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Influence of the Use of Statin on the Stability of Erythrocyte Membranes in Multiple Sclerosis

Mariana Vaini de Freitas · Marcela Ramos de Oliveira · Diogo Fernandes dos Santos · Rita de Cássia Mascarenhas Netto · Sheila Bernardino Fenelon · Nilson Penha-Silva

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Abstract Multiple sclerosis (MS) probably occurs by oxidative, inflammatory and autoimmune mechanisms. This study investigated the influence of statin on the stability of erythrocyte membranes in MS patients. The population was composed of one group with simvastatin therapy (20 mg/day), another group without statin therapy and a healthy control group. The stability of erythrocytes was evaluated by the half-transition points, H_{50} and D_{50}, obtained from the curves of hemolysis induced by hypotonic shock and ethanol action, respectively. Erythrocytes of MS patients were less stable against lysis by both chaotropes. This behavior may be merely a consequence of the lifestyle of MS patients or it may be intrinsically associated with the conjunct of factors responsible for the development of the disease. The use of statin by MS patients was associated with lower levels of LDL and total cholesterol, as expected, and with higher stability of erythrocytes against ethanol compared to the values of untreated MS patients.

Keywords Cholesterol · Erythrocyte · Hemolysis · Membrane · Multiple sclerosis · Statin · Stability

M. V. de Freitas · R. de Cássia Mascarenhas Netto · N. Penha-Silva (✉)
Instituto de Genética e Bioquímica, Universidade Federal de Uberlândia, Av. Pará, 1720, Uberlândia, MG 38400-902, Brazil
e-mail: nspenha@ufu.br

M. R. de Oliveira · D. F. dos Santos · S. B. Fenelon
Faculdade de Medicina, Universidade Federal de Uberlândia, Av. Pará, 1720, Uberlândia, MG 38400-902, Brazil

Introduction

Multiple sclerosis (MS) is a degenerative disease characterized by defects in the myelin membrane of the neural cells, and it seems to develop by oxidative, inflammatory and autoimmune mechanisms (Knight 1977; Hauser and Goodkin, 1998) in genetically vulnerable individuals, influenced by factors associated with nutrition (Swank et al. 1952; Schwarz and Leweling 2005).

MS is possibly associated with a disproportion in the nature of the fatty acids of the membrane phospholipids (Swank et al. 1952; Schwarz and Leweling 2005), which makes sense since a high $\omega 6$:$\omega 3$ polyunsaturated fatty acid (PUFA) ratio is associated with exacerbation of inflammation and autoimmunity (Calder 2005; Koch et al. 2006). Since PUFAs are very vulnerable to oxidation, which can generate imbalance in the nature of the membrane fatty acids, this means that the membrane contains the elements associated with the manifestation of the triad of degeneration (oxidation, inflammation and autoimmunity).

The exercise of membrane function depends on the structural homeostasis of this biological complex. There is a point of critical fluidity where the membrane congregates the necessary stability for its preservation and a necessary level of elasticity so that the cells suffer the conformational changes demanded for the exercise of their complex functions (Sinensky 1974).

To keep this critical fluidity, cells use several homeostatic mechanisms. These mechanisms include the action of solutes that promote the organization of the membrane (such as water and several osmolytes) and even the solutes that can promote its disorganization (such as urea and ethanol) (Cunha et al. 2007; Penha-Silva et al. 2008).

The circulating lipids are also important homeostatic agents. A lower content of unsaturated fatty acids in

relation to saturated fatty acids in phospholipids is a characteristic that decreases membrane fluidity. On the other hand, a higher content of unsaturated fatty acids in relation to saturated fatty acids in phospholipids is a characteristic that increases membrane fluidity. Membrane fluidity is also regulated by changes in cholesterol concentration. Membrane fluidity decreases with an increase in cholesterol content (Sinensky 1974).

Unbalance in membrane homeostasis can be promoted by a chronic increase in low-density lipoprotein cholesterol (LDL-C) and/or low levels of high-density lipoprotein cholesterol (HDL-C), resulting in degenerative manifestations, such as those associated with aging (Penha-Silva et al. 2007), atherosclerosis (Rosenson and Tangney 1998) and MS (Nue 1983; Navarro and Segura 1988; Alberts et al. 1992), conditions that have been related to the triad oxidation, inflammation and autoimmunity.

Statins are drugs that congregate hypocholesterolemic, antioxidant, anti-inflammatory and immunomodulatory properties (Sellner et al. 2008). Although the use of statins in the treatment of MS has not yet passed the stage of investigation, it provides promising expectations (Oliveira et al. 2007).

The need to elucidate the processes involved in the relationship between the causes and effects of MS requires the expansion of scientific research (Schwarz and Leweling 2005). To put statins in the context of membrane homeostasis and investigate the implications of the disease in other cells of the body, in addition to neural, seems to be a valid path to expand the research.

Due to previous experience in studying the behavior of the erythrocyte membrane (Cunha et al. 2007; Penha-Silva et al. 2007, 2008; De Freitas et al. 2008), this work evaluates the influence of a typical statin, simvastatin (Corsini et al. 1999), on the stability of the membrane of red blood cells (RBCs) in patients with MS.

Materials and Methods

Population

This work was previously approved by the local institutional ethics committee. The problem population was constituted of 16 women suffering from MS (26–58 years old). They were divided into two groups, one treated with 20 mg/day of simvastatin for 2.66 ± 1.03 years ($n = 6$) and another who were not using statin ($n = 10$). All patients were under standard treatment with interferon-beta. None of them had been taking the drug natalizumab, which alters erythrocyte functions (Lindberg et al. 2008). A control group consisted of six healthy women with BMI and age similar (28–56 years) to those of the problem group, all of them without routine use of medication and chronic consumption of ethanol. None of the volunteers had comorbidities, especially those known to affect the erythrocytes, such as hemoglobinopathies. A signed informed consent was obtained from each volunteer.

Blood Sample Collection

Blood samples (4 ml) were collected by intravenous puncture, after nocturnal fasting (8–14 h), in evacuated tubes containing 1 g dl^{-1} K_4EDTA.

Determination of the Osmotic Fragility of Human Erythrocytes

We prepared two sets of Eppendorf flasks containing 1-ml aliquots of 0–0.9% NaCl, which were preincubated at 37°C for 10 min, and added 10 µl of blood. After homogenization and incubation of the tubes for 20 min at 37°C, they were centrifuged at 3,000×g for 10 min. The erythrocyte lysis was followed by measuring absorbance of supernatants at 540 nm (A_{540}). Absorbance values were plotted against NaCl concentration and fitted to the sigmoidal regression curve given by the Boltzmann equation:

$$A_{540} = \frac{A_{min} - A_{max}}{1 + e^{(S - H_{50})/dS}} + A_{max} \quad (1)$$

where A_{min} and A_{max} represent the minimum and maximum values of absorbance of the sigmoid, respectively; S is NaCl concentration; H_{50} is the NaCl concentration capable of promoting 50% of hemolysis; and dS is the amplitude of the sigmoidal transition between A_{max} and A_{min}. The percentage of hemolysis in each assay tube was calculated by the equation

$$\text{Hemolysis } (\%) = \frac{A}{A_{max}} \times 100\% \quad (2)$$

Determination of the Stability of Human Erythrocytes against Ethanol

We prepared two sets of Eppendorf flasks containing 1-ml aliquots of 2–24 ml of ethanol per deciliter of solution in saline, which were preincubated at 37°C for 10 min, and added 10 µl of blood. After homogenization and incubation of the tube for 20 min at 37°C, they were centrifuged at 3,000 × g for 10 min at room temperature. The erythrocyte lysis was followed by measuring absorbance of the supernatants at 540 nm (A_{540}). Absorbance values were plotted against ethanol concentration and fitted to the sigmoidal regression curve given by the Boltzmann equation:

$$A_{540} = \frac{A_{min} - A_{max}}{1 + e^{(D - D_{50})/dD}} + A_{max} \quad (3)$$

where D is the concentration of ethanol, D_{50} represents the concentration of ethanol capable of promoting 50% of hemolysis and dD is the amplitude of the variation in concentration of ethanol in the sigmoidal transition between A_{min} and A_{max}. The percentage of hemolysis in each assay tube was calculated by equation 2.

Determination of Hematological and Biochemical Variables

Hematological variables were determined using an automated hematology analyzer (Cell-Dyn 3700; Abbott Laboratories, Santa Clara, CA).

The reference values (adult feminine population) used were erythrocyte (4.3–5.0 million/mm^3), hemoglobin (12.0–15.5 g%), hematocrit (35–45%), mean cell volume (MCV, 82–98 fl), total cholesterol (excellent <200 and high >239 mg/dl), LDL-C (excellent <100 and high >160 mg/dl), VLDL-C (excellent <100 and high >160 mg/dl), HDL-C (low <40 and desirable >40 mg/dl) and triglycerides (excellent <150 and high >200 mg/dl).

Statistical Analyses of Experimental Data

The data and statistics were analyzed using software package OriginPro 8.0 (Microcal, Northampton, MA). Analysis of the line of sigmoidal regression was considered significant at $P < 0.05$. Comparison of the data was made by analysis of variance, using Tukey's post-hoc test.

Results

The scores of the Expanded Disability Status Scale (EDSS) and the values of some hematological and biochemical variables of MS patients with and without treatment with simvastatin were compared (Table 1). EDSS scores and the values of hematological variables were not significantly different between groups. However, significant differences were observed in the levels of LDL- and total cholesterol.

Hemolysis against hypotonicity (osmotic fragility) and action of ethanol are shown in Figs. 1 and 2, respectively, in the three experimental groups (control group and groups of MS patients with and without treatment with simvastatin). The data of all patients in each group were submitted to simultaneous fittings only to illustrate the degree of homogeneity of results.

Blood samples were individually analyzed, with determination of their respective values of H_{50} and D_{50}. The average values of these parameters are shown in Table 2. H_{50} values were significantly higher in MS patients without statin therapy (0.472 ± 0.013 g dl^{-1} NaCl) than in the volunteers of the control group (0.427 ± 0.015 g dl^{-1} NaCl). H_{50} values were not significantly different in MS patients on statin therapy (0.457 ± 0.013 g dl^{-1} NaCl) compared to the other groups (MS patients without statin therapy and control group). This means that the MS patients had erythrocytes that were less stable against hypotonicity than volunteers who do not have the illness and that the statin therapy was not capable of increasing the membrane stability of erythrocytes against hypotonicity to the point of making it similar to the membrane stability present in the volunteers of the control group.

D_{50} values were significantly lower in the group of MS patients without statin therapy (13.87 ± 0.74% v/v ethanol) than in the control group (15.38 ± 0.24% v/v ethanol), which means that the MS patients had erythrocytes that were less stable against the chaotropic action of ethanol than those of the control group. D_{50} values of MS patients using statin treatment (15.20 ± 0.15% v/v ethanol) were not significantly different from those of the control group, but they were significantly higher than those of MS patients without statin therapy. This means that treatment with statin increased the stability of erythrocytes in patients with MS against the chaotropic action of ethanol, making it similar to the stability of membrane in the control group.

Table 1 Comparison of EDSS scores and hematological and biochemical variables in MS patients with and without simvastatin treatment

Variables	With treatment ($n = 6$)	Without treatment ($n = 10$)	P
EDSS (1–10)	3.83 ± 0.82	4.25 ± 0.92	
Erythrocytes (millions/mm^3)	4.58 ± 0.38	4.67 ± 0.38	
Hemoglobin (g%)	14.03 ± 0.76	14.50 ± 0.96	
Hematocrit (%)	41.67 ± 2.50	43.20 ± 2.45	
MCV (fl)	91.72 ± 2.62	90.10 ± 1.09	
Total cholesterol (mg/dl)	146.12 ± 25.28	206.11 ± 62.69	*
Triglycerides (mg/dl)	123.83 ± 20.57	114.55 ± 29.48	
HDL-C (mg/dl)	51.33 ± 10.14	49.07 ± 11.65	
VLDL-C (mg/dl)	25.95 ± 4.79	25.07 ± 11.088	
LDL-C (mg/dl)	77.43 ± 16.79	118.83 ± 40.59	*

* Statistically significant difference ($P < 0.05$) between groups (ANOVA)

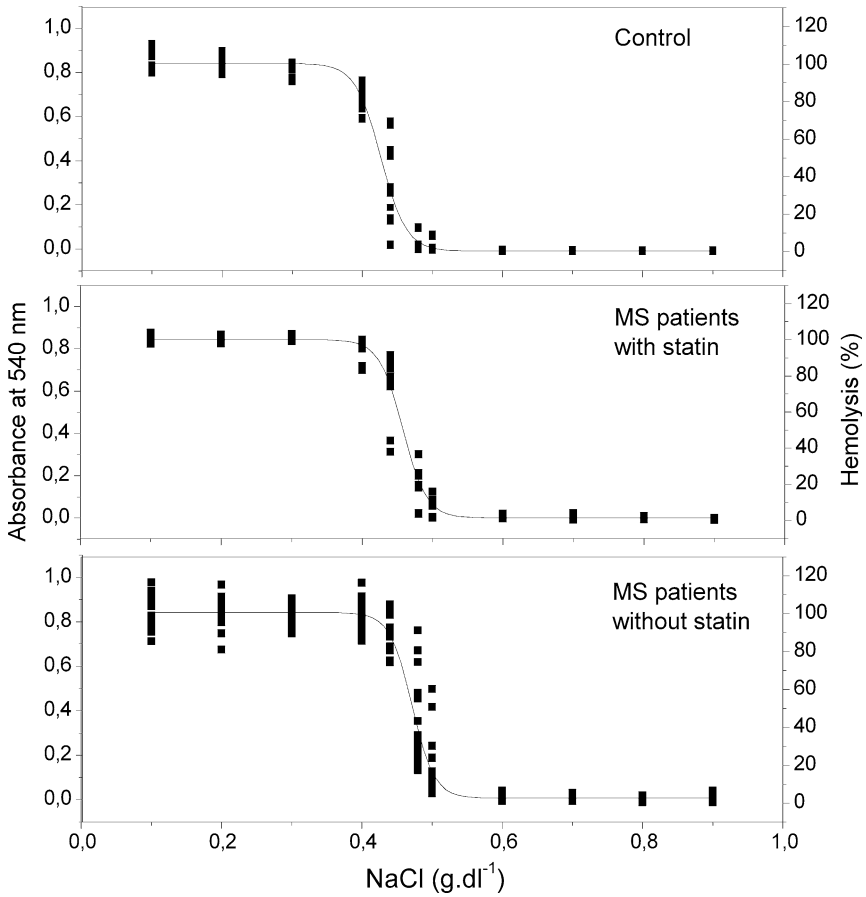

Fig. 1 Profiles of simultaneous adjustment of osmotic fragility of erythrocytes in the control group and in the groups of MS patients with and without statin treatment

Figure 3 shows the possible mechanisms that could affect the structural homeostasis of membranes in neural cells and in erythrocytes of MS patients.

Discussion

In this work, hypotonic shock and the chaotropic action of ethanol were chosen to analyze the stability of human erythrocytes.

The data in Table 2 show that MS patients without statin therapy had significantly higher values of H_{50} than those determined in the control group. This means that it was not necessary to decrease more substantially the NaCl concentration to produce hemolysis or, in other words, that the erythrocytes of MS patients were less resistant to osmotic lysis.

In fact, Caspary et al. (1967) also reported an increase in the osmotic fragility of erythrocytes during the acute phase of the disease. Kurantsin-Mills and collaborators (1982) found an absence of alteration in nonhospitalized patients and an increase in the osmotic fragility of erythrocytes in internal patients. Enhancement of the mechanic fragility of erythrocytes was also reported by Schauf et al. (1980).

As the values of D_{50} were significantly smaller for MS patients than for patients who did not have the disease (Table 2), this means that the MS patients had erythrocytes that were less stable against the chaotropic action of ethanol.

Besides this, statin therapy was associated with higher values of D_{50} in MS patients, which means higher stability against ethanol (Table 2). Since the values of D_{50} were not significantly different in the group of patients with MS treated with statins compared to the control group (Table 2), this means that the use of statin was able to restore the stability of erythrocytes against ethanol in patients with MS. This result indicates an association between the blood cholesterol decrease and the reestablishment of membrane homeostasis.

In fact, a rise in cholesterol content is a known cause of reduction in membrane fluidity. Erythrocytes are cells particularly sensitive to modifications in the extracellular cholesterol concentration (Cooper et al. 1975; Schick and Schick 1985). Cholesterol accumulates in the cell membrane, making it more rigid (Chabanel et al. 1983; Koter et al. 2002). The excess of plasmatic cholesterol modifies the properties of erythrocytes and can contribute to an increase in blood viscosity, reduction of oxygen quantity

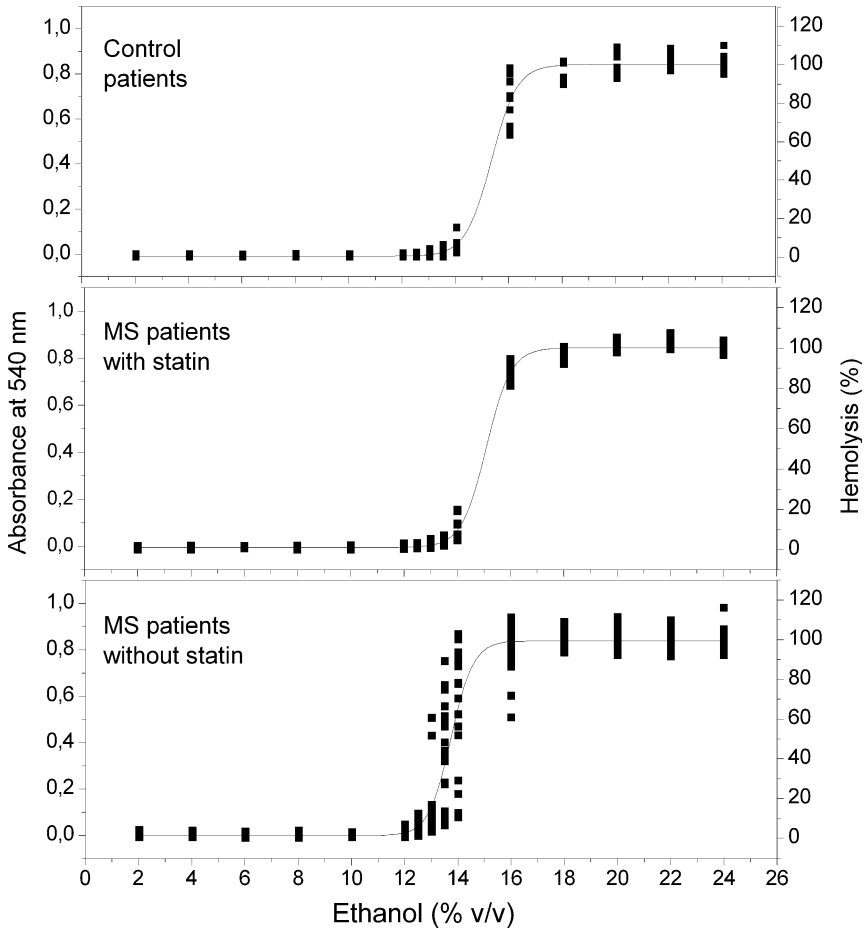

Fig. 2 Profiles of simultaneous adjustment of erythrocyte stability against ethanol in the control group and in the groups of MS patients with and without statin therapy

Table 2 Values of H_{50} and D_{50} (mean ± SD) in control group and subgroups of MS patients with and without simvastatin treatment

Variables	Control ($n = 6$)	With treatment ($n = 6$)	Without treatment ($n = 10$)
H_{50} (g/dl NaCl)	0.427 ± 0.015	0.457 ± 0.013*	0.472 ± 0.013*
D_{50} (% v/v ethanol)	15.38 ± 0.24	15.20 ± 0.15**	13.87 ± 0.74*,**

* $P < 0.05$ compared to control group (Tukey post-hoc test), ** $P < 0.05$ compared to the other MS group (Tukey post-hoc test)

and endothelial dysfunction. LDL-C is incorporated into the cellular membrane (Martinez et al. 1996), decreasing membrane fluidity and erythrocyte deformability (Cooper 1977; Muller et al. 1990; Dwight et al. 1996), compromising cellular functions (Sinensky 1974; Cribier et al. 1993).

Statin therapies have been associated with several hemorheological benefits, which comprise improvement in the blood microcirculation through an influence on blood viscosity and the structure of the erythrocyte membrane (Martinez et al. 1996) and improvement in endothelial function (Vaughan et al. 1996) through increased membrane fluidity of the endothelial cells, thus favoring functionality.

Significant reduction in the membrane cholesterol content was associated with treatment with lovastatin (Martinez et al. 1996) and with pravastatin (Lijnen et al. 1994). The reduction in membrane cholesterol must have been determinative in the increase of erythrocyte membrane fluidity observed by Levy and collaborators (1992) and Martinez and collaborators (1996) after treatment with lovastatin, although modifications in erythrocyte membrane fluidity have not been observed after using simvastatin by some authors (van Doormaal et al. 1989; Dwight et al. 1996). On the other hand, Coccia and collaborators (2007) found increased membrane fluidity after use of simvastatin for 3 weeks. Another group of researchers reported a course with two phases in simvastatin treatment: reduction of lipidemia until the end of the first month of treatment and increase in the membrane fluidity of erythrocytes 4–6 months after the beginning of therapy (Rabini et al. 1993).

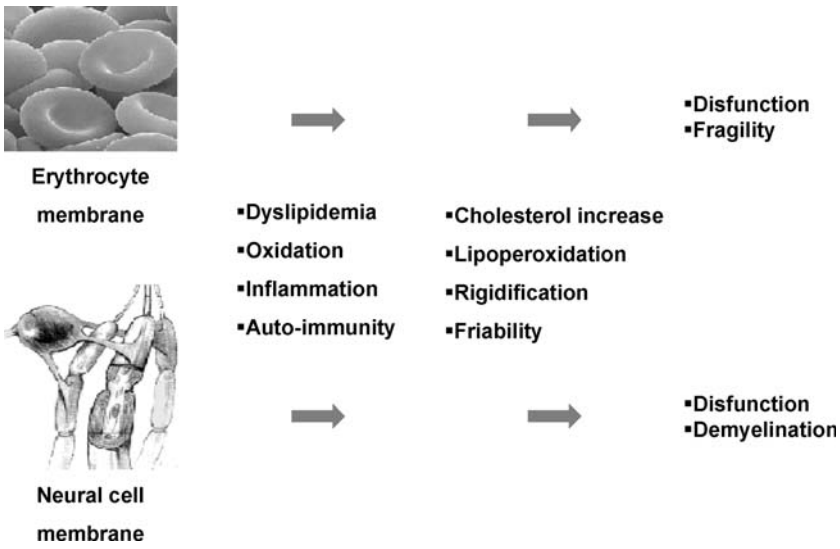

Fig. 3 Common putative mechanisms that could affect structural homeostasis of membranes in neural cells and in erythrocytes of patients with MS

As the patients of the present study were being treated with statins for 2.66 ± 1.03 years, they certainly would have already reached the stage of increase in fluidity of membrane, which results in increased stability of the membrane against ethanol, as in fact was observed in the MS patients treated with simvastatin (Table 2).

This change in membrane behavior coincides with the effect observed in the levels of blood cholesterol in patients group treated with statin. As expected, LDL- and total cholesterol were significantly lower in the treated group (Table 1), which certainly would decrease the availability of cholesterol for captation by extrahepatic tissues.

However, the increase in membrane stability against ethanol observed in the treated patients was not observed in the membrane stability against hypotonic shock (Table 2). This behavior may be a consequence of the small size of the population but also of some difference in the mechanism of lysis promoted by ethanol in relation to hypotonic lysis. Ethanol has a recognized protein-denaturing action (Fonseca et al. 2006), which is not necessarily involved in hypotonic lysis, where the erythrocytes absorb water, swell and suffer membrane rupture (Jain 1973).

The hypercholesterolemia we observed in MS patients (Table 1) agrees with literature reports. In MS, abnormality in lipid homeostasis is not restricted to myelin but also affects lipidemia (Cunnane et al. 1989; Alberts et al. 1992; Comoglu et al. 2004). Demyelination of MS plaques also releases cholesterol and fatty acid in the cerebrospinal fluid (Nue 1983; Navarro and Segura 1988; Cunnane et al. 1989; Cherayil 1990; Alberts et al. 1992).

In experimental animals and humans, hyperlipidemia has been associated with inflammation and oxidant signaling in the brain (Mattson et al. 2003; White et al. 2009). A failure in cholesterol homeostasis can be the primary cause of many neurodegenerative diseases (Joseph et al. 1998; Koudinov and Koudinova 2005), and it would be certainly connected to the changes in erythrocyte stability observed here for MS.

The results observed in this work seem to be expressive and reflect a characteristic of MS. They cannot be explained by differences in the hematological parameters (erythrocyte and hemoglobin amount, hematocrit and MCV) between MS patients and healthy volunteers since they were not significantly different between those groups (Table 1).

MCV changes would in fact be responsible for the stability differences between groups (Jain 1973). Although the occurrence of macrocytosis during the acute phase of the illness has been reported by Caspary and collaborators (1967), this characteristic was not observed by Grasso and collaborators (1992).

Another factor that could contribute to the membrane behavior changes in MS is oxidative stress. Oxidation markers have been found to be raised in the plasma of MS patients (Naidoo and Knapp 1992; Glabinski et al. 1993; Besler and Comoglu, 2003) and in the CNS of animals with experimental autoimmune encephalomyelitis (MacMicking et al. 1992). In individuals suffering from MS there is DNA damage by reactive oxygen species (ROS), surely associated with neurodegeneration (Vladimirova et al. 1999; Lu et al. 2000).

MS is characterized by the excessive presence of inflammatory infiltrations consisting mainly of lymphocytes and macrophages in the CNS (Frohman et al. 2006; Lev et al. 2007; Schreibelt et al. 2007). Products of lipid peroxidation have been found in many inflammatory processes (Kwiatkowsha et al. 1999). During inflammation, macrophages promote oxidative attack and can damage surrounding cells (Besler and Comoglu 2003). This oxidative attack causes alterations in the structural and

functional organization of cellular membranes, including reduction of membrane fluidity, increase in permeability, inactivation of enzymes and decrease in the content of essential fatty acids (Kopff et al. 1993; van Ginkel and Sevanian 1994). Formation of malondialdehyde (MDA), a lipoperoxidation product, is associated with the rigidification of erythrocyte membrane, thus decreasing its morphological plasticity (Pfafferott et al. 1982).

An important property of the statins is the antioxidant activity observed in humans and experimental animals (Aviram et al. 1998; Tong et al. 2009). According to Koter and collaborators (2002), treatment with atorvastatin was associated with decreased lipoperoxidation in patients with hypercholesterolemia.

Since a significant reduction in the activity of glutathione peroxidase was described in erythrocytes of MS patients (Shukla et al. 1977), which represents a decrease in antioxidant defense, it seems reasonable to assume that simvastatin therapy contributed to the protection of erythrocytes against oxidation by ROS, leading to improvement in membrane homeostasis in comparison to MS patients who were not using statins.

Despite the benefits reported, the risks associated with statin therapies need to be carefully analyzed by health professionals since positive correlations have been found between low serum cholesterol levels and death due to noncardiovascular causes, such as cancer, accident, depression and suicide (Herrinton and Friedman 1995).

The results we observed in this work and a broad group of literature evidence here discussed agree with the idea that the same conjunct of factors (dyslipidemia, oxidation, inflammation and autoimmunity), putatively involved in MS progression, would also be related at least partially to the degeneration of the erythrocyte membrane (Fig. 3), for reasons that would include genetic inheritance, feeding and lifestyle.

References

Alberts JJ, Marcovina SM, Christensen RH (1992) Lecithin cholesterol acyltransferase in human cerebrospinal fluid: reduced level in patients with multiple sclerosis and evidence of direct synthesis in the brain. Int J Clin Lab Res 22:169–172

Aviram M, Rosenblat M, Bisgaier CL, Newton RS (1998) Atorvastatin and gemfibrozil metabolites, but not the parent drugs are potent antioxidants against lipoprotein oxidation. Atherosclerosis 138:271–280

Besler HT, Comoglu S (2003) Lipoprotein oxidation, plasma total antioxidant capacity and homocysteine level in patients with multiple sclerosis. Nutr Neurosci 6:189–196

Calder PC (2005) Polyunsaturated fatty acids and inflammation. Biochem Soc Trans 33:423–427

Caspary EA, Sewell F, Field EJ (1967) Red blood cell fragility in multiple sclerosis. BMJ 2:610–611

Chabanel A, Flamm M, Sung KL, Lee MM, Schachter SL, Chien S (1983) Influence of cholesterol content on red cell membrane viscoelasticity and fluidity. Biophys J 44:171–176

Cherayil GD (1990) Effects of in vitro hyperthermia on fatty acids of red blood cells and plasma lipids from patients with multiple sclerosis. J Neurol Sci 95:141–151

Coccia R, Spadaccio C, Foppoli C, Perluigi M, Covino E, Lusini M, Chello M (2007) The effect of simvastatin on erythrocyte membrane fluidity during oxidative stress induced by cardiopulmonary bypass: a randomized controlled study. Clin Ther 29:1706–1717

Comoglu S, Yardimci S, Okcu Z (2004) Body fat distribution and plasma lipid profiles of patients with multiple sclerosis. Turk J Med Sci 34:43–48

Cooper RA (1977) Abnormalities of cell-membrane fluidity in pathogenesis of disease. N Engl J Med 297:371–377

Cooper RA, Arner EC, Wiley JS, Shattil JS (1975) Modifications of red cell membrane structure by cholesterol-rich lipid dispersion. J Clin Invest 55:115–125

Corsini A, Bellosta S, Baetta R, Fumagalli R, Paoletti R, Bernini F (1999) New insights into the pharmacodynamic and pharmacokinetic properties of statins. Pharmacol Ther 84:413–428

Cribier S, Morrot G, Zachowski A (1993) Dynamics of the membrane lipid phase. Prostaglandins Leukot Essent Fatty Acids 48:24–32

Cunha CC, Arvelos LR, Costa JO, Penha-Silva N (2007) Effects of glycerol on the thermal dependence of the stability of human erythrocytes. J Bioenerg Biomembr 39:341–347

Cunnane SC, Ho SY, Dore-Duffy P, Ells KR, Horrobin DF (1989) Essential fatty acid and lipid profiles in plasma and erythrocytes in patients with multiple sclerosis. Am J Clin Nutr 50:801–806

De Freitas MV, Netto RCM, Huss JCC, De Souza TMT, Costa JO, Firmino CB, Penha-Silva N (2008) Influence of aqueous crude extracts of medicinal plants on the osmotic stability of human erythrocytes. Toxicol In Vitro 22:219–224

Dwight JF, Mendes-Ribeiro AC, Hendry BM (1996) Effects of HMG-CoA reductase inhibition on erythrocyte membrane cholesterol and acyl chain composition. Clin Chim Acta 256:53–63

Fonseca LC, Corrêa NCR, Garrote-Filho MS, Cunha CC, Penha-Silva N (2006) Effects of the solvent composition on the stability of proteins in aqueous solutions. Quim Nova 29:543–548

Frohman EM, Racke MK, Raine CS (2006) Multiple sclerosis—the plaque and its pathogenesis. N Engl J Med 354:942–955

Glabinski A, Tawsek NS, Bartosz G (1993) Increased generation of superoxide radicals in the blood of MS patients. Acta Neurol Scand 88:174–177

Grasso MG, Colleluori A, Millefiorini E, Pozzilli C (1992) Lack of association between macrocytosis and multiple sclerosis. J Neurol Neurosurg Psychiatry 55:1096–1097

Hauser SL, Goodkin DE (1998) Multiple sclerosis and other demyelinating diseases. In: Fauci AS, Braunwald E, Isselbacher KJ, Wilson DJ, Martin JB, Kasper DL, Longo SL (eds) Harrison's principles of internal medicine, 14 edn. McGraw-Hill, New York, pp 2452–2461

Herrinton LJ, Friedman GD (1995) Serum cholesterol concentration and risk of brain cancer. BMJ 310:367–368

Jain NC (1973) Osmotic fragility of erythrocytes of dogs and cats in health and in certain hematologic disorders. Cornell Vet 63: 411–423

Joseph JA, Denisova N, Fisher D, Bickford P, Prior R, Cao G (1998) Age-related neurodegeneration and oxidative stress: putative nutritional intervention. Neurol Clin 16:747–755

Knight S (1977) Cellular immunity in multiple sclerosis. Br Med Bull 33:45–50

Koch M, Ramsarannsing GSM, Fokkema MR, Heersema DJ, Keyser JD (2006) Erythrocyte membrane fatty acids in benign and progressive forms of multiple sclerosis. J Neurol Sci 244:123–126

Kopff M, Zakrzewska I, Czernicki J, Klem J, Strzelczyk M (1993) Red blood cell adenosine deaminase activity in multiple sclerosis. Clin Chim Acta 214:97–101

Koter M, Broncel M, Chojnowska-Jezierska J, Klikczynska K, Franiak I (2002) The effect of atorvastatin on erythrocyte membranes and serum lipids in patients with type-2 hypercholesterolemia. Eur J Clin Pharmacol 58:501–506

Koudinov AR, Koudinova NV (2005) Cholesterol homeostasis failure as a unifying cause of synaptic degeneration. J Neurol Sci 229(230):233–240

Kurantsin-Mills J, Samji N, Moscarello MA, Boggs JM (1982) Comparison of membrane structure, osmotic fragility, and morphology of multiple sclerosis and normal erythrocytes. Neurol Res 7:1523–1540

Kwiatkowsha S, Piasecka G, Zieba M, Piotrowski W, Nowak D (1999) Increased serum concentrations of conjugated dienes and malondialdehyde in patients with pulmonary tuberculosis. Respir Med 93:272–276

Lev N, Gilgun-Sherki Y, Offen D, Melamed E (2007) The role of oxidative stress in the pathogenesis of multiple sclerosis: current state. In: Qureshi GA, Parvez SH (eds) Oxidative stress and neurodegenerative disorders. Elsevier, New York, pp 283–295

Levy Y, Leibowitz R, Aviram M, Brook JG, Cogan U (1992) Reduction of plasma cholesterol by lovastatin normalizes erythrocyte membrane fluidity in patients with severe hypercholesterolaemia. Br J Clin Pharmacol 34:427–430

Lijnen P, Celis H, Fagard R, Staessen J, Amery A (1994) Influence of cholesterol lowering on plasma membrane lipids and cationic transport system. J Hypertens 12:59–64

Lindberg RL, Achtnichts L, Hoffmann F, Kuhle J, Kappos L (2008) Natalizumab alters transcriptional expression profiles of blood cell subpopulations of multiple sclerosis patients. J Neuroimmunol 194:153–164

Lu F, Selak M, O'connor J, Croul S, Lorenzana C, Butunoi C, Kalman B (2000) Oxidative damage to mitochondrial DNA and activity of mitochondrial enzymes in chronic active lesions of multiple sclerosis. J Neurosci 177:95–103

MacMicking JD, Willenborg DO, Weidemann MJ, Rockett KA, Cowden WB (1992) Elevated secretion of reactive nitrogen and oxygen intermediates in hyperacute experimental autoimmune encephalomyelitis: enhancement by the soluble products of encephalitogenic T cells. J Exp Med 176:303–307

Martinez M, Vaya A, Marti R, Gil L, Lluch J, Carmena R, Aznar J (1996) Erythrocyte membrane cholesterol/phospholipid changes and haemorheological modifications in familial hypercholesterolemia treated with lovastatin. Thromb Res 83:375–388

Mattson MP, Duan W, Guo Z (2003) Meal size and frequency affect neuronal plasticity and vulnerability to disease: cellular and molecular mechanisms. J Neurochem 84:417–431

Muller S, Ziegler O, Donner M, Drouin P, Stoltz JF (1990) Rheological properties and membrane fluidity of red blood cells and platelets in primary hyperlipoproteinemia. Atherosclerosis 83:231–237

Naidoo R, Knapp ML (1992) Studies of lipid peroxidation products in cerebrospinal fluid and serum in multiple sclerosis. Clin Chem 38:2449–2454

Navarro X, Segura R (1988) Plasma lipids and their fatty acid composition in multiple sclerosis. Acta Neurol Scand 78:152–157

Nue IS (1983) Essential fatty acids in the serum and cerebrospinal fluid of multiple sclerosis patients. Acta Neurol Scand 7:151–163

Oliveira MR, Santos DF, Fenelon SB, Penha-Silva N (2007) Uso de rosuvastatina em esclerose múltipla. Rev Neurosci 15:246–250

Penha-Silva N, Firmino CB, De Freitas Reis FG, Huss JCC, De Souza TMT, De Freitas MV, Netto RCM (2007) Influence of age on the stability of human erythrocyte membranes. Mech Ageing Dev 128:444–449

Penha-Silva N, Arvelos LR, Cunha CC, Aversi-Ferreira TA, Gouvêa-e-Silva LF, Garrote-Filho MS, Finotti CJ, Bernardino-Neto M, De Freitas Reis FG (2008) Effects of glycerol and sorbitol on the thermal dependence of the lysis of human erythrocytes by ethanol. Bioelectrochemistry 73:23–29

Pfafferott C, Meiselman HJ, Hochstein P (1982) The effect of malonyldialdehyde on erythrocyte deformability. Blood 59: 12–15

Rabini RA, Polenta M, Staffolani R, Tocchini M, Signori R, Testa I, Mazzanti L (1993) Effect of hydroxymethylglutaryl-CoA reductase inhibitors on the functional properties of erythrocytes membranes. Exp Mol Pathol 59:51–57

Rosenson RS, Tangney CC (1998) Antiatherothrombotic properties of statins: implications for cardiovascular event reduction. JAMA 279:1643–1650

Schauf CL, Frischer H, Davis FA (1980) Mechanical fragility of erythrocytes in multiple sclerosis. Neurology 30:323–325

Schick BP, Schick PK (1985) Cholesterol exchange in platelets, erythrocytes and megakaryocytes. BBA Lipids Lipid Metabol 833:281–290

Schreibelt G, Van Horssen J, Van Rossum S, Dijkstra CD, Drukarch B, Vries HE (2007) Therapeutic potential and biological role of endogenous antioxidant enzymes in multiple sclerosis pathology. Brain Res Rev 56:322–330

Schwarz S, Leweling H (2005) Multiple sclerosis and nutrition. Mult Scler 11:24–32

Sellner J, Greeve I, Findling O, Grandgirard D, Leib SL, Mattle HP (2008) Atorvastatin does not alter serum levels of sCD95 and sCD95L in multiple sclerosis. Clin Exp Immunol 152:280–284

Shukla VKS, Jensen GE, Clausen J (1977) Erythrocyte glutathione peroxidase deficiency in multiple sclerosis. Acta Neurol Scand 56:542–550

Sinensky M (1974) Homeoviscous adaptation. A homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. Proc Natl Acad Sci USA 71:522–525

Swank RL, Lerstad O, Strom A, Backer J (1952) Multiple sclerosis in rural Norway its geographic and occupational incidence in relation to nutrition. N Engl J Med 246:722–728

Tong X, Nicolakakis N, Fernandes P, Ongali B, Brouillette J, Quirion R, Hamel E (2009) Simvastatin improves cerebrovascular function and counters soluble amyloid-beta, inflammation and oxidative stress in aged APP mice. Neurobiol Dis 35:406–414

van Doormaal JJ, Bos WJ, Muskiet FA, Doorenbos H (1989) Simvastatin influence linoleic acid metabolism. Pharm Weekbl Sci 11:134–135

van Ginkel G, Sevanian A (1994) Lipid peroxidation induced membrane structural alterations. Methods Enzymol 233:273–288

Vaughan CJ, Murphy MB, Buckley BM (1996) Statins do more than just lower cholesterol. Lancet 348:1079–1082

Vladimirova O, Lu FM, Shawver L, Kalman B (1999) The activation of protein kinase C induces higher production of reactive oxygen species by mononuclear cells in patients with multiple sclerosis than in controls. Inflamm Res 48:412–416

White CL, Pistell PJ, Purpera MN, Gupta S, Fernandez-Kim S, Hise TL, Keller JN, Ingram DK, Morrison CD, Bruce-Keller AJ (2009) Effects of high fat diet on Morris maze performance, oxidative stress, and inflammation in rats: contributions of maternal diet. Neurobiol Dis 35:3–13