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Serum and intraerythrocyte antioxidant enzymes and lipid peroxides in children with migraine

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Abstract:
The oxidant-antioxidant balance disorders underlie a number of acute and chronic diseases of the central nervous system (CNS). It is believed that oxidative stress plays a role in the pathogenesis of migraine.
The study objective was to assess the processes of lipid peroxidation with malondialdehyde (MDA) as its major indicator and to determine the activities of antioxidant enzymes: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-R) in the serum and erythrocytes of patients at developmental age with migraine with and without aura.
The study group consisted of 34 patients at the age of 10–18 years (mean ± standard deviation: 14.04 ± 2.29 years), suffering from migraine. The control group included 38 patients, aged 4–17 years (mean age 12.11 ± 3.46).
MDA concentration and activities of SOD, GSH-Px and GSSG-R were determined in serum and erythrocytes of all the patients. In the migraine group, the MDA levels in serum and erythrocytes were statistically significantly lower than in control subjects ($p < 0.001$). In the migraine group, serum GSH-Px activity was significantly higher ($p < 0.05$). The GSSG-R activity in the erythrocytes of migraine children was significantly higher compared to controls ($p < 0.001$). SOD activity was decreased and GSH-Px was increased (non-significantly) in erythrocytes of migraineurs.
Our results confirm the disturbances of lipid peroxidation processes in migraine and suggest the activation of antioxidant mechanisms. Its important indicator seems to be the increase in the GSSG-R activity in the erythrocytes and the GSH-Px activity in serum between migraine attacks. Further studies are necessary.

Key words:
migraine, headaches, antioxidant enzymes, lipid peroxides

Introduction

The oxidant-antioxidant balance is an important component of homeostasis in the organism. In physiological conditions, very reactive products are constantly being formed in each aerobic organism. These products are referred to as reactive oxygen species, reactive nitrogen species or oxygen free radicals. At the same time, mechanisms of antioxidant defense are being triggered [l7, 33].

In recent years, the involvement of free radical reactions both in physiological and pathological processes has aroused keen interest. It is difficult to think of a disease in the etiology of which free radicals would not be involved. However, it is still unclear and controversial whether oxidative stress is a cause or a consequence of illness [12].

The oxidant-antioxidant balance disorders underlie a number of acute and chronic diseases of the central nervous system (CNS). A high content of polyunsatu-

rated fatty acids, particularly of arachidonic acid in the cellular membranes, increases the likelihood of peroxidation. Malondialdehyde (MDA) is one of the most frequently used indicators of lipid peroxidation (and a very toxic compound).

It is believed that oxidative stress plays a certain role in the pathogenesis of epilepsy. A number of studies have been conducted on the processes of lipid peroxidation and the activities of antioxidant enzymes in epileptic children [1, 26, 27]. Studies in our laboratory show increased MDA levels in erythrocytes and serum of epileptic patients of developmental age, which may indicate intensification of membrane lipid peroxidation [27]. Epileptic children show disturbances in antioxidant balance in the serum and erythrocytes that is manifested as a decreased activity of superoxide dismutase (SOD), increased activity of glutathione peroxidase (GSH-Px) and unchanged glutathione reductase (GSSG-R) [27]. These results may indicate activation of the antioxidant mechanisms in response to increased release of free radicals and excessive use of enzymes in defense against free radicals.

Although migraine is a long known pathology accompanying mankind from the dawn of history, its etiology and pathomechanism remain unclear. It is now believed that migraine is associated with inborn predisposition to hypersensitive neurovascular reactions that may be induced by specific factors or may result from cyclic changes in the CNS [11]. Every individual has an inborn migraine threshold with the degree of sensitivity depending on the balance between stimulation and inhibition at different levels of the nervous system. This balance depends, among other things, on the efficiency of oxidative phosphorylation in mitochondria, on the function of ion channels and on the levels of magnesium, stimulatory amino acids, monoamines and opiates [14]. In this chain of factors, oxygen free radicals and antioxidant systems may also play a major role, especially since migraine and epilepsy, both being seizure-like disorders, have much in common.

The study objective was to assess the processes of lipid peroxidation with MDA as its major indicator, and to determine the activities of antioxidant enzymes: SOD, GSH-Px and GSSG-R in serum and erythrocytes of patients at developmental age with migraine with and without aura. Therefore, in the present study we investigated whether the concentration of MDA, a marker of lipid peroxidation, changes in serum and erythrocytes in migraine and whether the activities of antioxidant enzymes (SOD, GSH-Px, GSSG-R) undergo any changes in this disease.

Elucidation of the mechanisms responsible for migraine seizures and acquiring of better knowledge of the role of oxygen free radicals and antioxidants in the pathomechanism of migraine may create new possibilities for more effective treatment of this disease.

Materials and Methods

Patients

The study group consisted of 34 patients, suffering from migraine with and without aura, hospitalized in the Department of Pediatric Neurology and Rehabilitation, Medical University of Białystok. The patients were at the age of 10–18 years (mean ± standard deviation: 14.04 ± 2.29 years). The group comprised of 13 girls and 21 boys. Migraine was diagnosed based on the criteria of the International Headache Classification [13]. In our migraine group, seven patients only had been taking any drugs before blood sampling: 2 – flunarizine, 1 – piracetam, 1 – oxcarbamazepine, 1 – paracetamol, 1 – metamizol.

The reference group (control) included 38 patients of the Department of Pediatric Neurology and Rehabilitation (22 boys and 16 girls), aged 4–17 years (mean age 12.11 ± 3.46), non-epileptic, with a negative history of migraine, vascular diseases, brain tumors or progressive degenerative processes. Children hospitalized in the Department due to lumbar-sacral aches and functional disorders were included in this group.

Patients in both groups underwent detailed pediatric and neurological examinations in order to find focal CNS damage. All the children had basic laboratory tests: morphology of venous blood, platelet count, transaminases, bilirubin, ammonium, glucose, electrolytes (Na, K, Ca, P, Cl, Mg), urea, creatinine, uric acid, proteinogram, cholesterol, urinalysis. Patients with significantly abnormal laboratory findings indicating liver dysfunction and lipid metabolism disorders were excluded from the study.

All the aura-free migraine patients underwent neuroimaging diagnostic procedures to exclude organic changes in CNS: magnetic resonance imaging (MRI) or computed tomography (CT) of the head, while those with aura had magnetic resonance angiography (MRA) of the cerebral vessels done.

Methods

MDA concentration and activities of SOD, GSH-Px and GSSG-R were determined in serum and erythrocytes of all the patients. Biological material for analysis was obtained simultaneously with collecting blood samples for routine laboratory diagnostic tests. In migraine patients, blood was taken between headaches, i.e. at least 24 h after the last headache attack.

Determinations of MDA level and activities of SOD, GSH-Px and GSSG-R were performed in the Research Laboratory of the Department of Pediatric Neurology, Medical University of Białystok, according to methods described in our previous papers [1, 2, 27]. MDA level was determined using the method of Buege and Aust [5]. SOD activity was assessed by the method of Sykes et al. [30], glutathione peroxidase by the method of Paglia and Valentine [21] and glutathione reductase according to Mize and Langdon [18]. In short, all enzymatic activities and MDA concentration were measured using spectrophotometry. The study protocols were approved by the local ethics committee.

Statistical analysis

For statistical analysis, computer program STATISTICA 6.0 for Windows was employed. Liliefors' normality test was used to examine the type of data distribution. As most of the results obtained in the study showed deviation from normal distribution, the nonparametric Wilcoxon's paired test, was used. Differences were considered statistically significant when $p < 0.05$.

Results

In all migraine and control patients, the routine blood analyses and neuroimaging findings (CT, MR, Angio-MR) were normal. Serum activities of SOD, MDA, GSH-Px and GSSG-R in the migraine children and in the control group are presented in Table l. In migraine, serum GSH-Px activity was significantly higher ($p < 0.05$) and MDA concentration was significantly lower as compared to the control ($p < 0.001$). No statistically significant difference between the activities of SOD and GSSG-R in serum of migraineurs was observed.

The activities of SOD, GSH-Px and GSSG-R and the level of MDA in the erythrocytes of migraine children and in the control group are shown in Table 2. In the migraine group, the MDA level in erythrocytes was also statistically significantly lower than in the control subjects ($p < 0.001$). The GSSG-R activity in the erythrocytes of migraine children was significantly higher as compared to the control ($p < 0.001$). SOD activity was decreased and GSH-Px was increased (not significantly) in erythrocytes of migraineurs.

Tab. 1. Mean values ± standard deviation of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-R) in serum of migraine and control children

	Migraine children (N = 34)	Control group (N = 38)
SOD activity (J/ml)	3.60 ± 0.82	3.66 ± 0.75
GSH-Px activity (nmol/ml)	305.07 ± 58.05*	255.31 ± 74.61
GSSG-R activity (nmol/ml)	27.16 ± 6.90	26.24 ± 8.54
MDA concentration (nmol/ml)	2.43 ± 0.31***	3.55 ± 0.83

* $p < 0.05$ *vs.* control values; *** $p < 0.001$ *vs.* control values

Tab. 2. Mean values ± standard deviation of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-R) in the erythrocytes of migraine and control children

	Migraine children (N = 34)	Control group (N = 38)
SOD activity (J/ml)	1.47 ± 0.78	1.79 ± 1.27
GSH-Px activity (nmol/ml)	319.65 ± 37.24	293.14 ± 48.28
GSSG-R activity (nmol/ml)	35.44 ± 11.17***	19.41 ± 8.09
MDA concentration (nmol/ml)	2.20 ± 0.37***	3.46 ± 0.89

*** $p < 0.001$ *vs.* control values

Discussion

Headache is a major symptom of migraine. However, the feeling of pain is a complex phenomenon, and its pathogenesis and association with aura are still controversial issues. There are a number of hypotheses

and many attempts have been made to combine them all into one theory that would assume the involvement of brain vessels and trigeminal nerve [11, 19]. The vascular theory of migraine seizure pathogenesis is the most widely accepted. Implication of other possible factors, genetic or chemical, does not exclude this theory, on the contrary, may complement it. Stimulation of the trigeminal nerve occurs both *via* the neuronal and *via* the chemical pathway, through serotonin, histamine and prostaglandins. Migraine attack-inducing factors can act directly on these chemical mediators or *via* the nervous system mediators. One of the hypotheses of the origin of headache in migraine is that of neurogenic inflammation of dura mater presented by Moskowitz et al. [19]. According to that model, central stimulation in the trigeminal nerve endings causes an antidromic release of substance P, calcitonin gene-related peptide (CGRP) and neurokin A, which increase permeability of vascular walls, dilate them with a likely involvement of nitric oxide (NO) and enhance the action of blood-derived factors, such as histamine and serotonin. This leads to inflammatory reactions and blood vessel edema, i.e. aseptic inflammation of arteries [22]. Moreover, inflammatory mediators increase sensitivity of nociceptors. However, as confirmed by investigations conducted with antagonists of substance P, endothelins and with agonists of 5-HT_{1D} receptors, which do not stop migraine seizures, the neurogenic inflammation itself does not explain the migraine-type headaches. Besides, neither magnetic resonance with the use of gadolinium nor retinal angiography during migraine seizures show extravasations due to extravascular permeation [16]. Thus, other factors should be considered in the pathogenesis of migraine headache.

Recently, it has been suggested that oxidative stress caused by free radicals may play a role in migraine pathogenesis [6]. The activity of free radicals in migraine can be associated with NO, which markedly dilates cerebral vessels, is a nociceptive neurotransmitter and as one of the major factors regulating cerebral flow plays a key role in the trigeminovascular mechanism of migraine [28]. Markedly elevated levels of NO and its metabolites in blood and platelets between seizures [9, 29], which increase significantly during migraine attack [24], seem to confirm the involvement of this molecule in the pathogenic chain. Peroxides, which open calcium-dependent potassium canals by distending brain arteries, are another factor of brain flow autoregulation cooperating with NO [34]. Increased blood flow through cerebral cortex leads to stimulation of the periaqueductal grey matter [35]. In such situation, brain structures may be potentially exposed to damage by free radicals due to repeated episodes of hyperoxia during migraine seizures. Migraine sufferers can be more vulnerable to toxic effects of free radicals. The mechanism of one of the drugs used in the prevention of migraine, flunarizine, is based on its free radical scavenging action which reduces oxidative stress [7].

Therefore, some attempts have been made to use free radical scavengers to prevent migraine. The application of a complex of antioxidant vitamins, e.g. vitamin C and E, has brought promising results [6].

The hypothesis of oxidative stress in migraine has been put forward for a few years [8, 23, 32]. However, due to very high reactivity of free radicals, it is difficult to obtain a direct evidence to support their involvement in this pathology. Studies performed to assess lipid peroxidation and activity of antioxidant enzymes in migraine patients are very few and seem to provide divergent results [8, 23, 25, 32]. Some of them have reported increased serum levels of thiobarbituric acid reactive substances [32], changes in platelet SOD in migraine with aura [23] or higher levels of NO metabolites in migraine [8]. In a group of 28 migraine sufferers, the activities of SOD and GSH-Px in erythrocytes were significantly higher as compared to the control group and patients with tension-type headaches [4]. However, researchers from India, in a group of 55 migrainous patients found no significant differences in the activities of catalase, SOD, GSH-Px in neutrophils or platelet SOD as compared to the control group [25]. The activities of the antioxidant enzymes did not correlate with age, disease duration or the length of time since the last attack.

In our study, MDA concentration was significantly lower in plasma and erythrocytes. Moreover, activities of antioxidant enzymes: GSH-Px and GSSG-R in erythrocytes and GSH-Px in plasma were elevated significantly in migraine. GSH-Px is the most important antioxidant enzyme that protects cells in the CNS. It could suggest the activation of antioxidant defense, which reduces the processes of lipid peroxidation, represented by MDA. This elevated activity of antioxidant defense system is a bit unclear and difficult to explain in comparison with other findings of earlier studies [4, 23, 32]. They found the increase in free radical concentration and oxidative stress in migraineurs. However, the activities of enzymes varied

in different compartments. Activity of SOD was lower in platelets [23] and erythrocytes [4], but there were no significant changes of SOD and GSH-Px concentration in neutrophils [25]. These results could suggest that the increase in free radicals in platelets should activate the antioxidant defense reactions, for example – in erythrocytes. However, the lack of significant changes in platelet SOD activity, reported in other study [25], shows dynamic character of the oxidant-antioxidant balance in migraine. Moreover, this balance could depend on time after migraine attack, connected with an increase in cerebral blood flow and brain stimulation. In our study, the processes of lipid peroxidation were examined minimum after 48 h. We did not find in literature complex studies, which would analyze the dynamic changes in oxidant-antioxidant balance during migraine attack and after it.

Furthermore, the drugs used in migraine have been postulated to be an important factor affecting the oxidant-antioxidants balance. The major classes of the medications for migraine prevention are beta blockers, calcium channel blockers, tricyclic antidepressants, anticonvulsants and nonsteroidal anti-inflammatory drugs (NSAIDs). There are multiple mechanisms of action upon which the preventive agents act. Free radical scavengers, like flunarizine, may provide a potential molecular basis for prophylactic antimigraine therapy by neutralizing nitric oxide overproduction and possibly preventing formation of highly toxic peroxide [7]. There have been conflicting reports as to whether NSAIDs such as acetylsalicylic acid are effective in protecting neurons against neurotoxicity. Acetaminophen has been shown to rescue neuronal cells from mitochondrial redox impairment, lipoperoxidative products and MDA generation [3]. Furthermore, acetaminophen also reduced the cytoplasmic accumulation of peroxides. Acetylsalicylic acid and acetaminophen inhibit lipid peroxidation and cell damage, *in vivo*, in the rat hippocampus [15]. Few anticonvulsants, such as valproate, gabapentin, topiramate, have been approved for migraine prevention [36]. Piracetam therapy also may be beneficial in the treatment of migraine [31]. Research findings concerning the effect of antiepileptic drugs on lipid peroxidation processes in erythrocytes and serum are discordant. In our previous studies, MDA concentration was elevated, GSH-Px and GSSG-R activities were increased, and SOD activity was decreased in erythrocytes of patients receiving antiepileptic polytherapy [27]. However, we observed an increase in MDA concentration and a decrease in GSSG-R and SOD activities in serum of children with chronic antiepileptic treatment [1]. These results suggest that antimigraine treatment may also modify the oxidant-antioxidant balance. We did not find any significant effect of the used drugs on the enzymatic activities.

Research findings concerning the oxidant-antioxidants balance in migraine are conflicting for many other reasons. Important factors could be age of patients, duration of treatment and doses of drugs. Furthermore, some studies assessed the activity of antioxidant enzymes in serum, other in erythrocytes, platelets and neutrophils. There are very few data analyzing and comparing lipid peroxidation processes in more compartments. Furthermore, a relationship between food consumption and migraine has been widely suggested [6]. Free radical scavengers may provide a potential molecular basis for prophylactic antimigraine therapy. Vitamins C and E, flavonoids, antioxidant enzyme cofactors, like zinc and selenium are well established natural dietary antioxidants with widely accepted health benefits. Some data suggest that antioxidant therapy may be beneficial also in the treatment of migraine possibly reducing headache frequency and severity [6]. The nutritional traditions are different in different populations, so natural antioxidant consumption could be changed. The methods of assessment of lipid peroxidation are conflicting.

Nilsen et al. [20] analyzed the effect of gender and environmental factors on MDA concentration in adults. They found a higher MDA concentration in plasma of women compared with men. Different results were shown by Diaz et al. [10], who did not find any differences in plasma activity of MDA, GSH-Px, NO and total antioxidant status between men and women.

The alternative interpretation is also possible. The results may suggest that changes in antioxidant enzymes are the cause rather than the consequence of altered MDA. Reduced SOD and increased GSH-Px would be expected to result in the accumulation of O_2^- and deficiency of H_2O_2. H_2O_2 may be a major inducer of lipid peroxidation, since it is converted to highly reactive hydroxyl radical. The decrease in H_2O_2 could reduce MDA formation. In addition, H_2O_2 at physiological concentration induces vasodilation, whereas O_2^- induces vasocostriction by scavening NO.

Our results confirm the disturbances of lipid peroxidation processes in migraine. They suggest the activation of antioxidant mechanisms. The increase in the GSSG-R activity in the erythrocytes and the GSH-Px activity in serum between migraine attacks seems to be its important indicator. Further studies are needed for a better understanding of these metabolic reactions.

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