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REVIEW ARTICLE

Fatty Acids and Antioxidants in Multiple Sclerosis: Therapeutic Role of GEMSP

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Abstract: Multiple sclerosis is a high-frequency neurological disorder in young adults. Although there are some genetic and environmental factors that have been related to the onset of the disease, these are still not completely understood and nowadays multiple sclerosis can neither be prevented, nor its symptom effectively treated due to disease heterogeneity. For this reason, the search of prognostic factors and new therapeutic compounds for MS has long aroused among clinicians and researchers. Among these therapeutic compounds, GEMSP, which consists of a mixture of functional constituents as fatty acids, antioxidants, free radical scavengers and amino acids linked individually to poly-L-Lysine (PL), is emerging as a promising drug for MS treatment. Pre-clinical studies using GEMSP have demonstrated that this drug strongly inhibits brain leukocyte infiltration and completely abolishes experimental autoimmune encephalomyelitis. In addition, in an open clinical trial in humans treated with GEMSP, in 72% of the cases, a positive evolution of the state of the MS patients treated with GMSP was observed. In this review a biochemical characterization of main constituents of GEMSP, which include fatty acids as oleic acid, linoleic acid or azelaic acid and the antioxidants alpha-tocopherol or ascorbic acid, will be provided in order to understand their proved therapeutic effects in MS.

Keywords: Multiple sclerosis, GEMSP, fatty acids and antioxidants.

1. INTRODUCTION

Multiple sclerosis (MS) is the most frequent disabling neurological disease in young adults. During the disease evolution, several independent processes are involved, such as inflammation, demyelination, neurodegeneration, gliosis and repair, which are responsible for the heterogeneity and individual variability in the expression of the disease, the prognosis and the response to treatment. Clinically, main characteristics of MS disease are: sensory focality, motor focality (paraparesis, hemiparesis), spasticity, balance disorders, visual disturbances such as loss of vision or double vision (diplopia) or sphincter dysfunction. These episodes of neurological alteration are called "relapses". Throughout life, the person suffering from MS can present relapses with a frequency that varies greatly from one person to another and that is impossible to predict. The recurrence of relapses is one of the factors that contributes to the accumulation of disability. In fact, this disease is the leading cause of non-traumatic disability in young adults in Europe and North America.

In 1996, The US National Multiple Sclerosis Society (NMSS) Advisory Committee on Clinical Trials in Multiple Sclerosis defined the clinical subtypes of MS. The main subtypes of MS are relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP), and progressive relapsing (PR) [1]. Clinically, RR represents the initial inflammatory phase, characterized by reversible symptom with neurological dysfunction, followed by periods of remission. Approximately 40-50% of these patients progress to SP, where the disease gradually evolves from intermittent relapses to a constant progressive worsening, resulting in permanent disability due to massive axonal loss. The PP is the most severe subtype, already affecting approximately 10% of all cases, and manifesting itself with progressive degeneration without any remission [2]. The PR manifests itself with a progression of the disease along with the recurrent relapses throughout it; this subtype of the disease is the least frequent. The possibility of relapses or radiological activity in progressive forms, whether primary or secondary, has recently led to the use of the term "progressive and active multiple sclerosis" to select those patients who may still benefit from treatments that modify the course of the disease [3].

The first symptoms of the disease appear in most cases between 20 and 40 years and is more frequent in women. The etiology is unknown. There are some genetic and environmental factors that are related to the disease, but these are still not well determined and today MS cannot be predicted or prevented. The diagnostic criteria are based to a large extent on the findings of the cerebral and medullary MRI that show that there are areas of inflammation in the myelin, in a characteristic location and number. Relapses are treated with corticosteroids at high doses, and since 1993 maintenance treatments have been available to reduce the frequency and severity of relapses, first-line (interferon beta, glatiramer acetate and new immunomodulators) [4] and second line, in patients without response to first line treatments or with very aggressive disease at the beginning (natalizumab, fingolimod, alemtuzumab, cladribine and ocrelizumab) [5]. Recently, ocrelizumab has also been approved for progressive forms [6]. Although these drugs have been proven to be effective in the MS treatment, they have important side effects.

Natalizumab is a humanized monoclonal antibody that inhibits lymphocyte migration via the blood brain barrier (BBB) by blockage of an integrin adhesion molecule. Natalizumab reduces disease activity and prevents disability progression, although it produces the following side effects in 1/100 patients: pharyngitis, urinary tract, infection, urticarial, cephalgia, dizziness, nausea, vomiting, arthralgia, fever, and rigidity Moreover, progressive multifocal leukoencephalopathy (PML) has been reported in 3.4/1000 patients [7]. PML is caused by an infection of glial cells in the CNS white matter by JC-virus [8]. Fingolimod is a structural analogue of

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sphingosine derived from fungal metabolites. It can cross the BBB and directly influence central nervous system pathogenesis in RRMS. Fingolimod acts primarily by reversibly retaining circulating central memory T cells and naive T cells in lymph nodes, thereby reducing the recirculation of autoreactive lymphocytes to the central nervous system (CNS) [9]. Remarkably, it has been described that fingolimod can also produce macular oedema and other ocular adverse effects [10]. Pan-lymphocyte-depleting anti-CD52 monoclonal antibody Alemtuzumab is licensed to treat relapsing forms of MS on the basis of the trial results. These have demonstrated its efficacy compared to interferon ß-1a and acceptable safety [11] [12], despite the frequent occurrence (30-35% of patients) of secondary autoimmunity. Muraro et al (2018) [13] re-viewed the adverse effects of Alemtuzumab and suggested the need of an active post-marketing surveillance. Cladribine is a nucleoside analogue of deoxyadenosine that depletes lymphocytes. It is admin-istered as a ten day-course of 1.75 mg/kg per year for two years. On concomitant administration of Cladribine, there is an increased rate of viral infections, and malignancies could be more frequent in patients treated with Cladribine, and because of its action mecha-nism, adverse effects on human gametogenesis could be expected. Ocrelizumab is a monoclonal antibody that depletes CD20 B-cells. Its most common side effect was infusion-related reaction, but it can also produce upper respiratory tract or herpesvirus infections [14].

In the search to find better treatments for MS, GEMSP is emerging as a novel therapeutic compound for MS, according to its reported beneficial effects in pre-clinical and clinical studies [15, 16]. Experimental autoimmune encephalomyelitis (EAE) is the main animal model used to study MS. Pre-clinical studies using GEMSP have demonstrated that this drug strongly inhibits brain leukocyte infiltration and completely abolishes EAE episodes and clinical scores. Molecular analyses also showed that GEMSP preserves myelin integrity [15]. In an open clinical trial in humans (phase IIa), after six months of treatment with GEMSP the results showed that 55% of the patients maintained a stable Expanded Disability Status Scale (EDSS) value and 18% of them had a decreases EDSS value, instead of a normal progression of 0.25 points on the mean EDSS scale [17]. Moreover, when the study was extended to 193 patients with MS, EDSS value was significantly lower than in the control group and the health improvement of MS group compared with control group was 24% higher [16].

GEMSP consists of a mixture of functional polypeptides: fatty acids, antioxidants, free radical scavengers and amino acids linked individually to poly-L-Lysine (PL), (see Table **1**).

In this review, we will discuss the individual efficacy of the GEMSP components in order to understand the effect of this therapy on MS patients.

FATTY ACID ADMINISTRATION TO MS TREATMENT

Meta-analysis of epidemiological data has demonstrated a relation between MS mortality and dietary fat. Intake of saturated fatty acids, mainly in animal fat products, correlates positively with MS mortality [18]. An increased risk of MS was found to be associated with high energy and animal food intake. The same study also revealed a protective effect of other nutrients, including vegetable protein, dietary fiber, cereal fiber, vitamin C, thiamine, riboflavin, calcium and potassium [19]. Why the prevalence of MS is higher in developed countries? Is the diet, between others environmental factors, which play a strategic role in the development of MS? There are many reviews trying to elucidate the role of diet in pathology of MS. Here we focus on the effect of a mediterranean diet and analyse the role of fatty acids in the disease. Main components of olive oil, oleic acid (OA) and phenols, have a wide variety of beneficial health effects, not only for MS. Cerebral ischemia, spinal cord injury, Huntington's disease, Alzheimer's disease or Parkinson's disease are pathologies where olive oil can have neuroprotective effects. Nowadays, many of the neuroprotective effects of olive oil have been characterised, but the mechanism behind these actions have not been fully elucidated. In an experimental model of MS, the

Table 1. Therapeutic indications of compounds constituents on GEMSP.

Compounds	Constituents in GEMSP	Therapeutic indications
Oleic acid	Azelaic Acid - PL - Oleic Acid	Neuroprotective Antioxidant activity Neurotrophic factor Remyelination
	T-T-Farnesyl-L.Cysteine - PL - Oleic Acid	
	Oleic Acid - PL - Palmitic Acid	
	Oleic Acid - PL - Myristic Acid	
	Oleic Acid - PL - Linoleic Acid	
	Oleic Acid - PL - Palmitoleic Acid	
	Cholesterol -PL - Oleic Acid	
	Oleic Acid - PL - Thioctic Acid	
Linoleic acid	Linoleic Acid – PL	Inmune-mediated MS Anti-inflammatory Reduced severity and duration of relapses
Azelaic acid	Azelaic Acid - PL - Oleic Acid	Antibacterial Antioxidant activity Anti-inflammatory
	Azelaic Acid - PL - Palmitoleic Acid	
α-Tocopherol	α-Tocopherol-succinate – PL	Antioxidant activity
Ascorbic Acid	Ascorbic Acid – PL	Antioxidant activity

gastric administration of olive oil or OA reduced the degree of lipid and protein oxidation and increased glutathione peroxidase, making it a diet-based mechanism for enhancing protection against oxidative damage [20]. The OA is one of the main component of myelin sheaths. Trépanier et al.[21] have evaluated the concentration of oleic acid after demyelination in a cuprizone model. The major finding of this study is that OA concentration is decreased following cuprizone administration and recovers with remyelination. Although, the biological relevance of OA for MS disease progression remains to be verified. Previous works showed that OA is a neurotrophic factor which induces neuronal differentiation [22, 23]. The OA is synthetized and released by astrocytes through a mechanism that included the transcytosis of albumin through the endoplasmic reticulum [24]. This OA could also be captured by oligodendrocytes for their own remyelination in MS. Hence, Lipidosin, a long-chain acyl-CoA synthetase activity, is increased in astrocytes in the area or remyelination following experimental demyelination induced by the administration of cuprizone to mice [25]. Moreover, in peripheral nervous system there are evidences of a translational or posttranslational regulation of stearoil-CoA desaturase-2 (enzyme limiting the synthesis of oleic acid) similar to myelin proteins [26].

There are several lines of evidence indicating that during brain growth and myelination, the major saturated and monounsaturated fatty acids in brain lipids are exclusively produced locally by novo biosynthesis. However, the n-6 and n-3 polyunsaturated fatty acids (PUFAs) must be transported and delivered to the brain by highly specific mechanisms [27]. Several models regarding the uptake of PUFA by the brain have been proposed. In one of them, lipoproteins containing PUFA, LDL, VLDL and HDL, along with lisophosphatidylcholine and unesterified fatty acid, enter the endothelium via receptor mediated transport. The esterified PUFA are then hydrolysed within the cell via fatty acid transport protein. They are selectively incorporated into the brain where they can be esterified to phospholipids or undergo other forms of metabolism. In another proposed model, unesterified PUFA enter the brain either via protein-mediated transport, or passive diffusion [28].

Considering these anti-inflammatory properties of PUFAs, numerous reviews have cited these agents as potential therapeutic compounds for immune-mediated disorders as MS [29, 30]. Linoleic acid (n-6) (LA), which is included in GEMSP, is converted into different metabolites through various enzymatic processes. Eventually it can be converted to arachidonic acid, a key intermediate in eicosanoids biosynthesis [31]. The anti-inflammatory effects of LA might include the competitive inhibition of arachidonic acid [32-34]. The production of anti-inflammatory prostaglandins E1 and E2, which are derived from the dihomo-γ-linoleic acid, can inhibit the production of proinflammatory cytokines, such as interleukin-2 and interfereon γ (IFN-γ) [35-37]. Several studies in patients with MS showed a reduction of T-cell proliferation in patients receiving PUFA supplementation [38], as well as a decrease in the levels of proinflammatory cytokines, such as IL-2, tumour necrosis factor (TNF) and IFN-γ [39, 40]. Small randomized trials of LA supplements in patients with RRMS showed a reduction in the severity and duration of relapses, [41, 42] although oppositely a different study with 69 patients with progressive MS did not find an effect of LA supplementation on disability accrual [43]. These apparently opposite results in LA effect may be due to the advanced stage of the disease, indicating that supplementation with LA may be effective at early stages of the disease, but not at later stages.

Another fatty acid which is included in GEMSP formulation is azelaic acid (Aze A). Aze A is a naturally occurring dicarboxylic acid produced by Malassezia furfur (fungus of the normal flora on the skin people) and found in whole grain cereals, rye, barley and animal products. Aze A possesses antibacterial, keratolytic, comedolytic, and anti-oxidant activity [44]. Aze A also possesses a direct anti-inflammatory effect due to its scavenger activity of reactive oxygen species. This drug is used topically to reduce inflammation associated with acne and rosacea [45]. However, Daverat et al. [46] have reported the existence of anticonjugated Aze A antibodies in serum of patients with MS. Interestingly, concentration of Aze A antibodies was higher in the serum of patients in acute relapse than in patients with the progressive form, and also higher than the concentration in the serum of patients with other neurological diseases or healthy subjects.

ROLE OF ANTIOXIDANTS IN MS

Antioxidants play an important role in the evolution of MS, since they are necessary for inhibiting the oxidation of essential fatty acids by free radicals in membrane phospholipids and, therefore, protect the integrity of myelin. Oxidative damage to the central nervous system can be caused by the release of large amounts of iron from damaged cells and low levels of antioxidants [47].

There are endogenous and exogenous antioxidants; and the latter are the ones that contribute to the diet. Different authors have studied the activity of these endogenous antioxidants in patients with MS:

Glutathione reductase is an enzyme which catalyzes the reduction of oxidized glutathion (GSSG) to reduced glutathione (GSH). This will be used by the glutathione peroxidase for the reduction of reactive oxygen species peroxide and lipoperoxides. In MS, as in other neurodegenerative diseases, there is a decrease in the levels of GSH, which leads to a lower antioxidant capacity [48].

Superoxide dismutase (SOD) catalyzes the transformation of free superoxide radical in hydrogen peroxide, a less harmful free radical. The measurement of its activity in erythrocytes obtained from patients in different clinical phentoypes of neuroinflammation is considered a potential marker of oxidative stress intensity in neuroinflammmation and disease severity [49].

Quinone oxidoreductase 1 (NQO1) is an antioxidant enzyme that catalyzes the reduction of various quinones to their corresponding hydroquinones using both NADH and NADPH as donors. This enzyme is found strongly upregulated in astrocytes of active MS lesions [50, 51], probably trying to counteract the negative effects of reactive species.

In people with MS and in animal models of MS, it has been observed the production of large amounts of peroxynitrite and superoxide, which are highly toxic to neurons [52]. In addition, the antioxidant activity is significantly lower in the plasma of patients with MS, since there are reduced levels of vitamin E, ubiquinol and glutathione peroxidase [53].

Alpha-tocopherol and ascorbic acid are administered in GEMSP therapy due to antioxidant vitamins, such as alpha-tocopherol, beta-carotene, retinol, and ascorbic acid, are decreased in the serum of patients with MS during a relapse or outbreak. This decrease is related to an increased oxidative rate as a result of higher lipid peroxidation [54]. These signs of oxidative stress suggest that a diet rich in vitamin E and selenium may help to inhibit the progression of the disease [55].

There are also different spices with antioxidant properties. Previous study in a mouse model showed that saffron addition can be effective in the treatment of experimental autoimmune encephalomyelitis, delaying the onset of the disease and reducing the clinical symptoms. Histological examination of brain tissue from mice treated with saffron showed less leukocyte infiltration [56]. Saffron has also shown a beneficial effect in another degenerative disease such as Alzheimer's. In patients who received saffron, cognitive function was significantly improved [57].

CONCLUSION

In conclusion, GEMSP, with high levels of fatty acids as oleic acid, linoleic acid or azelaic acid and the antioxidants as alpha-tocopherol or ascorbic acid in its composition, displays potent anti-

inflammatory and anti-oxidant properties, providing beneficial effects on MS patients. A broad majority of them show an improvement or deceleration of the disease. There is an additional advantage related to its administration via the sublingual route and crossing BBB. These reasons together make GEMSP an emerging and promising drug for MS treatment.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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