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ALTERATIONS IN THE CHOLINESTERASE AND ADENOSINE DEAMINASE ACTIVITIES AND INFLAMMATION BIOMARKER LEVELS IN PATIENTS WITH MULTIPLE SCLEROSIS

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Abstract—Multiple sclerosis (MS) is one of the main chronic inflammatory diseases of the CNS that cause functional disability in young adults. It has unknown etiology characterized by the infiltration of lymphocytes and macrophages into the brain. The aim of this study was to evaluate the acetylcholinesterase (AChE) activity in lymphocytes and whole blood, as well as butyrylcholinesterase (BChE) and adenosine deaminase (ADA) activities in serum. We also checked the levels of nucleotides, nucleosides, biomarkers of inflammation such as cytokines (interleukin (IL)-1, IL-6, interferon (IFN)-γ, tumor necrosis factor-alpha (TNF-α) and IL-10) and C-reactive protein (CRP) in serum from 29 patients with the relapsing-remitting form of MS (RRMS) and 29 healthy subjects as the control group. Results showed that AChE in lymphocytes and whole blood as well as BChE, and ADA activities in serum were significantly increased in RRMS patients when compared to the control group ($P < 0.05$). In addition, we observed a decrease in ATP levels and a significant increase in the levels of ADP, AMP, adenosine and inosine in serum from RRMS patients in relation to the healthy subjects ($P < 0.05$). Results also demonstrated an increase in the IFN-γ, TNF-α, IL-1, IL-6 and CRP ($P < 0.05$) and a significant decrease in the IL-10 ($P < 0.0001$) in RRMS patients when compared to control. Our results suggest that alterations in the biomarkers of inflammation and hydrolysis of nucleotides and nucleosides may contribute to the understanding of the neurological dysfunction of RRMS patients.

Key words: multiple sclerosis, cholinesterases, lymphocytes, adenosine deaminase, inflammation, cytokines.

INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease characterized by neurodegeneration and neuroinflammation in the CNS (Darvesh et al., 2010). In the MS, it occurs as the aggregation of multiple plates of demyelination in the brain and spinal cord white matter (Minguetti, 2001). MS presents episodes of neurological dysfunction with variable remission (Costa et al., 2005) and it is considered one the most common causes of neurological disability in young adults (Reipert, 2004; Jordy et al., 2008) and the incidence is higher in women than in men (Koch-Henriksen and Sorensen, 2011). It can be present in different forms, such as primary progressive (PPMS), secondary progressive (SPMS), progressive relapsing (RPMS) and relapsing-remitting (RRMS), which is the most prevalent form (80% cases) (Compston and Coles, 2008; Das, 2012).

The most commonly used drugs for the treatment of relapsing forms of MS are interferon β (IFN-β) and glatiramer acetate; however, the mechanisms of action of these immunomodulatory drugs in MS are different (Milo and Panitch, 2011). The treatment with glatiramer acetate is considered a good option for RRMS patients; nevertheless, it is recommended that the treatment is modified if the frequency or severity of clinical relapses becomes worse (Jalilian et al., 2012).

The etiology of MS remains unclear; however studies have demonstrated that autoimmune T cells, targeting

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Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; ADA, adenosine deaminase; BChE, butyrylcholinesterase; BuSCh, butyrylthiocholine iodide; CRP, C-reactive protein; DTNB, 5,5′-dithio-bis-2-nitrobenzoic acid; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; HPLC, high-pressure liquid chromatography; IFNs, interferons; IL, interleukin; MS, multiple sclerosis; NTPDase, ectonucleoside triphosphate diphosphohydrolase; PPMS, primary progressive form of MS; RRMS, relapsing-remitting form of MS; SPMS, secondary progressive form of MS; TNF, tumor necrosis factor-alpha.

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myelin components play a role in the mediating inflammatory process, particularly in the early stages of RRMS (Lassmann, 2005). This dysfunction in the immune system keeps T lymphocytes activated crossing the blood–brain barrier and developing a harmful action against myelin and oligodendrocytes (Aktas et al., 2007).

Several mediators are able to modulate the actions of lymphocytes; among these we can highlight acetylcholine (ACh). This molecule synthesized and released from lymphocytes is considered an immunomodulatory agent (Kawashima and Fujii, 2003a,b). According to experiments in humans, it acts in various nonneuronal cells, such as in the immune system and blood cells (De Almeida and Saldanha, 2010). Recently it was demonstrated that it acts as an anti-inflammatory agent and thus induces a decrease in the production of pro-inflammatory cytokines by macrophages (De Oliveira et al., 2012).

The homeostatic control of ACh is performed by different enzymes, among them acetylcholinesterase (AChE, EC 3.1.1.7), which is responsible for the degradation of ACh into metabolites choline and acetate (Jaques et al., 2011a,b). In addition, butyrylcholinesterase (BChE, EC 3.1.1.8) is an enzyme capable of hydrolyzing a variety of esters including ACh (Darvesh et al., 2010). Several studies have shown that the activities of these enzymes are altered in inflammatory diseases (Bencherif et al., 2011; De Oliveira et al., 2012), such as Alzheimer's disease and diabetes mellitus (Das, 2007).

Apart from the involvement of ACh in pro- and anti-inflammatory events, ATP and adenosine also contribute to the fine-tuning of inflammatory and immune responses (Bours et al., 2006). ATP act as a pro-inflammatory agent, whereas, its breakdown product, adenosine, exhibits potent anti-inflammatory and immunosuppressive action (Linden, 2006; Zimmermann, 2008; Ferrero, 2011; Gombault et al., 2012).

The extracellular levels of ATP and adenosine are controlled by cell surface ectoenzymes such as ectonucleoside triphosphate diphosphohydrolase (NTPDase), 5′-nucleotidase and adenosine deaminase (ADA). NTPDase is involved in the breakdown of ATP and ADP to AMP which is hydrolyzed by ecto-5′-nucleotidase to adenosine (Gessi et al., 2007; Schetinger et al., 2007; Yegutkin, 2008). ADA catalyzes the irreversible deamination of adenosine to inosine (Franco et al., 1997). These enzymes in association constitute a highly organized cascade, which has a very important role in the control of the immune and inflammatory responses (Yegutkin, 2008). The ADA is considered a key enzyme in purine metabolism; it acts by inactivating the adenosine and its regulation happens through the cytokines on the cell surface during T cell activation (Cordero et al., 2001).

During MS a chronic inflammatory process occurs leading to the production of a variety of pro-inflammatory cytokines, such as interleukins (IL-1 and IL-6), tumor necrosis factor-alpha (TNF-α), and IFNs. Moreover, a reduction in anti-inflammatory cytokines such as IL-10 possibly occurs, and this cytokine imbalance concerning pro- and anti-inflammatory leads to an increased production of free radicals (Das, 2012). C-reactive protein (CRP) is an acute phase protein synthesized in the liver and it can be used as a potential marker of inflammatory activity and prognosis in MS (Soilu-Hänninen et al., 2005). Also, its production is induced by cytokines such as IL-6, IL-1 and TNF-α (Gabay and Kushner, 1999; Sellner et al., 2008).

Previous studies from our laboratory have demonstrated alterations in the AChE activity in brain from rats submitted to demyelization (Mazzanti et al., 2006, 2007, 2009) and in the ectonucleotidase activities in lymphocytes and platelets from patients with MS (Spanevello et al., 2010a,b) demonstrating the importance of purinergic and cholinergic signaling in this pathological condition. Due to the fact that cholinesterases as well as ADA control the levels of two important immunomodulatory molecules, ACh and adenosine, and considering that the mechanisms in relation to the pathogenesis of MS are poorly understood the aim of this study was evaluated the AChE, BChE and ADA activities in lymphocytes, whole blood and serum from patients with RRMS. In addition, the levels of adenine nucleotides and nucleosides, cytokines and CRP were also assessed in order to cooperate for a better understanding of the inflammatory and immune process in the RRMS.

EXPERIMENTAL PROCEDURES

Study population

The sample consisted of 29 patients with MS and 29 healthy subjects as a control group. The diagnosis of MS was based on the McDonald criteria (McDonald et al., 2001), and all patients had the relapsing-remitting form (RRMS). Most patients were treated with current immunosuppressive therapy. The general characteristics of the patients are shown in Table 1. All subjects gave written informed consent to participate in this study and the Human Ethics Committee of the Health Science Center from the Federal University of Santa Maria approved the protocol under number 23081.007854/2007-44. Twelve milliliters of blood was obtained from each patient and used for lymphocyte preparation and other biochemical determinations. The same procedure was carried out for the control group.

Isolation of mononuclear cells from human blood

Mononuclear leukocytes were isolated from human blood collected with EDTA and separated on Ficoll-Histopaque density gradients as described by Böyum (1968). Despite the fact that the methodology described above is employed for separating mononuclear cells, the study performed by Jaques et al. (2011a,b) demonstrated that there is a high incidence of lymphocytes (95%) in these samples and the amount of monocytes is practically insignificant. For this reason we treat the samples as containing only lymphocytes. Lymphocyte viability and integrity were confirmed by determining the percentage of cells, excluding 0.1% Trypan Blue and measuring