monitoring of the quality of scoring.

In conclusion, training evaluators in scoring oral mucositis has a significant improvement on the outcome of mucositis evaluation.

REFERENCES

1. Elting LS, Cooksley C, Chambers M, Cantor SB. The burdens of cancer therapy - Clinical and economic outcomes of chemotherapy-induced mucositis. *Cancer* 2003;98:1531-1539.
2. Sonis ST, Oster G, Fuchs H, Bellm LA, Bradford WZ, Edelsberg J, Hayden V, Eilers J, Epstein JB, LeVeque FG, Miller C, Peterson DE, Schubert MM, Spijkervet FKL, Horowitz M. Oral mucositis and the clinical and economic outcomes of hematopoietic stem-cell transplantation. *J Clin Oncol* 2001;19:2201-2205.
3. Rubenstein EB, Peterson DE, Schubert M, Keefe D, McGuire D, Epstein J, Elting LS, Fox PC, Cooksley C, Sonis ST. Clinical practice guidelines for the prevention and treatment of cancer therapy-induced oral and gastrointestinal mucositis. *Cancer* 2004;100:2026-2046.
4. Cooper JS, Pajak TF, Forastiere AA, Jacobs J, Campbell BH, Saxman SB, Kish JA, Kim HE, Cmelak AJ, Rotman M, Machtay M, Ensley JF, Chao KSC, Schultz CJ, Lee N, Fu KK. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. *N Engl J Med* 2004;350:1937-1944.
5. Muller MJ, Szegedi A. Effects of interrater reliability of psychopathologic assessment on power and sample size calculations in clinical trials. *J Clin Psychopharmacol* 2002;22:318-325.
6. Kobak KA, Engelhardt N, Williams JBW, Lipsitz JD. Rater training in multicenter clinical trials: Issues and recommendations. *J Clin Psychopharmacol* 2004;24:113-117.
7. Parulekar W, Mackenzie R, Bjarnason G, Jordan RCK. Scoring oral mucositis. *Oral Oncol* 1998;34:63-71.
8. Sonis ST, Eilers JP, Epstein JB, LeVeque FG, Liggett Jr WH, Mulagha MT, Peterson DE, Rose AH, Schubert MM, Spijkervet FKL, Wittes JP. Validation of a new scoring system for the assessment of clinical trial research of oral mucositis induced by radiation or chemotherapy. Mucositis Study Group. *Cancer* 1999;85:2103-2113.
9. Anonymous. Handbook for reporting results of cancer treatment. *WHO Offset Publ* 1979;48:15-22.
10. Kobak KA, Lipsitz JD, Feiger A. Development of a standardized training program for the Hamilton Depression Scale using internet-based technologies: results from a pilot study. *J Psychiatr Res* 2003;37:509-515.

4 Quantification of oral mucositis due to radiotherapy by determining viability and maturation of epithelial cells

M.A. Stokman
F.K.L. Spijkervet
A.N.M. Wymenga
F.R. Burlage
W. Timens
J.L.N. Roodenburg
E.G.E. de Vries

Journal of Oral Pathology & Medicine 2002;31:153-157

SUMMARY

Background An in vitro assay has been developed for quantitative assessment of chemotherapy induced oral mucositis. In the present study this method was evaluated for assessment of irradiation mucositis at a cellular level.

Patients and methods Ten patients with head and neck cancer participated in this study. All patients were treated with conventional fractionated curative postoperative radiotherapy. Prior to, and weekly during, the irradiation course, oral washings were obtained to determine viability of epithelial cells by trypan blue dye exclusion. Maturation of epithelial cells was assessed from smears in buccal mucosa of these patients (Papanicolaou staining). The viability data were compared with the WHO score for mucositis.

Results Epithelial cell viability increased during the first three weeks of radiation (P=0.04), and was seen earlier than the subjective mucosal changes with the WHO score. Cell maturity shifted from immature and intermediate to mature (P=0.03).

Conclusions The cell viability assay can be considered as an objective method for following the development of irradiation mucositis, and seems to be more sensitive during the first three weeks of irradiation than the clinical WHO scoring method.

INTRODUCTION

Oral mucositis is the inflammatory reaction of the oral mucosa due to radiotherapy and/or chemotherapy. Oral complications are inevitable sequelae of head and neck irradiation, involving pseudomembranous and ulcerative mucositis in about 80% of the patients.[1] It is often painful, afflicts oral functioning such as swallowing and eating, diminishes the quality of life and can be dose-limiting.[2] Ionizing radiation causes damage of rapidly proliferating cells of the basal cell layer of the epithelium. The severity of mucositis due to radiotherapy depends on the type of radiation, fractionation schedule, total cumulative dose and the volume of irradiated tissue.[3-5]

During a conventional curative radiation schedule the first signs of mucositis - a white discoloration or erythema - will appear after 10 Gy radiotherapy. These signs are caused by a higher degree of hyperkeratinization and vascular dilatation, respectively. The more severe stages of mucositis, i.e. pseudomembranes and ulceration, occur after 30 Gy. These signs are due to further breakdown of the mucous membrane together with vascular changes.[1-3,6]

The biological basis of mucositis is the sterilization of proliferating cells in the germinative layer of the epithelium. Their gradual proliferative failure causes a deficit in the cellular supply of the functional layers. Due to the natural loss of the superficial layer by mechanical wear and tear, hypoplasia of the epithelium and eventually ulcerative mucositis develops.[7-9]

At a cellular level, the development of mucositis due to radiation is only partially understood. Recently a hypothesis was proposed, describing the development of mucositis in four consecutive phases, namely the inflammatory/vascular phase, the epithelial phase, the ulcerative/bacterial phase and the healing phase.[10]

To compare outcomes of preventive strategies or treatment modalities an objective, reliable and reproducible scoring system would be helpful. Most available scoring systems are clinical observational scores based on a combination of local mucositis parameters (signs) together with general complaints such as pain and effect on eating.[11] Differences in definition and operationalization of these general complaints hamper proper comparison of the outcomes using these scoring systems. Some other scoring systems appeared to be unreliable and not reproducible, while some are difficult to use in a clinical setting.[11] Of the available scoring systems, the WHO score and the NCI score are most frequently applied. However, these scoring

systems are based on a combination of local mucositis signs and general complaints. Thus, the same difficulties apply to these two methods.[11-13]

With Sonis's hypothesis[10] for mucositis development and new perspectives of preventive strategies in mind, a method for quantifying mucositis at a cellular level is demanded.

To investigate mucositis objectively and on a cellular level, Wymenga and colleagues developed an in vitro assay for chemotherapy induced mucositis.[14] The aim of the present study was to investigate whether this method can be used to quantify mucositis in head and neck cancer patients who received radiotherapy and to compare the results with the WHO scoring system.

PATIENTS AND METHODS

From February 1997 till December 1997, consecutive patients with oral, oral-pharyngeal or salivary gland squamous cell malignancies were considered for entering the study. Eligible were patients if they received postoperative radiotherapy as follows: (1) with a conventional fractionation of 2 Gy per day with a 6 MV accelerator and (2) with a minimum accumulative dose of 50 Gy according the ICRU 50 recommendations. Patients were not eligible with: (1) an oral mucosa defect other than related to tumour surgery, (2) a need for an obturator or resection prosthesis, (3) treated with antibiotics for an infection in the 2 week period before the start of irradiation, (4) oral candidiasis or acute periodontitis, or (5) nasogastric tube feeding at the start of irradiation.

The evaluation period enclosed the whole period of 6 week period of irradiation.

From the 11 patients included in this study one patient quitted the radiotherapy treatment after 2 weeks and was excluded from the study. The 10 remaining patients evaluated in this study comprised seven men and three women, with a mean age of 59.2 (range, 42-77 years). Nine patients had a squamous cell carcinoma, (seven oral, and two in the tonsil area), and one patient had an adenocarcinoma of the parotid gland.

According to our supportive oral care protocol all patients were evaluated for potential risk factors for oral complications, such as exacerbation of peri-apical and periodontal infections, by means of a

thorough oral and dental evaluation, including a radiographic examination.[15] All potential risk factors were appropriately eliminated before start of radiotherapy. The supportive oral care regimen consisted of a daily standard protocol of cleansing the oral cavity by spraying with saline by the dental hygienist, and rinsing of the mouth by the patients themselves with a salt-baking soda solution at least eight times a day. Edentulous patients were not allowed to wear their dentures during the course of radiotherapy. In this study, five patients were edentulous, three were dentate and two were partially dentate.

The Medical Ethical Board Committee approved the study and all patients gave written informed consent.

A control group, which consist of 10 healthy volunteers, seven men and three women, with a mean age of 54.3 (range, 48-63 years), was evaluated for comparison of the baseline outcome of epithelial cell maturation and viability.

Scoring mucositis

Prior to, and at the end of each week of radiation, an oral washing, as well as a buccal smear with a cytobrush, were obtained for epithelial cell determination of viability and maturation. At the same time, mucositis was clinically evaluated using the WHO toxicity grading.[12] Mucosal epithelial cells were obtained twice from the control group, at a weekly interval, for measurement of viability and maturation.

To acquire an oral washing, patients gargled and rinsed their mouth with 10 ml sterile saline for 30 sec, and spat out into a tube. This expectorate was centrifuged within 10 min after collection (190 g, 10 min, room temperature) and the supernatant was discarded. The fluid was washed with 10 ml saline and centrifuged again to eliminate salivary fibers. Pellets were resuspended in 1 ml RPMI 1640 medium (Gibco, Paisley, UK) containing 5% fetal calf serum. Subsequently, 50 µl suspension and 50 µl trypan blue dye (0.4% in 0.15 m NaCl) were combined and immediately transferred to a hemocytometer. Cell counts were performed, after which the percentage viable cells and total cells number were calculated. Determination of the viability was performed independently from the clinical mucositis scoring.

Buccal mucosa smears were put on glass-slides and stained according to Papanicolaou. Epithelial cell morphology was assessed after collection

of all slides at the end of study period.[16] The assessment was a blinded and randomized, and was performed by one observer (M.A.S.). Orange stained cells were classified as mature, while blue/green stained cells were categorized as immature cells. Cells with a partly orange and partly green appearance were graded as intermediate cells. Percentage of mature, intermediate and immature cells was determined from each smear.

Mucositis was evaluated clinically on a weekly basis, according to the WHO toxicity grading, in which grade 0 = normal, no mucositis; grade 1 = soreness and erythema; grade 2 = erythema, ulcers, can eat solids; grade 3 = ulcers, requires liquid diet only; grade 4 = alimentation not possible.[12]

Statistical analysis

Statistical analysis included: Spearman rank correlations and Wilcoxon signed rank test. A P value < 0.05 was considered significant.

RESULTS

The percentage viable cells at baseline in the patient group (46.2% ± 18%) did not differ from controls (47.8% ± 12%).

During the first 3 weeks of radiotherapy the viability increased significantly (P=0.04) from 46.2 (± 18%) to 62.9 (± 19%) (Figure 1).

The mean percentage of mature cells increased during radiotherapy and this increase was significant (P=0.03) after 2 weeks of treatment compared to the baseline outcome. The mean percentage of intermediate and immature cells decreased, although this was not significant (Figure 2).

The WHO mucositis score is shown in figure 3. At the end of the first week, three patients experienced mucositis grade 1. After three weeks, nine patients had mucositis grade 2 or worse.

Figure 4 shows the course of mucositis using the WHO score (grade 3, 4) compared with the outcome of the cell viability scores (≥ 5% compared with baseline). The change in the cell viability score preceded the change in the WHO score during the first 3 weeks. However, the correlation between these two scores was not significant in week 1 and 3 (P=0.09, P=0.6 respectively). In week 2 there was a significant correlation (P=0.04).

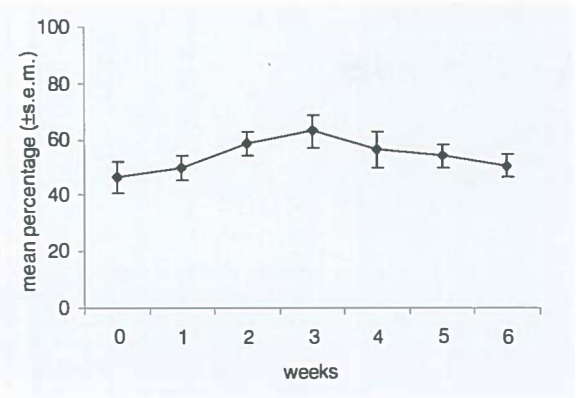


Figure 1. Mean percentage (± sem) viable epithelial cells in an oral washing before and during radiotherapy

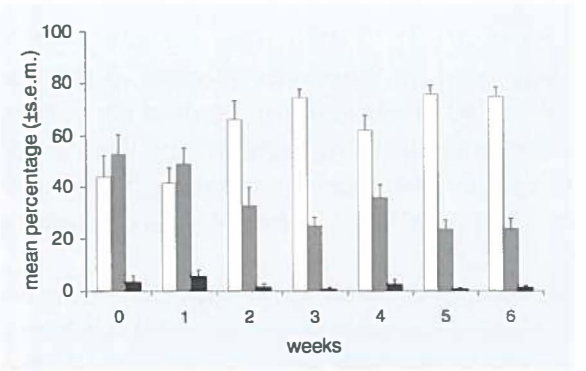


Figure 2. Morphology of buccal epithelial cells stained according to Papanicolaou, mean percentage (± sem) of mature cells (white), intermediate (grey) and immature cells (black) at baseline and during treatment

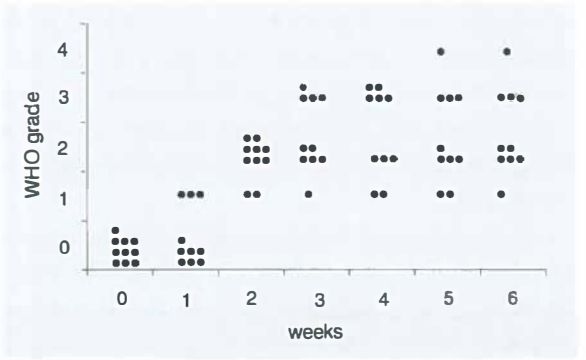


Figure 3. Mucositis score according to the WHO grading system before and during treatment

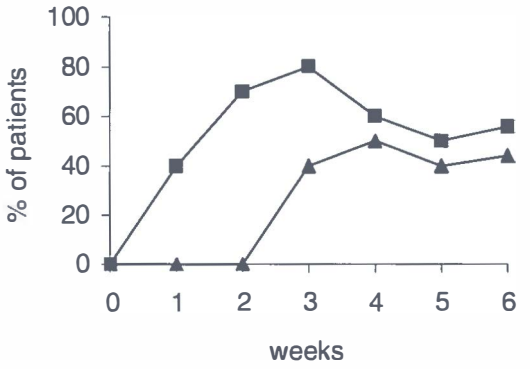


Figure 4. Percentage of patients with an increased cell viability (≥ 5% compared with baseline ■) and a WHO mucositis score grade 3 and 4 (▲) at baseline and during the radiotherapy

DISCUSSION

This study shows that the assay used, in which the changes of the oral epithelium at the cellular level can be quantified, can be used for scoring mucositis in head and neck cancer patients during radiotherapy. The clinical course of mucositis in these patients, who received standard oral care, followed an expected pattern, with over 80% of the patients developing severe mucositis after 3 weeks.[1]

The observed increase in percentage of viable epithelial cells during the first three weeks occurred due to desquamation of the non-vital upper oral epithelial layer, which is thought to be the basic process during irradiation at a mucosal level.[3] Thus, whereas at baseline, many superficial non-vital epithelial cells are obtained with oral washing and cytobrush, during radiotherapy, which causes desquamation, these sampling methods yield mostly suprabasal (viable) epithelial cells. The increase in percentage of viable cells reflects the epithelial phase of mucositis described by Sonis.[10] Earlier reports showed that after three weeks of irradiation the normal architecture of the mucosa is disturbed and leakage and disruption in the basal membrane develops.[3] This phenomenon almost certainly explains the decrease in viability after three weeks.

In addition to the increase in percentage of viable epithelial cells during the first three weeks, changes in the morphology of the epithelial cells were also detected. A shift from immature and intermediate to more mature

epithelial cells was observed during the first three radiotherapy weeks. These changes possibly reflect the changes at the basal cell layer, i.e. disturbed cell proliferation of the epithelial layer due to radiotherapy.[10]

In contrast, a decrease in the percentage of mature cells and an increase in the percentage of immature epithelial cells was reported after high-dose chemotherapy.[14] This difference may indicate different mechanisms of radiotherapy and chemotherapy at the oral mucosal level, one such difference being the treatment regime. In general, chemotherapy has a discontinuous effect due to a different schedule of administration. By contrast, the radiotherapy regime used in this study was a continuous process, on a daily basis for a period of 6-7 weeks.[17] Wymenga and colleagues evaluated patients during one chemotherapy cycle.[14] The radiation patients were evaluated during the whole six-week treatment period. Silverman and colleagues describes lesions due to chronic irritation that shed predominantly mature squamous cells.[18] This probably explains the difference between the results from chemotherapy patients and radiotherapy patients. Another explanation relates to the effect of radiotherapy has on the cell. The cell is damaged by radiation and loses its reproductive integrity. A possible consequence for the cell may be the inability to divide, while remaining physiologically functional for a long period.[19] This could result in the cell migrating to the surface, and remaining there to maturate. Furthermore, residual mitotic activity of irreversible damaged cells, designated as abortive divisions, is supposed to significantly contribute to overall cell production.[20]

The increase in percentage of viable epithelial cells is seen earlier in the treatment regime than the clinical mucositis observed using the WHO score. This suggests that, during the early treatment phase, the former method is more sensitive in detecting mucosal changes. After three weeks, the percentage of viable epithelial cells decreases, while the WHO score does not diminish. The viable cell method can therefore only be used for scoring the course and development of mucositis during the first three weeks of radiation. The development of severe pseudomembranes/ulcerative mucositis during head and neck irradiation is at its maximum three weeks from the onset of radiotherapy. During this period, the viable cell method can closely follow the course of mucositis. The results of this study support the idea that this method could facilitate the evaluation of new preventive strategies at a cellular level. This could be of value for future studies evaluating preventive strategies.

REFERENCES

1. Spijkervet FKL, van Saene HKF, Panders AK, Vermey A, Mehta DM. Scoring irradiation mucositis in head and neck cancer patients. *J Oral Pathol Med* 1989;18:167-171.
2. Berger AM, Kilroy TJ. Oral complications. In: DeVita VT, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology*. Philadelphia: Lippincott-Raven, 1997;2714-2725.
3. Baker DG. The radiobiological basis for tissue reactions in the oral cavity following therapeutic X-irradiation. A review. *Arch Otolaryngol* 1982;108:21-24.
4. Dutreix J, Tubiana M, Wambersie A, Malaise E. The influence of cell proliferation in tumours and normal tissues during fractionated radiotherapy. *Eur J Cancer* 1971;7:205-213.
5. van der Schueren E, van der Bogaert W, Kian Ang.K. Radiotherapy with multiple fractions per day. In: Steel GG, Adams GE, Peckham MJ, eds. *The biological basis of radiotherapy*. Amsterdam: Elsevier, 1983;195-210.
6. van der Schueren E, van der Bogaert W, Vanuytsel L, van Limbergen E. Radiotherapy by multiple fractions per day (MFD) in head and neck cancer: acute reactions of skin and mucosa. *Int J Radiat Oncol Biol Phys* 1990;19:301-311.
7. Dorr W, Kummermehr J. Accelerated repopulation of mouse tongue epithelium during fractionated irradiations or following single doses. *Radiother Oncol* 1990;17:249-259.
8. Dorr W. Repopulation in mouse oral mucosa: treatment splits. *Radiother Oncol* 1994;33:139-147.
9. Dorr W. Radiobiological models of normal tissue reactions. *Strahlenther Onkol* 1998;174 Suppl 3:4-7.
10. Sonis ST. Mucositis as a biological process: a new hypothesis for the development of chemotherapy-induced stomatotoxicity. *Oral Oncol* 1998;34:39-43.
11. Parulekar W, Mackenzie R, Bjarnason G, Jordan RCK. Scoring oral mucositis. *Oral Oncol* 1998;34:63-71.
12. Anonymous. Handbook for reporting results of cancer treatment. *WHO Offset Publ* 1979;48:15-22.
13. Anonymous. Oral complications of cancer therapies: diagnosis, prevention, and treatment. National Institutes of Health. *Conn Med* 1989;53:595-601.
14. Wymenga ANM, Spijkervet FKL, Timens W, Timmer-Bosscha H, Sluiter WJ, Mulder NH. A new in vitro assay for quantitation of chemotherapy-induced mucositis. *Br J Cancer* 1997;76:1062-1066.
15. Jansma J, Vissink A, Spijkervet FKL, Roodenburg JLN, Panders AK, Vermey A, Szabo BG, Gravenmade EJ. Protocol for the prevention and treatment of oral sequelae resulting from head and neck radiation therapy. *Cancer* 1992;70:2171-2180.
16. Papanicolaou GN, Traut HF. Diagnosis of uterine cancer by the vaginal smears. *New York: Commonwealth Fund*, 1943.
17. Sonis ST. Oral Complications. In: Holland JF, Bast Jr RC, Morton DL, Frei III E, Kufe DW, Weichselbaum RR, eds. *Cancer Medicine*. Philadelphia: Williams&Wilkins, 1997;3255-3264.
18. Silverman S, Becks H, Farber SM. The diagnostic value of intraoral cytology. *J D Res* 1958;37:195-205.

19. Hellman S. Principles of cancer management: radiation therapy. In: DeVita VT, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncoloy*. Philadelphia: Lippincott-Raven, 1997;307-332.

20. Dorr W, Weber FM. Effect of changing weekly dose on accelerated repopulation during fractionated irradiation of mouse tongue mucosa. *Int J Radiat Biol* 1995;67:577-585.

5

The role of oral mucositis on the systemic inflammation parameter IL-8 in febrile neutropenic cancer patients

M.A. Stokman
C.S.M. Oude Nijhuis
F.K.L. Spijkervet
E.S.J.M. de Bont
P.U. Dijkstra
S.M.G.J. Daenen
J.A. Gietema
W.T.A. van der Graaf
H.J.M. Groen
E. Vellenga
W.A. Kamps

Cancer Investigations (accepted 2005)

SUMMARY

Background Cancer patients treated with cytostatic drugs often develop oral mucositis, considered to be a mucosal injury in which various cytokines, e.g. interleukin 8 (IL-8), may play a role. Plasma IL-8 is a systemic inflammatory response parameter. This study investigated whether oral mucositis affects plasma IL-8 levels in febrile neutropenic cancer patients.
Patients and methods Patients (n=57) who were hospitalized with chemotherapy induced neutropenic fever were scored for oral mucositis on the second day of hospitalization according to a validated oral mucositis assessment scale (OMAS) and WHO toxicity grading.

Patients (n=20) with a clinical sepsis or local bacterial infection were excluded from this evaluation. The remaining 37 patients were divided in a group with and without oral mucositis.
Results The difference in plasma IL-8 level between patients with and without mucositis was not significant (P=0.7). Similar no difference was observed in the degree and duration of granulocytopenia.
Conclusion These results indicate that low-grade oral mucositis is not related to the systemic plasma IL-8 level in febrile neutropenic cancer patients without a clinical sepsis or local bacterial infection.

INTRODUCTION

Fever is among the first signs of bacterial infection in neutropenic patients. Classical inflammatory signs such as erythema, pain and swelling are often less overt in neutropenic cancer patients due to impaired immune defence mechanisms.[1]

Blood plasma levels of TNF-α and IL1-β are often low whereas the levels of IL-6 and IL-8 increase in the peripheral blood during systemic infection in cancer patients with chemotherapy induced neutropenia.[2-4] The standard therapy for patients with fever and chemotherapy induced neutropenia is hospitalization and intravenous administration of broad-spectrum antibiotics on empirical grounds although a considerable number of these patients probably have no bacterial infection. It would be a great advantage if a simple method, such as the measurement of IL-8 level in combination with a physical examination, could distinguish patients with febrile neutropenia and low risk of infection who would need no hospitalization with no or restricted use of antibiotics.[5]

Oral mucositis is a sequelae in patients treated with chemotherapy, which can occur in 40% to 100% of the patients.[6-8] Development of mucositis, with the bacterial colonization of the damaged epithelium, provides an opportunity for bacteria or their products to leak through the injured mucosa, which may further stimulate the release of inflammatory cytokines such as IL-8.[9,10] However, the relationship between a systemic inflammation parameter, such as plasma IL-8 level, and mucositis has not been studied before. In the development of a risk assessment model with the plasma IL-8 level as parameter it is important to investigate the relationship between oral mucositis and the plasma IL-8 level in patients without documented infections.

A possible relationship has been described between the development of mucositis and herpes simplex infection (HSV) as well as the contribution of HSV reactivation to unexplained fever in neutropenic cancer patients.[11-13]

The main purpose of this study was to investigate the relationship between oral mucositis and systemic IL-8 levels in neutropenic cancer patients with fever without a local bacterial infection or a clinical sepsis. In addition, the relationship was analyzed between mucositis and neutropenia, duration of hospitalization and fever, and herpes simplex virus re-activation in this population.

PATIENTS AND METHODS

Included in the present study were all consecutive cancer patients (children and adults) who developed febrile neutropenia after outpatient chemotherapy and were eligible for a risk assessment study provided they were hospitalized on Sunday to Friday.[5] Chemotherapy related neutropenia was defined as granulocytes < $0.5x10^9$/L or leukocytes < $1x10^9$/L and fever as axillary body temperature >38.5 °C once or 38.0 - 38.5 °C 3 times over a 6-hours observation period.[2] Patients already receiving antibiotics were excluded, as were patients after autologous or allogeneic bone marrow transplantation. The use of selective gut decontamination or *Pneumocystic carinii* pneumonia prophylaxis (oral trimethoprim-sulfamethoxazole 3/15 mg per kg/day three days a week) were no exclusion criteria.

At presentation a physical examination was performed in the enrolled patients. Neutropenia was determined by absolute neutrophil or leukocyte counts and paranasal sinus and chest radiographs were obtained. Cultures of blood and sites that were suspect for infection were collected. In addition EDTA blood specimens were collected to measure IL-8 plasma concentrations. Patients were scored for mucositis and a sample from the oropharynx was taken for detection of a possible herpes simplex virus infection (HSV). Total days of hospitalization, days of fever, and days of neutropenia were recorded. The Medical Ethical Committee approved the study and all patients and/or their parents dependent of age of the patients, gave written informed consent.

Clinical sepsis

Clinical sepsis was defined as follows: systolic blood pressure ≤ 90 mmHg in adults or < -2 standard deviations (SD) for age in children, or both heart rate > 100/min ánd respiratory rate > 20/min in adults or both heart and respiratory > +2 SD for age in children, according to systemic inflammatory response syndrome (SIRS) criteria.[14]

Mucositis

The mucositis was scored according the Oral Mucositis Assessment Score (OMAS) and WHO score without knowledge of patients characteristics and blood results on the second day of hospitalization. Two scoring systems were used to be able to compare outcomes with findings from the literature.

The OMAS mucositis score measures 9 sites in the mouth for erythema, pseudomembranes or ulcerations, scoring range 0-5.[15] The WHO mucositis score is as follows: grade 0 = normal, no mucositis; grade 1 = soreness and erythema; grade 2 = erythema, ulcers, can eat solids; grade 3 = ulcers, requires liquid diet only; grade 4 = alimentation not possible.[16]

Measurement of plasma IL-8

Plasma IL-8 concentrations were determined using a chemiluminescent immunoassay system according to the manufacturer's instructions (Immulite, Diagnostic Products Corporation, Los Angeles, USA).[2]

Herpes Simplex virus detection

The viral cultures were taken with the swab technique, and transported in Gly-medium for laboratory culturing. Standard viral culture techniques were used. In patients with mucositis the swab was obtained from the pseudomembranes and in patients without mucositis the swab was obtained from the oropharynx.

Statistical analysis

Only the first febrile episode of each patient was used in the statistical analysis because repeated observations within the same subject cannot be considered as independent. The Mann-Whitney U test was used to analyze the differences in mucositis, the IL-8 plasma level, leukocyte and neutrophil counts, duration of hospitalization, fever and neutropenia between the patients with and without mucositis. Chi-square test was used to analyze the differences in gender, type of cancer and viral cultures between the patients with and without mucositis. Spearman's correlation coefficient was calculated for correlation between the different outcome variables. Statistical tests were carried out two sided at a significance level of 5%.

RESULTS

From May 1999 until January 2002, 57 patients were included, in whom 73 episodes of chemotherapy related neutropenia and fever were observed. Only the first febrile episode of every patient was used for this analysis. From the 57 patients, 20 patients were diagnosed with signs of a clinical

sepsis (n=7) or a local bacterial infection (n=13). Local bacterial infections comprised sinusitis (n=2), skin and soft tissue infections (n=5), urinary tract infections (n=2), otitis (n=2), parotitis (n=1) and dental infection (n=1). None of these patients had a positive blood culture. To avoid influence of clinical sepsis or local bacterial infection on the plasma IL-8 level, the results of the 20 patients diagnosed with a local bacterial infection or clinical sepsis were not used in the analysis. Patients characteristics of the remaining 37 are shown in table 1. Patients with mucositis were significantly older than

Table 1. Patient characteristics of the patients without a clinical sepsis or local bacterial infection (n=37) in relation to the presence of oral mucositis

	mucositis (n = 19)	no mucositis (n = 18)	*P*-value
Age in yrs median (IQR)	37 (12-49)	8 (4-27)	0.02
*Gender: male/female	11/8	8/10	0.52
*Type of cancer: hematologic (n=13) solid (n=24)	 62% 46%	 38% 54%	0.50
Leukocytes at admission x 10^9/L, median (IQR) (n)	0.4 (0.2-0.65) (10)	0.25 (0.13-0.58) (12)	0.44
Absolute neutrophil count at admission x 10^9/L, median (IQR) (n)	0.05 (0.01-0.09) (9)	0.11 (0.08-0.28) (6)	0.06
Days with neutropenia, median (IQR)	5 (4.0-6.3)	4 (1.5-6.5)	0.30
Days of fever, median (IQR)	1 (1.0-2.3)	1 (1.0-2.0)	0.96
Days of hospitalization, median (IQR)	6 (3.8-8.3)	5.5 (3.0-7.3)	0.68
Mucositis score OMAS mean ± SD WHO 0 (n) WHO 1 (n) WHO 2 (n) WHO 3 (n) WHO 4 (n)	 0.7 ± 0.9 0 6 9 0 4	 0 18 0 0 0 0	
*Viral cultures (n) positive/negative	12 3/9	10 0/10	0.21
IL-8 level pg/ml median (IQR)	70 (29-175)	58.5 (30.8-154.3)	0.70

IQR= inter-quartile range.
*All differences between the groups were analyzed using Mann Whitney U test for independent samples except * in which chi-square test was used*

patients without mucositis (P=0.02). No correlation was found between age and the plasma IL-8 level (r=0.08, P=0.6). The variables gender and type of tumour did not differ significantly between patients with and without mucositis.

Mucositis

The mean OMAS mucositis score in the total group (n=37) was 0.3 (SD 0.7); 18 patients had no mucositis whereas 19 patients developed mucositis (mean OMAS score was 0.7 (SD 0.9)). From the 19 patients with mucositis, 13 patients developed pseudomembranes or ulcerations and had WHO score grade ≥ 2 (Table 1). Comparison of the scoring methods for mucositis showed a high correlation between the OMAS en WHO score (ρ=0.95, P=0.003).

From 22 patients (12 patients with mucositis and 10 patients without mucositis) a viral microbiological sample was taken to detect a herpes simplex virus infection. A positive test for HSV was found in three patients, all three had mucositis (25%). Two of the patients had an ulcerative mucositis (Table 1).

IL-8

The median plasma IL-8 level for patients with mucositis was 70 pg/ml (inter-quartile range 29-175) and for patients without mucositis 58.5 pg/ml (inter-quartile range 30.8-154.3). No significant difference in plasma IL-8 level between patients with and without mucositis was found (P=0.7) (Figure 1, Table 1).

Mucositis in relation to duration of fever, hospitalization and neutropenia

No significant differences were found between patients with and without mucositis for the duration of fever, hospitalization and neutropenia (Table 1).

DISCUSSION

This study shows that the plasma IL-8 level as a parameter of systemic inflammation in cancer patients with febrile neutropenia is not influenced by low-grade oral mucositis.

The plasma IL-8 level was used as parameter for systemic inflammation since in earlier studies it was found that the plasma IL-8 level was a more sensitive parameter in cancer patients with chemotherapy induced neutro-

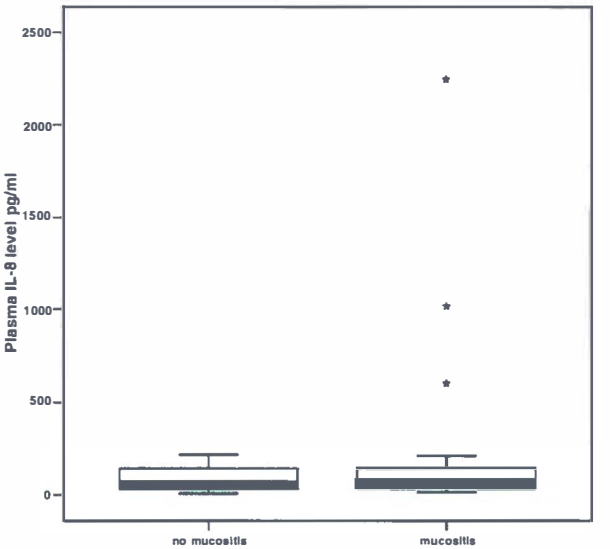


Figure 1. Box-and-whisker plot of the plasma IL-8 levels for the group of patients with and without mucositis

penia than e.g. IL-6.[2,17] Additionally, measurement of plasma IL-8 levels at the onset of fever in neutropenic patients is a valuable and sensitive tool to define a group of low-risk patients.[2,4,5,17] The current study was part of a risk assessment study in which the plasma IL-8 level was used as a parameter for systemic inflammation in febrile neutropenic patients.[5] In the development of such a risk model it is important to investigate the impact of oral mucositis on the plasma IL-8 level and whether oral mucositis must be considered as a local infection. Mucositis in neutropenic cancer patients can be a potential portal of entry for the endogenous oral flora leading to bacteremia or sepsis.[11,18-20] In this study no significant difference was found in the plasma IL-8 level between patients with and without mucositis and therefore mucositis should not be considered as a local infection in this patient population.

No influence of mucositis on the IL-8 parameter was found. However, the overall severity of mucositis in this patient population was low with a mean mucositis score (OMAS) of 0.3 (n=37). In an earlier observational study for development of mucositis, 75% of the patients developed mean levels of mucositis > 1 (OMAS) during bone marrow transplantation.[21] The relative

low mucositis scores in this study might explain that mucositis did not affect the plasma IL-8 levels. This finding supports the hypothesis of Sonis (2004) who assumed that the release of cytokines starts in the primary damage response but the massive release of cytokines occurs in the ulcerative phase.[10]

In this study in cancer patients treated at the outpatient department, oral mucositis was not related to duration of fever, hospitalization or neutropenia. This contrasts with patients undergoing hematopoietic stem-cell transplantation. In that patient population oral mucositis is positively correlated with the risk of infection, duration of hospitalization and death.[21] This difference in the role of oral mucositis may be explained by differences in intensity of chemotherapy. In the study of Sonis and colleagues (2001) more than 80% of the patients developed ulcerations while in the present study only 35% of the patients presented with ulcerations.[21]

A possible relationship between HSV and oral mucositis has been previously proposed.[12] It was concluded that oral ulcers are associated with HSV infection and in some cases probably caused by the virus. In the current study, only three patients (25%) in the group with mucositis and no patients in the group without mucositis had a positive HSV culture. The number of positive HSV infections was too small to detect such an association with mucositis. Therefore, further studies in larger populations are needed to understand the role of HSV in mucositis and fever of unknown origin (FUO) in neutropenic patients.

In conclusion, this study shows no relationship between low-grade oral mucositis and the plasma IL-8 level in febrile neutropenic cancer patients without clinical sepsis or local bacterial infection. Oral mucositis should therefore not be considered as a focus of infection in a risk group assessment based on IL-8 in out clinic patients with fever during chemotherapy induced neutropenia.

REFERENCES

1. Sickles EA, Greene WH, Wiernik PH. Clinical presentation of infection in granulocytopenic patients. *Arch Intern Med* 1975;135:715-719.

2. de Bont ESJM, Vellenga E, Swaanenburg JCM, Fidler V, Visser-van Brummen PJ, Kamps WA. Plasma IL-8 and IL-6 levels can be used to define a group with low risk of septicaemia among cancer patients with fever and neutropenia. *Br J Haematol* 1999;107:375-380.

3. Lehrnbecher Th, Venzon D, Haas Md, Chanock SJ, Kuhl J. Assessment of measuring circulating levels of interleukin-6, interleukin-8, C-reactive protein, soluble Fc gamma receptor type III, and mannose-binding protein in febrile children with cancer and neutropenia. *Clin Infect Dis* 1999;29:414-419.

4. Kern WV, Heiss M, Steinbach G. Prediction of gram-negative bacteremia in patients with cancer and febrile neutropenia by means of interleukin-8 levels in serum: targeting empirical monotherapy versus combination therapy. *Clin Infect Dis* 2001;32:832-835.

5. Oude Nijhuis CSM, Kamps WA, Daenen SMGJ, Gietema JA, van der Graaf WTA, Groen HJM, Vellenga E, Fidler V, de Bont ESJM. No antibiotics for selected febrile neutropenic cancer patients might be safe and feasible. *Proc Am Soc of Clin Oncol* 22,2961.2003.

6. Sonis ST. Oral Complications. In: Holland JF, Bast Jr RC, Morton DL, Frei III E, Kufe DW, Weichselbaum RR, eds. *Cancer Medicine*. Philadelphia: Williams&Wilkins, 1997;3255-3264.

7. Pico JL, Avila-Garavito A, Naccache P. Mucositis: its occurrence, consequences, and treatment in the oncology setting. *Oncologist* 1998;3:446-451.

8. Woo SB, Sonis ST, Monopoli MM, Sonis AL. A longitudinal study of oral ulcerative mucositis in bone marrow transplant recipients. *Cancer* 1993;72:1612-1617.

9. Blijlevens NM, Donnelly JP, de Pauw BE. Mucosal barrier injury: biology, pathology, clinical counterparts and consequences of intensive treatment for haematological malignancy: an overview. *Bone Marrow Transplant* 2000;25:1269-1278.

10. Sonis ST. The pathobiology of mucositis. *Nature Reviews Cancer* 2004;4:277-284.

11. Kubesova H, Penka M, Kral Z, Adam Z, Vasova I, Tomiska M, Vorlicek J. Herpes Simplex Infection as possible etiology for febrile neutropenia and mucositis in patients treated for hematological malignancies. *Acta Med Austriaca* 1998;25:57-60.

12. Bergmann OJ, Mogensen SC, Ellegaard J. Herpes simplex virus and intraoral ulcers in immunocompromised patients with haematologic malignancies. *Eur J Clin Microbiol Infect Dis* 1990;9:184-190.

13. Rasoul-Rockenschaub S, Zielinski CC, Muller C, Tichatschek E, Popow-Kraupp T, Kunz C. Viral reactivation as a cause of unexplained fever in patients with progressive metastatic breast cancer. *Cancer Immunol Immunother* 1990;31:191-195.

14. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RMH, Sibbald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest* 1992;101:1644-1655.

15. Sonis ST, Eilers JP, Epstein JB, LeVeque FG, Liggett Jr WH, Mulagha MT, Peterson DE, Rose AH, Schubert MM, Spijkervet FKL, Wittes JP. Validation of a new scoring system for the assessment of clinical trial research of oral mucositis induced by radiation or chemotherapy. Mucositis Study Group. *Cancer* 1999;85:2103-2113.

16. Anonymous. Oral complications of cancer therapies: diagnosis, prevention, and treatment. National Institutes of Health. *Conn Med* 1989;53:595-601.

17. Schonbohn H, Schuler M, Kolbe K, Peschel C, Huber C, Bemb W, Aulitzky WE. Plasma levels of IL-1, TNF alpha, IL-6, IL-8, G-CSF, and IL1-RA during febrile neutropenia: results of a prospective study in patients undergoing chemotherapy for acute myelogenous leukemia. *Ann Hematol* 1995;71:161-168.

18. Bochud PY, Calandra T, Francioli P. Bacteremia due to viridans streptococci in neutropenic patients: a review. *Am J Med* 1994;97:256-264.

19. Ruescher TJ, Sodeifi A, Scrivani SJ, Kaban LB, Sonis ST. The impact of mucositis on alpha-hemolytic streptococcal infection in patients undergoing autologous bone marrow transplantation for hematologic malignancies. *Cancer* 1998;82:2275-2281.

20. Donnelly JP, Muus P, Schattenberg A, de Witte T, Horrevorts A, de Pauw BE. A scheme for daily monitoring of oral mucositis in allogeneic BMT recipients. *Bone Marrow Transplant* 1992;9:409-413.

21. Sonis ST, Oster G, Fuchs H, Bellm LA, Bradford WZ, Edelsberg J, Hayden V, Eilers J, Epstein JB, LeVeque FG, Miller C, Peterson DE, Schubert MM, Spijkervet FKL, Horowitz M. Oral mucositis and the clinical and economic outcomes of hematopoietic stem-cell transplantation. *J Clin Oncol* 2001;19:2201-2205.

6

Oral mucositis and selective elimination of oral flora in head and neck cancer patients receiving radiotherapy. A double blind randomized clinical trial

M.A. Stokman
F.K.L. Spijkervet
F.R. Burlage
W.L. Manson
E.G.E. de Vries
J.L.N. Roodenburg

British Journal of Cancer 2003;88:1012-1016

SUMMARY

Background Mucositis is an acute inflammation of the oral mucosa because of radiotherapy and/or chemotherapy. All patients receiving radiotherapy in the head and neck region develop oral mucositis.
The aim of this study was to analyze the effects of selective oral flora elimination on radiotherapy induced oral mucositis, in a double blind, randomized, placebo-controlled trial.
Patients and methods Sixty-five patients with a malignant tumor in the head and neck regions to be treated with primary curative or postoperative radiotherapy participated in this study. The patients received either the active lozenges of 1 g containing polymyxin E 2 mg, tobramycin 1.8 mg and amphotericin B 10 mg (PTA) (33 patients) or the placebo lozenges (32 patients), four times daily during the full course of radiotherapy. Mucositis, changes in the oral flora, quality of feeding and changes of total body weight were assessed.
Results Mucositis score did not differ between the groups during the first 5 weeks of radiotherapy. Nasogastric tube feeding was needed in six patients (19%) of the placebo group and two patients (6%) of the PTA group (P=0.08). Mean weight loss after 5 weeks of radiation was less in the PTA group (1.3 kg, SD 3.0) than in the placebo group (2.8 kg, SD 2.9) (P=0.05). Colonization index of *Candida* species and Gram-negative bacilli was reduced in the PTA group and not in the placebo group (P<0.05). No effect on other micro-organisms was detected.
Conclusion Selective oral flora elimination in head and neck irradiation patients does not prevent the development of severe mucositis.

INTRODUCTION

Radiotherapy in head and neck cancer patients can induce oral mucositis, which is an acute inflammation of the oral mucosa. Until now no effective intervention has been developed to prevent oral mucositis in radiotherapy.[1] This prevention is even more relevant now because altered fractionation schedules for the treatment of head and neck malignancies induce more severe mucositis.[2] All patients receiving radiotherapy in the head and neck region develop oral mucositis to some extent, depending on radiation schedule, radiation field, radiation volume and cumulative dose.[3] Clinically, mucositis appears in a conventional radiation scheme after a cumulative radiation dose of 10-20 Gy as a white discoloration of the mucosa because of hyperkeratinization. The next stage is a deepening erythema followed by the development of pseudomembranes and ulcerations. Severe mucositis, appearing as pseudomembranes, will develop at the end of the third week of radiation, after about 30 Gy.[4,5] Prevention of severe mucositis is important because mucositis affects the patient's feeding status, physical and mental well being and it can influence the course of radiotherapy.[1] Further oral pain because of mucositis has a serious impact on the quality of life of patients.[3]

Several mechanisms are supposed to play a role in the development of mucositis: changes at the cellular level of the basal cell layer, inflammatory process in the epithelium and influence of bacteria on mucosal surface. Changed oral flora, colonizing the oral mucosa, may aggravate the mucosa reaction because of radiation.[6] The carriage and colonization of aerobic Gram-negative bacilli are thought to play a role in the pathogenesis of irradiation mucositis.[7] A hypothesis has been proposed on the development of mucositis in four consecutive phases, in which the ulcerative/bacterial phase is thought to play a role in the development of fibrous pseudomembranes of the oral mucosa.[8] A pilot study in 15 patients reported the protective effect of an antibiotic lozenge for selective elimination of the oral flora.[9] Less severe mucositis and a less mean mucositis score compared to a historical control group was observed. None of the PTA (polymyxin E 2 mg, tobramycin 1.8 mg and amphotericin B 10 mg) treated patients needed nasogastric tube feeding. In a cohort study including 36 patients, it was found that PTA lozenges may reduce irradiation mucositis.[10] In contrast, randomized studies reported conflicting effects on mucositis by selective oral flora elimination.[11-13]

The aim of this study was to evaluate in a randomized, double blind, placebo-controlled trial the effects of selective oral flora elimination on the development of irradiation induced oral mucositis, feeding, weight loss and colonization of aerobic Gram-negative bacilli and yeast.

PATIENTS AND METHODS

Protocol

Patients with a malignant tumor in the head and neck regions to be treated with primary curative or postoperative radiotherapy were eligible for this study. Inclusion criteria for the study were: external bilateral irradiation via parallel-opposed portals by a linear accelerator (4-6 Mev), fractionation of 2 Gy daily, five times a week, with a prescribed dose of at least 50 Gy and at least 50% of the oral mucosa in the field of radiation. The dose specification was in line with ICRU 50 recommendations.[14]

Criteria for exclusion were: (1) an oral mucosa defect other than related to tumor surgery; (2) need for an obturator or resection prosthesis and; (3) treatment with antibiotics for an oral infection the last 2 weeks before the start of irradiation.

As a standard procedure all patients were evaluated before radiation treatment for potential risk factors for oral complications by means of a thorough oral and dental evaluation, including a radiographic examination. All potential risk factors were eliminated appropriately before the start of radiotherapy. The supportive oral care regimen consisted of a daily protocol of cleansing the oral cavity by means of spraying with saline by the dental hygienist, and mouth rinsing by the patients with a salt-baking soda solution at least eight times a day to remove sticky saliva and debris. Dentate patients applied a neutral fluoride gel every second day with custom made trays and edentulous patients were not allowed to wear their dentures during the course of radiotherapy.[15]

The Medical Ethical Committee approved the study and all eligible patients gave written informed consent.

Assignment

The eligible patients were randomized to receive active lozenges of 1 g containing polymyxin E 2 mg, tobramycin 1.8 mg and amphotericin B 10 mg

(PTA) or placebo lozenges. The ingredients of the placebo lozenge were identical with the PTA lozenge except the active drugs. The colour, taste and form of the PTA and placebo lozenges were identical as well. Randomization was performed by the hospital pharmacist according to a computer-generated, randomized allocation schedule. Patients, clinicians, dental hygienists and microbiologists were blind for who was taking antibiotics. The patients used a PTA or placebo lozenge four times daily starting the first day of irradiation during the total radiation period.

Assessments

The study period included only the first 5 weeks of radiation because of the wide range of field changes above 50 Gy of radiation. During the study period mucositis, feeding and body weight scores were performed at the start of radiotherapy and twice weekly (Monday-Thursday). The assessments were performed by an assigned dental hygienist. For each patient a mean weekly score was calculated on basis of these two scores. These mean scores were used for further statistical analyses.

Twice weekly (Monday-Thursday) and two times before the start of radiation oral washings were obtained to examine the oral flora for Gram-negative bacilli, *Candida* species, viridans streptococci, *Enterococci*, *Staphylococcus aureus* and coagulase-negative *Staphylococci*.

Mucositis

The mean mucositis was scored by using qualitative and quantitative parameters.[4] Four different local signs of mucositis (k) might be distinguished: 1 = white discoloration; 2 = erythema; 3 = formation of pseudomembranes; 4 = ulceration. Mucositis of the oral cavity was determined for maximally eight distinguishable irradiated areas of the mouth: buccal mucosa (left and right), soft and hard palates, dorsum and border of the tongue (left and right) and the floor of the mouth. The degree of mucositis of each area was scored according to the local signs of mucositis. The length (E) of the local sign of mucositis was measured: 1 = ≤ 1 cm; 2 = 1-2 cm; 3 = 2-4 cm; 4 = ≥ 4 cm. The degree of mucositis was defined as the product of the values k and E. The mucositis score was defined as the mean of the scores assigned to the irradiated areas.

The mucositis was also scored according to the WHO score (grade 0 = normal, no mucositis; grade 1 = soreness and erythema; grade 2 = erythema,

ulcers, can eat solids; grade 3 = ulcers, requires liquid diet only; grade 4 = alimentation not possible).[16]

Feeding
The quality of feeding was scored (0 = normal, no changes; 1 = symptoms without medication; 2 = symptoms with medication; 3 = liquid diet only; 4 = nasogastric tube feeding) and body weight was determined. Afterwards the changes in weight were scored.

Microbiological methods
To acquire an oral washing, patients gargled and rinsed their mouth with 10 ml sterile saline for 30 sec, and spit it into a sterile vial.

One ml of the sample was diluted in 9 ml of Brain Heart infusion (BHI) (Oxoid, Basingstoke, England) and this suspension was serially diluted in BHI. The suspensions were than plated out onto 5% sheep blood agar, McConkey-3 agar (Oxoid) and Yeast morphology agar (Merck, Darmstadt, Germany). The agar plates and BHI broth cultures tubes were incubated overnight aerobically at 37 °C. If an agar plate did not show growth and the corresponding BHI broth culture of the dilution series did show turbidity, then this suspension was plated again onto the agars mentioned above. With this enrichment step even low numbers of *Candida* species and Gram-negative bacilli could be detected.[17] By reading and counting the plates after incubation the viable numbers of microorganisms per ml was estimated. The identification was performed by standard microbiological techniques.

Definitions
Carriage of a particular microorganism was defined as the condition in which a patient showed a minimum of two consecutive oral washings positive for that organism.

Colonization index of the oral cavity was defined as the sum of logarithms of the concentrations of a particular microorganism isolated from 1 ml of oral-washing specimens divided by the number of oral washings.

Statistical analysis
Sample size calculation of this study was based on the study by Spijkervet.[9] A two-sided α of 5% and a power of 80% were used. Additionally, a 50% reduction of mucositis in the PTA group was determined as clinical relevant with a normal incidence of mucositis of 80%. Based on these

assumptions, 27 patients in each group would be sufficient. Intention-to-treat analysis was performed. The difference of drop-outs between both groups was analyzed using Fisher's exact test. The results were analyzed, with respect to mean mucositis, the loss of weight, and colonization numbers for five different microorganisms (t-test for independent samples) and the WHO mucositis score and feeding (Mann-Whitney U test). Two-sided tests, performed at the 5% level of significance, were used.

Table 1. Patient characteristics

Patients characteristics	Placebo (n = 32)	PTA (n = 33)	*P*-value
*Age mean ± SD (yrs)	54 (10.8)	56 (12.5)	0.36
Gender: male / female (n)	22/10	24/9	0.79
Tumor site			
Oral cavity (n)	17	23	0.41
Oropharynx (n)	10	8	
Hypopharynx (n)	1	1	
Unknown primary (n)	4	1	
Histology			
Squamous (n)	31	32	1.00
Other (n)	1	1	
T-stage			
T1 (n)	4	5	0.25
T2 (n)	6	5	
T3 (n)	8	8	
T4 (n)	9	14	
Unknown (n)	5	1	
N stage			
N0 (n)	10	14	0.12
N1 (n)	6	10	
N2a (n)	2	1	
N2b (n)	9	3	
N2c (n)	1	3	
N3 (n)	4	2	
Surgery			
Yes (n)	21	27	0.17
No (n)	11	6	
Dentures			
Yes (n)	20	25	0.29
No (n)	12	8	

*All differences between the groups were analyzed using chi square test except * in which t-test was used*

RESULTS

Patients characteristics are shown in table 1. From January 1994 to February 1997, 65 patients were included, 33 patients received PTA lozenges and 32 the placebo lozenges. Out of the 65 included patients, 58 patients were evaluable for the total evaluation period of 5 weeks. Seven patients (11 %) dropped out earlier from the study, five of the PTA group (15 %) and two of the placebo group (6%). The difference of dropouts between both groups was not significant. One patient (PTA) developed a skin reaction, unlikely caused by the PTA lozenges, and one patient (placebo) could not suck the lozenges because of the tumor surgery of his tongue. The other 5 dropped out for reasons not related to one of the lozenges. Of the seven dropouts, one patient stopped after 1 week, two patients after 2 weeks, one patient after 3 weeks and three patients after 4 weeks radiation.

Mucositis

The mean mucositis was the same in the PTA group and the placebo group during the study period ($P>0.2$) (Figure 1). During the 5-week observation period 89% of the patients in the PTA group developed pseudomembranes and in the placebo group 94%. The mucositis according the WHO score did not differ throughout the study period between both groups ($P>0.5$). In the PTA group 80% of the patients developed grade 3 and 4 mucositis according the WHO score, and in the placebo group 90%. The appearance of pseudomembranes was for both groups at a similar radiation status; after 30 Gy of radiation.

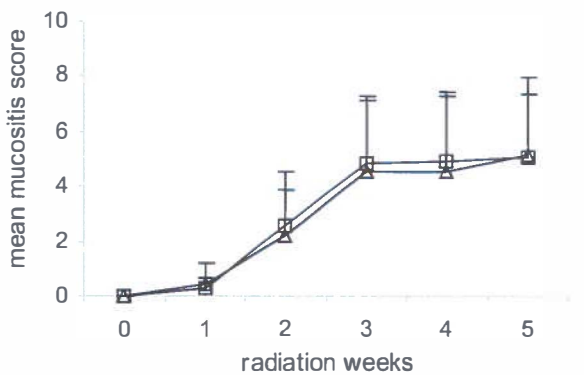


Figure 1. The mean mucositis score (±SD) for the PTA group (□) and the placebo group (△)

Feeding

Six patients (19%) in the placebo group (n=32) and two patients (6%) in the PTA group (n=33) needed nasogastric tube feeding during the evaluation period (P=0.08).

Body weight

The mean weight loss after 5 weeks of radiation was less in the PTA group by 1.3 kg (SD 3.0) than in the placebo group 2.8 kg (SD 2.9) (P=0.05).

Microorganism

For viridans streptococci, *Enterococci*, *Staphylococcus aureus* and coagulase-negative *Staphylococci*, a similar pattern of carriage and colonization index was found in both groups.

The colonization index and carriage of *Candida* species at baseline was equal in both groups (P>0.8). During the first two radiation weeks the colonization index for *Candida* species showed an increase in the placebo group and a decrease in the PTA group. After 2 weeks, an increase in both groups was found but the difference between the two groups remained significant during the total study period (P<0.05).

During the first 4 weeks a significant difference was found for the carriage of *Candida* species (P<0.03) (Figure 2).

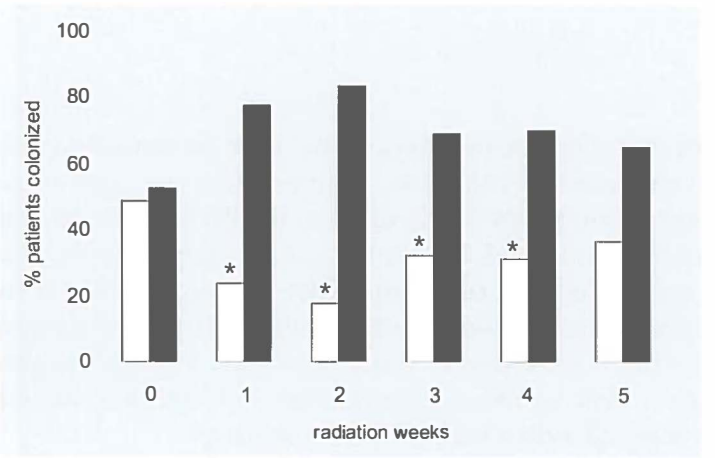


*Figure 2. Carriage of the oropharynx for Candida species for the PTA group (white) and the placebo group (black). * represents a significant difference between the PTA and placebo group*

The colonization index and carriage of aerobic Gram-negative bacilli at baseline was equal in both groups (P=0.9). During the radiation period the colonization index in the PTA group was less than in the placebo group, but the difference was only significant in the second week of radiation (P=0.05).

During the first 2 weeks the carriage of aerobic Gram-negative bacilli was reduced in the PTA group (P<0.04). In weeks 3-5 the difference was no longer significant (Figure 3).

All results are summarized in table 2.

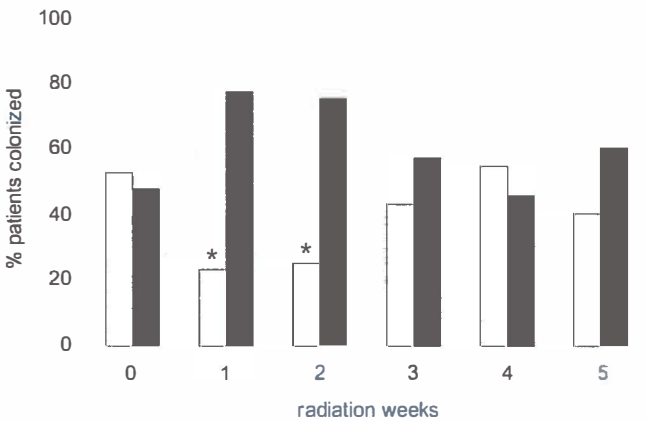


*Figure 3. Carriage of the oropharynx for aerobic Gram-negative bacilli for the PTA group (white) and the placebo group (black). * represents a significant difference between the PTA and placebo group*

DISCUSSION

In this study, no effect of selective oral flora elimination on mucositis was observed. The development of mucositis follows the same pattern as reported in an earlier cohort study.[18] According to the WHO score 80% of the patients in the PTA group and 90% in the placebo group developed mucositis grade 3 and 4. This severity of mucositis is in accordance with the outcomes of Okuno and colleagues.[11] In other studies, different outcomes are reported. A significant reduction of mucositis in the PTA group was found by two groups.[9,10] Both studies are nonrandomized clinical trials and the PTA group is compared with a historical control group.

A reduction in mucositis distribution and affected area, dysphagia and weight loss in the PTA group is reported by Symonds and colleagues.[12]

Table 2. Results of the PTA-placebo group for mean mucositis, weight loss, carriage and colonization index of *Candida* species and aerobic Gram-negative bacilli

Week	0		1		2		3		4		5	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Mean mucositis												
PTA	0	0	0.5	0.7	2.2	1.7	4.6	2.7	4.5	2.7	5.0	2.3
placebo	0	0	0.3	0.4	2.6	2.0	4.8	2.3	4.9	2.5	5.2	2.8
Weight loss												
PTA	0	0	-0.3	1.0	0.4	1.3	0.6	2.1	1.0	2.7	1.3	3.0
placebo	0	0	0.3	0.9*	0.6	1.2	1.3	1.7	2.2	2.3	2.8	2.9*
Candida col. index												
PTA	1.0	1.3	0.4	0.8	0.5	0.9	0.6	0.9	0.8	1.5	1.0	1.5
placebo	1.1	1.3	1.2	1.6*	1.6	1.7*	1.7	1.7*	2.0	1.7*	1.9	1.8*
Candida carriage												
PTA (%)	48		23.5		17.6		31.8		30.8		36	
Placebo (%)	52		76.5#		82.4#		68.2#		69.2#		64	
aerobic Gram neg bac. col. index												
PTA	0.7	1.2	0.4	0.8	0.4	0.9	0.6	1.2	0.5	1.0	0.4	0.9
Placebo	0.7	1.4	0.8	1.1	0.9	1.1*	0.8	1.0	0.8	1.4	0.8	1.5
aerobic Gram neg bac. carriage												
PTA (%)	52.4		23.1		25		42.9		54.5		40	
Placebo (%)	47.6		76.9#		75#		57.1		45.5		60	

** represent a significant difference between the PTA and placebo group using an independent sample t-test, # represent a significant difference between the PTA and placebo group using chi-square test*

From a total of 221 patients in that study, 98 (44 %) patients had a larynx carcinoma (PTA = 57, placebo = 41). Of these patients the radiation field included only a minor part of the oral mucosa, in which mucositis could develop. In the study of Okuno and colleagues only a subjective patient-reported amelioration of mucositis was reported but no reduction was found in clinically observed mucositis.[11] The PTA group (n = 54) in that study consisted of an unblinded (n = 29) and a blinded (n = 26) group. Only in the unblinded PTA group the mean mucositis, reported by the patients was lower than the placebo group. Recently, it was shown in a randomized study including 77 patients that selective oral flora elimination does not reduce radiation mucositis.[13] A problem in that study is the short evaluation time of only the first 3 weeks of radiotherapy. Whereas normally development of severe mucositis starts after 3 weeks of radiation.[2]

A complicating factor in comparing outcomes from different studies is the assessment method of mucositis. All studies used different scoring methods. Therefore, two scoring methods were used in the current study. The WHO score is a widely accepted method, but this score is a combination of local mucositis signs and general complaints.[16] The other scoring method in the current study is based only on mucosal signs of mucositis.[4] It therefore provides a more precise estimation of the mucositis development at the mucosal level. It further makes a comparison possible with outcomes of earlier publications.[4,19] For future studies, we recommend the use of the OMAS score from the mucositis study group because this scoring method is a reliable, well validated and widely accepted method.[20] This scoring method was published later than the start of the current study and was therefore not used as scoring method in this study.

In the current study, patients who received PTA lozenges had less weight loss than patients receiving placebo lozenges, assuming a better feeding status of the PTA-group patients (mean difference 1.5 kg). Owing to the minimal effect of PTA on the mucositis level, we found the feeding outcome of minor clinical relevance.

In our study, carriage and colonization of aerobic Gram-negative bacilli and *Candida* species decreased in the PTA group but was not totally eradicated. These findings are in line with the findings of other studies.[9,10,12,13] Based on these findings and the development and severity of mucositis it can be concluded that the presence of *Candida* species and aerobic Gram-negative bacilli has no influence on the development of radiation induced mucositis. The increase of the carriage and colonization of *Candida* species and aerobic Gram-negative bacilli after 3 weeks of radiation may be explained by the development of xerostomia, which makes dissolving of the lozenges more difficult. Wijers and colleagues tried to overcome this problem by using a paste instead of a lozenge. However, the paste appeared to be an unsuccessful form of application because already after randomization 32% of the patients refused further participation and 77% of the patients dropped out after four study weeks because of bad taste and unpleasant sensation of the paste texture in the mouth.[13]

In conclusion, PTA lozenges have a positive effect on the quality of feeding and amount of weight loss but cannot prevent severe mucositis. The presence of *Candida* species and aerobic Gram-negative bacilli has no effect on the development and severity of radiation induced mucositis.

Based on our findings of this randomized clinical trial, we do not recommend this type of supportive care for the reduction or prevention of radiation mucositis.

REFERENCES

1. Sutherland SE, Browman GP. Prophylaxis of oral mucositis in irradiated head-and-neck cancer patients: a proposed classification scheme of interventions and meta-analysis of randomized controlled trials. *Int J Radiat Oncol Biol Phys* 2001;49:917-930.
2. Kaanders HAM, van der Kogel AJ, Ang KK. Altered fractionation: limited by mucosal reactions? *Radiother Oncol* 1999;50:247-260.
3. Miralbell R, Allal AS, Mermillod B, Pastoors B. The influence of field size and other radiotherapy parameters on acute toxicity in pharyngolaryngeal cancers. *Strahlenther Onkol* 1999;175:74-77.
4. Spijkervet FKL, van Saene HKF, Panders AK, Vermey A, Mehta DM. Scoring irradiation mucositis in head and neck cancer patients. *J Oral Pathol Med* 1989;18:167-171.
5. Baker DG. The radiobiological basis for tissue reactions in the oral cavity following therapeutic X-irradiation. A review. *Arch Otolaryngol* 1982;108:21-24.
6. van Saene HKF, Martin MV. Do microorganisms play a role in irradiation mucositis? *Eur J Clin Microbiol Infect Dis* 1990;9:861-863.
7. Bernhoft CH, Skaug N. Oral findings in irradiated edentulous patients. *Int J Oral Surg* 1985;14:416-427.
8. Sonis ST. Mucositis as a biological process: a new hypothesis for the development of chemotherapy-induced stomatotoxicity. *Oral Oncol* 1998;34:39-43.
9. Spijkervet FKL, van Saene JJM, Panders AK, Vermey A, Mehta DM. Mucositis prevention by selective elimination of oral flora in irradiated head and neck cancer patients. *J Oral Pathol Med* 1990;19:486-489.
10. Kaanders HAM, Pop LAM, Muytjens HL, Bruaset I, van Daal WAJ. Topical administration of antimicrobial agents to prevent irradiation mucositis of the oral cavity and oropharynx: A pilot study. *Rad Oncol Inves* 1995;2:283-290.
11. Okuno SH, Foote RL, Loprinzi CL, Gulavita S, Sloan JA, Earle J, Novotny PJ, Burk M, Frank AR. A randomized trial of a nonabsorbable antibiotic lozenge given to alleviate radiation-induced mucositis. *Cancer* 1997;79:2193-2199.
12. Symonds RP, McIlroy P, Khorrami J, Paul J, Pyper E, Alcock SR, McCallum I, Speekenbrink ABJ, McMurray A, Lindemann E, Thomas M. The reduction of radiation mucositis by selective decontamination antibiotic pastilles: a placebo-controlled double-blind trial. *Br J Cancer* 1996;74:312-317.
13. Wijers OB, Levendag PC, Harms ER, Gan-Teng AM, Schmitz PI, Hendriks WD, Wilims EB, van der Est H, Visch LL. Mucositis reduction by selective elimination of oral flora in irradiated cancers of the head and neck: a placebo-controlled double-blind randomized study. *Int J Radiat Oncol Biol Phys* 2001;50:343-352.
14. Anonymous. ICRU Report 50: Prescribing, recording and reporting photon beam therapy. Bethesda (MD): *ICRU Publications* 1993.

15. Jansma J, Vissink A, Spijkervet FKL, Roodenburg JLN, Panders AK, Vermey A, Szabo BG, Gravenmade EJ. Protocol for the prevention and treatment of oral sequelae resulting from head and neck radiation therapy. *Cancer* 1992;70:2171-2180.

16. Anonymous. Handbook for reporting results of cancer treatment. *WHO Offset Publ* 1979;48:15-22.

17. Spijkervet FKL, van Saene HKF, Panders AK, Vermey A. Colonisation index of the oral cavity: a novel technique for monitoring colonisation defence. *Microb Ecol Health Dis* 1989;2:145-151.

18. Spijkervet FKL, Panders AK, Vermey A, van Saene JJM, Mehta DM, Fidler V. Effect of chlorhexidine rinsing on the oropharyngeal ecology in patients with head and neck cancer who have irradiation mucositis. *Oral Surg Oral Med Oral Pathol* 1989;67:154-161.

19. Parulekar W, Mackenzie R, Bjarnason G, Jordan RCK. Scoring oral mucositis. *Oral Oncol* 1998;34:63-71.

20. Sonis ST, Eilers JP, Epstein JB, LeVeque FG, Liggett Jr WH, Mulagha MT, Peterson DE, Rose AH, Schubert MM, Spijkervet FKL, Wittes JP. Validation of a new scoring system for the assessment of clinical trial research of oral mucositis induced by radiation or chemotherapy. Mucositis Study Group. *Cancer* 1999;85:2103-2113.

7

Clinical effects of flurbiprofen tooth patch on radiation induced oral mucositis. A pilot study

M.A. Stokman
F.K.L. Spijkervet
F.R. Burlage
J.L.N. Roodenburg

Supportive Care in Cancer 2005;13:42-48

SUMMARY

Background Mucositis is an oral sequelae of radiotherapy. In the development of mucositis several mechanisms play a role, such as inflammation and the effect of radiation on the high proliferation rate of oral basal epithelial cells. Therefore, administration of a drug with anti-inflammatory and antiproliferative properties might delay the disorder and/or alleviate the severity of oral mucositis. The aim of this pilot study was to evaluate the effect of flurbiprofen in a tooth patch on the development, severity and duration of pseudomembranous mucositis in patients treated with curative head and neck radiotherapy.

Patients and methods The study group comprised 12 patients with a malignant tumor in the head and neck region to be treated with primary curative or postoperative radiotherapy. Patients applied once a day before sleep a flurbiprofen tooth patch to a natural tooth or upper denture during the full course of radiotherapy, starting 1 week before the onset of radiotherapy. Oral mucositis, pain, feeding, body weight and viability and maturation of epithelial cells were assessed. The results were compared with the findings in a historical control group.

Results No differences were found for severity and duration of pseudomembranous mucositis between the two groups. The onset of pseudomembranous/ulcerative mucositis occurred later in the flurbiprofen group (14.6 ± 3.8 days, mean ± SD) than in the historical control group (11 ± 3.5 days; $P<0.05$).

Conclusion This study shows that the flurbiprofen 15 mg tooth patch cannot prevent the development of pseudomembranous mucositis and has no influence on the duration of oral mucositis.

INTRODUCTION

Oral mucositis is an inflammation of the oral mucosa due to radiotherapy in patients with head and neck cancer.[1] Until now no effective intervention has been developed to prevent oral mucositis following radiotherapy.[2,3] However, in a previous study benzydamine in an oral rinse has been shown to improve the ulcer-free rate and to diminish the incidence of ulceration and erythema in patients undergoing conventional curative radiotherapy.[4] Changing radiotherapy schedules or combining with chemotherapy induces more severe oral mucositis and makes its prevention more important.[5]

Ionizing radiation damages rapidly proliferating cells, such as epithelial tumor cells and layer cells of the oral epithelium. The development and severity of irradiation mucositis depends on type of radiation, fractionation schedule, total cumulative dose, and irradiated tissue volume.[6] During curative radiation without preventive strategies the first sign of mucositis can be objectively identified after delivery 10 Gy of radiation as a white discoloration or erythema of the mucosa. The more severe stages are clinically seen after delivery 30 Gy of radiation as the formation of pseudomembranes and ulceration. About 80% of patients receiving radiotherapy (cumulative dose of 50-70 Gy) are likely to develop ulceration/pseudomembranes.[1,7]

The biological basis of mucositis is the sterilization of proliferating cells in the germinative layer of the epithelium. Their gradual proliferative failure causes a deficit in the cellular supply of the functional layers. Due to the natural loss of the superficial layer by mechanical wear and tear, hypoplasia of the epithelium and eventually ulcerative mucositis develops.[8]

The development of mucositis due to radiation is only partially understood at cellular level. Five consecutive phases have been suggested recently in the development of mucositis: (1) initiation, (2) primary damage response, (3) signal amplification, (4) ulceration, and (5) healing.[9]

Prevention of mucositis is the best treatment option. Nowadays, therapy is based on symptomatic treatment with the use of analgesics, oral rinses, topical application of disinfecting agents, topical/systemic application of antibiotics. The current state of knowledge of the biological basis of mucositis has led to the introduction of the following experimental drugs for prevention of mucositis:

- Anti-inflammatory agents, such as benzydamine [9];
- Modulators of bone marrow and/or epithelial cell differentiation such as granulocyte-macrophage colony-stimulating factor (GM-CSF) [10];
- Drugs protecting damaged oral mucosa from the effects of antineoplastic therapy such as sucralfate[11], amifostine[12], prostaglandin E_2[13];
- Antimicrobial agents such as a decontamination antibiotic lozenge[14], povidone iodine.[15]

Most drugs are administered as tablets or by topical oral application (mouth rinse, ointment or lozenge). A few drugs, such as amifostine are administered parenterally. Studies of these drugs are either still in early clinical phases, have yielded inconclusive results, and/or have demonstrated severe adverse events, such as vomiting and hypotension.[16]

Radiation injury to the epithelium, causing mucositis, is associated with production of active oxygen and cytokines (such as IL-1 and IL-6). During this process cyclooxygenase 2 (COX-2) is induced and has been found to be responsible for the synthesis of prostaglandins which cause the separation of tight cellular junctions and increase in vascular permeability.[17] Flurbipofen is an efficient inhibitor of COX-2, and might delay or prevent mucositis.[18] Secondly, flurbiprofen has an antiproliferative activity by inhibition of normal cell proliferation. A drug with anti-inflammatory and antiproliferative properties is a promising option to delay the development and/or alleviate the severity of radiation mucositis.[19]

A drug delivery system (Perio Products, Jerusalem, Israel) has been developed consisting of a tooth patch that can be affixed to a tooth (buccal side) without an adhesive, and delivers the active agent to the oral mucosa.[20] Initial in vitro studies with a flurbiprofen tooth patch showed that this drug is released from the patch over a period of several hours. The potential efficacy of a flurbiprofen tooth patch on mucositis has been studied in a hamster model of radiation induced mucositis. Tooth patches containing 15 mg flurbiprofen, or placebo, were inserted into hamster cheek pouches, one on day -5 or -3 and/or one on day 0 immediately following acute irradiation of the cheek pouches with a dose of 35 Gy. Starting from 2 weeks after irradiation, the clinical severity and duration of oral mucositis were significantly reduced in hamsters which had received one flurbiprofen patch 5 days before radiation, as compared to placebo or no drug treatment (data not shown; personal communication, J. Loewenstein).

The aim of this study was to evaluate the effects of the flurbiprofen tooth patch on the development, severity and duration of oral mucositis in patients treated with curative head and neck radiation.

PATIENTS AND METHODS

Patient selection

Patients with a malignant tumor in the head and neck region to be treated with primary curative or postoperative radiotherapy were eligible for this study. Inclusion criteria for the study were: conventional, fractionated, postoperative radiotherapy to the oropharyngeal region using a 6 MeV linear accelerator. The dose was calculated using computerized planning, fractionation of 2 Gy daily, five times a week, for a total cumulative irradiation dose of 60-70 Gy, with at least half of the oral cavity within in the radiation field. The dose specification was in line with ICRU 50 recommendations.[21]

Criteria for exclusion were: history of allergy to flurbiprofen, aspirin or another NSAIDS; an oral mucosal defect other than related to tumor surgery; treated with chemotherapy concurrently or within 4 weeks before the start of radiotherapy; treatment concomitantly with NSAID's or diuretics; alcohol (> 3 units daily) or drug abuse; general health condition with active bleeding gastric ulcer, or significant hepatic, neurological, psychiatric, endocrine disease.

As a standard procedure, all patients were evaluated before radiation treatment for potential risk factors for oral complications by means of a thorough oral and dental evaluation, including a radiographic examination. All potential risk factors were eliminated appropriately before the start of radiotherapy. The supportive oral care regimen consisted of a daily cleansing of the oral cavity with a saline spray administered by a dental hygienist and mouth rinsing by the patients themselves with a salt/baking soda solution at least eight times a day to remove sticky saliva and debris. Dentate patients applied a neutral fluoride gel every second day using custom-made trays.[22] Patients had a natural tooth or an upper denture to which a tooth patch could be affixed to the buccal side.

The Medical Ethics Committee approved the study and all eligible patients gave written informed consent.

Treatment

Each eligible patient applied a flurbiprofen (15mg) tooth patch once a day before sleep at night to the same natural tooth or the upper denture to the buccal side. Patients administered the patches themselves starting 1 week before the start of radiotherapy, and on each following night. The medication was applied until completion of the course of radiotherapy. Drug treatment was discontinued at the first onset of ulceration/pseudomembrane formation because of possible negative effects of a NSAID on ulcers of the mucosa. During every study visit the patient was questioned regarding adverse effects of the flurbiprofen tooth patch. All patients started the radiotherapy at the same weekday (Monday). The total study duration for each enrolled patient was 14 weeks.

Treatment evaluation

During the study period evaluation was performed 1 week prior to the start of radiotherapy and thrice weekly (Monday, Wednesday, Friday) during 6 weeks of radiotherapy. Afterwards two follow up visits at a 1-week interval were scheduled. Mucositis, oral pain, and pain on swallowing and feeding were scored. Body weight scores were obtained once a week. Prior to, and at the end of each week of radiation, an oral washing, as well as a buccal smear with a cytobrush, were carried out for determination of epithelial cell viability and maturation.[23]

Mucositis

The mucositis was scored according the Oral Mucositis Assessment Scale (OMAS) and WHO score. OMAS evaluates nine regions of the oral cavity for erythema and the presence and size of pseudomembranes or ulcerations. The value of OMAS at any given assessment is obtained by summing the erythema and ulceration/pseudomembrane subscores at each site and then averaging these scores across all nine sites. The mean score has a range from 0-5.[24] The WHO mucositis score is as follows: grade 0 = normal, no mucositis; grade 1 = soreness and erythema; grade 2 = erythema, ulcers, can eat solids; grade 3 = ulcers, requires liquid diet only; grade 4 = alimentation not possible.[25]

Pain

Oral pain and pain on swallowing were scored subjectively on 100-mm visual analogue scales (VAS) ranging from no pain (0 mm) to intolerable

pain (100 mm), and no impact on swallowing (0 mm) to impossible to swallowing (100 mm) respectively.

Feeding

The global assessment of eating function was scored on a four-level scale: 1 = normal food, 2 = soft food only, 3 = liquids only and 4 = no oral food intake possible. The total body weight was assessed once a week, beginning at start of radiotherapy until the study end visit.

Viability of mucosal epithelial cells and maturation

To acquire an oral washing, patients gargled and rinsed their mouth with 10 ml sterile saline for 30 sec, and spat out into a tube. This expectorate was centrifuged within 10 min after collection (190 g, 10 min, room temperature) and the supernatant was discarded. The fluid was washed with 10 ml saline and centrifuged again to eliminate salivary fibers. Pellets were resuspended in 1 ml RPMI 1640 medium (Gibco, Paisley, UK) containing 5% fetal calf serum. Subsequently, 50 µl suspension and 50 µl trypan blue dye (0.4% in 0.9% NaCl) were combined and immediately transferred to a hemocytometer. Cells were counted, after which the percentage of viable cells and total number of cells were calculated.

Buccal mucosa smears were put on glass-slides and stained according to the procedure described by Papanicolaou and Traut.[26] Epithelial cell morphology was assessed after collection of all slides at the end of study period. The assessment was blinded and randomized, and was performed by one observer (M.A.S.). Orange-stained cells were classified as mature, while blue/green-stained cells were categorized as immature cells. Cells with a partly orange and partly green appearance were graded as intermediate. The percentages of mature, intermediate and immature cells were determined from each smear.

Historical control

The historical control group consisted of ten patients, seven men and three women, aged 59.2 ± 11.7 (mean±SD) (Table 1).[23] Nine patients had a squamous cell carcinoma, (seven oral, and two in the tonsil area), and one patient had an adenocarcinoma of the parotid gland. The patients were radiated according the same inclusion criteria, and the supportive oral care regimen was the same as used in the current study.

Table 1. Patient characteristics

Patients characteristics	Flurbiprofen (n=12)	Historical control (n=10)	*P*-value
*Age ± SD (yrs)	57.7 (13.5)	59.2 (11.7)	0.73
Gender: male / female (n)	8 / 4	7 / 3	0.68
Tumor site			
Oral cavity (n)	7	7	0.45
Oropharynx (n)	4	2	
Unknown primary (n)	1	0	
Salivary Gland Tumor	0	1	
Histology			
Squamous (n)	10	9	1.00
Other (n)	2	1	
T-stage			
T1 (n)	0	3	0.34
T2 (n)	6	3	
T3 (n)	1	1	
T4 (n)	4	2	
Unknown (n)	1	1	
N-stage			
N0 (n)	7	4	0.60
N1 (n)	2	2	
N2a (n)	0	0	
N2b (n)	1	1	
N2c (n)	1	0	
N3 (n)	0	2	
Unknown (n)	1	1	
Dentures			
Yes (n)	8	5	1.00
No (n)	4	5	
Operation			
Yes (n)	9	9	0.37
No (n)	3	1	

*All differences between the groups were analyzed using chi-square test except * in which t-test was used*

Statistical analysis

Student's t-test for independent samples was used to analyze the differences between flurbiprofen-treated patients and the historical controls with respect to OMAS score, loss of weight, oral pain and pain on swallowing. The Mann-Whitney U test was used to analyze the differences in WHO mucositis score, feeding, viability and maturation of epithelial cells between the flurbiprofen-treated patients and the historical controls. Spearman's correlation was used

to evaluate the relationship between mucositis, oral pain and pain on swallowing. To compare the viability of epithelial cells and maturation before and during radiation treatment, the Wilcoxon's signed ranks test for paired samples was used. Two-sided P values <0.05 were considered statistically significant.

RESULTS

During 15 months, 24 patients were screened from among whom 12 patients fulfilled the inclusion criteria. The patients characteristics are shown in table 1.

None of the patients applied the flurbiprofen 15 mg tooth patch during the whole radiation period due to development of pseudomembranous/ulcerative mucositis at an early stage during radiotherapy. The application period after start of radiotherapy was 14.6 ± 3.8 days (mean±SD) and was in accordance with the mean onset of pseudomembranous/ulcerative mucositis. In comparison, the mean onset of pseudomembranes in the historical control group was 11 ± 3.5 days. The difference between the historical control and flurbiprofen groups was significant ($P<0.05$).

No adverse events were reported during the evaluation period for the flurbiprofen tooth patch.

Mucositis

The mean OMAS mucositis scores are shown in figure 1. During the first 2 weeks, the mucositis developed gradually, but after this period a steep increase in mucositis was observed. During the first 2 weeks, the development and degree of mucositis was lower in the flurbiprofen group than in the historical control group (Figure 1). After 2 weeks of radiation the difference in the mean OMAS score between the flurbiprofen and historical control groups was significant ($P=0.007$). After 3 weeks of radiation the OMAS mucositis score was not significantly different between the two groups.

The WHO mucositis score for the flurbiprofen-treated patients followed the same pattern as the OMAS mucositis score. A steep increase in the mucositis score was seen after 2 weeks of radiation, with a stabilization of the severity during the rest of the radiation period, and a slight decrease after cessation of the radiotherapy. All study patients showed a WHO mucositis score of at least 2.

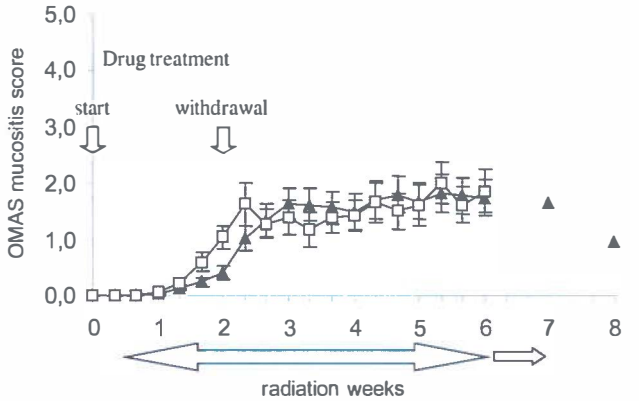


Figure 1. OMAS mucositis scores (± sem) for the flurbiprofen group (▲) and the historical control group (□)

Pain

The increase in oral pain and pain on swallowing followed closely the OMAS mucositis score. A steep increase in pain was seen between week 2 and 3, the period of development of the severe forms of mucositis. No correlation was found between mucositis, oral pain and pain on swallowing. Oral pain and pain on swallowing were higher in the flurbiprofen group than in the historical control group. This difference was significant only after the second radiation week (P=0.03).

Feeding and Body weight

All patients had a global assessment of eating function ≥ 2 after 2 weeks of radiotherapy. A mean weight loss of 3.2 kg was observed between the start of radiotherapy (mean body weight 77.9 kg) and after 6 weeks of radiotherapy (mean body weight 74.7 kg). At the study end visit the mean body weight loss was 4.6 kg (mean body weight 73.3 kg) from the start of radiotherapy. No significant differences were found between the flurbiprofen and historical control groups in feeding and weight loss.

Viability and maturation of epithelial mucosal cells

The percentage of viable epithelial cells at baseline was 52.7% ± 9.1%. During the first week the percentage of viable epithelial cells decreased, after which viability was variable in character. No significant differences were found

Table 2. OMAS scores, oral pain, pain on swallowing, weight, viability and maturation of epithelial cells in the flurbiprofen and control groups

Week (cumulative radiation dose, Gy)	0 (0)		1 (10)		2 (20)		3 (30)		4 (40)		5 (50)		6 (60)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
OMAS														
Flurbiprofen	0	0	0.03	0.06	0.4	0.4	1.6	0.1	1.5	1.1	1.7	1.1	1.7	1.2
Control	0	0	0.07	0.13	1.0	0.6	1.4	0.9	1.4	0.9	1.6	1.2	1.9	1.1
Oral Pain														
Flurbiprofen	0.2	0.3	0.4	0.5	2.4	2.2	3.3	2.2	3.8	2.6	4.5	2.7	4.6	3.4
Control	0.2	0.6	0.1	0.3	0.6	0.8*	1.4	2.2	1.4	2.7	2.0	3.2	2.2	3.3
Pain on swallowing														
Flurbiprofen	0.7	1.7	0.5	0.6	2.5	2.3	3.1	2.5	3.6	2.8	4.2	3.0	3.9	3.2
Control	0.1	0.3	0.1	0.3	0.8	1.1*	1.6	2.2	1.7	3.0	2.0	3.3	1.6	3.2
Weight loss														
Flurbiprofen	0	0	0.6	1.4	0.8	1.6	1.5	1.9	2.3	2.5	3.2	3.4		
Control	0	0	0.1	0.2	1.1	1.0	2.4	1.6	2.9	2.1	4.0	2.5		
Viability of epithelial cells %														
Flurbiprofen	52.7	9.1	46.9	16.5	55.0	9.0	58.1	12.2	50.9	13.5	58.8	20.0	52.8	15.3
Control	46.2	17.8	49.7	14.0	58.6	13.2	62.9	19.2	56.2	20.0	54.1	13.0	50.6	13.9
Mature Epithelial cells %														
Flurbiprofen	39.8	35.2	45.3	27.2	55.2	29.6	70.4	16.2	71.1	32.8	69.2	33.7	71.6	26.8
Control	43.9	25.8	41.6	17.5	65.7	22.6	74.2	10.0	61.6	17.7	75.3	11.1	74.4	10.8
Intermediate epithelial cells %														
Flurbiprofen	33.1	14.7	31.6	18.0	29.8	16.2	18.9	7.5	14.1	12.1	15.9	9.0	19.3	15.5
Control	52.7	22.2	49.1	18.5	32.8	22.4	25.1	10.0	35.9	16.1#	24.0	10.9	24.2	10.9
Immature epithelial cells %														
Flurbiprofen	20.3	16.6	15.9	17.5	15.3	19.0	7.2	8.4	0.7	1.6	1.6	2.4	2.8	3.6
Control	3.5	7.8	5.8	7.8	1.5	3.6	0.7	1.8	2.6	5.7	0.8	1.2	1.4	1.6

** Significant difference between the flurbiprofen and control groups by an independent sample t test*
 # Significant difference between the flurbiprofen and control groups by the Mann-Whitney U test

between the baseline and the other radiation weeks. Neither was a significant difference found between the flurbiprofen and the historical control group.

The mean percentage of mature cells increased during radiotherapy, but this increase was only significant at the end of third radiation week compared to the baseline value (P=0.03). The mean percentage of intermediate cells decreased and was significant for the third, fourth and fifth radiation week compared to the baseline outcome (P=0.02, 0.03, 0.04 respectively). The mean percentage of immature cells also decreased and was significant after the third radiation week until the end of the radiation period compared to the baseline value (P<0.04). The only significant difference between the flurbiprofen and historical control groups was found for the intermediate cells during the fourth week of radiation.

The results for the flurbiprofen and control groups are summarized in table 2.

DISCUSSION

The primary endpoint of this study was prevention of pseudomembranous mucositis. The study showed that the flurbiprofen 15 mg tooth patch only delayed and did not prevent development of pseudomembranous mucositis.

The process of development of mucositis is thought to be a five-phase process at the cellular level. The reducing influence of a NSAID could be expected during the initial phase of inflammation and vascular reactions due to the radiation. Flurbiprofen is an efficient inhibitor of COX-2 which is induced during inflammatory processes. It has been shown in an animal model that after radiation, COX-2 is expressed at high levels on days 10 and 16, especially in submucosal fibroblasts and endothelium. The kinetics of COX-2 parallel mucositis severity. It was concluded that COX-2 is not a primary driver of radiation injury, but instead plays an amplifying role.[27] These outcomes might explain the delayed initial development of irradiation mucositis during the first 2 weeks in patients treated with flurbiprofen 15 mg tooth patches compared to a historical control group.[23] However, in the current study application of flurbiprofen tooth patches was discontinued on pseudomembrane/ulceration formation. Because the level of expression of COX-2 increases during ulceration, it can be suggested that a longer application period of flurbiprofen should be evaluated.

Flurbiprofen is a drug with antiproliferative properties. It could therefore potentially be used to delay the development of mucositis by its ability to attenuate the high rate of basal epithelial cell proliferation even prior to the initiation of antineoplastic therapy. This could be the explanation for the delay in the development of pseudomembranes in the flurbiprofen group. The assessment of viability and maturation of epithelial cells cannot explain the delay at a cellular level because no differences were found in viability or maturation of epithelial cells between the flurbiprofen and control groups.

The onset of pseudomembranous/ulcerative mucositis in this study was significantly later in the flurbiprofen group than in the historical control group where no preventive measures were tested. However, the clinical relevance of this finding is disputable because in none of the flurbiprofen treated patients development of pseudomembranes was prevented. Furthermore, no effect was found on the duration of mucositis. This is in contrast to the findings in the hamster model in which a reduction in severity and duration was found. In the human situation, radiation is on a daily basis, five times per week, whereas in the animal situation the effects of a single radiation were studied. The proliferation of epithelial cells failed during radiotherapy, resulting in a loss of the basal layer after 3 weeks. Apparently starting the flurbiprofen tooth patch 1 week before the start of radiotherapy did not influence the susceptibility of the epithelial cells to radiation damage, as might have been expected from the hamster study.

The effect of the flurbiprofen tooth patch was found to be minimal, probably because of the small sample size, the single tested dosage and the application period. In this study no serious side effects related to the drug were seen. The outcome of this study together with previously reported findings indicate that a higher dosage or longer application period should be investigated in a future study.

The flurbiprofen group experienced more pain than the control group. The baseline scores for oral pain and pain on swallowing in the flurbiprofen group were already higher. At the end of the second radiation week the mucositis was significantly lower in the flurbiprofen group but pain was significantly higher. No correlation was found between mucositis, oral pain and pain on swallowing. However, in contrast, in the validation study of the OMAS score, mucositis, oral pain and pain on swallowing were strongly correlated.[24] No rational explanation for this phenomenon can be suggested.

This study showed no effect of the flurbiprofen tooth patch on severity and duration of oral mucositis due to radiation. Only a delayed development

of pseudomembranous/ulcerative mucositis was seen in the flurbiprofen group compared to a historical control group.

ACKNOWLEDGEMENT

The authors are grateful for support from Perio Products, Ltd, in supplying the flurbiprofen tooth patches.

REFERENCES

1. Spijkervet FKL, van Saene HKF, Panders AK, Vermey A, Mehta DM. Scoring irradiation mucositis in head and neck cancer patients. *J Oral Pathol Med* 1989;18:167-171.
2. Sutherland SE, Browman GP. Prophylaxis of oral mucositis in irradiated head-and-neck cancer patients: a proposed classification scheme of interventions and meta-analysis of randomized controlled trials. *Int J Radiat Oncol Biol Phys* 2001;49:917-930.
3. Clarkson JE, Worthington HV. Interventions for preventing oral mucositis for patients with cancer receiving treatment (Cochrane Review). *Cochrane Library*. Chichester, UK: John Wiley & Sons, Ltd, 2004.
4. Epstein JB, Silverman S Jr, Paggiarino DA, Crockett S, Schubert MM, Senzer NN, Lockhart PB, Gallagher MJ, Peterson DE, LeVeque FG. Benzydamine HCl for prophylaxis of radiation-induced oral mucositis: results from a multicenter, randomized, double-blind, placebo- controlled clinical trial. *Cancer* 2001;92:875-885.
5. Trotti A, Bellm LA, Epstein JB, Frame D, Fuchs HJ, Gwede CK, Komaroff E, Nalysnyk L, Zilberberg MD. Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: a systematic literature review. *Radiother Oncol* 2003;66:253-262.
6. Miralbell R, Allal AS, Mermillod B, Pastoors B. The influence of field size and other radiotherapy parameters on acute toxicity in pharyngolaryngeal cancers. *Strahlenther Onkol* 1999;175:74-77.
7. Baker DG. The radiobiological basis for tissue reactions in the oral cavity following therapeutic X-irradiation. A review. *Arch Otolaryngol* 1982;108:21-24.
8. Dorr W. Radiobiological models of normal tissue reactions. *Strahlenther Onkol* 1998;174 Suppl 3:4-7.
9. Sonis ST. The pathobiology of mucositis. *Nature Reviews Cancer* 2004;4:277-284.
10. Saarilahti K, Kajanti M, Joensuu T, Kouri M, Joensuu H. Comparison of granulocyte-macrophage colony-stimulating factor and sucralfate mouthwashes in the prevention of radiation-induced mucositis: A double-blind prospective randomized phase III study. *Int J Radiat Oncol Biol Phys* 2002;54:479-485.
11. Meredith R, Salter M, Kim R, Spencer S, Weppelmann B, Rodu B, Smith J, Lee J. Sucralfate for radiation mucositis: results of a double-blind randomized trial. *Int J Radiat Oncol Biol Phys* 1997;37:275-279.
12. Buntzel J, Kuttner K, Frohlich D, Glatzel M. Selective cytoprotection with amifostine in concurrent radiochemotherapy for head and neck cancer. *Ann Oncol* 1998;9:505-509.

13. Matejka M, Nell A, Kment G, Schein A, Leukauf M, Porteder H, Mailath G, Sinzinger H. Local benefit of prostaglandin E2 in radiochemotherapy-induced oral mucositis. *Br J Oral Maxillofac Surg* 1990;28:89-91.

14. Stokman MA, Spijkervet FKL, Burlage FR, Dijkstra PU, Manson WL, de Vries EGE, Roodenburg JLN. Oral mucositis and selective elimination of oral flora in head and neck cancer patients receiving radiotherapy: a double-blind randomised clinical trial. *Br J Cancer* 2003;88:1012-1016.

15. Rahn R, Adamietz IA, Boettcher HD, Schaefer V, Reimer K, Fleischer W. Povidone-iodine to prevent mucositis in patients during antineoplastic radiochemotherapy. *Dermatology* 1997;195 Suppl 2:57-61.

16. Rubenstein EB, Peterson DE, Schubert M, Keefe D, McGuire D, Epstein J, Elting LS, Fox PC, Cooksley C, Sonis ST. Clinical practice guidelines for the prevention and treatment of cancer therapy-induced oral and gastrointestinal mucositis. *Cancer* 2004;100:2026-2046.

17. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, van de Putte LBA, Lipsky PE. Cyclooxygenase in biology and disease. *FASEB J* 1998;12:1063-1073.

18. Vane JR, Botting RM. Anti-inflammatory drugs and their mechanism of action. *Inflamm Res* 1998;47:S78-S87.

19. Lozanski G, Ballou SP, Kushner I. Effect of flurbiprofen on cytokine production by human monocytes and U-937 and THP-1 cell lines. *J Rheumatol* 1992;19:921-926.

20. Zevin S, Swed E, Cahan C. Clinical effects of locally delivered nicotine in obstructive sleep apnea syndrome. *Am J Ther* 2003;10:170-175.

21. Anonymous. ICRU Report 50: Prescribing, recording and reporting photon beam therapy. *Bethesda (MD): ICRU Publications*, 1993.

22. Vissink A, Burlage FR, Spijkervet FKL, Jansma J, Coppes RP. Prevention and treatment of the consequences of head and neck radiotherapy. *Crit Rev Oral Biol Med* 2003;14:213-225.

23. Stokman MA, Spijkervet FKL, Wymenga ANM, Burlage FR, Timens W, Roodenburg JLN, Vries EGEd. Quantification of oral mucositis due to radiotherapy by determining viability and maturation of epithelial cells. *J Oral Pathol Med* 2002;31:153-157.

24. Sonis ST, Eilers JP, Epstein JB, LeVeque FG, Liggett Jr WH, Mulagha MT, Peterson DE, Rose AH, Schubert MM, Spijkervet FKL, Wittes JP. Validation of a new scoring system for the assessment of clinical trial research of oral mucositis induced by radiation or chemotherapy. Mucositis Study Group. *Cancer* 1999;85:2103-2113.

25. Anonymous. Oral complications of cancer therapies: diagnosis, prevention, and treatment. National Institutes of Health. *Conn Med* 1989;53:595-601.

26. Papanicolaou GN, Traut HF. Diagnosis of uterine cancer by the vaginal smears. *New York: Commonwealth Fund*, 1943.

27. Sonis ST, O'Donnell KE, Popat R, Bragdon C, Phelan S, Cocks D, Epstein JB. The relationship between mucosal cyclooxygenase-2 (COX-2) expression and experimental radiation-induced mucositis. *Oral Oncol* 2004;40:170-176.

8 Outcome of local application of amifostine (WR-1065) on epirubicin induced oral mucositis. A phase II study

M.A. Stokman
F.M. Wachters
P. Koopmans
J.G.M. Burgerhof
H.J.M. Groen
F.K.L. Spijkervet
D.R.A. Uges
G.A.P. Hospers

Anticancer Research 2004;24:3262-3267

SUMMARY

Background Intravenous administration of amifostine reduces chemotherapy induced toxicity. Preclinical experiments showed a reduction in radiation induced mucositis after local application of the active metabolite of amifostine (WR-1065). This study evaluated the effect of local application of WR-1065 on chemotherapy induced oral mucositis.

Patients and Methods Non-small cell lung cancer patients treated with gemcitabine and epirubicin every 3 weeks for a maximum of five cycles were included. WR-1065 was administered during the second and third cycle as an oral rinse. Oral mucositis evaluation included WHO toxicity grading, a validated oral mucositis assessment scale (OMAS), and a questionnaire.

Results Twenty-four patients were evaluated for at least one control and one rinse cycle. Mucositis scores, pain and feeding difficulties increased from day 1 to day 15, and were not significantly different between control and rinse cycles. Local application of WR-1065 leads to detectable quantities of WR-1065 in epithelial mucosa cells. A negative correlation between WR-1065 concentration and OMAS score was found.

Conclusion No clinical detectable influence of WR-1065 on oral mucositis was found.

INTRODUCTION

Oral mucositis is an inflammatory-like change of the oral mucosa due to cytotoxic chemotherapy or radiotherapy. The type of antineoplastic treatment and patient related factors influence the incidence and severity of oral mucositis. The onset is usually between 5 and 8 days after chemotherapy and the duration is highly variable. Especially in patients receiving high-dose chemotherapy followed by bone marrow or peripheral stem cell transplantation, mucositis can be dose limiting.[1] The pathogenesis of oral mucositis is not fully understood, but is thought to involve direct and indirect mechanisms. Direct toxic effect of cytostatic agents on rapidly dividing cells of oral epithelium can result in mucosal atrophy, erythema and ulceration. Indirect stomatotoxic effects are caused by release of inflammatory mediators, loss of protective salivary constituents, and therapy induced neutropenia. Bacteria, fungi and viruses can superimpose secondary infections of the damaged mucosa. Mucositis is proposed to develop in four consecutive phases: 1) the inflammatory/vascular phase (release of free radicals and cytokines); 2) the epithelial phase (reduced epithelial renewal) with atrophy and ulceration; 3) the ulcerative/bacterial phase (colonization mixed flora, causing release of endotoxines) with further tissue damage by stimulation of cytokines; 4) the healing phase.[2]

Mucositis causes major discomfort in patients. Pain and restriction of normal feeding and drug intake are the most important discomforts. In severe stages of mucositis secondary infection of mucosal ulcers can provide a port of entry for microorganisms into the circulation, which can lead to life-threatening septicaemia, especially in myelosuppressed patients. It is worthwhile to investigate strategies to prevent oral mucositis since the actual regimens for mucositis prevention are mainly palliative. Local and systemic analgesics are applied for pain relief, while antimicrobial agents are applied for bacterial or fungal infections or for prevention.

Cytoprotective agents, such as amifostine (Ethyol®, WR-2721), can reduce the toxicity induced by radiotherapy or chemotherapy. Amifostine is a prodrug, which is active as a protective agent when dephosphorylated by alkaline phosphatase to its active metabolite WR-1065. WR-1065 is preferentially taken up into normal rather than neoplastic cells because of the higher alkaline phosphatase activity, better vascularization and higher pH of normal tissue. Once inside the cell, WR-1065 protects against

chemotherapy and radiation induced damage by scavenging free radicals, donating hydrogen ions to free radicals, depleting oxygen, and direct binding and inactivating cytotoxic drugs.[3,4] Intravenous administration of amifostine provides protection against a broad range of cytotoxic agents. Reduction in hematological or non-hematological toxicity is described for cisplatin, carboplatin, doxorubicin, paclitaxel, and 5-fluorouracil.[5-8] Prevention of mucositis is mainly described in head and neck cancer patients treated with radiotherapy.[9-11]

Preclinical experiments showed a reduction in radiation induced mucositis after local application of amifostine. Topical application of 50 mg amifostine to the buccal mucosa in mice reduced the severity of radiation induced oral mucositis, without any toxicity.[12] However, no prevention against radiation induced proctitis and colitis was found after rectal administration of amifostine doses between 100 and 450 mg/enema.[13] Reasons for failure to protect the rectosigmoid mucosa may be related to the method of administration (the rectum was not empty), and the relatively long period (45 minutes) between application of amifostine and the onset of radiation.

A pilot study in patients with stage IIIb or IV non-small cell lung cancer (NSCLC) evaluated the feasibility of 125 mg WR-1065 as a mouthwash. Before administration, 200 mg WR-2721 was ex vivo converted to WR-1065. No systemic side-effects were observed and WR-1065 was well tolerated. In this study, WR-1065 was detectable in washed, isolated, vital mouth mucosa cells at a concentration of 3.7-19.9 ng/10^5 cells.[14] Previous in vitro experiments showed that a cellular concentration of 13 ng WR-1065 in 10^5 mouth mucosa cells induced radioprotection.[15,16] Taken together, these findings suggest that 200 mg WR-2721 (10 ml 0.09 M) or 125 mg WR-1065 (10 ml 0.09 M) can be safely administered, and the reached cytoprotective concentration achieved might be effective in the treatment of oral mucositis.

The aim of this phase II trial was to evaluate the effect of local application of WR-1065, the active compound of amifostine, on oral mucositis in NSCLC patients treated with epirubicin and gemcitabine. The effect of WR-1065 on mucositis was the primary end point of this study. The secondary end point was to determine WR-1065 in oral epithelial mucosa cells.

PATIENTS AND METHODS

Patient selection

Patients (≥18 years) with a histologically or cytologically proven diagnosis of unresectable stage III or IV NSCLC were eligible for this study. Exclusion criteria were: child bearing potential without effective means of contraception; pregnancy or lactation; presence of other malignancies; presence of ulcerative lesions in the oral cavity or any grade of mucositis in the last 4 days; Sjögren's syndrome; infection requiring systemic antibiotics within the previous 14 days; significant renal dysfunction (serum creatinine levels > 1.5 times the normal acceptable range (62-106 µmol/L) or creatinine clearance less than 50 ml/minute); hematological disorders (leukocytes < $3.0x10^9$/L, neutrophils < $1.5x10^9$/L, Hb < 5.0 mmol/L); use of topical oral disinfectants within the previous two weeks; or use of other investigational drugs within the previous 30 days. The medical ethics review committee approved the protocol and all eligible patients gave written informed consent before study entry.

Treatment

Gemcitabine (1125 mg/m²) was administered in 250 mL of 0.9% NaCl by a 30-minute infusion on days 1 and 8 of each 21-day cycle. Epirubicin (100 mg/m², in 50 mL of 0.9% NaCl) was given as an intravenous bolus injection within 5 minutes on day 1 (after gemcitabine administration). Treatment consisted of a maximum of five cycles and was stopped earlier in case of tumor progression, intolerable toxicity or patient's request. Ondansetron 8 mg and dexamethason 8 mg were used as antiemetics twice a day on days 1, 2, and 8. During the second and third cycle, WR-1065 (10 ml, 20 mg/mL) was administered as an oral rinse. Before administration, amifostine (WR-2721) was converted to WR-1065 ex vivo (WR-2721 at 37 °C for 1 hour, pH 3.5), because alkaline phosphatase concentrations in the mouth are low. The oral rinse was used 15 minutes before and 5 minutes after epirubicin infusion. Patients were instructed to rinse and gargle for 1 minute and subsequently to spit out.

Treatment evaluation

During the study period, evaluation was conducted on days 1, 8 and 15 of each cycle by a dental hygienist or a physician. Oral mucositis was evaluated using World Health Organization (WHO) toxicity grading and the Oral

Mucositis Assessment Scale (OMAS).[17] OMAS evaluates nine regions of the oral cavity for erythema and the presence and size of pseudomembranes or ulcerations. The value of OMAS at any given assessment is obtained by summing the erythema and ulceration/pseudomembrane subscores at each site and then averaging these scores across all nine sites. The OMAS score ranges from 0-5.

On days 1, 8, and 15 of each cycle patients filled in a questionnaire. This questionnaire consisted of 100 mm visual analogue scales for oral pain (ranging form 'no pain, 0 mm' to 'very severe pain, 100 mm') and swallowing difficulty (ranging from 'no impact on swallowing, 0 mm' to 'extreme impact on swallowing, 100 mm') and one multiple-choice question about eating function (with four levels of functioning: 'normal', 'soft foods only', 'liquids only', or 'no oral intake possible').

For determination of the cell viability of oral mucosa cells, an oral washing was performed before administration of WR-1065 and cytostatics, on days 1, 8 and 15 of each cycle.[18,19] To obtain an oral washing, patients gargled and rinsed their mouth for 30 seconds with 10 mL sterile 0.9% NaCl solution and than spat it out into a tube. This expectorate was centrifuged within 10 min of collection (190 g, 10 min, 4°C) and the supernatant was removed. The cell suspension was washed with 10 mL of 0.9% NaCl and centrifuged again to eliminate salivary fibers. Cell pellets were resuspended in 1 mL RPMI (Gibco, Paisley, UK) containing 5% fetal calf serum. Subsequently, 50 µL suspension and 50 µL trypan blue dye (0.4% in 0.9% NaCl) were mixed and cell counts were performed to calculate the percentage of viable cells.

For determination of WR-1065, an oral washing was obtained before and 15 minutes after treatment with WR-1065 during the second and third cycle. Subsequently, the expectorate was subdivided into two portions. For determination of cell viability 1 mL was used. The remaining 9 mL was centrifuged and washed according to the above mentioned procedure. Thereafter, the cell pellet was stored at -80°C. WR-1065 concentrations were determined by high performance liquid chromatography as described by Korst.[20]

Statistical analysis

Based on the results of a phase II study with epirubicin and gemcitabine every 3 weeks, WHO grade 2-3 mucositis is expected to occur in the first and/or fourth cycle (control cycles) in 35% of patients.[21] The incidence in

the second and/or third cycle (rinse cycles) is expected to be 10%. Based on the McNemar test on discordance, 20 patients are needed to detect a 25% difference with a power of 85% and a two-sided significance level of 5%. With a drop out of about 15%, 25 patients have to be included.

Data are analyzed using a linear mixed effects model. Respondent and cycle number are considered to be nested factors, days are modeled linear within cycles. When a response variable had a skewed distribution, the data was log-transformed. Summary measures were made and compared for cycles with and without mouthwash. To exclude the impact of the order of the control and rinse cycle in relation to the response variables, a cross-over design was used for analysis. Therefore, twelve patients with a control cycle followed by a rinse cycle, and twelve patients with a rinse cycle followed by a control cycle were selected. Spearman's correlation coefficient was calculated for correlation of WR-1065 concentration and mucositis.

RESULTS

Patient characteristics and treatment

From December 2000 till April 2002, 29 patients were included. Due to discontinuation of chemotherapy, five patients dropped out earlier. The remaining 24 patients had at least two evaluable chemotherapy cycles, one with WR-1065 rinse and one without. The patients evaluated in this study comprised 20 men and 4 women, with a mean age of 61 years (range 46-73).

Because not all patients had four evaluable cycles, the first chemotherapy cycle was used for all patients as a control cycle. The cycle with the fewest missing data was used as WR-1065 rinse cycle; 19 times cycle two, 4 times cycle three and 1 time cycle five.

Mucositis

During the first cycle, 21% of the patients experienced mucositis WHO grade 2. The mean mucositis score increased from day 1 to day 15 in both the control and rinse cycle (Table 1). During the total treatment period, none of the patients experienced WHO mucositis score grade 3 and 4. No significant differences in WHO and OMAS mucositis score were found between control and rinse cycles.

Table 1. OMAS and WHO mucositis scores, pain, difficulties on swallowing and feeding in control and rinse cycles (n=24)

		Day 1	Day 8	Day 15
OMAS (mean)	Control	0	0.18	0.19
	Rinse	0.03	0.26	0.26
WHO (median)	Control	0	1	1
	Rinse	0	0.5	1
Pain (mean, mm)	Control	0.1	7.2	24.6
	Rinse	4.6	10.2	23.1
Difficulties on swallowing (mean, mm)	Control	1.4	10.3	17.5
	Rinse	4.8	8.9	16.2
Feeding (median)	Control	1	1	1
	Rinse	1	1	1

Pain and feeding

A significant difference for pain was found between the consecutive days within the same cycle, with increasing pain in time ($P< 0.01$). No significant difference for pain was found between the control and rinse cycle (Table 1). For difficulties in swallowing and eating function, no significant differences were found between the control and rinse cycle (Table 1).

The results of the crossover design are shown in Table 2A and B. These data suggest a possible influence of the sequence and cycle number of the control and rinse cycle. However, analysis of the data summarized in table 2A and B with use of linear mixed effects model shows no significant differences between the control and rinse cycle for all response variables (OMAS and WHO mucositis score, pain, swallowing and feeding).

Viability of mucosal epithelial cells

The mean percentage viable epithelial cells at the start of the control cycles (56 ± 18%) was comparable with this percentage at the start of the rinse cycles (56 ± 14%). No significant differences were found between the consecutive days within the same cycle. Nor was a significant difference found in the viability of mucosal epithelial cells between the control and rinse cycle (Table 3).

Table 2A. Results of the crossover design (n=12), in order control-rinse for the variables OMAS and WHO mucositis scores, pain, difficulties on swallowing and feeding in control and rinse cycles

		Day 1	Day 8	Day 15
OMAS (mean)	Control	0	0.18	0.13
	Rinse	0.03	0.28	0.26
WHO (median)	Control	0	1	1
	Rinse	0	0.5	1.5
Pain (mean, mm)	Control	0.1	10.5	18.4
	Rinse	5	16.3	28.4
Difficulties on swallowing (mean, mm)	Control	0.1	16.3	16.7
	Rinse	8.1	12.4	20.1
Feeding (median)	Control	1	1	1
	Rinse	1	1	1.5

Table 2B. Results of the crossover design (n=12), in order rinse-control for the variables OMAS and WHO mucositis scores, pain, difficulties on swallowing and feeding in control and rinse cycles

		Day 1	Day 8	Day 15
OMAS (mean)	Rinse	0.02	0.24	0.21
	Control	0.05	0.21	0.49
WHO (median)	Rinse	0	0	0
	Control	0	0.5	1
Pain (mean, mm)	Rinse	4	5.2	14.9
	Control	2.1	6.7	24.4
Difficulties on swallowing (mean, mm)	Rinse	1.4	4.8	10.4
	Control	2.2	5.1	22.7
Feeding (median)	Rinse	1	1	1
	Control	1	1	1

Table 3. Percentage viable epithelial cells

	Day 1		Day 8	Day 15
	before rinsing	after rinsing		
Control (SD)	56 (18.0)	-	59 (18.8)	56 (10)
Rinse (SD)	56 (14.1)	53.6 (20.0)	62 (12.7)	58 (11.2)

Determination of presence of WR-1065

It was possible to determine the presence of WR-1065 in washed, isolated, vital mouth mucosa cells in 22 out of 24 patients. The cell pellet was lost in one patient; another patient had an interfering analytical peak, which made determination of WR-1065 impossible. The median cellular concentration of WR-1065 was 11.9 ng/10^5 viable cells (range 0.09-3821.7 ng/10^5). Eleven patients had a mean cellular concentration < 13 ng/10^5 viable cells. A significant negative correlation was found between the concentration of WR-1065 and the OMAS mucositis score ($r = -0.54$, $P=0.012$).

DISCUSSION

This study shows no clinical effect of local application with WR-1065 on oral mucositis in patients with unresectable stage III or IV NSCLC. Nevertheless a significant negative correlation was found between the WR-1065 concentration in mouth mucosa cells and the OMAS mucositis score, meaning that a higher cellular concentration of WR-1065 leads to less mucositis.

The cellular concentration of WR-1065 found in mouth mucosa cells after the first mouthwash with 125 mg WR-1065 has a large range (0.09 to 3821.7 ng/10^5). In vitro studies showed that after a five-minute incubation period with 4 mmol/l WR-1065, a concentration of 13 ng/10^5 cells was detectable and effective.[16] By adding 4 mmol/l amifostine in combination with alkaline phosphatase to V79-171 cells, 40% of the cells were protected from damage caused by radiotherapy (8 Gy).[15] In the current study, a cellular concentration of WR-1065 < 13 ng/10^5 (range 0.09-7.1) was found in 11 patients. This concentration was lower than the concentration found by Calabro-Jones and colleagues, which was necessary for a protecting effect.

Another reason for finding no clinical effect on mucositis and the low cellular concentrations might be the uptake of WR-1065. Following intravenous administration, amifostine is rapidly cleared from the plasma.[22] The rapid clearance of amifostine is largely due to the fast conversion of amifostine to its active metabolite, WR-1065. An animal study showed that maximum tissue concentrations of WR-1065 occurred within 5 to 15 minutes after amifostine injection.[23] Based on these results, amifostine should be applied a short period (15 to 30 minutes) before chemotherapy. Therefore, WR-1065 was given 15 minutes before epirubicine infusion in this study.

The uptake of WR-1065 is dependent of the temperature, the pH and contact time.[16] The inter-individual differences in cellular concentrations of WR-1065 might be caused by differences in oral temperature and pH. Although patients were instructed not to eat or drink during the epirubicine infusion, a physiological difference in the pH of saliva could result in different WR-1065 concentrations.

The low incidence of the experienced mucositis might be another reason for finding no clinical effect. Only 21% of the patients experienced WHO grade 2 mucositis during the first cycle, though at least 35% of grade 2 or more was expected.[21] During the total treatment period, none of the patients experienced a WHO mucositis grade 3 and 4. While in the study of Wachters and colleagues 12% of the patients experienced WHO mucositis grade 3 and 2% grade 4.[24] The low cellular concentration of WR-1065, together with the relatively low severity of mucositis, may be the reason for not finding a significant clinical effect on the presence of mucositis.

The results of this study indicate that, for prevention of oral mucositis, a higher cellular concentration of WR-1065 is necessary. Therefore, future studies should aim on a higher cellular concentration of WR-1065. This could probably be realized by a higher rinsing frequency, extended rinsing time and/or increasing the concentration of WR-1065. Another aspect is the evaluation of the pH value of saliva in patients. It might be interesting to better characterize the influence of the pH value on the uptake of WR-1065 into mouth mucosa cells.

In conclusion, local application of WR-1065 is feasible and leads to detectable quantities of WR-1065 in washed, isolated, vital mouth mucosa cells. An effective cellular concentration of WR-1065 was found in only 50% of the patients. A negative correlation was found between the concentration WR-1065 and the OMAS mucositis score. No clinical detectable difference in mucositis between the control and rinse cycle was found. The low cellular concentration of WR-1065 and the low incidence and severity of mucositis might be the reason for finding no clinical effect of WR-1065.

ACKNOWLEDGMENT

The authors are grateful for support from the Schering Plough Corporation who supplied amifostine.

REFERENCES

1. Berger AM, Kilroy TJ. Oral complications. In: DeVita VT, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology*. Philadelphia: Lippincott-Raven, 1997;2714-2725.
2. Sonis ST. Mucositis as a biological process: a new hypothesis for the development of chemotherapy-induced stomatotoxicity. *Oral Oncol* 1998;34:39-43.
3. Culy CR, Spencer CM. Amifostine: an update on its clinical status as a cytoprotectant in patients with cancer receiving chemotherapy or radiotherapy and its potential therapeutic application in myelodysplastic syndrome. *Drugs* 2001;61:641-684.
4. Hospers GAP, Eisenhauer EA. The sulfhydryl containing compounds WR-2721 and glutathione as radio- and chemoprotective agents. A review, indications for use and prospects. *Br J Cancer* 1999;80:629-638.
5. Capizzi RL. Amifostine reduces the incidence of cumulative nephrotoxicity from cisplatin: Laboratory and clinical aspects. *Semin Oncol* 1999;26:72-81.
6. Hartmann JT, von Vangerow A, Fels LM, Knop S, Stolte H, Kanz L, Bokemeyer C. A randomized trial of amifostine in patients with high-dose VIC chemotherapy plus autologous blood stem cell transplanation. *Br J Cancer* 2001;84:313-320.
7. Kemp G, Rose P, Lurain J, Berman M, Manetta A, Roullet B, Homesley H, Belpomme D, Glick J. Amifostine pretreatment for protection against cyclophosphamide-induced and cisplatin-induced toxicities: results of a randomized control trial in patients with advanced ovarian cancer. *J Clin Oncol* 1996;14:2101-2112.
8. Planting AST, Catimel G, de Mulder PHM, de Graeff A, Hoppener F, Verweij J, Oster W, Vermorken JB. Randomized study of a short course of weekly cisplatin with or without amifostine in advanced head and neck cancer. *Ann Oncol* 1999;10:693-700.
9. Bourhis J, De Crevoisier R, Abdulkarim B, Deutsch E, Lusinchi A, Luboinski B, Wibault P, Eschwege F. A randomized study of very accelerated radiotherapy with and without amifostine in head and neck squamous cell carcinoma. *Int J Radiat Oncol Biol Phys* 2000;46:1105-1108.
10. Koukourakis MI, Kyrias G, Kakolyris S, Kouroussis C, Frangiadaki C, Giatromanolaki A, Retalis G, Georgoulias V. Subcutaneous administration of amifostine during fractionated radiotherapy: a randomized phase II study. *J Clin Oncol* 2000;18:2226-2233.
11. Schonekas KG, Wagner W, Prott FJ. Amifostine—a radioprotector in locally advanced head and neck tumors. *Strahlenther Onkol* 1999;175 Suppl 4:27-29.
12. Dendale R, Bourhis J, Diawara O, Eschwege F, Habboubi N, Guichard M. Effect of systemic and topical administration of amifostine on radiation-induced mucositis in mice. *Proc Am Soc of Clin Oncol* 16, 64. 1997.
13. Montana GS, Anscher MS, Mansbach CM, Daly N, Delannes M, Clarke-Pearson D, Gaydica EF. Topical application of WR-2721 to prevent radiation-induced proctosigmoiditis. A phase I/II trial. *Cancer Cytopathology* 1992;69:2826-2830.
14. Theunissen DJ, Koopmans P, Hospers GAP, Stokman MA, Uges DRA. Op zoek naar een succesvolle toepassing van WR-1065. (In search of a successful application of WR-1065. Active metabolite of amifostine in the prevention of mucositis). *Pharm Weekbl* 2000;135:566-570.
15. Calabro-Jones M, Fahey RC, Smoluk GD, Ward JF. Alkaline phosphatase promotes radioprotection and accumulation of WR-1065 in V79-171 cells incubated in medium containing WR-2721. *Int J Radiat Biol* 1985;47:23-27.

16. Calabro-Jones M, Aguilera JA, Ward JF, Smoluk GD, Fahey RC. Uptake of WR-2721 derivatives by cells in culture: identification of the transported form of the drug. *Cancer Res* 1988;48:3634-3640.

17. Sonis ST, Eilers JP, Epstein JB, LeVeque FG, Liggett Jr WH, Mulagha MT, Peterson DE, Rose AH, Schubert MM, Spijkervet FKL, Wittes JP. Validation of a new scoring system for the assessment of clinical trial research of oral mucositis induced by radiation or chemotherapy. Mucositis Study Group. *Cancer* 1999;85:2103-2113.

18. Wymenga ANM, Spijkervet FKL, Timens W, Timmer-Bosscha H, Sluiter WJ, Mulder NH. A new in vitro assay for quantitation of chemotherapy-induced mucositis. *Br J Cancer* 1997;76:1062-1066.

19. Stokman MA, Spijkervet FKL, Wymenga ANM, Burlage FR, Timens W, Roodenburg JLN, de Vries EGE. Quantification of oral mucositis due to radiotherapy by determining viability and maturation of epithelial cells. *J Oral Pathol Med* 2002;31:153-157.

20. Korst AE, van der Vijgh WJ. High-performance liquid chromatographic assay using electrochemical detection for the combined measurement of amifostine, WR 1065 and the disulfides in plasma. *J Chromatogr B Biomed Sci Appl* 1997;693:167-174.

21. van Putten JWG, Eppinga P, Erjavec Z, de Leede G, Nabers J, Smeets JB, Sleijfer DT. Activity of high-dose epirubicin combined with gemcitabine in advanced non-small-cell lung cancer: a multicenter phase I and II study. *Br J Cancer* 2000;82:806-811.

22. Shaw LM, Turrisi AT, Glover DJ, Bonner HS, Norfleet AL, Weiler C, Kligerman MM. Human pharmacokinetics of WR-2721. *Int J Radiat Oncol Biol Phys* 1986;12:1501-1504.

23. Utley JF, Seaver N, Newton GL, Fahey RC. Pharmacokinetics of WR-1065 in mouse tissue following treatment with WR-2721. *Int J Radiat Oncol Biol Phys* 1984;10:1525-1528.

24. Wachters FM, van Putten JWG, Kramer H, Erjavec Z, Eppinga P, Strijbos JH, de Leede GPJ, Boezen HM. First-line gemcitabine with cisplatin or epirubicin in advanced non-small-cell lung cancer: a phase III trial. *Br J Cancer* 2003;89:1192-1199.

9

Summary, considerations and future perspectives

Samenvatting, overwegingen en toekomstige ontwikkelingen

SUMMARY

Oral mucositis induced by radiotherapy or chemotherapy is a frequently occurring toxicity in patients with cancer. It is a painful side-effect and restricts oral functions, such as speech, swallowing and chewing. Oral mucositis is associated with an increase in the number of systemic infections, days in hospital and overall costs. All these aspects can limit the cancer therapy and can have a negative impact on cancer patients health-related quality of life.

The aim of this thesis is to investigate new options for evaluation and intervention of cancer therapy induced oral mucositis.

Chapter 2 describes the outcomes of meta-analyses which evaluate the effectiveness of interventions for the prevention of oral mucositis in cancer patients. A literature search was performed for randomized controlled clinical studies between 1966-2004 aiming at prevention of mucositis, in cancer patients, undergoing head and neck radiation, chemotherapy, or chemoradiation. In order for a study to be eligible for the meta-analyses the control group had to consist of a placebo, no intervention or another intervention group. Mucositis was scored either by the WHO, or the NCI-CTC score, or the absence or presence of ulcerations, or the presence or absence of grade 3 and 4 mucositis. The meta-analyses included 45 studies fulfilling the inclusion criteria, in which 8 different interventions were evaluated i.e: - the antiseptic agents chlorhexidine and iseganan; - the anti-microbial agents PTA (polymyxin E, tobramycine and amphotericin B); - the hematopoietic growth factors: granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF); - oral cooling with ice cubs; - the mouth coating agent sucralfate; - the amino acid glutamine; and the cytoprotective agent amifostine. Four interventions showed a significant preventive effect on the development or severity of oral mucositis; PTA, GM-CSF/G-CSF, oral cooling with ice cubs and amifostine. To date, no single intervention completely prevents oral mucositis, so combined preventive therapy strategies seems to be required to ensure more successful outcomes.

Chapter 3 describes the study that analyzed the effect of training on concordance of evaluators in scoring oral mucositis in a multicenter trial. In the assessment of new agents for prevention of mucositis the inter-evaluator variability needs to be minimized. This would likely best be

accomplished by training of evaluators. To evaluate the effect of training, training meetings were organized, as start up of a phase III multicenter trial. Evaluators were informed about intention of the study, the mechanisms of development and clinical appearance of mucositis, and were trained in scoring mucositis according the Oral Mucositis Assessment Score (OMAS). The effect of the training was tested by a pre- and post-training test. The test consisted of 15 slides with clinical pictures of mucositis. The evaluator had to score mucositis of the depicted region for ulceration and erythema. Of each evaluator the scores of the pre- and post-training were compared to the reference standard. During 8 months, six meetings were organized where in total 65 evaluators were trained (mean 11 per training, range 8-15). Comparing the mean performance of the evaluators, the mean evaluator's percentage correctly scored slides according the OMAS increases significantly for both ulceration (47 to 69%) and erythema (63 to 77%). Comparing the results of the evaluators of scoring the absence or presence of ulceration and/or erythema, the correctly scored slides increased significantly between pre- and post-test training for both ulceration from 83% to 92% and erythema from 79% to 85%. Training evaluators in scoring oral mucositis has a significant improvement on the outcome of mucositis evaluation. Therefore training must be incorporated in the organization of clinical studies in multicenter trials using oral mucositis as a primary endpoint.

In **chapter 4** an in vitro assay is described for quantitative assessment of radiotherapy induced oral mucositis. Assessment of mucositis in clinical trials is mostly based on observational scores on mucosal changes, subjective complaints and impaired function. This study attempted to investigate an objective mucositis assessment at a cellular level and to compare the results with the WHO scoring method. Ten patients, treated for head and neck cancer, participated in this study. All patients were treated with conventional fractionated curative postoperative radiotherapy. Prior to, and weekly during, the irradiation course, oral washings were obtained to determine viability of epithelial cells by trypan blue dye exclusion. Maturation of epithelial cells was assessed from smears in buccal mucosa of these patients (Papanicolaou staining). The cell viability data were compared with the WHO score for mucositis. Epithelial cell viability increased during the first three weeks of radiation. This was seen earlier than the subjective mucosal changes with the WHO score. This suggests that, during the early treatment phase, the former method is more sensitive in detecting mucosal changes.

In this period also the cell maturity shifted from immature and intermediate to mature. After three weeks, the percentage of viable epithelial cells decreases, while the WHO score does not diminish. The cell viability method can therefore only be used for scoring the course and development of mucositis during the first three weeks of radiation. The results of this study support the idea that the cell viability assay can be considered as an objective method for following the development of irradiation mucositis, and seems to be more sensitive during the first three weeks of irradiation than the clinical WHO scoring method.

In **chapter 5** the relationship is investigated between oral mucositis and plasma cytokine interleukin-8 (IL-8) levels in neutropenic cancer patients with fever without a local bacterial infection or a clinical sepsis. In neutropenic cancer patients, oral mucositis is a potential portal of entry for the indigenous oral flora leading to bacteremia or sepsis. Fever is one of the first clinical signs of bacterial infection in these patients. The standard therapy for patients with fever and chemotherapy induced neutropenia is hospitalization and intravenous administration of broad-spectrum antibiotics. A risk assessment model has been developed, using objective clinical parameters and the plasma IL-8, to select patients with febrile neutropenia at low risk for bacterial infection. Plasma IL-8 is considered to be a systemic inflammatory response parameter to foci of infection. Oral mucositis is found to be a mucosal injury in which various cytokines, e.g. IL-8 may play a role. Patients (n=57) who were hospitalized with chemotherapy induced neutropenic fever were scored for oral mucositis on the second day of hospitalization according to the validated oral mucositis assessment scale (OMAS) and WHO toxicity grading. Patients (n=20) with a clinical sepsis or local bacterial infection were excluded from this evaluation. The remaining 37 patients were divided in a group with and without oral mucositis. The difference in plasma IL-8 level between patients with and without mucositis was not significant. In addition no difference was observed in the degree and duration of granulocytopenia. These results indicate that oral mucositis is not related to the systemic plasma IL-8 level in febrile neutropenic cancer patients without a clinical sepsis or local bacterial infection.

Chapter 6 describes the results of a double-blind placebo controlled study, evaluating the effects of selective oral flora elimination on radiotherapy induced oral mucositis. Changed oral flora, colonizing the oral mucosa, may aggravate the mucosa reaction induced by radiation. The

carriage and colonization of aerobic Gram-negative bacilli are thought to play a role in the pathogenesis of irradiation mucositis. In this study 65 patients were entered, with a malignant tumor in the head and neck regions, who were treated with primary curative or postoperative radiotherapy. The patients received either the active lozenges of 1 g containing polymyxin E 2 mg, tobramycin 1.8 mg and amphotericin B 10 mg (PTA) (33 patients) or the placebo lozenges (32 patients), four times daily during the full course of radiotherapy. Mucositis, changes in the oral flora, quality of feeding and changes of total body weight were assessed. Mucositis score did not differ between the groups during the first 5 weeks of radiotherapy. Nasogastric tube feeding was needed by six patients (19%) of the placebo group and two patients (6%) of the PTA group. Mean weight loss after 5 weeks of radiation was less in the PTA group (1.3 kg, SD 3.0) than in the placebo group (2.8 kg, SD 2.9). Colonization index of *Candida* species and Gram-negative bacilli was reduced in the PTA group and not in the placebo group ($P<0.05$). No effect on other microorganisms was detected. It was concluded that selective oral flora elimination in head and neck irradiation patients does not prevent the development of severe mucositis.

Chapter 7 reports the results of a pilot study evaluating the effect of flurbiprofen in a tooth patch on the development, severity and duration of pseudomembranous oral mucositis in patients treated with curative head and neck radiotherapy. Administration of flurbiprofen, a drug with anti-inflammatory and antiproliferative properties might delay the development of mucositis and/or alleviate its severity. The study group comprised 12 patients with a malignant tumor in the head and neck region who were treated with primary curative or postoperative radiotherapy. Patients applied once a day before sleep a flurbiprofen tooth patch to a natural tooth or upper denture starting 1 week before the onset of radiotherapy and during the full course of radiotherapy. Oral mucositis, pain, feeding, body weight and viability and maturation of epithelial cells were assessed. The results were compared with the findings in a historical control group. No differences were found for severity and duration of pseudomembranous mucositis between the two groups. The onset of pseudomembranous/ulcerative mucositis occurred later in the flurbiprofen group than in the historical control group ($P<0.05$). This study shows that, beside the latter onset, the flurbiprofen 15 mg tooth patch cannot prevent the development of pseudomembranous mucositis and has no influence on the duration of oral mucositis.

In **chapter 8** the results of a phase II study evaluating the effect of local application of the active metabolite of amifostine (WR-1065) on chemotherapy induced oral mucositis are reported. Cytoprotective agents, such as amifostine (Ethyol®, WR-2721), can reduce the toxicity induced by radiotherapy or chemotherapy. Amifostine is a prodrug, which is active as a protective agent when dephosphorylated by alkaline phosphatase to its active metabolite WR-1065. Once inside the cell, WR-1065 protects against chemotherapy and radiation induced damage by scavenging free radicals, donating hydrogen ions to free radicals, depleting oxygen, and direct binding and inactivating cytotoxic drugs. Preclinical experiments showed a reduction in radiation induced mucositis after local application of the active metabolite of amifostine (WR-1065). Non-small cell lung cancer patients treated with gemcitabine and epirubicin every 3 weeks for a maximum of five cycles were included. WR-1065 was administered during the second and third cycle as an oral rinse. Oral mucositis evaluation included WHO toxicity grading, the validated oral mucositis assessment scale (OMAS), and a questionnaire. Twenty-four patients were evaluated for at least one control and one rinse cycle. Mucositis scores, pain and feeding difficulties increased from day 1 to day 15, and were not significantly different between control and rinse cycles. Local application of WR-1065 leads to detectable quantities of WR-1065 in epithelial mucosa cells. A negative correlation between WR-1065 concentration and OMAS score was found. No clinical detectable influence of local application of WR-1065 on oral mucositis was observed.

CONSIDERATIONS AND FUTURE PERSPECTIVES

To date, despite the current understanding of the complex development of oral mucositis in cancer patients, no intervention is available for a complete prevention of oral mucositis. Interventions that target one specific process as part of the mucositis pathobiology process are shown to be largely ineffective.[1] Future studies in prevention of oral mucositis should aim multiple biological targets of the mucositis process either by an intervention with multiple mechanism effects or combination of interventions. The effect of local application of amifostine, a free radical scavenger and pro-inflammatory cytokine reducer, did not shown to be clinically effective in oral mucositis prevention (chapter 8). This could be due to the low available concentration of amifostine in epithelial mucosa cells. Future studies should aim for a

higher cellular concentration of amifostine, possibly realized by a higher rinsing frequency, extended rinsing time and/or increasing concentration of amifostine in the application fluid.

Because of the partial preventive effect on oral mucositis by selective flora elimination and the beneficial effect on the development of mucositis by growth factors, further insight in combination of these interventions is of high interest for new strategies in mucositis prevention.

In chapter 7 a delay was found in the development of ulcerative oral mucositis with application of an anti-inflammatory drug aiming cyclooxygenase-2 (COX-2) suppression. However, this intervention was discontinued with the onset of ulcerations. The effects of a longer application period of these anti-inflammatory drugs should be considered because the level of expression of COX-2 increases within endothelial cells and fibroblasts during ulcerations.[2]

In many available mucositis intervention studies the primary endpoint is prevention of oral mucositis. However, prevention of the ulcerative phase of oral mucositis seems to be more important. Oral ulcerations represent the most painful and unpleasant stage of mucositis for patients, and for the neutropenic patients there is an increased risk for bacteremia and sepsis. Therefore, intervention studies should evaluate the development and duration of ulcerations as primary endpoint.

Possibilities to predict risks for development of mucositis or systemic effects of mucositis in cancer patients would represent a great advantage. Therapy related factors such as chemotherapeutic agent choice, radiation dose and schedule, together with patient related variables such as nutritional status, age and gender are known to influence mucositis.[3] It is not possible yet to identify individual risk for development of mucositis. The developed risk assessment model for bacterial infections and complications using the plasma interleukin-8 (IL-8) and objective clinical parameters allows selection of a low-risk group who could withhold antibiotics and early hospital discharge.[4] In chapter 5 is shown that oral mucositis does not influence the plasma IL-8 level in febrile neutropenic cancer patients without a clinical sepsis or local bacterial infection and therefore should not be considered as a local infection in this patient population. However, the overall severity of mucositis was low in the studied patient population. Further studies should be initiated to show whether these results also hold in febrile neutropenic cancer patients with severe mucositis.

Most clinical intervention studies on oral mucositis prevention mandate larger sample sizes than are available at single study sites. For the needed multicenter trials, one of the major concerns is the establishment of adequate inter-evaluator reliability with regards to mucositis evaluation. To increase the inter-evaluator reliability in multicenter trials start up training meetings are necessary to standardize evaluators to the same method of scoring and baseline knowledge.[5] Chapter 3 describes the short term results of the impact of training on the evaluators in scoring oral mucositis. The study showed that training of evaluators in scoring oral mucositis has a positive significant influence on the outcome of mucositis evaluation. Further studies are necessary not only to confirm these results but also to evaluate the effect of training in time and the post training monitoring of the quality of scoring during intervention studies.

Progress in the treatment of cancer by innovations in radiotherapy concepts or new cancer drugs may be accompanied by earlier and more severe side-effects. Supportive care in cancer, aiming for prevention and management of adverse effects of cancer and its treatment across the entire continuum of a patients illness, will play a more prominent role in the treatment of cancer patients. It is identified as fifth dimension in cancer therapy in addition to surgery, radiation therapy, chemotherapy, hormonal therapy and immunotherapy.[6] From the WHO report on strategies to improve and strengthen cancer control programs it is concluded that patients who have cancer, besides an optimal cancer treatment within a multi-disciplinary team approach, requires supportive care throughout the treatment to ensure an adequate quality of life.[7] Team approach can provide a comprehensive care for the cancer patient. Specialists (medical, surgical, radiation, oncologist) together with several dedicated paramedical specialists needs to be a member of the multidisciplinary team. The dental hygienist has a pre-eminent background for providing oral supportive care. This profession should develop training and research programs for dedicated dental hygienists not only because implementation of preventive strategies for oral mucositis in cancer patients is complicated but also to be able to support new developments.

SAMENVATTING

Mucositis is een ontstekingachtige verandering van het slijmvlies van mond en keel. Het is een veel voorkomende bijwerking van radiotherapie als behandeling van kwaadaardige tumoren in het hoofdhals gebied. Ook bij behandeling met chemotherapie van deze tumoren in het hoofdhals gebied of van kwaadaardige afwijkingen elders in het lichaam kan deze aandoening van het mondslijmvlies optreden. Mucositis is zeer pijnlijk en belemmert de normale functies van mond en keel, zoals spreken, drinken, kauwen, slikken en voedselopname. Mucositis is weliswaar van tijdelijke aard, maar veroorzaakt, in periode van aanwezigheid, zeer veel last bij deze, vaak toch al ernstig zieke patiënten en heeft daardoor een sterk negatief effect op de kwaliteit van leven. Mucositis wordt bovendien beschouwd als bron van systemische infecties bij patiënten, die behandeld worden met cytostatica. Deze complicaties leiden tot verlenging van de ziekenhuisopname en werken kosten verhogend. Bovendien kunnen ze zo ernstig zijn dat de behandeling van de patiënt met een kwaadaardige aandoening in gedrang komt, doordat men gedwongen wordt de dosis van de cytostatica te verlagen of de behandeling geheel te stoppen. Uit bovenstaande blijkt dat er een grote behoefte bestaat aan de mogelijkheid tot preventie van mucositis.

In dit proefschrift wordt een onderzoek beschreven naar nieuwe mogelijkheden voor de beoordeling en de preventie van mucositis van het mondslijmvlies.

Hoofdstuk 2. Om inzicht te verkrijgen in de effectiviteit van interventies op de preventie van mucositis van het mondslijmvlies werd een literatuur onderzoek volgens moderne methoden uitgevoerd, waarbij gebruik werd gemaakt van meta-analyses. Bij het literatuuronderzoek werd gezocht naar gerandomiseerde gecontroleerde klinische studies, die gepubliceerd zijn tussen 1966-2004 en waarin als doel werd aangegeven preventie van mucositis van het mondslijmvlies. Patiënten uit deze studies werden behandeld met radiotherapie op het hoofdhals gebied, of chemotherapie, of chemoradiatie. Belangrijke voorwaarde was dat de mate van mucositis was bepaald met de WHO of de NCI-CTC scoringsmethode, met vermelding van de af- c.q. aanwezigheid van ulceraties of de af- c.q. aanwezigheid van graad 3 en 4 mucositis. Vijfenveertig studies voldeden aan bovengenoemde inclusie criteria. Van deze studies was het mogelijk voor 8 verschillende locale of systemisch werkende preventieve middelen (interventies) meta-analyses

uit te voeren. Van deze 8, bleken er 4 een significant gunstig effect te hebben bij de preventie van mucositis of bij de vermindering van de ernst van mucositis van het mondslijmvlies. Deze middelen zijn: PTA (polymyxine E, tobramycine, amfotericine B), de hematopoëtische groeifactoren granulocyt macrofaag koloniestimulerende factor (GM-CSF) en de granulocyt koloniestimulerende factor (G-CSF), koelen met ijsblokjes en amifostine. Voor zover kan worden nagegaan is er tot op heden in de literatuur geen methode beschreven, waarmee mucositis volledig kan worden voorkomen.

Hoofdstuk 3. Een belangrijk probleem bij de opzet van een klinisch onderzoek van geneesmiddelen is het verkrijgen van voldoende proefpersonen. Daarom moet vaak gekozen worden voor een multicenter onderzoek. Een probleem daarbij vormt de uniformiteit van de beoordeling van mucositis door de verschillende beoordelaars. Het is te verwachten dat een goede vooropleiding van deze beoordelaars de eenheid in beoordeling zal verbeteren. In welke mate dit mogelijk is wordt in dit hoofdstuk aangegeven. Om het effect van de opleiding, waaraan beoordelaars van diverse centra deelnamen, te evalueren werden er voorafgaand aan het eigenlijke onderzoek opleidingsbijeenkomsten gehouden, als start van een fase III studie. Tijdens deze opleidingsbijeenkomsten werden toekomstige beoordelaars geïnformeerd over de opzet van de studie, de ontwikkelingsmechanismen en klinische verschijningsvormen van mucositis. Bovendien werden de deelnemers getraind in het scoren van mucositis volgens de OMAS scoringsmethode. Het effect van de opleiding werd geëvalueerd door de deelnemers een test af te nemen voor en na de training. Het opleiden van beoordelaars in het scoren van mucositis van het mondslijmvlies leidde tot een significante verbetering van de uitkomsten van de mucositis evaluatie. De conclusie is dan ook dat opleiding van de beoordelaars geïntegreerd moet worden in de organisatie van studies, waarbij de klinische beoordeling wordt uitgevoerd door verschillende beoordelaars. Dit geldt des te meer als het al of niet aanwezig zijn van mucositis symptomen wordt gebruikt voor het beoordelen van resultaten van maatregelen met als doel het voorkomen van mucositis.

Hoofdstuk 4. Voor de vroegtijdige diagnostiek en preventie van mucositis is het nodig objectieve maatstaven te vinden voor vroege stadia van mucositis met nog weinig klinische symptomen. Getracht wordt hierin door middel van een laboratorium onderzoek verbetering aan te brengen. In dit hoofdstuk worden twee cytologische methoden beschreven, waarmee

geprobeerd wordt de ernst van mucositis van het mondslijmvlies, veroorzaakt door radiotherapie op het hoofdhals gebied, objectief te kwantificeren. De eerste methode berust op het bepalen van het percentage vitale epitheelcellen van het mondslijmvlies, in een mondspoelsel, met behulp van een celkleuring met trypaanblauw, te beginnen voorafgaand aan de bestraling en daarna wekelijks tijdens de periode radiotherapie. Bij de tweede methode wordt de mate van rijping van de epitheelcellen van het wangslijmvlies beoordeeld met behulp van een uitstrijkje gevolgd door een kleuring volgens Papanicolaou. Het onderzoek werd uitgevoerd bij 10 patiënten, die postoperatief werden bestraald in het hoofdhals gebied, volgens het principe van de conventionele, gefractioneerde, in opzet curatieve radiotherapie. De gegevens over de mate van vitaliteit van de epitheelcellen van de mond mucosa werden vergeleken met de uitkomsten van de WHO mucositis score. Het percentage losgeraakte vitale epitheelcellen van het wangslijmvlies toonde een duidelijke stijging tijdens de eerste drie weken van de radiotherapie. Deze verandering trad in een vroeger stadium op dan de klinische waarneembare veranderingen van het mondslijmvlies, zoals deze werden gescoord op basis van de WHO mucositis score. Deze bevindingen wekken de indruk dat, gedurende de eerste fase van de radiotherapie, de cytologische methode gevoeliger is om initiële veranderingen van het mondslijmvlies aan te tonen dan de klinische methode. In dezelfde periode verschoof bovendien de mate van rijping van de cellen van het wangslijmvlies van onrijpere vormen naar rijpere vormen. Drie weken na aanvang van de bestraling verminderde het percentage losse vitale cellen weer, terwijl de bevindingen op basis van de WHO mucositis score niet verminderden. De bepaling van het percentage vitale epitheelcellen is hierdoor alléén zinvol voor het scoren en het beoordelen van de ontwikkeling van mucositis tijdens de eerste drie weken van de radiotherapie. De resultaten van dit onderzoek ondersteunen de hypothese, dat deze cytologische methode voldoende objectief is om de beginnende ontwikkeling van een bestralingsmucositis te kunnen volgen. Vooral gedurende de eerste 3 weken van de radiotherapie is deze methode gevoeliger dan de WHO mucositis scoringsmethode.

Hoofdstuk 5. Bij kanker patiënten met neutropenie tengevolge van cytostatica toediening kan mucositis van het mondslijmvlies mogelijk een porte d'entrée vormen voor lichaamseigen bacteriën, waardoor een algemene bacteriële infectie of sepsis kan ontstaan. Het optreden van koorts bij deze

patiënten, met een sterk verminderde weerstand, wordt beschouwd als het eerste teken van een ernstige algemene bacteriële infectie. Het standaard beleid in deze situatie is om de patiënt op te nemen in het ziekenhuis en te behandelen door intraveneuze toediening van een breedspectrum antibioticum. Om overbehandeling te voorkomen bestaat er in de kliniek, behoefte om uit deze patiëntenpopulatie, een groep patiënten te selecteren met een laag risico voor een algemene infectie en daardoor het toedienen van antibiotica en het aantal klinische opnames te beperken. De waarde van de interleukine-8 (IL-8) spiegel in het bloed gecombineerd met objectieve gegevens uit lichamelijk onderzoek zou bij deze selectie een rol kunnen spelen. Mucositis bestaat uit een beschadiging van het mondslijmvlies die gepaard gaat met een ontsteking, waarbij verschillende cytokines, zoals IL-8, een rol zouden kunnen spelen. De hoogte van de IL-8 spiegel in het bloed zou hierdoor kunnen worden beïnvloedt. Deze mogelijke invloed van mucositis werd onderzocht bij kankerpatiënten met koorts en neutropenie, echter zonder algemene bacteriële infectie en klinische symptomen van sepsis. Bij patiënten (n=57), die chemotherapie hadden ondergaan en opgenomen werden vanwege neutropenie en koorts, werd op de tweede dag van de ziekenhuisopname de mucositis gescoord volgens de OMAS score en met behulp van de WHO scorings-methode. Patiënten (n=20) met duidelijke klinische symptomen van sepsis of een algemene bacteriële infectie werden uitgesloten van deze evaluatie vanwege de zeer waarschijnlijke invloed van deze situaties op de IL-8 spiegel. De resterende 37 patiënten werden verdeeld in een groep met, en een groep zonder mucositis. Er was geen verschil in de hoogte van de IL-8 spiegel tussen patiënten met en die zonder mucositis en er bestond geen relatie met de ernst en duur van de granulocytopenie. Deze resultaten laten zien dat mucositis van het mondslijmvlies als zodanig geen invloed heeft op de hoogte van de plasma spiegel van IL-8 bij neutropene kankerpatiënten met koorts bij wie geen duidelijke sepsis of een algemene bacteriële infectie is aan te tonen.

Hoofdstuk 6. In de literatuur worden verschillende factoren beschreven die een rol zouden kunnen spelen bij het zich ontwikkelen van mucositis. Hiertoe worden gerekend: een stoornis in de regeneratie van de mucosa door beschadiging van de basale sneldelende laag van epitheelcellen, het optreden van een ontstekingsproces in het epitheel, en de invloed van bacteriën op het mucosa oppervlak. Wanneer deze infectie zou kunnen worden voorkomen c.q. behandeld zou het lijden van de patiënt aanzienlijk vermin-

derd kunnen worden. Daarom werd een onderzoek gedaan naar de invloed van langdurige toediening van een combinatie preparaat van polymyxine E, tobramycine en amfotericine B (PTA). In dit hoofdstuk worden de resultaten beschreven van een dubbelblind, placebo gecontroleerd onderzoek, naar het effect van selectieve eliminatie van de mondflora met PTA zuigtabletten op een door radiotherapie geïnduceerde mucositis. Dit onderzoek betrof in het totaal 65 patiënten met een kwaadaardige tumor in het hoofdhals gebied, die behandeld werden door middel van een bestraling die primair als curatie was bedoeld of patiënten die een postoperatieve bestraling kregen ter preventie van eventuele recidieven. Deze totale groep werd onderverdeeld in twee gelijkwaardige subgroepen van respectievelijk 33 en 32 patiënten. De patiënten van de eerste subgroep kregen, gedurende de gehele periode van radiotherapie vier keer daags een zuigtablet van 1 gram die 2 mg polymyxine E, 1,8 mg tobramycine en 10 mg amfotericine B (PTA) (33 patiënten) bevatte. De patiënten van de tweede subgroep kregen een identieke tablet zonder werkzame bestanddelen (placebo) (32 patiënten). Van beide groepen werden de volgende punten geëvalueerd: het zich ontwikkelen van mucositis, veranderingen in de samenstelling van de mondflora, voedingsproblemen en veranderingen in het lichaamsgewicht. Er werd gedurende de eerste 5 weken van radiotherapie tussen beide groepen geen verschil in mucositis score waargenomen. In de placebogroep was, in verband met de ernst van de mucositis, sondevoeding nodig geweest bij zes patiënten (19%) en in de PTA groep bij twee patiënten (6%). Het gemiddelde gewichtsverlies na 5 weken bestraling was in de PTA groep geringer (1,3 kg, SD 3,0) dan in de placebogroep (2,8 kg, SD 2,9). De kolonisatie indices van *Candida* en Gram-negatieve bacteriën was lager in de PTA groep dan in de placebogroep ($P<0.05$). Voor de andere micro-organismen werd geen verschil gevonden. Op grond van deze uitkomsten kan worden geconcludeerd dat het zich ontwikkelen van een ernstige mucositis bij patiënten met een maligne tumor in het hoofdhals gebied, die bestraling krijgen, niet kan worden voorkomen door een selectieve reductie van de mondbacteriën. De symptomen van de optredende mucositis lijken echter milder.

Hoofdstuk 7. Flurbiprofen heeft ontstekingsremmende en antiproliferatieve eigenschappen en zou daardoor de ernst van mucositis van het mondslijmvlies kunnen verminderen. Bij het zoeken naar medicamenteuze behandeling c.q. preventie van mucositis zou deze stof misschien uitkomst kunnen bieden. Daarom werd een pilot studie gedaan naar de werkzaam-

heid van flurbiprofen, waarbij het effect wordt geëvalueerd van flurbiprofen, in een nieuw ontwikkelde tandkleeftablet, op de ontwikkeling, ernst en duur van de door radiotherapie geïnduceerde pseudomembraneuze mucositis. De testgroep bestond uit 12 patiënten met een kwaadaardige tumor in het hoofdhals gebied die behandeld werden met primaire curatieve of post-operatieve radiotherapie. Om een indruk te krijgen van de lokale werking plakten patiënten gedurende de gehele bestralingsperiode, een nieuw ontwikkelde tandkleeftablet met 15 mg flurbiprofen één keer per dag, voor het slapen, op een natuurlijk gebitselement of op de bovenprothese. Mucositis van het mondslijmvlies, pijn, voeding, lichaamsgewicht en vitaliteit en maturatie van epitheel cellen van het wangslijmvlies werden geëvalueerd. De resultaten werden vergeleken met een historische controlegroep. Er werden geen verschillen gevonden tussen beide groepen voor wat betreft de ernst en duur van pseudomembraneuze mucositis. Wel ontstond de mucositis later in de flurbiprofen groep dan in de historische controlegroep ($P<0.05$). Deze studie toont aan dat de flurbiprofen tandkleeftablet, in deze dosering en met deze toedieningswijze althans, het optreden van mucositis niet kan voorkomen en geen invloed heeft op de duur ervan.

Hoofdstuk 8. Amifostine is een organisch thiosulfaat, dat in het dier-model cytotoxische schade, die wordt geïnduceerd door radiotherapie en of door chemotherapie, kan voorkomen. Er werd daarom een fase II studie uitgevoerd met lokale applicatie van de actieve metaboliet van amifostine (WR-1065), met als doel een door chemotherapie geïnduceerde mucositis te voorkomen, bij patiënten, die behandeld werden voor een niet-kleincellig longcarcinoom. Deze patienten werden 3 wekelijks behandeld met de cytostatica gemcitabine en epirubicine met een maximum van vijf kuren. WR-1065 werd tijdens de tweede en derde kuur als mondspoelmiddel toegediend. De mucositis van het mondslijmvlies werd geëvalueerd volgens de WHO toxiciteitschaal, de OMAS en een vragenlijst. Vierentwintig patiënten werden voor ten minste 1 controle kuur en 1 kuur, waarbij gespoeld werd met WR-1065, geëvalueerd. De mucositis scores, pijn en voedingsproblemen verergerden van dag 1 tot dag 15. Er was geen significant verschil te zien tussen de controle kuur en de kuur, waarbij met WR-1065 werd gespoeld. Lokale toediening van WR-1065 leidde wel tot detecteerbare hoeveelheden van WR-1065 in cellen van het mondslijmvlies. Er werd een negatieve correlatie gevonden tussen de WR-1065 concentratie en de OMAS score. Er werd geen klinisch waarneembare gunstige invloed van WR-1065

op het optreden van mucositis van het mondslijmvlies waargenomen, althans met deze onderzoeksmethode.

OVERWEGINGEN EN TOEKOMSTIGE ONTWIKKELINGEN

Ondanks de huidige inzichten in de ontstaanswijze van mucositis van het mondslijmvlies, is er tot nu toe nog geen effectieve methode beschikbaar, waarmee het optreden van mucositis kan worden voorkomen. De medicamenten, gericht op een specifiek onderdeel van het ontwikkelingsproces van mucositis zijn tot nu toe grotendeels ineffectief gebleken.[1] Toekomstig onderzoek naar de mogelijkheden van preventie van mucositis van het mondslijmvlies zou gericht kunnen zijn op een selectie van één of meer effectieve methoden o.a. op basis van variaties in concentraties en toedieningswijzen. Het zou ook gericht kunnen zijn op methoden, die gelijktijdig werkzaam zijn op verschillende onderdelen van het ontwikkelingsproces van mucositis.

Lokale applicatie van amifostine, dat vrije radicalen zou wegvangen en pre-ontstekingscytokines zou reduceren, liet in ons onderzoek klinisch geen significant gunstig effect zien ten aanzien van de preventie van mucositis (hoofdstuk 8). Dit kan echter een gevolg zijn van een te lage beschikbare concentratie van amifostine in de epitheelcellen van het mondslijmvlies. Toekomstig onderzoek zou dan ook gericht kunnen zijn op het effect van hogere concentraties van amifostine in de epitheelcellen. Dit zou mogelijk gerealiseerd kunnen worden door de mond vaker te spoelen, de tijdsduur van het spoelen te verlengen en/of de concentratie van amifostine in het spoelmiddel te verhogen.

Zoals reeds eerder gesteld valt er vermoedelijk ook winst te behalen uit een geschikte combinatie van geneesmiddelen. Een voorbeeld hiervan zou het volgende kunnen zijn. Gelet op het gunstige, reducerende effect van een selectieve flora eliminatie, met name van aërobe Gram negatieve bacteriën en schimmels op de symptomen van mucositis en de remmende werking van groeifactoren op de ontwikkeling van mucositis zou verder inzicht in het effect van een combinatie van beide medicaties van groot belang kunnen zijn voor het ontwikkelen van nieuwe strategieën ter preventie van mucositis.

In hoofdstuk 7 wordt een vertragend effect op de ontwikkeling van ulceratieve mucositis beschreven van het toedienen van een ontsteking-

remmend medicament (flurbiprofen), met als doel het verlagen van de expressie van cyclooxygenase-2 (COX-2). Tijdens dit onderzoek werd de toediening gestopt, wanneer, ondanks de medicatie, toch ulceraties zichtbaar werden. Een langere toedieningsperiode zou overwogen kunnen worden, omdat de concentratie van COX-2 in endotheel cellen en fibroblasten toeneemt bij de aanwezigheid van ulceraties.[2] Ook hier kan worden gedacht aan een combinatie, n.l. met een selectieve flora eliminatie.

In veel onderzoeken met betrekking tot mucositis is het primaire einddoel de volledige preventie van mucositis. Doch wanneer dit voor alsnog niet haalbaar lijkt, zou het onderzoek thans primair het meest effectief gericht kunnen worden op methoden, waarmee de kans groot lijkt dat de ontwikkeling van mucositis afgeremd wordt en de duur van de ulceratieve vorm beperkt blijft. De aanwezigheid van ulceraties van het mondslijmvlies zijn, namelijk voor de patiënten, de meest pijnlijke en belastende fase van mucositis. Voor de patiënten met een neutropenie is er bovendien in dit stadium een verhoogd risico op het optreden van een algemene bacteriële infectie en sepsis.

Het zou ook een grote stap voorwaarts zijn als de kansen op de ontwikkeling van mucositis of van de systemische gevolgen van mucositis te voorspellen zouden zijn. Het is gebleken dat aan de therapie gerelateerde factoren, zoals de keuze van de cytostatica en de toedieningswijze, de bestralingsdosis en het bestralingsschema, samen met aan de patiënt gerelateerde factoren, zoals voedingsstatus, leeftijd en geslacht, de ontwikkeling van mucositis kunnen beïnvloeden.[3] Het is helaas nog niet mogelijk om de risicofactoren die de ontwikkeling van mucositis nadelig beïnvloeden bij elk individu afzonderlijk te bepalen. Het ontwikkelde risicomodel, waarvan de plasma interleukine-8 (IL-8) spiegel wordt gecombineerd met objectieve gegevens van het lichamelijk onderzoek van de patiënt, biedt een mogelijkheid om een groep van patiënten met een maligniteit met koorts en neutropenie te selecteren, bij wie slechts een gering risico bestaat op het ontwikkelen van een algemene bacteriële infectie.[4] In hoofdstuk 5 wordt aangetoond dat de aanwezigheid van mucositis van het mondslijmvlies als zodanig geen invloed heeft op de plasma IL-8 spiegel bij patiënten met een maligniteit, gecombineerd met neutropenie en koorts, bij wie geen klinische sepsis of een algemene bacteriële infectie bestaat. Mucositis dient daarom in deze patiënten populatie niet te worden beschouwd als risicovolle locale ontsteking op basis van infectie. Toekomstige studies zouden uitgevoerd moeten worden

om te beoordelen of deze resultaten ook gelden bij kankerpatiënten met neutropenie, met koorts en met ernstige mucositis.

Voor vele klinische onderzoeken op het gebied van mucositis preventie geldt dat er grotere groepen patiënten nodig zijn dan gewoonlijk op één onderzoekslocatie aanwezig zijn. Dit maakt combinatie van identieke patiënten groepen van verschillende locaties noodzakelijk. Deze zogenaamde multicenter studies vereisen het tot stand brengen van een adequate inter-beoordelaars betrouwbaarheid, gericht op de mucositis evaluatie. Om deze betrouwbaarheid te optimaliseren in multicenter studies is het noodzakelijk om, voorafgaand aan de start het onderzoek, opleidingsbijeenkomsten te organiseren. Het onderzoek, beschreven in hoofdstuk 3, laat zien dat het trainen van beoordelaars in het scoren van mucositis de uitkomsten van de mucositis evaluatie significant beter gelijkwaardig worden. Verder onderzoek is noodzakelijk, niet alleen voor bevestiging van de gevonden resultaten, maar ook om de invloed van de factor tijd op de kwaliteit van de trainings-resultaten te evalueren. De kwaliteit dient na de training bewaakt te worden, opdat de scoringsresultaten gedurende de interventiestudie gelijkwaardig zullen blijven tussen de verschillende onderzoekscentra.

De vooruitgang in de behandeling van maligne aandoeningen, bijvoorbeeld door innovaties op het gebied van bestralingstherapieën of krachtiger geneesmiddelen tegen bepaalde maligne afwijkingen kunnen vergezeld gaan van een hogere toxiciteit, meer weefselbeschadiging en in een vroeger stadium optreden of ontstaan van ernstige bijwerkingen. Additionele zorg, gericht op de preventie en behandeling van deze bijwerkingen, zal als onderdeel van de totale behandeling, zeer waarschijnlijk steeds belangrijker worden. Deze additionele zorg is erkend als de vijfde dimensie in de kanker-behandeling, naast de chirurgie, radiotherapie, chemotherapie, hormoon- en immuno-therapie.[6] In het WHO rapport van 2004, over strategieën ter verbetering en ondersteuning van kankertherapieën, wordt geconcludeerd, dat, naast een optimale kankertherapie, binnen een multidisciplinaire team-benadering, ook optimale additionele zorg moet worden geboden, om een goede kwaliteit van leven van de patiënt te waarborgen.[7] Alleen een dergelijke teambenadering, met een goede onderlinge communicatie, maakt een alles-omvattende zorg voor de kankerpatiënt mogelijk. Dit houdt in, dat naast specialisten, die bij de directe behandeling van de patiënt betrokken zijn (artsen, chirurgen, radiotherapeuten, oncologen) ook vertegenwoordigers van verschillende ondersteunende beroepen deel moeten uit maken van

het multidisciplinaire team. De mondhygiënist is hiervan een voorbeeld. Na een speciale gedifferentieerde opleiding kan de mondhygiënist uitstekend additionele mondzorg geven aan oncologische patiënten en deelnemen aan onderzoek op dit gebied en zo in de nabije toekomst een bijdrage leveren aan de behandeling en/of preventie van mucositis.

In de werkgroep hoofdhals oncologie van het UMCG is deze problematiek reeds vroegtijdig onderkend. Binnen de afdeling Kaakchirurgie is men reeds in 1985 begonnen met onderzoek naar methoden, die het lijden van deze patiënten, die behandeld worden voor kwaadaardige tumoren in het hoofdhals gebied te verminderen.[8]

Ditzelfde geldt ook voor het optreden van mucositis bij patiënten, die met cytostatica worden behandeld voor een maligne aandoening elders in het lichaam. De zorg voor preventie en behandeling van mucositis berust in de grote oncologische centra grotendeels bij speciaal daartoe opgeleide mondhygiënisten. Het is vooral binnen deze beroepsgroep, waar grote behoefte bestaat aan gevalideerde kennis op dit gebied. Kennis, die vooral te verwerven is, door het zelf doen van wetenschappelijk onderzoek, vooral ook omdat men zelf het beste op de hoogte is met de klinische problematiek van deze patiënten.

De medische c.q. tandheelkundige professie zal voor mondhygiënisten die betrokken zullen worden bij de additionele zorg voor kankerpatiënten speciale opleidings- en onderzoeksprogramma's moeten ontwikkelen, niet alleen omdat de implementatie van preventieve strategieën voor mucositis van het mondslijmvlies ingewikkeld is, maar ook om daarnaast nieuwe ontwikkelingen op dit gebied beter te kunnen evalueren en ondersteunen.

REFERENCES - REFERENTIES

1. Rubenstein EB, Peterson DE, Schubert M, Keefe D, McGuire D, Epstein J, Elting LS, Fox PC, Cooksley C, Sonis ST. Clinical practice guidelines for the prevention and treatment of cancer therapy-induced oral and gastrointestinal mucositis. *Cancer* 2004;100:2026-2046.
2. Sonis ST, O'Donnell KE, Popat R, Bragdon C, Phelan S, Cocks D, Epstein JB. The relationship between mucosal cyclooxygenase-2 (COX-2) expression and experimental radiation-induced mucositis. *Oral Oncol* 2004;40:170-176.
3. Barasch A, Peterson DE. Risk factors for ulcerative oral mucositis in cancer patients: unanswered questions. *Oral Oncol* 2003;39:91-100.
4. Oude Nijhuis CS. Fever and neutropenia in cancer patients; a risk-adapted approach (dissertation). Groningen, The Netherlands: University Medical Center Groningen; 2004.

5. Kobak KA, Engelhardt N, Williams JBW, Lipsitz JD. Rater training in multicenter clinical trials: Issues and recommendations. *J Clin Psychopharmacol* 2004;24:113-117.

6. Rosenbaum EH, Rosenbaum I, Andrews A, Spiegel D. Fifth dimension cancer supportive care. *URL http://www.cancersupportivecare.com*, 2005.

7. World Health Organisation. Strategies to improve and strenghten cancer control programmes in Europe. Geneva; 2004.

8. Spijkervet FKL. Irradiation mucositis and oral flora (dissertation). Groningen, The Netherlands: University Medical Center Groningen; 1989.

Dankwoord

Toen ik in 1988 op de afdeling Kaakchirurgie als mondhygiënist kwam werken, had ik niet kunnen bedenken dat ik ooit nog eens een proefschrift zou schrijven. Na 9 jaar patiëntenzorg zocht ik in mijn werk een nieuwe uitdaging, waarvan het ondersteunen van onderzoek een van de mogelijkheden was. Na het participeren in een multicenter onderzoek, als een van de beoordelaars van mucositis en als datamanager, kwam daarna de mogelijkheid om zelf, parttime, onderzoek te doen. Het ging om projectmatig klinisch onderzoek, waarbij het contact met de patiënten, wat belangrijk voor mij is, bleef bestaan. Zo kwam het, dat na een drietal projecten er ineens gesproken werd over promoveren. Is dat mogelijk als mondhygiënist? Ja dus, maar dit proefschrift zou er niet gekomen zijn zonder de hulp van velen.

Door het uitvoeren van onderzoek binnen de verschillende afdelingen van een academisch ziekenhuis en waar je daarnaast, als mondhygiënist, het werkveld hebt, is er een lange lijst van personen die op enige wijze hebben bijgedragen tot de voltooiing van mijn proefschrift. Graag wil ik dan ook iedereen bedanken die mij heeft geïnspireerd, ondersteund, geadviseerd en gestimuleerd bij het onderzoek. Enkelen wil ik bij name noemen.

Grote bewondering heb ik voor alle kankerpatiënten die een behandeling moesten ondergaan met radiotherapie of chemotherapie, maar die desondanks aan de diverse onderdelen van mijn onderzoek hebben meegewerkt. Ik wil dan ook graag alle patiënten bedanken die, ongeacht de vaak kapotte monden, toch weer wilden spoelen, slijmvliescellen wilden afstaan, medicamenten wilden gebruiken en klinische inspectie van de mond toelieten. Zonder hun bijdragen was dit onderzoek niet mogelijk geweest.

Prof. dr. J.L.N. Roodenburg, hooggeleerde promotor, beste Jan. Jij was een van de leden van de sollicitatiecommissie die mij in 1987 aannam op de afdeling Mondziekten, Kaakchirurgie & Bijzondere Tandheelkunde. Ik heb me altijd afgevraagd of het dragen van het legergroene pak dat ik toen droeg, invloed heeft gehad op de uitkomst. De persoonlijke gesprekken, jouw steun en wijze van coachen heb ik altijd erg gewaardeerd. Je betrokkenheid bij "onze" patiënten verdient veel respect. Daarnaast stel ik het op prijs dat je "de mondhygiënist", als paramedische beroepsbeoefenaar, altijd als gelijkwaardige (gespreks)partner hebt beschouwd bij de behandeling van de oncologische patiënt. Bedankt voor jouw begeleiding bij het onderzoek en voor mijn persoonlijke ontwikkeling in de uitvoering van mijn vak als mondhygiënist.

Prof. dr. E.G.E. de Vries, hooggeleerde promotor, beste Liesbeth. Je heldere visie op het onderzoek en het vermogen om dingen weer op een rij te krijgen, is geweldig. Meerdere promovendi hebben dat in het verleden genoemd, maar jouw snelle beoordeling van de manuscripten is duizelingwekkend. Op het moment dat ik dacht, even "rust" te hebben, omdat het manuscript naar de medeauteurs was, kwam ik bij jou bedrogen uit. Bedankt voor de snelle correcties, zelfs van de referentielijsten, en je opbouwende kritiek. Daarnaast wil ik je bedanken voor het vertrouwen in mij. Het is een voorrecht dat jij mijn promotor wilde zijn.

Dr. F.K.L. Spijkervet, zeergeleerde copromotor, lieve Fred. Door het beoordelen en scoren van je onderzoekspatiënten kwam ik in aanraking met het doen van onderzoek. Je expertise op het gebied van de mucositis, was reden voor diverse farmaceutische firma's om onze afdeling te benaderen voor het uitvoeren van onderzoeken op het gebied van mucositis. Door jouw toedoen heb ik de mogelijkheid gekregen de verschillende onderzoeken uit te voeren. Je ondersteuning bij het leren presenteren en het schrijven van artikelen is met geen pen te beschrijven. Bedankt voor alle inspirerende gesprekken en vele discussies. Onze intensieve samenwerking leidde niet alleen tot dit proefschrift, maar ook tot onze liefde voor elkaar. Ik had, 18 jaar geleden, nooit kunnen denken dat jij nu én copromotor én echtgenoot zou zijn. Bedankt voor alle fijne momenten en dat we nog lang van elkaar mogen genieten.

Prof. dr. L.G.M. de Bont, hooggeleerde professor, beste Lambert. Je managementkwaliteiten en vooruitziende blik op zaken, met een scherp oog voor de dingen die ertoe doen, wekken veel respect en waardering. Zonder jouw inspirerende inbreng was de wetenschappelijke ontwikkeling die onze afdeling doormaakt niet daar, waar die nu is. Ik wil je bedanken voor de mogelijkheid die ik kreeg om binnen de afdeling Kaakchirurgie onderzoek te doen. Daarnaast bood je mij de gelegenheid naar diverse nationale en internationale congressen te gaan om de resultaten van mijn onderzoek te presenteren.

De heer R.M. Rolvink, beste Richard. Jij bent degene, die ervoor heeft gezorgd dat ik de afdeling niet verliet, toen ik minder wilde gaan werken. Daarnaast gaf je mij, later, mede de mogelijkheid en de ruimte om mij verder te ontwikkelen in het onderzoeksveld. Wie had toen kunnen denken dat deze twee cruciale punten zo'n enorme invloed op mijn verdere leven zouden hebben. Ik wil je bedanken voor het feit dat je me niet hebt laten

gaan, alsook voor alle ondervonden steun en de vele persoonlijke gesprekken die we hadden. Ik heb veel van je geleerd.

Prof. dr. J.A. Langendijk en Prof. dr. I. van der Waal, hooggeleerde leden van de beoordelingscommissie. Ik wil u bedanken, als gewaardeerde leden van de beoordelingscommissie, voor de snelle beoordeling van het manuscript.

Prof dr. J.B. Epstein. It is a great privilege that a person with such a well recognized reputation in the supportive oral care field is willing to come all the way from Chicago to be opponent for the defence of my thesis. Thanks for being member of my thesis review committee and the quick judgement. Our personal contact at the different supportive care meetings and your humoristic views have been an indispensable inspiration for me.

Prof. dr. G. Boering, hooggeleerde professor, lieve Geert. Jij was, als hoofd van de afdeling, voorzitter van de sollicitatiecommissie die mij aannam in 1987. Daar ben ik je nog altijd erkentelijk voor. Het werken op een afdeling Mondziekten, Kaakchirurgie & Bijzondere Tandheelkunde van een academisch ziekenhuis is een uitdaging gebleken en een goede plek om je vakinhoudelijk en als persoon te verrijken en te ontwikkelen. Ik heb je altijd gewaardeerd voor je betrokkenheid bij de diverse disciplines van de afdeling. Ook na je emeritaat zijn we elkaar niet uit het oog verloren en je bent me bij het onderzoek van waardevolle adviezen blijven voorzien.

Alle medeauteurs wil ik bedanken voor hun energieke inzet en de prettige samenwerking om de manuscripten tot een publiceerbare versie te maken.

Dr. P.U. Dijkstra, beste Pieter. Jouw relativerende kijk op het doen van onderzoek en het beoordelen van wel of niet significante verschillen is voor mij een enorme steun geweest. Bedankt voor alle waardevolle en opbeurende gesprekken over de statistische uitkomsten. Je motto: "Denk erom, je moet ook blijven genieten" is een waarheid als een koe en dat kan ik nu beamen.

Drs. F.R. Burlage, beste Fred. Bedankt voor alle prettige jaren van samenwerking bij de behandeling van de hoofdhals oncologische patiënten. Jij hebt het mede mogelijk gemaakt dat diverse onderzoeken op de afdeling Radiotherapie konden worden uitgevoerd. Jouw onderzoek naar de functie van de speekselklieren na hoofdhals bestraling laat zien hoezeer jij als radiotherapeut betrokken bent bij de "oral supportive care" van deze patiënten.

Drs. F.M. Wachters en Dr. C.S.M. Oude Nijhuis, beste Floris en Claudi. Ik wil jullie bedanken voor de goede samenwerking en dat jullie de metingen

deden als ik niet aanwezig was, zodat het onderzoek gecontinueerd kon worden.

Dr. E.S.J.M. de Bont, beste Eveline. Jij bent de drijfveer achter de IL-8 studie. Bedankt voor de prettige discussies over de opzet van de studie en het uiteindelijke manuscript. Het is ons toch gelukt, het is geaccepteerd.

Dr. G.A.P. Hospers, beste Geke. Bedankt voor je vertrouwen dat je de amifostine studie door mij liet uitvoeren, voor de gesprekken en voor het uitschrijven van de formulieren voor de medicatie.

Dr. H.M. Boezen, drs. J.G.M. Burgerhof en drs. J.P. Schouten, beste Marike, Hans en Jan. Bedankt voor jullie statistische analyses. Jullie inbreng was iedere keer van onschatbare waarde.

Medewerkers van de afdeling Kaakchirurgie, de sectie Bijzondere Tandheelkunde, de afdeling Radiotherapie en de afdeling Kinderoncologie. Bedankt voor jullie interesse in mijn onderzoek, de prettige werksfeer en de plezierige samenwerking.

Mijn collega-mondhygiënisten, de dames J.M. Ekelhoff, H. van der Lei-Groenewegen, G.M. Meijer en C.H.J.M. Zegger, beste Judith, Hester, Marieke en Carla. Jullie zorgden voor een stabiele factor in de mondhygiënische behandeling van onze patiënten. Mede hierdoor kreeg ik de afgelopen jaren de gelegenheid mijn proefschrift af te schrijven. Ik ben jullie daar zeer erkentelijk voor. De collegialiteit, goede samenwerking, vriendschap en vakinhoudelijke expertise doen me beseffen dat we een goed team vormen waar ik nog jaren mee hoop te kunnen samenwerken.

Medewerkers van het Lab, voormalig H0. Bedankt voor de gezelligheid. Speciale dank voor Dr. H. Timmer en Dr. J. Meijer, beste Hetty en Coby, voor jullie bereidwilligheid mij op het gebied van de laboratoriumproeven bij te staan.

Medewerkers van het Interne Dagcentrum. Bedankt voor jullie medewerking bij de amifostine studie en het steeds weer op tijd piepen als er een patiënt moest spoelen.

Medeonderzoekers en oud-onderzoekers van de derde verdieping. Bedankt voor de vele gezellige koffieuurtjes, de taartjes, de adviezen en de onderzoeksondersteuning.

De heer H. de Jonge, beste Harry. Bedankt voor de ondersteuning op financieel en computergebied. Jij zult vaak gedacht hebben "daar heb je d'r weer", als de computer het weer eens liet afweten. Het was je nooit teveel.

De dames van het secretariaat Kaakchirurgie en Medische Oncologie, K. Wolthuis, N.E. Jaeger, L. Kempers en G.H. Beuker, beste Karin, Nienke,

Lisa en Gretha. Bedankt voor jullie secretariële ondersteuning, gezelligheid en belangstelling.

Drs. B. van Minnen, beste Baucke. Vier jaar lang was jij mijn kamergenoot, medeonderzoeker, het luisterende oor en de helpende hand. Bedankt voor de vele kopjes koffie die je voor me haalde, de gezelligheid, de afleiding, de humor en de vele gesprekken niet alleen over onderzoek, maar ook over de alledaagse dingen. Fijn dat je mijn paranimf wilt zijn.

Mevrouw H. van der Lei-Groenewegen, lieve Hester. In 1993 kwam jij het team van de mondhygiënisten versterken. Tijdens ons congresbezoek, in 1995 naar Japan, ontstond er tussen ons een hechte vriendschap. We hebben in de afgelopen 10 jaar veel samen meegemaakt. Bedankt voor al die keren dat je me hebt bijgestaan. Je bent er altijd voor me en daarom waardeer ik het zeer dat je vandaag aan mijn zijde staat. De kwaliteit van vriendschap is niet afhankelijk van de kwantiteit, maar gelukkig komt er nu weer meer tijd om samen leuke dingen te gaan doen.

Lieve Karin, Lars en Erik, bedankt dat jullie mij altijd het gevoel geven dat ik erbij hoor.

Lieve ouders, ook al deed ik atheneum B, ik wilde bij nader inzien geen academische studie volgen. Toen ik aangaf, nadat ik gezakt was, meteen naar de opleiding mondhygiëne te gaan, was dat voor jullie geen punt van discussie. Jullie steunden mij in mijn keuze en daar ben ik jullie nog steeds dankbaar voor. Daarnaast wil ik jullie bedanken voor de plezierige jeugd die ik heb gehad en het gevoel dat bij ons thuis zoveel mogelijk was. Fijn, dat jullie er altijd voor me waren en zijn.

List of Co-Authors

Boezen, H.M.	Department of Epidemiology and Bioinformatics University Medical Center Groningen, The Netherlands
Bont, E.S.J.M. de	Department of Pediatric Oncology University Medical Center Groningen, The Netherlands
Burgerhof, J.G.M.	Department of Epidemiology and Bioinformatics University Medical Center Groningen, The Netherlands
Burlage, F.R.	Department of Radiotherapy University Medical Center Groningen, The Netherlands
Daenen, S.M.G.J.	Department of Internal Medicine, Division of Hematology University Medical Center Groningen, The Netherlands
Dijkstra, P.U.	Department of Oral and Maxillofacial Surgery Department of Rehabilitation University Medical Center Groningen, The Netherlands
Gietema, J.A.	Department of Internal Medicine, Division of Medical Oncology University Medical Center Groningen, The Netherlands
Graaf, W.T.A. van der	Department of Internal Medicine, Division of Medical Oncology University Medical Center Groningen, The Netherlands
Groen, H.J.M.	Department of Internal Medicine, Division of Pulmonary Oncology University Medical Center Groningen, The Netherlands
Hospers, G.A.P.	Department of Internal Medicine, Division of Medical Oncology University Medical Center Groningen, The Netherlands
Kamps, W.A.	Department of Pediatric Oncology University Medical Center Groningen, The Netherlands
Koopmans, P.	Department of Pharmacy and Toxicology University Medical Center Groningen, The Netherlands
Manson, W.L.	Department of Microbiology University Medical Center Groningen, The Netherlands

Oude Nijhuis, C.S.M. Department of Pediatric Oncology
University Medical Center Groningen, The Netherlands

Roodenburg, J.L.N. Department of Oral and Maxillofacial Surgery
University Medical Center Groningen, The Netherlands

Schouten, J.P. Department of Epidemiology and Bioinformatics
University Medical Center Groningen, The Netherlands

Sonis, S.T. Division of Oral Medicine
Brigham and Women's Hospital
Boston, United States

Spijkervet, F.K.L. Department of Oral and Maxillofacial Surgery
University Medical Center Groningen, The Netherlands

Timens, W. Department of Pathology
University Medical Center Groningen, The Netherlands

Uges, D.R.A. Department of Pharmacy and Toxicology
University Medical Center Groningen, The Netherlands

Vellenga, E. Department of Internal Medicine, Division of Hematology
University Medical Center Groningen, The Netherlands

Vries, E.G.E. de Department of Internal Medicine, Division of Medical Oncology
University Medical Center Groningen, The Netherlands

Wachters, F.M. Department of Internal Medicine, Division of Pulmonary Oncology
University Medical Center Groningen, The Netherlands

Wymenga, A.N.M. Department of Internal Medicine, Division of Medical Oncology
Medisch Spectrum Twente, The Netherlands

Curriculum Vitae

The author of this thesis was born in Amsterdam, the Netherlands, on December 14th, 1963. She graduated as a dental hygienist at Stichting Opleiding Mondhygiëne in Utrecht in 1985. She graduated from high school (VWO), that she started at Andreas College in Drachten and completed at Fries Avondcollege in Leeuwarden, in 1989. Between 1985 and 1988 she worked as dental hygienist in a private practice and orthodontic practice. From 1988-1999 she was appointed as dental hygienist at the department of Oral and Maxillofacial Surgery (Head: Prof. G. Boering DDS, PhD (till 1994), Prof. L.G.M. de Bont DDS, PhD (from 1994)) at the University Medical Center Groningen (formerly University Hospital Groningen). Since 1999, she is appointed as part-time dental hygienist and part-time clinical researcher in the field of oral supportive care in cancer at the same department.

In 2005 she received the research price of best clinical publication 2003-2004 of "Vereniging voor Biologie van de Mond", Netherlands Institute for Dental Sciences.

Address for correspondence
M.A. Stokman
University Medical Center Groningen
Department of Oral and Maxillofacial Surgery
P.O. Box 30.001
9700 RB Groningen
The Netherlands

Phone: +31 50 3613840
Fax: +31 50 3611136
E-mail: m.a.stokman@kchir.umcg.nl