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Lipid peroxidation in the pathogenesis of neuroborreliosis

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ABSTRACT

This study analyzed the onset of lipid peroxidation (LPO) in neuroborreliosis and the effects of ceftriaxone therapy on LPO. Twenty-two patients with early neuroborreliosis and 22 healthy subjects were studied. LPO in the cerebrospinal fluid (CSF), as well as the plasma and urine was estimated by the levels of reactive aldehydes: 4-hydroxynonenal (4-HNE), 4-hydroxyhexenal, malondialdehyde, and 4-oxo-nonenal, F_2-isoprostanes and A_4/J_4-neuroprostanes (NPs). The plasma level of 4-HNE-protein adducts arachidonic acid (AA), docosahexaenoic acid (DHA) and vitamin E was determined. Additionally, enzymatic activities of phospholipase A_2 (PLA_2), platelet-activating factor acetylhydrolase (PAF-AH) and glutathione peroxidase (GSH-Px) were determined. A decrease of AA, DHA levels and GSH-Px activity in plasma was associated with a significant increase of aldehydes in the CSF, plasma and urine. Similarly, the increase of F_2-isoprostanes and NPs in the CSF and plasma was associated with the decreased activity of PLA_2 and PAF-AH. Ceftriaxone therapy cured patients and reduced the levels of F_2-isoprostanes, NPs and reactive aldehydes. However, the activities of PLA_2 and PAF-AH increased. Pathophysiological association of neuroborreliosis with systemic LPO was revealed. Effective antibiotic therapy attenuated LPO. Biomarkers of LPO could be useful to monitor the onset of neuroborreliosis and show the effectiveness of pharmacotherapy.

1. Introduction

Ticks (*Ixodidae*) are arachnids responsible for the transmission of many species of bacteria, protozoa, viruses and rickettsias pathogenic to humans and animals. Among the microorganisms transmitted by ticks, spirochete *Borrelia burgdorferi* is the main cause of human infection, especially in central Europe. It is a causative agent of Lyme disease, which is the most common tick-borne disease in the northern hemisphere. In the last several years, a significant increase in patients suffering from Lyme disease has been observed. Every year there are 85,000 new cases in Europe and 15–20,000 in North America. The highest morbidity rate is observed in Estonia, Lithuania, Sweden, Austria, Czech Republic, Slovenia and Poland. In Poland in particular, Lyme disease is considered a very serious medical problem.

In the pathogenesis of Lyme disease, inflammatory and immunological mechanisms play a dominant role. *B. burgdorferi* causes damage to various organs and systems, most frequently of the skin, nervous system, heart, joints and eyes. The most dangerous of the Lyme disease manifestation is neuroborreliosis, which is associated with *B. burgdorferi* invasion in the central nervous system (CNS). Biological markers causing nerve cell

Abbreviations: 19:0 PC, 1,2-dinonadecanoyl-*sn*-glycero-3-phosphocholine; 4-HHE, 4-hydroxyhexenal; 4–HNE, 4-hydroxynonenal; 4-ONE, 4-oxo 2-nonenal; NPs, A_4/J_4-neuroprostanes; 8-isoPGF$_{2\alpha}$, 8-isoprostanes; AA, arachidonic acid; BHT, butylhydroxytoluene; BSA, bovine serum albumin; CNS, central nervous system; CSF, cerebrospinal fluid; DHA, docosahexaenoic acid; PLA_2, phospholipase A_2; ELISA, enzyme-linked immunosorbent assay; EM, erythema migrans; ESR, erythrocyte sedimentation rate; FAMES, fatty acid methyl esters; FID, flame ionization detector; GSH, glutathione; GSH-Px, glutathione peroxidase; HPLC, high-performance liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; LPO, lipid peroxidation; LCMS, liquid chromatography with mass spectrometry; MDA, malondialdehyde; MRM, multiple reaction monitoring; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; Nrf2, nuclear factor (erythroid-derived 2)-like 2; *O*-PFB-oxime, O-pentafluorobenzyl-oxime; *O*-PFB-oxime-TMS, O-pentafluorobenzyl-oxime-trimethylsilane; PAF-AH, platelet-activating factor acetylhydrolase; PBS, phosphate buffered saline; PPARs, peroxisome proliferator-activated receptors; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; SIM, selected ion-monitoring; STAT3, *signal transducer and activator of transcription 3*; TBE, tick-borne encephalitis; TLC, thin layer chromatography; UPLC, ultra-performance liquid chromatography

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damage of neuroborreliosis are chinolin acid and L-kynurenine [1–3]. Increased concentrations of these molecules in the CSF of patients with neuroborreliosis and encephalopathy were observed [4]. In primary peripheral blood macrophages, an elevated activity of all enzymes in the kynurenine pathway has been reported. Brain tissue infiltration during the inflammatory process leads to a local increase in the concentration of indoleamine 2,3-dioxygenase, 3-kynurenine hydroxylase, kynureninase and 3-hydroxyantranilic oxidase. An increase in the concentration of chinolin acid in the CNS has been observed [5]. Intensive reactive oxygen species (ROS) generation and, as a consequence, oxidative modifications of cell components are promoted. In addition to protein and DNA modifications, damage to membrane phospholipids also affects the nervous system. As nerve cells have the largest cell body and membrane surface area, they are especially vulnerable to oxidation. The cerebellum requires a relatively large amount of oxygen, which stimulates the production of ROS. Nerve cells are susceptible to ROS action because of the high content of phospholipid polyunsaturated fatty acids, primarily AA and DHA. Brain metabolism is characterized by mechanisms promoting ROS generation, such as the oxidation of neurotransmitters [6]. Like other macrophages, some glial cells can either directly or through cytokine activation produce superoxide anion, nitric oxide and hydrogen peroxide [7]. Moreover, some brain regions have a high concentration of non-heme iron ions that catalyze reactive hydroxyl radical formation from hydrogen peroxide [8]. However, brain antioxidant defenses provide only modest protection [9]. In particular, levels of catalase and glutathione peroxidase, which are enzymes participating in phospholipid protection, are low in most brain regions and consequently in the CSF [10,11]. ROS may participate in non-specific lipid peroxidation through chain reactions of lipid radicals. These react with oxygen to form products that are diverse and depend on the substrate oxidized.

Not fully clarified pathogenesis of neuroborreliosis and a lack of proper diagnosis make that search for indicators, which could help predict the course and consequences of neuroborreliosis are necessary. Discovery of neuroborreliosis biomarkers may aid diagnosis and facilitate an understanding of disease progression.

The purpose of this study was to determine if the development of neuroborreliosis affects neuronal phospholipids peroxidation. The level of polyunsaturated fatty acids (PUFAs) cyclization and fragmentation products in CSF, plasma and urine were checked. The changes in phospholipid unsaturated fatty acid levels and antioxidants protecting PUFAs against peroxidation - glutathione peroxidase activity and vitamin E concentration were determined. Additionally, the efficacy of antibiotic therapy on lipid peroxidation was estimated.

2. Materials and methods

Plasma, urine and CSF samples were collected from a group of 22 patients (8 female and 14 male) with a mean age of 50 ± 17 years old (21–83) treated in the Department of Infectious Diseases and Neuroinfections, Medical University of Bialystok, Poland. Ten (4 female and 6 male) of the 22 examined patients showed a higher cytosis and presented an inflammatory process in the CNS at admission. These patients took part in follow-up examinations to determine the effects of antibiotic treatment on the intensity of lipid peroxidation.

All patients were inhabitants of north-eastern Poland, where Lyme disease is endemic. Diagnosis of neuroborreliosis was confirmed by epidemiological anamnesis, clinical manifestation and serological examinations. The results showed detection of anti/ *B. burgdorferi* IgM and IgG antibodies in enzymes by an ELISA (enzyme-linked immunosorbent assay) (*Borrelia* recombinant IgG and IgM High Sensitivity, Biomedica, Austria). In all cases, ELISA results were confirmed by Western blot. Additionally, immunoblot tests in IgM and IgG were conducted to estimate intrathecal synthesis of antibodies (Virotech, Germany). We diagnosed definitive neuroborreliosis by clinical picture, lymphocytary pleocytosis in CSF and intrathecal immunoglobulin synthesis. In addition, probable neuroborreliosis was diagnosed via clinical history and at least one of the following: lymphocytary pleocytosis in the CSF, erythema migrans (EM) > 5 cm in diameter and prompt clinical response to antibiotic treatment. Anamnesis, laboratory tests, and serological tests for *B. burgdorferi s.l.* and CSF examinations were performed.

Thirteen (59%) patients were inhabitants of cities, and 9 patients (41%) were country inhabitants. In all patients, based on serological tests from serum and cerebrospinal fluid, tick-borne encephalitis (TBE) was excluded. Epidemiological anamnesis revealed previous tick bites in 11 (50%) patients. Only 4 (18%) patients had EM. Twelve (54%) patients complained about headaches, and 2 (9%) complained about vertigo. Joint pain was observed in 4 (18%) patients, and fever in 5 (22%). The mean duration of fever was 1.8 ± 2.8 days. With physical examination, 12 (54%) patients showed positive meningeal signs. The mean duration of hospitalization was 23 ± 6 days.

All patients were classified as early neuroborreliosis (stage II), defined as signs and symptoms lasting fewer than 6 months.

In the group of 22 patients with neuroborreliosis, 16 (72%) had facial nerve palsy, and 1 (45%) had bilateral palsy. All patients with facial nerve palsy were unable to raise an eyebrow, complained about weakness of facial muscles, and had incomplete eye closure as well as mouth asymmetry.

All patients (100%) with neuroborreliosis had lymphocytary meningitis. The mean cytosis of CSF was 114 ± 95 cells/mm^3 (max-289 cells/mm^3; min-11 cells/mm^3) with the mean lymphocyte percentage of 86 ± 11.6%. The mean protein concentration was 92 ± 48 mg/dl. Bannwarth's syndrome was diagnosed in 7 (31%) patients.

All of the patients had anti-*B. burgdorferi* serum antibodies, with a mean titer of IgM-24 ± 19 BBU/ml and for IgG-55 ± 23 BBU/ml. All results were confirmed by Western blot. Ten patients (45%) had intrathecal synthesis of IgM antibodies, and 12 (54%) had intrathecal synthesis of IgG antibodies in the cerebrospinal fluid. Both were positive in 4 patients (10%).

Significant increases in inflammatory parameters were observed. Mean erythrocyte sedimentation rate (ESR) was 24 ± 19 mm per hour, CRP- 5 ± 6 mg/dl, and WBC-8300 ± 3000/ml (Table 1).

Nine out of 10 patients participating in follow-up examinations were treated with intravenous ceftriaxone (Ceftriaxon MIP; MIP Pharma Poland; 2 g/day) for 21 ± 7 days leading to complete recovery. In one case, oral doxycycline (Doxycyclinum TZF; Polfa Tarchomin Poland; 2×100 mg/day) was used due to a penicillin allergy, also with positive effect. One patient experienced severe sequelae or persistence of symptoms (tetraparesis). Significantly decreased cytosis and protein content were observed in neuroborreliosis patients after antibiotic treatment (Table 2). However, at the follow-up examinations no cases of CSF parameters were normalized.

While recording disease history, particular attention was given to the current use of certain medications (anticoagulants and antiplatelet drugs) and comorbidities (liver, kidney or cardiovascular diseases, cancer, respiratory disorders, and diabetes).

The plasma and urine control group consisted of 22 healthy subjects (8 female and 14 male) with an average age of 49 years (21–76). The exclusion criteria were as follows: pregnancy; lack of written consent; and recent treatment with certain medications including nonsteroidal anti-inflammatory drugs, steroids and oral contraceptives. Control cerebrospinal fluid was received from 19