ELSEVIER

Journal of the Neurological Sciences 244 (2006) 123 – 126

Journal of the Neurological Sciences

www.elsevier.com/locate/jns

Erythrocyte membrane fatty acids in benign and progressive forms of multiple sclerosis

Marcus Koch *, Geeta S.M. Ramsaransing, M. Rebecca Fokkema, Dorothea J. Heersema, Jacques De Keyser

Department of Neurology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands

Received 14 June 2005; received in revised form 2 December 2005; accepted 12 January 2006
Available online 6 March 2006

Abstract

Background: There is no good explanation why a proportion of patients with multiple sclerosis (MS) have a relatively benign form of the disease. An imbalance between saturated and unsaturated fatty acids (FA) might influence the disease course of MS.
Aim: To assess whether the erythrocyte membrane fatty acid composition, which is a biological marker of long term dietary FA consumption, is different between patients with benign and progressive MS.
Methods: The erythrocyte membrane FA composition was measured by gas chromatography in 23 healthy controls, 27 patients with benign MS, 32 patients with secondary progressive MS and 23 patients with primary progressive MS. None of the patients was following a special diet.
Results: No significant differences in levels of saturated and unsaturated FA or in omega-3- and omega-6-polyunsaturated FA were found between controls and patients with the different subtypes of MS.
Conclusion: Our data suggest that factors other than dietary fatty acid consumption are responsible for the different disease courses of MS.

Keywords: Multiple sclerosis; Benign multiple sclerosis; Polyunsaturated fatty acids

1. Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system of unknown origin. Since the middle of the previous century many investigations have tried to link MS incidence and dietary habits [1]. Especially the dietary fatty acids (FA) became the focus of interest in many experimental and clinical studies.

For many years it has been suspected that MS might be associated with an imbalance between unsaturated and saturated FA: an increase in mono- and polyunsaturated FA (MUFA, PUFA) and a decrease in saturated FA (SFA) are believed to reduce inflammation and disease severity. Accordingly there is a large variety of special diets and dietary supplements designed to increase the ingested amount of MUFA and PUFA in patients with MS.

Whereas measurements of serum FA in serum are heavily influenced by day to day changes in FA ingestion, erythrocyte membrane FA composition is a measure of the long term dietary FA intake over the past several months. In this study, we used capillary gas chromatography to measure the relative amounts of PUFA, MUFA and SFA of the erythrocyte membranes of healthy controls and MS patients with different clinical subtypes of MS, none of whom was following a special diet. Capillary gas chromatography is the standard measurement technique for the investigation of fatty acids in biological tissues. It has been shown previously to be highly reliable and reproducible [2–4]. Our aim was to assess whether there is an association

* Corresponding author. Tel.: +31 50 3612430; fax: +31 50 3611707.
E-mail address: m.w.koch@neuro.umcg.nl (M. Koch).

0022-510X/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jns.2006.01.010

Table 1
List of FA measured in this study

SFA	MUFA	PUFA
C14:0 (myristate)	C18:1,ω9 (oleic acid)	C18:2,ω6 (linoleic acid)
C16:0 (palmitate)	C18:1,ω7 (vaccenic acid)	C18:3,ω6 (gamma linolenic acid)
C18:0 (stearate)	C20:1,ω9 (gondoic acid)	C18:3,ω3 (alpha linolenic acid)
C20:0 (arachidic acid)	C24:1,ω9 (nervonic acid)	C20:2,ω6 (eicosadienoic acid)
C22:0 (behenic acid)		C20:3,ω9 (mead acid)
C24:0 (lignoceric acid)		C20:3,ω6 (eicosatrienoic acid)
C26:0 (hexacosanoic acid)		C20:4,ω6 (arachidonic acid)
		C20:5,ω3 (eicosapentanoic acid)
		C22:4,ω6 (docosatetraenoic acid)
		C22:5,ω6 (docosapentaenoic acid)
		C22:5,ω3 (docosapentaenoic acid)
		C22:6,ω3 (docosahexanoic acid)

between long term dietary FA consumption and the disease course of MS.

2. Patients and methods

2.1. Patients

All patients gave their written informed consent before inclusion into the study. The study was approved by the ethics committee of the University Medical Center Groningen. Exclusion criteria were a relapse or use of corticosteroids in the past 3 months, fever or infections, and pregnancy. Defined onset symptoms were used to define the year of onset.

23 healthy controls and 82 patients with MS participated in the study. None of the patients or control persons was following a special diet. Of the MS patients, 27 had relapsing–remitting MS with a benign disease course (BMS), 32 had secondary progressive MS (SPMS), and 23 had primary progressive MS (PPMS).

BMS was defined as a score on Kurzke's Expanded Disability Status Scale (EDSS) [5] of 3.0 or lower despite at least 10 years of disease duration. PPMS was defined as a disease course that was progressive from the outset without preceding relapses. Fourteen patients were using interferon β; no other immunomodulatory or immunosuppressive drugs were used.

2.2. Sample collection, processing and analysis

EDTA anticoagulated venous blood samples were obtained by venepuncture and immediately cooled in melting ice. Erythrocyte membrane FA were isolated from the blood samples as described previously [3]. Briefly, the blood samples were centrifuged, buffy coats and plasma were removed and the erythrocytes were washed three times in isotonic saline. The samples were resuspended to a haematocrite of 50%, and butylated hydroxytoluene (anti-oxidant) and margaric acid (C17:0, internal quantification standard) were added. FA methyl esters were prepared by acid-catalyzed transmethylation.

The relative amounts of the 23 FA listed in Table 1 were measured by gas chromatography as described previously [3]. Relative amounts of the individual FA were calculated assuming that equal peak areas correspond with equal weight amounts [6]. Data were expressed as mol%. For data analysis the measured relative amounts of individual FA were added to give grouped relative amounts for SFA, MUFA and PUFA. For further analysis PUFA were grouped into ω3-fatty acids (ω3FA) and ω6-fatty acids (ω6FA).

2.3. Statistics

The statistical significance of differences between the control group and the different subgroups of MS patients (BMS, PPMS and SPMS) was assessed with one-way analysis of variance. The unpaired *t*-test was used for comparisons of patients using and not using interferon β. The normal distribution of all measured data was ascertained using the Kolmogorov–Smirnov test. All statistical analyses were performed with the Graphpad Prism for Windows statistical software package version 4.00 (Graphpad Software, San Diego, USA).

Table 2
Demographic data of patients and control persons

Characteristics	Healthy controls	BMS	PPMS	SPMS
n	23	27	23	32
Sex, female/male	13/10	18/9	15/8	21/11
Age [years], median (range)	48 (37–65)	49 (28–70)	53 (36–69)	49 (27–70)
Disease duration [years], median (range)	–	21 (10–40)	12 (3–21)	19 (7–35)
Use of interferon, *n*	–	–	2	12
EDSS, median (range)	–	2.0 (0–3.0)	6.0 (4.0–8.5)	7.0 (3.0–8.0)

Table 3
Relative SFA, MUFA, PUFA, omega-3-FA, omega-6-FA contents for patients and control persons

	SFA [mol%]	MUFA [mol%]	PUFA [mol%]	ω3FA [mol%]	ω6FA [mol%]
Healthy controls	48.63±1.03	16.90±0.92	34.47±1.14	6.42±1.72	27.81±2.29
BMS	49.29±2.15	16.60±1.33	34.11±2.64	6.06±1.53	27.80±2.65
PPMS	49.34±1.39	17.02±0.85	33.64±1.56	6.38±1.45	27.01±2.05
SPMS	49.29±1.22	16.90±1.04	33.80±1.42	5.89±0.91	27.66±1.68
p	0.31	0.53	0.4	0.44	0.55

Values are means±S.D.

Statistical significance was taken to be at the 5% level ($p<0.05$).

3. Results

Demographic data of the patient and control group are given in Table 2. The control group and patient groups were well matched regarding age and sex. There were no significant differences in relative SFA, MUFA, PUFA, ω3FA and ω6FA contents between controls, BMS, PPMS and SPMS patients (Table 3). No significant differences in relative SFA, MUFA, PUFA, ω3FA and ω6FA content could be found between patients using or not using interferon β (data not shown).

4. Discussion

More than 50 years ago, Swank published an epidemiological study on the relation of nutrition and MS incidence in rural Norway. He found higher incidence of MS in areas with higher intake of saturated animal fats as compared to areas with a high consumption of sea fish [7]. As sea fish and fish oil are a natural source of PUFA, this finding led to the hypothesis that their consumption may have a preventive effect on the incidence MS and reduce disease severity. Later epidemiological studies on MS incidence and nutrition did not uniformly confirm Swank's original findings, and population-based case-controlled trials failed to show a relation between fat consumption and MS incidence [8–10].

Experimental studies showed low levels of the PUFA linoleic acid (C18:2,ω6) in blood, blood cells, CSF and brain tissue of patients with MS [11–15]. Studies using the animal model of MS, experimental allergic encephalitis (EAE), showed a protective effect of linoleic acid [16] and other ω6FAs [17] whereas a deficiency of linoleic acid increased the symptoms of EAE [18]. Accordingly, several therapeutic trials with PUFA have been conducted in patients with MS. In a meta-analysis of three randomized controlled clinical trials, linoleic acid treated patients had a significantly lower relapse severity and duration, although no influence on the relapse rate was found [19]. In previous studies examining the FA composition of erythrocyte membranes of patients with MS a decrease in PUFA and an increase in SFA levels were found [12,20].

As a consequence of these findings many special diets designed to decrease SFA ingestion and to increase MUFA and PUFA ingestion are available to MS patients, some of them at considerable cost.

With this study we tried to assess whether there were significant differences in erythrocyte membrane FA composition and thus long term FA consumption between healthy controls and patients with benign and progressive MS.

We found no significant differences in the relative amounts of SFA, MUFA, PUFA, ω3FA and ω6FA between healthy controls and patients with benign and progressive MS. We were unable to corroborate the findings of the cited earlier studies [12,20]. Our data suggest that factors other than dietary fatty acid consumption are responsible for the different disease courses of MS.

Acknowledgements

The technical assistance with the chromatographic measurements of Ms. I.A. Martini is gratefully acknowledged.

This work was supported by Multiple Sclerosis International (MSI), Amsterdam, The Netherlands.

References

[1] Schwarz S, Leweling H. Multiple sclerosis and nutrition. Mult Scler 2005;11:24–32.
[2] Jaeger H, Klor HU, Ditschuneit H. Automated glass capillary gas–liquid chromatography of fatty acid methyl esters with reference to *cis* and *trans* isomers. J Lipid Res 1976;17:185–90.
[3] Muskiet FA, van Doormaal JJ, Martini IA, Wolthers BG, van der SW. Capillary gas chromatographic profiling of total long-chain fatty acids and cholesterol in biological materials. J Chromatogr 1983;278:231–44.
[4] Traitler H. Recent advances in capillary gas chromatography applied to lipid analysis. Prog Lipid Res 1987;26:57–280.
[5] Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33:1444–52.
[6] Volmer M, Meiborg G, Muskiet FA. Simultaneous capillary gas chromatographic profiling of medium- and long-chain fatty acid methyl esters with split injection. Correction for injection-related discrimination by the 'bracketing' method. J Chromatogr 1988;434:385–94.
[7] Swank RL, Lerstad O, Strom A, Backer J. Multiple sclerosis in rural Norway its geographic and occupational incidence in relation to nutrition. N Engl J Med 1952;246:722–8.
[8] Zhang SM, Willett WC, Hernan MA, Olek MJ, Ascherio A. Dietary fat in relation to risk of multiple sclerosis among two large cohorts of women. Am J Epidemiol 2000;152:1056–64.

[9] Tola MR, Granieri E, Malagu S, Caniatti L, Casetta I, Govoni V, et al. Dietary habits and multiple sclerosis. A retrospective study in Ferrara, Italy. Acta Neurol (Napoli) 1994;16:189–97.
[10] Berr C, Puel J, Clanet M, Ruidavets JB, Mas JL, Alperovitch A. Risk factors in multiple sclerosis: a population-based case–control study in Hautes-Pyrenees, France. Acta Neurol Scand 1989;80:46–50.
[11] Navarro X, Segura R. Plasma lipids and their fatty acid composition in multiple sclerosis. Acta Neurol Scand 1988;78:152–7.
[12] Navarro X, Segura R. Red blood cell fatty acids in multiple sclerosis. Acta Neurol Scand 1989;79:32–7.
[13] Neu I, Woelk H. Investigations of the lipid metabolism of the white matter in multiple sclerosis: changes in glycero-phosphatides and lipid-splitting enzymes. Neurochem Res 1982;7:727–35.
[14] Fisher M, Johnson MH, Natale AM, Levine PH. Linoleic acid levels in white blood cells, platelets, and serum of multiple sclerosis patients. Acta Neurol Scand 1987;76:241–5.
[15] Alling C, Vanier MT, Svennerholm L. Lipid alterations in apparently normal white matter in multiple sclerosis. Brain Res 1971;35:325–36.
[16] Meade CJ, Mertin J, Sheena J, Hunt R. Reduction by linoleic acid of the severity of experimental allergic encephalomyelitis in the guinea pig. J Neurol Sci 1978;35:291–308.
[17] Harbige LS, Layward L, Morris-Downes MM, Dumonde DC, Amor S. The protective effects of omega-6 fatty acids in experimental autoimmune encephalomyelitis (EAE) in relation to transforming growth factor-beta 1 (TGF-beta1) up-regulation and increased prostaglandin E2 (PGE2) production. Clin Exp Immunol 2000;122:445–52.
[18] Clausen J, Moller J. Allergic encephalomyelitis induced by brain antigen after deficiency in polyunsaturated fatty acids during myelination. Is multiple sclerosis a nutritive disorder? Acta Neurol Scand 1967;43:375–88.
[19] Dworkin RH, Bates D, Millar JH, Paty DW. Linoleic acid and multiple sclerosis: a reanalysis of three double-blind trials. Neurology 1984;34:1441–5.
[20] Cherayil GD. Sialic acid and fatty acid concentrations in lymphocytes, red blood cells and plasma from patients with multiple sclerosis. J Neurol Sci 1984;63:1–10.