Peripheral Blood Mononuclear Cell Membrane Fluidity and Disease Outcome in Patients with Multiple Sclerosis

Gloudina M. Hon · Mogamat S. Hassan ·
Susan J. van Rensburg · Stefan Abel ·
Rajiv T. Erasmus · Tandi Matsha

Received: 8 February 2011 / Accepted: 1 June 2011 / Published online: 21 June 2011
© Indian Society of Haematology & Transfusion Medicine 2011

Abstract Immune cell membrane lipids are important determinants of membrane fluidity, eicosanoid production and phagocytosis and fatty acid metabolic abnormalities have been reported in immune cells from patients with multiple sclerosis. The aim of this study was to investigate the relationship between peripheral blood mononuclear cell membrane fluidity, permeability status, and disease outcome as measured by the Kurtzke expanded disability status scale. Phospholipids, fatty acids and cholesterol composition in peripheral blood mononuclear cells from 26 patients diagnosed with multiple sclerosis and 25 healthy control subjects were determined by colorimetric assay, gas chromatography and enzymatic assays, respectively. Membrane fluidity was calculated according to previously established formulae and correlated with C-reactive protein

and the Kurtzke expanded disability status scale. There were no significant differences in membrane lipids in peripheral blood mononuclear cells from patients and controls. However, correlation studies showed lipid metabolic abnormalities, which were reflected in significant correlations between membrane fluidity as measured by both its fatty acid and phospholipid compositions, and the functional system scores. C-reactive protein showed positive correlations with phosphatidylcholine, phosphatidylserine, phosphatidylinositol and total phospholipids in membranes from control subjects. Metabolic abnormalities, as well as correlations between membrane fluidity and the functional system scores, suggested the involvement of these immune cell membranes in the disease progression. Furthermore, the changed relationship between membrane phospholipids and C-reactive protein, which has been shown to correlate with infectious episodes and clinical relapse, could be an indication of immune cell dysfunction in patients with multiple sclerosis.

G. M. Hon · M. S. Hassan · T. Matsha
Department of Bio-Medical Sciences, Faculty of Health and
Wellness Science, Cape Peninsula University of Technology,
P. O. Box 1906, Bellville, Cape Town 7530, South Africa

S. J. van Rensburg
National Health Laboratory Services, Tygerberg Hospital,
Tygerberg, South Africa

S. Abel
PROMEC Unit and NIRU, South African Medical Research
Council, Tygerberg, South Africa

R. T. Erasmus
Division of Chemical Pathology, University of Stellenbosch,
Tygerberg Campus, Tygerberg, South Africa

T. Matsha (✉)
Biomedical Technology, Faculty of Health and Wellness
Science, Cape Peninsula University of Technology,
P. O. Box 1906, Bellville, Cape Town 7530, South Africa
e-mail: matshat@cput.ac.za

Keywords Multiple sclerosis · Fatty acids ·
Phospholipids · Membrane fluidity

Abbreviations

CRP	C-reactive protein
EDSS	Kurtzke expanded disability status scale
MS	Multiple sclerosis
PBMC	Peripheral blood mononuclear cells
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PS	Phosphatidylserine
PI	Phosphatidylinositol
PUFA	Polyunsaturated fatty acids
SATS	Saturated fatty acid
SM	Sphingomyelin

Introduction

The presence of peripheral blood mononuclear cells (PBMC) in brain tissue from patients with multiple sclerosis (MS) is well documented [1, 2]. Furthermore, polyunsaturated fatty acid (PUFA) metabolic abnormalities have been reported in these immune cells [3, 4]. PUFAs are precursors for inflammatory mediators of inflammation, the eicosanoids, but they are also structural fatty acids, and as such they supply structural integrity and various biological functions [2, 5]. Changes in the fatty acid composition may affect the function of membrane phospholipid molecules [2, 6]. Mammalian cell membranes consist of a double layer of phospholipids, into which fatty acids are incorporated, interspersed with cholesterol. Cells maintain a specific trans-membrane phospholipid distribution [7], with phosphatidylcholine (PC) and sphingomyelin (SM) contained on the outer leaflet of plasma membranes, and phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) on the inner leaflet [5, 8]. Loss of membrane phospholipid asymmetry is associated with many physiological and pathological phenomena [7] and changes in the membrane phospholipid composition can affect phagocytosis, T-lymphocyte signalling and antigen presentation [9]. Phagocytosis is an important mechanism in many cells for the elimination of micro-organisms or foreign particles, and membrane fluidity plays an important role in this process [10].

Polyunsaturated fatty acid metabolic alterations previously reported in the PBMC membranes from patients with MS [3, 4] together with other lipid abnormalities may have an effect on membrane fluidity [2, 6]. The aims of this study therefore was to investigate the relationship between PBMC membrane fluidity and permeability status, and disease outcome as measured by the Kurtzke expanded disability status scale (EDSS) [14] as well as the inflammatory process as measured by C-reactive protein (CRP).

Experimental Methods

Ethics Approval

Ethics approval for the study was obtained from the Health Sciences Research Ethics Committee (HSREC) of the Cape Peninsula University of Technology (CPUT). Patients with MS were contacted and recruited through the MS Society, Western Cape Branch, South Africa. Patients and control subjects gave informed written consent prior to their inclusion in the study.

Study Population

The study population consisted of 26 female Caucasians patients and 25 age- and gender matched female control subjects. All the patients were diagnosed by a neurologist based on clinical, laboratory and magnetic resonance imaging (MRI) findings. Patients on any fatty acid supplements, interferon or corticosteroid treatment or patients with a second disease were excluded from the study. The median (quartile range) for years since diagnoses was 9 (11) years and the median (quartile range) for the EDSS were 5.3 (3.0). Four of the patients were actively diseased, 11 had a relapse 5–12 months previously and 11 did not show any relapses for more than a year. Nine patients were using non-steroidal anti-inflammatory drugs (NSAIDs) and five patients were using immunosuppressive medication, while 12 patients used neither of these two types of medications.

Measurement of the Disability Status of Patients

The functional disability status of each patient was measured by a trained clinician using the Kurtzke expanded disability status scale (EDSS) [14]. The EDSS quantifies disability in eight functional systems (FS) and allows neurologists to assign a functional system score (FSS) in each of these. The disability scale for the EDSS are from 0 to 10, in which the 0 score indicates no disability at all and 10 indicates death due to MS. The FSS are from 0 to 5 or from 0 to 6. The EDSS and FSS at their highest (that is worse outcome) indicate the following: The EDSS: death due to MS; The pyramidal FSS: quadriplegia; The cerebellar FSS: the inability to perform coordinated movements due to ataxia; The brainstem FSS: inability to speak or swallow; The sensory FSS: essential loss of sensation below the head; The bowel and bladder FSS: loss of bowel and bladder function; The visual FSS: grade 5 plus maximal visual acuity of better eye of 20/60 or less; The cerebral FSS: dementia [14].

Blood Sample Processing and Analysis

Venous blood from consenting participants was collected into anti-coagulant EDTA tubes (Beckman Coulter, South Africa), and separated using histopaque-1077 separation medium as per manufacturer's instructions (Sigma-Aldrich, South Africa). The PBMCs were washed in 0.85% saline solution and stored at -80°C . Membrane phospholipids PC, PE, PS, PI and SM were determined using a colorimetric assay as previously described [15, 16] and quantified in $\mu\text{g Pi/mg protein}$. The membrane PC, PE, PS, PI and SM fatty acid compositions were measured by gas chromatography as previously described [17, 18] and

quantified in $\mu\text{g}/\text{mg}$ protein. Membrane cholesterol was determined according to an enzymatic assay [19] and quantified in $\mu\text{g}/\text{mg}$ protein. A bicinchoninic acid protein determination assay was used to determine the protein content [20]. Plasma CRP was determined using a Beckman nephelometer auto-analyser using reagents from Beckman, South Africa and was quantified in $\mu\text{g}/\text{ml}$ plasma.

Relationship Between Measured Lipids

Membrane fluidity was calculated as follows: membrane fatty acid saturation, as measured by the total saturated fatty acid (SATS) to total polyunsaturated fatty acid (PUFA) ratio [11–13]; membrane phospholipid asymmetry, as measured by the membrane saturated nature (phospholipid PC + SM/PE + PS ratio) [13]; the ordered-crystalline-phase to liquid-crystalline-phase lipids present, as measured by the phospholipid PE to PC ratio [11, 12] as well as the cholesterol to total phospholipid ratio [13]. Cholesterol was used as indications of both membrane fluidity and permeability [21, 22].

Statistical Analysis

A statistics programme, STATISTICA (STATISTICA 7, StatSoft Inc 1984–2004) was used to perform all statistical analyses. Descriptive data are presented as median (quartile range). For asymmetrical data Mann–Whitney *U* was used to compare distributions between the cases and control subjects. Correlations were calculated using Spearman's Rank correlation coefficient. In view of the small sample size, *P* values were corrected for multiple testing by Bonferroni. For correlations between the EDSS and total SATS to total PUFA ratios, phospholipids, as well as phospholipid to total phospholipid ratios: *P* value <0.004, between the EDSS and saturated nature (phospholipid PC + SM/PE + PS ratio), the ordered-to liquid-crystalline-phase lipids (phospholipid PE to PC ratio): *P* value <0.01, for correlations between lipids: *P* value <0.01, between the CRP and total SATS to total PUFA ratios, phospholipids, phospholipid ratios: *P* value <0.01 were considered as statistically significant.

Results

There were no significant differences between patients and controls in PBMC membrane total SATS, total PUFAs or the total SATS to total PUFA ratios. The membrane PC phospholipid to total phospholipid ratio was non-significantly lower and SM phospholipid to total phospholipid ratio non-significantly higher in patients with MS. The saturated nature (phospholipid PC + SM/PE + PS ratio) and the ordered-

liquid-crystalline-phase lipids (phospholipid PE to PC ratio) showed no difference between patients and controls.

Metabolic Relationship Between the Different Lipids Classes in Peripheral Blood Mononuclear Cell Membranes from Patients with Multiple Sclerosis and Control Subjects

In general, membrane total SATS showed highly significant positive correlations with membrane PUFAs in both study groups; the exception being PE SATS and PE PUFAs which did not show a significant correlation in the membranes from patients with MS (Fig. 1). After correction for multiple testing by Bonferroni, membrane phospholipids showed highly significant positive correlations with total phospholipids in both study groups ($R = 0.84$ (MS), 0.85

Fig. 1 **a** Significant positive correlation between PE saturated and polyunsaturated fatty acids in peripheral blood mononuclear cell membranes from control subjects: $R = 0.76$; $P = 0.00001^*$. **b** Non-significant correlation between PE saturated and polyunsaturated fatty acids in peripheral blood mononuclear cell membranes from patients with multiple sclerosis: $R = 0.33$; $P = 0.10$

Fig. 2 **a** Significant inverse correlation between the cerebral functional system score and the ratio PC saturated to polyunsaturated fatty acids in peripheral blood mononuclear cell membranes from patients with multiple sclerosis: $R = -0.51$; $P = 0.01$. **b** Significant positive correlation between the sensory functional system score and the ratio PS saturated to polyunsaturated fatty acids in peripheral blood mononuclear cell membranes from patients with multiple sclerosis: $R = 0.40$; $P = 0.04$

(controls), $P \leq 0.000001^*$ after correction for multiple testing by Bonferroni). PS phospholipids showed a positive correlation with PS SATS in the control group ($R = 0.53$, $P = 0.01$), but not in the patient group ($R = 0.16$, $P = 0.42$). Cholesterol showed positive correlations with PC and PI SATS, PC, and PE and PUFAs in both study groups ($P \leq 0.01$).

Correlations Between the Peripheral Blood Mononuclear Cell Membrane Lipids from Patients with Multiple Sclerosis and the Kurtzke Expanded Disability Status Scale

PBMC membrane total PUFAs from patients with MS showed an inverse correlation with the sensory

FSS ($R = -0.52$; $P = 0.007$). The PC SATS to PUFA ratio showed an inverse correlation with the cerebral FSS (Fig. 2a: $R = -0.51$; $P = 0.01$), while the PS and PI SATS to PUFA ratios showed positive correlations with the sensory FSS (Fig. 2b: $R = 0.40$; $P = 0.04$ and $R = 0.45$; $P = 0.03$, respectively). The SM to total phospholipid ratio and the saturated nature (phospholipid ratio PC + SM/PE + PS ratio) showed significant positive correlations with the sensory FSS ($R = 0.65$; $P = 0.0003^*$ and $R = 0.63$; $P = 0.001^*$, respectively). The PC and PS phospholipid to total phospholipid ratios showed inverse correlations with the sensory FSS ($R = -0.52$; $P = 0.01$ and $R = -0.44$; $P = 0.03$, respectively). The PI to total phospholipid ratio showed a positive correlation with the EDSS ($R = 0.47$; $P = 0.02$). Cholesterol showed no correlation with the EDSS or FSS. Significant P values after correction for multiple testing by Bonferroni are indicated with an *.

Correlations Between the C-reactive Protein and Peripheral Blood Mononuclear Cell Membrane Lipids from Patients with Multiple Sclerosis and Controls Subjects are Summarized in Table 1

The CRP showed positive correlations with the PS SATS to PUFA ratio in PBMC membranes from patients with MS, and positive correlations with PC, PS, PI and total phospholipids from control persons. It showed no correlation with the saturated nature (phospholipid PC + SM/PE + PS ratio), the ordered- to liquid-crystalline-phase lipids (phospholipid PE to PC ratio) or cholesterol from either the controls or patients.

Discussion

Results from this study showed that there were no significant differences in PBMC membrane lipids or membrane fluidity as measured by these lipids between patients with MS and control subjects. However, even though no differences were found, correlation studies demonstrated a disturbed metabolic relationship between these lipids, when compared to that of control subjects. Harbige and Sharief [3] used correlation studies to establish the metabolic relationship between membrane PUFAs. In this regard, the Rank R value is used as an indication of the degree of association between the values of two lipids. In the present study, the observed positive correlations in control subjects between SATS, PUFAs, phospholipids, and cholesterol were absent in the PBMC membranes from patients with MS. These lipid metabolic abnormalities could be expected to have had an effect on the behaviour of the lipid molecules, indirectly on membrane fluidity and permeability, and consequently on the functions of these immune cells

Table 1 Correlation between C-reactive protein and peripheral blood mononuclear cell membrane lipids from patients with multiple sclerosis and control subjects

	Controls N, 25		Patients with MS N, 26	
	<i>R</i>	<i>P</i> value	<i>R</i>	<i>P</i> value
Phospholipids				
PC	0.53	0.01*	−0.21	0.33
PE	0.16	0.48	−0.29	0.17
PS	0.55	0.01*	−0.21	0.33
PI	0.45	0.03	−0.16	0.46
SM	0.41	0.06	−0.19	0.37
Total phospholipids	0.50	0.02	−0.28	0.19

C-reactive protein was quantified in µg/ml plasma

* Significant *P* values: corrected for multiple testing by Bonferroni

such as eicosanoid production and phagocytosis. Phagocytosis is an important mechanism in cells for the elimination of micro-organisms or foreign particles, and in which process membrane fluidity plays an important role [10].

It is not clear from the results of this study whether these metabolic abnormalities are adjustments on a cellular level. Cells adjust their membrane lipids to adapt to changes in their environment [12, 13]. However, the disturbed metabolic relationship between membrane lipids from patients with MS were reflected in the positive correlations between membrane fluidity as measured by both its fatty acid saturation (PS and PI SATS to PUFA ratios) and saturated nature (phospholipid PC + SM/PE + PS ratio) and the sensory FSS, that is, higher saturation correlated with a worse outcome. The sensory FSS at its highest score (that is worse outcome), indicates essential loss of sensation below the head [14]. In contrast, membrane fluidity as measured by its fatty acid saturation (PC SATS to PUFA ratio) showed an inverse correlation with the cerebral FSS which is used to indicate dementia [14] and may therefore be affected differently by PBMC lipid composition and membrane fluidity. Furthermore, Chia et al. [11] used X-ray diffraction studies to investigate myelin from both healthy controls and patients with MS to show. The results of this study are however preliminary and need further investigation.

The CRP is an acute phase protein and has been used as a marker of inflammation, also in assessment of the degree of inflammation in patients with MS [23, 24] and has been shown to correlate with infectious episodes and clinical relapse in patients with MS [25]. The positive correlation between the CRP and membrane phospholipids from healthy controls reported in this study was absent in patients with MS. It has been suggested that the biological activities of CRP depend on its interaction with phospholipids of cell membranes [26]. Furthermore, phospholipids indirectly also play a role in the inflammatory process as it contains the *n*-6 and *n*-3 PUFAs which are mediators of inflammation. The

absence of correlation between the CRP and phospholipids in patients with MS needs further investigation to ascertain whether it contributes to the disease outcome.

Conclusions

The limitation of this study was that only female patients were used. The main strength of this study is however that neither the cases nor the controls were on any fatty acid supplements, and the patients were not on interferon or corticosteroid treatment. In conclusion, membrane fluidity as measured by both its fatty acid and phospholipid compositions, correlated with the FSS, more specifically the essential loss of sensation below the head (sensory FSS) and this could be due to the observed lipid metabolic abnormalities in PBMC membranes. Furthermore, the changed relationship between membrane phospholipids and C-reactive protein could be an indication of immune cell dysfunction in patients with multiple sclerosis, however this needs further investigations.

Acknowledgments We would like to extend our sincere gratitude to the following: The MS Society, Western Cape Branch, South Africa and sister Treska Botha for the recruitment of patients, Johanna van Wyk for technical support in the analysis of fatty acids, Dr Marius de Klerk for the measurement of the EDSS and FSS.

Conflict of Interest None.

References

1. Adams CWM (1977) Pathology of multiple sclerosis: progression of the lesion. *Br Med Bull* 33:15–20
2. Adibhatla RM, Hatcher JF (2007) Role of lipids in brain injury and diseases. *Future Lipidol* 2:403–422
3. Harbige LS, Sharief MK (2007) Polyunsaturated fatty acids in the pathogenesis and treatment of multiple sclerosis. *Br J Nutr* 98:S46–S53

4. Hon GM, Hassan M, Van Rensburg SJ et al (2009) Immune cell membrane fatty acids and inflammatory marker, C-reactive protein, in patients with multiple sclerosis. *Br J Nutr* 102:1334–1340
5. Caret RL, Denniston KJ, Topping JJ (1997) Lipids and their functions in biochemical systems. In: Wheatley CH (ed) *Principles and applications of inorganic, organic and biological chemistry*, 2nd edn. McGraw-Hill, Boston, pp 379–407
6. Horrobin DF (1999) The phospholipid concept of psychiatric disorders and its relationship to the neurodevelopmental concept of schizophrenia. In: Peet M, Glen I, Horrobin DF (eds) *Phospholipid spectrum disorder in psychiatry*. Marius Press, Lancashire, pp 3–16
7. Zwaal RFA, Schroit AJ (1997) Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. *Blood* 89:1121–1132
8. Barenholz Y, Thompson TE (1999) Sphingomyelin: biophysical aspects. *Chem Phys Lipids* 102:29–34
9. Calder PC (2007) Immunomodulation by omega-3 fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 77:327–335
10. De Pablo MA, De Cienfuegos GA (2000) Modulatory effects of dietary lipids on immune system functions. *Immunol Cell Biol* 78:31–39
11. Chia LS, Thompson JE, Moscarello MA (1984) Alteration of lipid-phase behavior in multiple sclerosis myelin revealed by wide-angle X-ray diffraction. *Proc Natl Acad Sci USA* 81:1871–1874
12. Williams EE (1998) Membrane lipids: what membrane physical properties are conserved during physiochemically-induced membrane restructuring? *Am Zool* 38:280–290
13. Allen HG, Allen JC, Boyd LC et al (2006) Determination of membrane lipid differences in insulin resistant diabetes mellitus type 2 in whites and blacks. *Nutrition* 22:1096–1102
14. Kurtzke JF (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 33:1444–1452
15. Itaya K, Ui M (1966) A new micromethod for the colorimetric determination of inorganic phosphate. *Clin Chim Acta* 14:361–366
16. Smuts CM, Weich HFH, Weight MJ et al (1994) Free cholesterol concentrations in the high-density lipoprotein subfraction-3 as a risk indicator in patients with angiographically documented coronary artery disease. *Coron Artery Dis* 5:331–338
17. Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–509
18. Van Jaarsveld PJ, Smuts CM, Tichelaar HY et al (2000) Effect of palm oil on plasma lipoprotein concentrations and plasma low-density lipoprotein composition in non-human primates. *Int J Food Sci Nutr* 51:S21–S30
19. Richmond W (1973) Preparation and properties of a cholesterol oxidase from *Nocardia* sp. And its application to the enzymatic assay of total cholesterol in serum. *Clin Chem* 19:1350–1356
20. Kaushal V, Barnes LD (1986) Effect of zwitterionic buffers on measurement of small masses of protein with bicinchoninic acid. *Anal Biochem* 157:291–294
21. Voet D, Voet JG (1995) Lipids and membranes. In: Voet D, Voet JG (eds) *Biochemistry*. John Wiley and Sons Inc, New York, pp 277–329
22. Barenholz Y (2002) Cholesterol and other membrane active sterols: from membrane evolution to “rafts”. *Prog Lipid Res* 41:1–5
23. Giovannoni G, Miller DH, Losseff NA et al (2001) Serum inflammatory markers and clinical/MRI markers of disease progression in multiple sclerosis. *J Neurol* 248:487–495
24. Sellner J, Greeve I, Mattle HP (2008) Atorvastatin decreases high-sensitivity C-reactive protein in multiple sclerosis. *Mult Scler* 14:981–984
25. Giovannoni G, Thorpe JW, Kidd D et al (1996) Soluble E-selectin in multiple sclerosis: raised concentrations in patients with primary progressive disease. *J Neurol Neurosurg Psychiatry* 60:20–26
26. Volanakis JE, Narkates AJ (1981) Interaction of C-reactive protein with artificial phosphatidylcholine bilayers and complement. *J Immunol* 126:1820–1825