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Chronic iron overload induces gender-dependent changes in iron homeostasis, lipid peroxidation and clinical course of experimental autoimmune encephalomyelitis

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ABSTRACT

To analyze iron- and gender-dependent mechanisms possibly involved in pathogenesis of multiple sclerosis (MS) in this study we evaluated the effects of iron overload (IO) on iron status and lipid peroxidation processes (LPO) in tissues of female and male DA rats during chronic relapsing experimental autoimmune encephalomyelitis, a well-established MS animal model.

Rats were treated by iron sucrose (75 mg/kg bw/day) or with saline solution during two weeks before the sensitization with bovine brain homogenate in complete Freund's adjuvant. Clinical signs of EAE were monitored during 29 days. Serum and tissues of CNS and liver were sampled before immunization and at day 13th post immunization (during acute phase of EAE). The determination of ferritin, iron, malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) and evaluation of histopathology were performed by ELISA, ICP spectrometry and immunohistochemistry.

Results showed that IO in female EAE rats accelerated the onset of disease. In contrast, in male rats it accelerated the progression of disease and increased the mortality rate. During acute phase of EAE female IO rats sequestered more Fe in the liver, spinal cord and in the brain and produced more ferritin than male EAE rats. Male rats, however, reacted on IO by higher production of MDA or 4-HNE in the neural tissues and showed greater signs of plaque formation and gliosis in spinal cord.

The data point to sexual dimorphism in mechanisms that regulate peripheral and brain iron homeostasis and imply that men and women during MS might be differentially vulnerable to exogenous iron overload.

1. Introduction

Multiple sclerosis (MS) is an inflammatory and degenerative disease characterized by damage of the myelin sheath that surrounds axons and of oligodendrocytes that produce myelin (Lassmann et al., 2007; Wekerle, 2008). It is polygenetically determined and influenced by genes within or outside the human leukocyte antigen (HLA/MHC) complex, as well as by epigenetic factors, such as DNA methylation, histone modifications and microRNA-based gene regulation that might be influenced also by lifestyle/environmental factors, such as smoking, nutrition, sun exposure/vitamin D and Epstein Barr virus infection (Brooks et al., 2010; Küçükali et al., 2015; Simpson et al., 2015). Furthermore, similarly to many other autoimmune diseases, MS is more common among women than men (Airas, 2015; Bove and Chitnis, 2014; Nicot, 2009).

Complex pathomechanisms leading to demyelination and neurodegeneration in MS are still unclear, but recent evidence emphasizes that one of the initial causes for neuronal death may be the deposition of iron in some regions of the brain (Ke and Qian, 2007; LeVine and Chakrabarty, 2004; Rouault and Cooperman, 2006; Stephenson et al., 2014; van Rensburg et al., 2012; Weigel et al., 2013). Its harmful effects are usually linked with the activation of Fe-dependent conversion of superoxide anion and hydrogen peroxide into the extremely reactive hydroxyl radical by Fenton and Haber–Weiss reaction (Halliwell and Gutteridge, 1984;

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Koskenkorva-Frank et al., 2013), since this may induce cell death owing to glutathione consumption, protein aggregation, lipid peroxidation, nucleic acid modification and mitochondrial dysfunction (Nunez et al., 2012; Urrutia et al., 2014). Moreover, the data implicate that iron deposits in active MS lesions contribute to the propagation of demyelination and neurodegeneration, since iron released from dying oligodendrocytes may induce an amplification of inflammatory oxidative burst within the central nervous system (CNS) after its liberation and subsequent accumulation within other microglia, astrocytes, or axons (Hametner et al., 2013). Iron-dependent events are, however, often regional, cellular and age sensitive (Connor et al., 2001; Thompson et al., 2001), and the excessive iron accumulation in the brain might not be a cause, but a consequence of the disease pathogenesis (Ke and Qian, 2007). Furthermore, it is important to realize that neurodegenerative disorders and some forms of MS might be induced also by iron deficiency, since the brain, like other organs, requires iron for mitochondrial respiration, oxidative phosphorylation and production of ATP, oxygen transportation, RNA and DNA repair, cell proliferation, cellular defenses, etc., as well as for myelin production and the synthesis and metabolism of neurotransmitters (van Rensburg et al., 2012). Particularly sensitive to iron deficiency are oligodendroglial precursor cells, which need iron for the maturation and oligodendrocytes, which have an extremely high energy requirements for producing and maintaining the myelin sheath (Schonberg et al., 2013). Owing to this, the therapeutic use of iron supplementation or iron-binding chelators in patients with MS is still under investigations (Weigel et al., 2013) and an individualized therapy based on genetic tests (Wekerle and Hohlfeld, 2010) and on biochemical determinations of iron and other biomarkers, such as vitamin B12 and vitamin D (van Rensburg et al., 2012) has been recommended.

Within this context and owing to high influence of gender on MS susceptibility and pathogenesis (Airas, 2015; Bove and Chitnis, 2014; Nicot, 2009), as well as on several aspects of iron metabolism (Gemmati et al., 2012; Qian and Shen, 2001; Rouault and Cooperman, 2006), in this study we tested the reactivity of female and male DA rats on iron overload prior to the immunization with encephalitogen and during the chronic-relapsing autoimmune encephalomyelitis (CR-EAE), an animal model that closely resembles the MS (Mix et al., 2010; Wekerle, 2008).

2. Materials and methods

2.1. Experimental animals

Experiments were performed on 54 male and 60 female Dark Agouti (DA) rats 7–8 weeks old. The animals were housed under standard conditions of light, temperature and humidity with unlimited access to food and water. Experimental procedures involving animals complied with Croatian laws and rules (NN 135/06; NN 37/13; NN 125/13; NN 055/2013) and with the guidelines set by European Community Council Directive (86/609/EEC). Experimental protocol was approved by the Ethical Committee of the University of Rijeka.

2.2. Iron treatment

Both male and female DA rats were randomly divided in two groups: iron treated group and control group (Table 1). Using a protocol described by Vu'o'ng Le et al. (Vu'o'ng Le et al., 2011) the experimental groups were intraperitoneally (i.p.) injected with iron – sucrose (Venofer, Vifor Pharma, France) at the dose of 75 mg/kg body weight/day for two consecutive weeks (6 times a week). Control groups were treated on the same way with physiological saline solution.

2.3. EAE induction and evaluation

Induction of CR-EAE was performed in female and male DA rats by bovine brain white matter homogenate emulsion (BBH) in the complete Freund's adjuvant (CFA) (Sigma, St. Louis, Mo., USA), as previously described (Jakovac et al., 2011). To each animal 2×0.1 mL emulsion was injected subcutaneously in each hind footpad. The evaluation of the clinical course was assessed daily using the following criteria: 0 – no symptoms; 1 – flaccid paralysis of tail; 2 – hind legs paresis; 3 – hind legs paralysis with incontinence and 4 – death of the animal.

Depending on the experimental design the animals were sacrificed by exsanguination before or after the induction of EAE (Table 1). The exsanguination was done in deep anesthesia, induced by combination of Ketamine (80 mg/kg) and Xylazine (5 mg/kg), given by intraperitoneal (i. p.) injection, according to the guidance of European Community Council Directive (86/609/EEC) and recommendation of National Centre for the Replacement, Refinement and Reduction of Animals in Research (www.nc3rs.org.uk).

2.4. Determination of serum ferritin and hepatic aminotransferases

Serum ferritin (SF), and aspartate and alanine aminotransferase (AST and ALT) activities were determined in blood collected by intracardial puncture. Blood was left clothing for two hours at room temperature, centrifuged on 2200 rpm for 15 min. Serum was removed, aliquoted and stored at −80 °C. For determination of SF (n = 6 for each group of animals) we used Ferritin (FTL) Rat ELISA

Table 1
Experimental design.

Groups	Gender	No of rats	Treatment	Immunization with BBH + CFA	Sacrification
A	Female rats	10	Fe-sucrose 75 mg/kg body weight/day or saline solution for 2 weeks	No	After 2 weeks of treatment
	Male rats	10	Fe-sucrose 75 mg/kg body weight/day or saline solution for 2 weeks	No	After 2 weeks of treatment
B	Female rats	37	Fe-sucrose 75 mg/kg body weight/day or saline solution for 2 weeks	Yes	During the first attack of EAE
	Male rats	30	Fe-sucrose 75 mg/kg body weight/day or saline solution for 2 weeks	Yes	During the first attack of EAE
C	Female rats	13	Fe-sucrose 75 mg/kg body weight/day or saline solution for 2 weeks	Yes	On the 29th post-immunization day
	Male rats	14	Fe-sucrose 75 mg/kg body weight/day or saline solution for 2 weeks	Yes	On the 29th post-immunization day