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ANTIOXIDATIVE ENZYME ACTIVITIES AND LIPID PEROXIDATION IN CHILDREN WITH INFLAMMATORY ENDOTHELIAL INJURY

AKTIVNOST ANTIOKSIDATIVNIH ENZIMA I PEROKSIDACIJA LIPIDA KOD DECE SA INFLAMATORNIM OŠTEĆENJEM ENDOTELA

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Summary: During the inflammatory process endothelial cells are activated and a proadherent ability is assumed. The synthesis of reactive oxygen metabolites, which follows the immunological processes, can cause oxidative damage to endothelial cells leading to the clinical expression of disease including a variety of skin manifestations. In this study the activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and the malondialdehyde concentration were examined in 36 children with inflammation-mediated damage to microvascular endothelial cells. On the basis of clinical manifestations the studied children were divided into 4 groups (1st group–macular skin manifestations, 2nd group–maculo-papular skin manifestations, 3rd group–papular skin manifestations, 4th group– erythematous skin manifestations). All the examined children showed symptoms of inflammation (mainly respiratory tract infections) with leukocytosis and monocytosis before actual skin manifestations took place. Superoxide dismutase activity was significantly decreased in three groups of patients, except in the group with erythematous skin manifestations. Catalase activity was significantly increased in all the groups compared to the control group. The values of malondialdehyde were significantly increased in the groups of children with maculopapular and erythematous skin manifestations. The results have confirmed the presence of a changed antioxidant enzyme pattern indicating oxidative stress during inflammatory endothelial cells injury. Malondialdehyde was not an adequate parameter in its evaluation.

Keywords: antioxidative enzymes, endothelial cell injury, inflammation, lipid peroxidation

Kratak sadržaj: Tokom inflamacije dolazi do aktivacije endotela i ispoljavanja njegove proadherentne sposobnosti. Sinteza reaktivnih metabolita kiseonika koja prati imunološke procese može pokrenuti oksidativno oštećenje mikrovaskularnih endotelnih ćelija, što se klinički manifestuje pojavom različitih kožnih manifestacija. U radu je praćena aktivnost antioksidativnih enzima (superoksid-dizmutaza, katalaza, glutation-peroksidaza) kao i vrednosti malondialdehida kod 36 dece sa inflamatornim oštećenjem endotelnih ćelija. Na osnovu prisutnih kliničkih manifestacija ispitanici su podeljeni u 4 grupe (I grupa–makulozne promene na koži, II grupa–makulo-papulozne, III grupa–papulozne i IV grupa–eritematozne promene na koži). Svi pacijenti su pre ispoljavanja manifestacija na koži imali simptome inflamacije (uglavnom infekcije respiratornih puteva) i kod svih su u krvnoj slici registrovane leukocitoza i monocitoza. U tri grupe ispitanika aktivnost superoksid-dizmutaze bila je značajno snižena, sem u grupi ispitanika sa eritematoznim promenama na koži, dok je aktivnost katalaze u svim ispitivanim grupama bila značajno povišena u odnosu na vrednosti u kontrolnoj grupi. Vrednosti malondialdehida bile su značajno povišene u grupi dece sa makulopapuloznim i eritematoznim kožnim promenama. Dobijeni rezultati potvrđuju prisustvo oksidativnog stresa tokom inflamatornog oštećenja endotelnih ćelija, ali se malondialdehid, kao parametar lipidne peroksidacije, nije pokazao adekvatnim za procenu inflamatornog endotelnog oštećenja.

Ključne reči: antioksidativni enzimi, oštećenje endotelnih ćelija, inflamacija, peroksidacija lipida

Introduction

There is growing evidence of the involvement of free radicals in disease processes. Free radicals can directly induce structural damage to every tissue in the body and cause tissue injury or even cell death (1). Oxidative stress can also contribute to disease

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generation via activation of the gene regulatory proteins (2).

An imbalance in the production of free oxygen/nitrogen species and the parameters of antioxidative protection is a significant factor in many diseases in childhood, including allergic and immunologic disorders (atopic dermatitis, bronchial asthma, chronic arthritis, Henoch–Schonlein purpura, Kawasaki disease, systemic lupus erythematosus and vasculitic syndrome) (3).

Endothelial cells control platelet adhesion, maintain the balance between prothrombotic and fibrinolytic activity, regulate vascular tone and play an important role in the inflammatory process through their ability to control the recruitment of leukocytes into inflammatory sites (4, 5). The capacity of the endothelium to produce nitric oxide (NO) is essential in the maintenance of vascular homeostasis, while the disturbance in NO production is a major contributor to the pathogenesis of vascular disease (6). During inflammation endothelial cells respond to chemokines and other proinflammatory mediators which modify the expression of adhesion molecules, leading to the recruitment of leukocytes and their transendothelial migration (5, 7). These proinflammatory mediators, such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and lipopolysaccharide (LPS), stimulate the expression of the inducible form of nitric oxide synthase in the microvascular endothelial cells to produce and release large amounts of NO (8, 9). On the other hand, the accumulation of polymorphonuclear leukocytes at inflammatory sites is followed by an excessive production of superoxide anion and other reactive oxygen species. These molecules produce an immunomodulatory effect by increasing the expression and releasing the inflammatory mediators (10).

Endothelial cells are continuously exposed to reactive oxygen species produced by activated inflammatory cells, smooth muscle cells, and endothelial cells themselves. The sources for ROS are enzymes that catalyze redox reactions, such as mitochondrial respiratory chain enzymes, cytosolic enzymes involved in lipid metabolism and membrane-associated enzymes such as NADPH oxidase. The last one, NADPH oxidase is the major source of superoxide anion in endothelial cells (11).

In addition to the ROS production rate, their levels and their effects are regulated by the antioxidant ability of biologic systems to neutralize, detoxify, and repair the cell damage caused by ROS. The cellular antioxidant systems include low-molecular weight antioxidants (e.g., ascorbic acid, glutathione, tocopherols, uric acid) and antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidases (GPx), and catalase. The antioxidant enzymes represent the first line of defense against toxic oxygen reactants by metabolizing them to innocuous byproducts. Therefore, the balanced interactions of these three enzymes are necessary to protect the cellular environment against the oxidative injury.

In the genesis of some skin eruptions, particularly in viral and bacterial infections, the most common pattern of reaction is the superficial, or even dermal, inflammatory infiltrate, while oxidative stress seems to be a major contributor to endothelial cell injury. Hence, we investigated the antioxidant enzyme activities and lipid peroxidation parameters in children with clinical manifestations of the endothelial injury.

Materials and Methods

This study included 36 children (aged from 1 to 18 years) with clinical manifestations consistent with vasculitis-like syndrome. Medical history of all patients showed frequent inflammatory events (mostly recurrent respiratory tract infections and/or reactive lymphadenopathy). The including criteria were age and signs of infection associated with the skin manifestations. None of the childred were under any treatment before the check-up and blood collection for analysis. On the basis of the actual skin manifestations the children were divided into 4 groups: 1st group–macular skin manifestations (8 children); 2nd group–maculo-papular skin manifestations (11 children); 3rd group–papular skin manifestations (12 children); 4th group–erythematous skin manifestations (5 children). The control group included 12 healthy children.

Biochemical analyses were performed in peripheral venous blood collected in vacutainer tubes. Hematological parameters were determined in whole blood, the levels of malondialdehyde (MDA) were measured in plasma, while the antioxidative enzyme activities were estimated in the hemolysate of washed erythrocytes stored at –20 °C until measurement.

The erythrocyte activities of superoxide dismutase and glutathione peroxidase were determined by Ransod and Ransel commercial tests (Randox Lab., Crumlin, UK) on the Beckman Synchron CX-5 autoanalyzer. The erythrocyte catalase activity was determined according to the method of Beutler (12). The concentration of malondialdehyde, a final end product of lipid peroxidation, was determined by the modified photometric method of Andreeva et al. (13) based on the reaction of malondialdehyde with thiobarbituric acid at high temperature, low pH and in the presence of iron.

Data are reported as mean $\pm$SD. The statistical significance of differences was estimated by using Student's t-test and Mann-Whitney U test.

The study has been approved by the Human Ethics Committee of the Medical Faculty in Niš. Informed consent was obtained from each child's parents before their participation in the study.

Results

The analysis of personal medical history data showed that all the examined children manifested symptoms of inflammation (such as respiratory tract infection and reactive lymphadenopathy) prior to the actual skin eruptions and manifestations. On the basis of the actual skin manifestations the children were divided into 4 groups: 1st group–macular skin manifestations (8 children; 6 boys and 6 girls, aged 6–18 years), 2nd group–maculo-papular skin manifestations (11 children; 5 boys and 2 girls, aged 1–18 years), 3rd group–papular skin manifestations (12 children; 8 boys and 4 girls, aged 1–17 years), 4th group–erythematous skin manifestations (5 children; 2 boys and 3 girls, aged 1–11 years), while the control group included 12 healthy children (7 boys and 5 girls, aged 1–11 years) (*Table I*).

All the patients showed leukocytosis and lymphocytosis, which were significant in the 2nd and 3rd group of children. Significant monocytosis was noted in all the groups and it was almost or even more than two-fold higher than in the control group of children (*Table II*).

The superoxide dismutase activity was significantly decreased in three groups of patients (the values of SOD activity were from 900±182.8 to 967±190.5 U/gHb), except in the 4th group (where the value of SOD activity was 967±190.5 U/gHb), compared to the control group (the SOD activity was 1126±144.1 U/gHb). The catalase activity was significantly increased in all the examined groups (the values were from 11.75±2.73 U/gHbx10^4, up to 13.04±3.0 U/gHbx 10^4) compared to the values in the control group of children (9.30±0.95 U/gHbx10^4). The activity of glutathione peroxidase did not show any significant changes among the studied groups. The values of malondialdehyde were significantly increased in the groups of children with maculo-papular and erythematous skin manifestations (3.25±0.66 μmol/L, and 3.13±0.78 μmol/L, toward 2.50±0.33 μmol/L in control group) (*Table III*).

Table I Clinical characteristics of the patients.

Group	Skin manifestation	n (boys/girls)	Age (years)
1st	macular	8 (6/2)	6–18
2nd	maculo-papular	11 (5/6)	1–18
3rd	papular	12 (8/4)	1–17
4th	erythematous	5 (2/3)	1–11
Control	–	12 (7/5)	1–15

Discussion

A number of etiological factors may induce endothelial cell damage and vasculitis-like syndrome via two basic immunopathologic mechanisms: the antigen–antibody imbalance or a cell-mediated process (8). In this study we focused on children with vascular endothelial damage followed by macules, papules, maculo-papular and erythematous skin

Table II Leukocytes, lymphocytes and monocytes values in the examined children.

	n	Le (%)	Ly (%)	Mo (%)
1st group	8	142.2 ± 68.6	124.1 ± 38.5	186.6 ± 68.4**
2nd group	11	137.7 ± 44.6*	138.8 ± 44.5*	207.8 ± 146.8*
3rd group	12	132.6 ± 35.2*	135.2 ± 32.6*	185.5 ± 73.5**
4th group	5	128.3 ± 52.2	110.4 ± 30.6	158.6 ± 102.4*
Control	12	104.2 ± 15.0	105.8 ± 19.9	89.0 ± 20.4

The values are given as the percentage of expected values for corresponding age ± SD.
*p<0.05 compared to the control group, **p<0.01 compared to the control group

Table III Antioxidant enzyme activities and parameter of MDA concentration in the examined groups of children.

	n	SOD (U/g Hb)	GPx (U/g Hb)	Catalase (U/g Hb x10^4)	MDA (μmol/L)
1st group	8	900 ± 182.8*	36.97 ± 5.41	12.27 ± 1.91**	2.47 ± 0.19
2nd group	11	927 ± 167.8**	37.04 ± 8.06	13.04 ± 3.0**	3.25 ± 0.66**
3rd group	12	912 ± 203.1*	36.84 ± 9.21	11.75 ± 2.73*	2.86 ± 0.67
4th group	5	967 ± 190.5	34.62 ± 8.09	11.78 ± 1.06**	3.13 ± 0.78**
Control	12	1126 ± 144.1	31.93 ± 4.62	9.30 ± 0.95	2.50 ± 0.33

The values are given as the percentage of expected values for corresponding age ± SD.
*p<0.05 compared to the control group, **p<0.01 compared to the control group

changes. All the examined patients showed signs of infection that preceded the actual skin eruptions, indicating the initiation of inflammatory events and setting the conditions for leukocyte adhesion to the endothelium and excess synthesis of free radicals, which can lead to damage and apoptosis of the endothelial cells. Oxidative damage to skin microvascular endothelial cells can be manifested as various skin eruptions, like those seen in our patients. Further, the significant changes in hematological parameters, especially the increase in monocytes number, confirmed an intense inflammatory event.

The now well recognised increase in oxidative stress plays an important role in the genesis of endothelial activation and dysfunction. Recent studies implicated NADPH oxidases as major sources of ROS involved in this abnormality (14, 15). Dysfunction of the endothelium encompasses both the abnormalities of endothelial-dependent vasodilator regulation and endothelial activation, and involves an increase in the endothelial–leukocyte interactions during pathophysiological inflammatory response. These changes in endothelial activation, among other effects, occur also in response to diverse stimuli including inflammatory cytokines (16). A major mechanism underlying ROS-dependent impairment of the endothelium is the superoxide-mediated inactivation of vasodilator nitric oxide, a reaction that generates peroxynitrite. There are several potential sources of superoxide in endothelial cells, but there is evidence which confirms an important role for NADPH oxidases as major sources of ROS involved in endothelial dysfunction, activation and redox signalling (17, 18). On the other hand, NADPH oxidases can cause the uncoupling of NOS secondary to oxidative degradation of the NOS cofactor, thereby leading to superoxide rather than NO generation.

In our patients significantly lower activity of superoxide dismutase was registered, which supported superoxide anion as a mediator of oxidative damage. Also, during inflammation superoxide anion can react with nitric oxide which is synthesized by activated inducible nitric oxide synthase forming peroxynitrite, a more powerful radical in the process of oxidative cell damage.

Reactive oxygen species can damage proteins, lipids and DNA, altering the cell's structure and function. Peroxidation of membrane-associated fatty acids and cholesterol will alter cell membrane fluidity and permeability and may eventually induce widespread membrane damage (1). Uncontrolled ROS production by activated inflammatory cells can lead to parenchymal, epithelial and endothelial cell injury. Cytokines, such as IFN-γ and IL-1, induce superoxide production by inflammatory cells and control their influx and activation (19). The adhesion of leukocytes to endothelial cells during inflammation depends on the expression of cell adhesion molecules which can be stimulated by bacterial lipopolysaccharides and by various proinflammatory cytokines. The adherence of leukocytes to the endothelial cells is also induced by ROS, and this effect is abolished by catalase but not superoxide dismutase (20).

All our patients showed significantly higher catalase activity, which can be important in the limitation of endothelial cells adhesion molecules expression and further progression of oxidative cell damage. The same antioxidant enzyme pattern disturbances as well as a significant increase in erythrocyte catalase activity and a significant decrease in erythrocyte SOD activity have been observed in almost all the patient groups. Previous investigations demonstrated that the same pattern of these enzymes is observed during hematological (21), neoplastic (22), ischemic (23), neurological (24) and systemic diseases (25).

On the other hand, superoxide anion participates in either the removal of radicals generated during lipid peroxidation, or the termination of the peroxidation process. Therefore, the low SOD activity can be considered useful because it provides a sufficient amount of superoxide anions to limit the process of lipid peroxidation and cell injury. However, significantly higher levels of MDA were registered only in the group of children with erythematous and maculo-papular skin changes. The results may indicate considerable endothelial cells damage in those patients, or maybe a widespread involvement of the endothelium in this type of skin manifestation. Although, we may also consider the possibility of endothelial cells injury in children with macular or papular skin manifestations due to mechanisms other than lipid peroxidation of oxidative cell damage.

In some studies it has been suggested that oxidative stress plays an important role in the pathogenesis of some skin diseases (26, 27) including some forms of urticaria, although systemic changes in antioxidant enzyme activity and lipid peroxidation have been demonstrated only in patients with physical urticaria (28), but not in patients with chronic idiopathic urticaria (29), and urticaria caused by nonsteroidal antiinflammatory drugs (30).

The results of this study related to the antioxidant enzymes have confirmed the importance of oxidative stress during inflammation, while the parameters of lipid peroxidation could not provide satisfactory and adequate information in the evaluation of inflammatory endothelial injury.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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