Chapter 10: The influence of inhaled corticosteroids and spacer devices on the growth of respiratory pathogenic microorganisms.

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Abstract

Introduction
Guidelines advise weekly cleansing of spacers one of the reasons being to prevent the spacers from becoming colonised with respiratory pathogens. Earlier work in clinical settings showed conflicting results.

Methods
Common respiratory pathogens and C. albicans were applied on petri dishes with and without inhaled corticosteroids and in three brands of spacer devices, with and without inhaled corticosteroids. Growth was measured.

Results
After 24 hours, S. aureus grew in 7 of 18 spacers (39%); P. aeruginosa in 12 out of 18 spacers (67%), and C. albicans survived in 5 of 18 spacers (28%). Microorganisms survived on petri dishes with fluticasone and beclomethasone, but not when budesonide was applied. One out of 30 metal Nebuhalers (3%) was colonised after 24 hours, whilst of 30 Volumatics 8 (27%) and Aerochambers 17 (57%) still had viable microorganisms. Application of inhaled steroids did not affect growth in the spacers.

Conclusions
The colonization of metal spacers is lower than of spacers made of polycarbonate or polyethylene. C. Albicans can survive in spacers. The survival of microorganisms in spacers is not influenced by inhaled corticosteroids.
Introduction

Pressurised metered dose inhalers (pMDI’S) in combination with spacers have gained wide popularity in the treatment of asthma in children. Most guidelines emphasize the use of the spacers to ensure optimal drug delivery.1,2 Spacers need maintenance and it is advised to clean them weekly.1 Incorrect cleansing of spacers or drying them with a towel, have the disadvantage of increasing static electric charge.3 Obviously, one of the reasons for regular cleansing is preventing microorganisms’ presence or even growth on the inside of the spacer. Knowledge on this issue has become even more important since recently an increase in hospitalisation for pneumonia was described in adults on inhaled corticosteroids (ICS).4 An explanation for this phenomenon has not been found, but theoretically, contaminated inhaler devices could play a role.

As far as we know, only two studies concerning the bacterial contamination of spacers have been published. In our previous study of spacers, used by outpatients, we found that 24 out of 64 (38%) were contaminated with non-pathogenic microorganisms, mostly Bacillus species; only one was contaminated with a Pseudomonas species.5 Cohen et al found bacterial contamination in 22 of 62 (36%) devices studied. However, they found P. aeruginosa, K. pneumoniae, and S. aureus in the spacers.6 The difference between the outcomes of the Cohen studies and ours prompted us to undertake a series of in vitro studies on the influence of ICS and spacer devices on the growth of respiratory pathogens. Since oral colonization with C. albicans is frequent in patients on ICS,7 we also included these. The aim was to study the survival of common respiratory pathogens on petri dishes and in three, widely available spacers, with and without commonly prescribed ICS sprayed on the inside.

Methods

Petri dishes

In the first set of experiments we grew microorganisms on petri dishes, with and without inhaled corticosteroids (ICS). Therefore we made suspensions with microorganisms, by diluting them to a concentration of 2 McFarland. This means that one millilitre of the suspension contains about 6,0 * 10⁶ Colony Forming Units (CFU). The microorganisms were commercially available (ATCC, LGC Promochem, Teddington UK). We studied P. aeruginosa (ATCC 27853); S. aureus (ATCC 25923); H. influenzae (ATCC 49766); S. pneumoniae (ATCC 49619); M. cattharalis (ATCC 25238); B. cepaciae (ATCC 25416) and C. albicans (ATCC 90028).

ICS were applied on polystyrene petri dishes (Sarstedt, Newton New Carolina). Fourteen doses of ICS
were sprayed on at a distance of 5 – 10 cm after shaking gently, according to instructions provided in the patient information. Three petri dishes were used for every combination of ICS and microorganism. Also, two petri dishes without ICS for each microorganism were used as controls. The ICS used were fluticasone 125 μg/dose; budesonide 200 μg/dose, and beclomethasone extra-fine solution aerosol 100 μg/dose. On every plate 40 μl (20 drops of 2 μl; containing about 24*10⁶ micro-organisms) suspension was added and the plates were covered to prevent dehydration by evaporation. The plates were kept at room temperature.

Three periods of culturing were studied. After 4, 12 or 24 h the cultures were swabbed with a cotton tipped brush and were inoculated onto plates, containing a medium, adjusted for each micro-organism studied. These were incubated for 24 hrs in a temperature of 37° C. Then the number of CFU’s was counted. According to our earlier study, we defined colonization when 50 or more CFU’s were found.

Spacers

In the second set of experiments we studied bacterial growth in spacers with and without ICS. The spacers studied were the Volumatic (GlaxoWellcomeKline, Zeist, Netherlands) in combination with fluticasone 125 μg/dose; the metal Nebuhaler (AstraZeneca, Zoetermeer, the Netherlands) in combination with budesonide 200 μg/dose and the Aerochamber plus (Trudell, London, Canada) in combination with beclomethasone fine particles 100 μg/dose.

The survival of the micro-organisms was studied after 4 hours and after 24 hours, and we studied all types of spacer as well as in combination with and without ICS. We chose to study survival in spacers after 4 and 24 hours and not after 12 hours. In the set of experiments with petri dishes we established that the growth of micro-organisms declined in a linear fashion. Because in the first set of experiments H. influenzae and S. pneumoniae did not grow, these were not included in this part of the study. Twelve hours prior to the experiments, the spacers were cleaned and dried according to the guidelines. Then 14 doses of steroids were sprayed into the spacer devices after shaking. After waiting for 30 minutes to allow the steroids to sediment, the spacers were inoculated with 40 μl of 2 McFarland. We choose 14 doses to mimic the amount of ICS after one week, because it is advised to clean the spacers once every week. We acknowledged that in daily life the ICS are partly removed from the spacers by inhalation, and therefore in this study the remaining amount in the spacer was exaggerated. This was done intentionally because we wanted to study the influence of ICS.

We sealed the spacers to prevent evaporation and kept them at room temperature (19° C) for 4 or 24 hours. Then the inner surfaces of the spacers were swabbed with cotton tipped brushes, inoculated
onto petri dishes, and cultured for 24 hours at a temperature of 37°C. Finally, the number of CFU’s was counted. Contamination was defined when > 50 CFU’s were found. To make comparison with the data of Cohen possible, computations were also made using their definition of contamination (> 100). Statistical analysis was performed using SPSS (version 13 for Windows), p < 0.05 was considered statistically significant. Chi-square tests were used.

**Results**

**Petri dishes.**

After 4 hours 49 of 77 dishes showed growth of micro-organisms (64%); after 12 hours 27 petri dishes (35%) were colonised and after 24 hours on 17 of the dishes viable micro-organisms were found (22%). Nine of these 17 were contaminated with S. aureus, with more than 500 CFU’s. H. influenzae en S. pneumoniae could only been harvested from the petri dishes after 4 hours (figure 1). Budesonide had, in contrast to beclomethasone and fluticasone, a significant negative effect on the viability of the micro-organisms even after 24 hours (figure 2).

![Figure 1: results of growth of microorganisms on petri dishes, with and without inhaled corticosteroids. Every microorganism was applied on petri dishes with inhaled corticosteroids, 3 dishes for each of the three steroids, and on 2 dishes without inhaled corticosteroids (in total 11 petri dishes per microorganism: 9 with a steroid, 2 without steroids). Presented are the numbers of petri dishes with colonization after 4, 12, and 24 hours.](image-url)
Spacers.

After 4 hours 60 out of 90 spacers (67%) were colonised and still yielded viable micro organisms. The number of CFU's varied from 0 to more than 1000. There were no differences between the three spacers. M. cattharalis had disappeared in all spacers but one. There were no differences between the spacers with and without ICS (64% vs. 69%).

After 24 hours 26 spacers (29%) still yielded viable micro organisms. In 8 spacers there were more than 1000 CFU's. Eight of the Volumatics (27%), 17 of the Aerochambers (57%) and one Nebuhaler (3%) were contaminated after 24 hours. The difference between the Nebuhaler on one hand and the Volumatic and Aerochamber on the other is statistically significant. Fifteen of the spacers with ICS (33%) were still contaminated after 24 hours, and 11 of the spacers without ICS (26%). P. aeruginosa was present after 24 hours in 12 of the 18 spacers (67%); S. aureus in 7; C. albicans in 5, M. cattharalis in 2 and B. cepaciae in none of the spacers (figures 3 and 4).

When the definition of Cohen for contamination was applied (>100 CFU's) 57 of the 90 spacer (63%) were found to be contaminated after 4 hours. There were no differences between the three spacers or between the spacers with and without ICS.

After 24 hours 19 spacers (21%) were contaminated: 5 of the Volumatics (17%), 13 of the Aerochambers (43%) and one Nebuhaler (3%). The number of contaminated Nebuhalers is statistically significantly lower than the numbers of contaminated Volumatics and Aerochambers. Twelve of the spacers with ICS (27%) were still contaminated after 24 hours, and 7 of the spacers without ICS (16%); this is

Figure 2: effect of application of 3 different inhaled corticosteroids on petri dishes on the survival of all microorganisms, compared with petri dishes without inhaled corticosteroids. The numbers of petri dishes with colonization after 4, 12, and 24 hours are presented.
not significant. *P. aeruginosa* colonised 11 of the 18 spacers (61%); *S. aureus* in 5; *C. albicans* in 3, *M. cattharalis* and *B. cepaciae* in none of the spacers.

![Figure 3: Survival of microorganisms in spacers with and without inhaled corticosteroids. Microorganisms were applied in three spacers of each type with inhaled corticosteroids and in three spacers of each type without inhaled corticosteroids, in total 18 spacers per microorganism. Presented are the numbers of contaminated spacers after 4 and 24 hours.](image)

![Figure 4: Survival of microorganisms in three brands of spacers, with and without inhaled corticosteroids applied, in total 30 spacers per type. The numbers of contaminated spacers after 4 and 24 hours are showed.](image)
Discussion

In this in vitro study we demonstrated that S. aureus and P. aeruginosa can survive in spacers and on petri dishes. Application of budesonide on petri dishes diminishes survival and the micro-organisms did not survive in the metal Nebuhaler, whether budesonide was applied or not made no difference. Using the cut-off levels as used by Cohen did not alter the interpretation of the results.

H. influenzae and S. pneumoniae did not survive on the petri dishes after 4 hours. This is not unexpected since it is known that H. influenzae only grows in special media and in a carbon dioxide enriched environment, and S. pneumoniae grows only in liquid media and requires a source of catalase, such as blood. That they did not survive on petri dishes without appropriate media suggests that these pulmonary pathogens will not grow in spacers. S. aureus and P. aeruginosa did survive, as well on the petri dishes as in the spacers. For patients with asthma these pathogens are probably not important. As far as we know pulmonary infections with these micro-organisms do not cause exacerbations of asthma. However, for patients with recurrent pulmonary infections this could be relevant, although we recognize that infections can recur by persistent intra-bronchially persistent bacteria. Oral colonization with C. albicans is frequent in patients on ICS, 4% to 13% of them develop clinical thrush.

We found that Candida could survive in spacers. Although host factors seem to be of influence, in the case of thrush spacer hygiene and cleaning could also be a factor.

Budesonide had a significant and relevant effect on the survival of micro-organisms in comparison with fluticasone and beclomethasone. We do not have an explanation for this. We were not aware of an inhibitory effect of ICS on bacterial growth. In a pilot study, we applied budesonide onto petri dishes with H. influenzae and found no influence. Also, we added increasing concentrations of prednisolone on cultures of H. influenzae, which did not affect survival, whilst amoxicillin, used as control, did. It could be that the excipient vehicle is more bactericidal than the propellants in other aerosols. This effect needs further investigation.

In this study, we only looked at bacteria and Candida species and not at viruses, as the route of transmission of viral infection is mostly via hand and mouth.

As we demonstrated the number of micro-organisms decreases with time. Therefore it seems reasonable to advice cleansing or handling the spacer just after use instead of cleansing and handling them just before use.

The difference between the survivals of micro-organisms in the Nebuhaler in comparison with the other devices is significant and probably clinically relevant. This has not been described before and
can not be explained by the effect of budesonide. The Volumatic is made of polycarbonate and the Aerochamber of polyethylene. We found no studies on the adherence and growth of micro-organisms on these materials without growth media. In studies with growth media, survival and adherence to metal as well as non-metal surfaces of micro-organisms has been described.\textsuperscript{12,13}

The experiments were done with the spacers sealed because we speculated that some patients leave the dosis aerosol on the inlets of the spacers and seal them by doing so. We acknowledge that in other cases the spacer will be left open. This will lead to evaporation and will further decrease the survival of microorganisms. Therefore, our study probably overestimates the survival. Cohen also found higher survival in a more humid environment and described that the spacers, blow-dried, were less contaminated.\textsuperscript{6}

**Clinical implications.**

After using spacers for inhalation or after cleaning, they should not be touched on the inside. After cleaning the spacer should be allowed enough time to dry. In cases of clinical thrush, spacer hygiene could be a factor of influence. Intensive cleansing of spacers could be important for patients with recurrent pulmonary infections. From a microbiological point of view, the metal spacer is superior to spacers made of polycarbonate and polyethylene. Inhaled corticosteroids appear not to affect bacterial growth in spacer devices.

*The manufacturers kindly provided the spacers; we bought the inhaled corticosteroids in the hospital pharmacy.*

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References.