Assessment of concentrations of oxidative stress parameters in children and teenagers with inflammatory bowel diseases

Ocena stężenia parametrów stresu oksydacyjnego u dzieci i młodzieży z nieswoistymi zapaleniami jelit

Rafał Czarnecki[1], Mieczysława Czerwionka-Szaflarska[1], Józef Kędziora[2], Jolanta Czuczejko[2]

[1]Chair and Department of Paediatrics, Allergology and Gastroenterology, Ludwik Rydygier Memorial *Collegium Medicum*, Bydgoszcz, Nicolaus Copernicus Memorial University, Toruń

[2]Chair and Department of Biochemistry, Ludwik Rydygier Memorial *Collegium Medicum*, Bydgoszcz, Nicolaus Copernicus Memorial University, Toruń

Przegląd Gastroenterologiczny 2009; 4 (4): 188–192

Key words: oxidative stress, inflammatory bowel diseases, children, teenagers.
Słowa kluczowe: stres oksydacyjny, nieswoiste zapalenia jelit, dzieci, młodzież.

Address for correspondence: Prof. Mieczysława Czerwionka-Szaflarska, MD, PhD, Chair and Department of Paediatrics, Allergology and Gastroenterology, Ludwik Rydygier Memorial *Collegium Medicum*, Bydgoszcz, Nicolaus Copernicus Memorial University, Toruń, 9 Maria Skłodowska-Curie Street, 85-094 Bydgoszcz, Poland, phone +48 52 585 48 50, e-mail: klped@cm.umk.pl

Abstract

Introduction: Oxidative stress is a state in which there occurs increased activity of oxygen free radicals that can result in irreversible lesions in the body and can contribute to tissue damage in different diseases. Free radicals formed in excess can damage cells, particularly endothelial cells of blood vessels, through initiation of a peroxidation process concerning polyunsaturated fatty acids (PFA) forming cell membranes. Moreover, free radicals increase aggregation and protein denaturation, resulting in humoral disturbances of immune mechanisms concerning the complement system and immunoglobulins.

Aim: The aim of the study was to measure the concentration of selected parameters of oxidative stress in children and teenagers with inflammatory bowel diseases.

Material and methods: Ninety five patients between 10 and 18 years of age (average age 14.9 years) were qualified for the study. Analysed patients were divided into the following groups: I – children and teenagers with Crohn's disease in remission (n = 14), II – children and teenagers with ulcerative colitis in remission (n = 13), III – comparative group, children and teenagers in whom the above-mentioned diseases and other chronic diseases were excluded, without features of inflammation (n = 68). The whole blood was collected from every patient at 8 a.m. The following substances were measured: concentrations of products of lipid peroxidation – compounds reacting with thiobarbituric acid (TBARS) (malonic dialdehyde) in erythrocytes, using the method of Placer *et al*., the whole blood concentration of anion radical peroxide released by activated granulocytes, using the method of Bellavite *et al*.,

Streszczenie

Wprowadzenie: Stres oksydacyjny jest stanem, w którym dochodzi do zwiększonej aktywności wolnych rodników tlenowych, co może prowadzić do nieodwracalnych zmian w organizmie oraz przyczyniać się do uszkodzenia tkanek w różnych stanach chorobowych. Wolne rodniki wytwarzane w nadmiarze mogą uszkadzać komórki, w szczególności śródbłonka naczyń krwionośnych poprzez inicjowanie procesu peroksydacji wielonienasyconych kwasów tłuszczowych (WKT) budujących błony komórkowe. Wolne rodniki wzmagają ponadto agregację i denaturację białka, w wyniku czego dochodzi do zaburzeń humoralnych mechanizmów odpornościowych układu dopełniacza i immunoglobulin.

Cel: Celem pracy było oznaczenie stężenia wybranych parametrów stresu oksydacyjnego u dzieci i młodzieży z nieswoistymi zapaleniami jelit.

Materiał i metody: Do badania zakwalifikowano 95 pacjentów w wieku 10–18 lat (średnia 14,9 roku). Badanych podzielono na następujące grupy: I – dzieci i młodzież z chorobą Leśniowskiego-Crohna w fazie remisji (n = 14), II – dzieci i młodzież z wrzodziejącym zapaleniem jelita grubego w fazie remisji (n = 13), III – grupa porównawcza – dzieci i młodzież, u których wykluczono powyższe stany chorobowe oraz inne choroby przewlekłe, bez cech ostrego stanu zapalnego (n = 68). Każdemu pacjentowi pobierano krew pełną o godzinie 8.00 rano. Oznaczono: stężenia produktów peroksydacji lipidów – związków reagujących z kwasem tiobarbiturowym (TBARS) (dialdehyd malonowy) w erytrocytach metodą Placera i wsp., stężenia anionorodnika ponadtlenkowego uwalnianego przez aktywowane granulocyty we krwi pełnej metodą Bellavite i wsp. oraz stężenia tlenku azotu we krwi reakcją Griessa wg Marletta i wsp.

blood concentration of nitrogen oxide, using the Griess reaction according to Marlett *et al*.
Results: Statistically significant differences concerning mean concentrations of malonic dialdehyde in group I in relation to the comparative group and in group II in relation to the comparative group were not proved. Statistically significant differences concerning mean concentrations of anion radical peroxide in group I in relation to the comparative group and in group II in relation to the comparative group were not observed. Similarly, statistically significant differences concerning mean concentrations of nitrogen oxide among patients from group I in relation to the comparative group and among patients from group II in relation to the comparative group were not proved.
Conclusions: On the basis of obtained results, it seems that pro-oxidative factors do not play an essential role in pathogenesis of inflammatory bowel diseases in children and teenagers.

Wyniki: Nie wykazano istotnych statystycznie różnic średnich stężeń dialdehydu malonowego w grupie I w stosunku do grupy porównawczej oraz w grupie II w stosunku do grupy porównawczej. Nie zaobserwowano statystycznie istotnych różnic średnich stężeń anionorodnika ponadtlenkowego w grupie I w stosunku do grupy porównawczej oraz w grupie II w stosunku do grupy porównawczej. Podobnie nie stwierdzono istotnej statystycznie różnicy w zakresie średnich stężeń tlenku azotu między pacjentami z grupy I w stosunku do grupy porównawczej oraz między pacjentami z grupy II w stosunku do grupy porównawczej.
Wnioski: Na podstawie uzyskanych wyników badań wydaje się, że czynniki prooksydacyjne nie odgrywają istotnej roli w patogenezie nieswoistych zapaleń jelit u dzieci i młodzieży.

Introduction

Oxidative stress is a state in which increased activity of oxygen free radicals occurs. It develops as a result of balance disturbances between production and removal of toxic oxygen derivatives. A substantial degree of balance disturbance in the system of oxidants and antioxidants can result in irreversible lesions in the body and can contribute to tissue damage in different diseases [1-3].

Free radicals (FR) are atoms or groups of atoms or molecules that have one or several free (not spare) electrons on their last orbital and it makes molecules significantly reactive. They are formed during processes of homological break concerning bonds in molecules of chemical compounds or as a result of electron transmission [4]. They join reactions with DNA, lipids, carbohydrates and proteins and damage them by electron collection, causing changes of their structure and function [5, 6].

The main free radicals are: anion radical peroxide (O_2^-), the most reactive and toxic hydroxide radical (hydroxylic) ($^\bullet OH$), hydrogen peroxide (H_2O_2) and singlet oxygen (1 O_2) (formed as a result of arousal of the oxygen molecule) and they share the name of reactive oxygen forms (ROF) [6].

The sources of ROF in physiological states are respiratory processes that proceed in mitochondria, where free radicals participate in biological reactions of oxidation and reduction in the respiratory chain and in regeneration concerning energy sources in the form of high-energy phosphorus compounds. Reactive oxygen forms that participate in the process of microorganism phagocytosis as an element of the NADPH oxidase system in cell membranes of phagocytes and neutrophils are a factor of organism defence. If more than enough of them are produced, they can damage cells, particularly endothelial cells of blood vessels and body tissues. Reactive oxygen forms initiate peroxidation of polyunsaturated and esterified fatty acids (PFA) creating cell membranes and they cause decay of lipid hydroxides. They contribute to accumulation of toxic metabolites of the peroxidation reaction and they induce generation of arachidonic acid metabolites, causing damage of cell membranes. Moreover, ROF increase aggregation and protein denaturation that results in humoral disturbances of immune mechanisms concerning the complement system and immunoglobulins [3].

The peroxidation process of free fatty acids proceeds as follows. An oxygen molecule is incorporated in the structure of membrane lipids. Radicals of lipid peroxides are formed and they can react with other unchanged lipids of cell membranes, forming next lipid radicals and lipid hydroperoxides. The presence of lipid hydroperoxides in membrane cells causes an increase of their permeability for protons and other ions, but in the case of intrinsic mitochondrial membranes it decreases the level of oxygen phosphorylation. Anion radical peroxide and iron ions are additional elements damaging cell membranes by participation in the process of lipid peroxidation of these membranes [7]. Malonic dialdehyde (MDA) is an indicator concerning process intensity of lipid peroxidation. Malonic dialdehyde is one of the final products of lipid peroxidation, often measured in many studies concerning problems of oxidative stress and lipid peroxidation [8-10].

Aim

The aim of the study was to measure concentrations of selected parameters of oxidative stress in children and teenagers with inflammatory bowel diseases.

Material and methods

Ninety five patients between 10 and 18 years of age (average age 14.9 years) diagnosed and treated in the Chair and Department of Paediatrics, Allergology and Gastroenterology of Ludwik Rydygier Memorial Collegium Medicum in Bydgoszcz were qualified for the study.

Analysed patients were divided into the following groups:

- I – children and teenagers with Crohn's disease in remission (n = 14),
- II – children and teenagers with ulcerative colitis in remission (n = 13),
- III – comparative group – children and teenagers in whom the above-mentioned diseases and other chronic diseases were excluded, without features of acute inflammation (n = 68).

Diagnosis of diseases and qualification for the groups were performed according to accepted diagnostic criteria.

The whole blood was collected from every patient at 8 a.m. Concentrations of products of lipid peroxidation were measured – compounds reacting with thiobarbituric acid (TBARS) (malonic dialdehyde – MDA) in erythrocytes, using the method of Placer *et al*., the whole blood concentration of anion radical peroxide released by activated granulocytes, using the method of Bellavite *et al*. and concentration of nitrogen oxide (NO) through determination of nitrites/nitrates concentrations in the blood, using the Griess reaction according to Marlett *et al*.

Results of the study were analysed statistically.

Results

Statistically significant differences concerning mean concentrations of malonic dialdehyde in group I in relation to the comparative group, similarly as in group II in relation to the comparative group, were not found. Precise data of this analysis are shown in Table I. Also statistically significant differences concerning mean concentrations of anion radical peroxide in group I in relation to the comparative group and in group II in relation to the comparative group were not observed (Table II). Also a statistically significant difference concerning mean concentrations of nitrogen oxide among patients from group I in relation to the comparative group and among patients from group II in relation to the comparative group was not proved. Precise data of this analysis are shown in Table III.

Discussion

Phagocytes are postulated to be the main source of oxygen free radicals in colitis, Crohn's diseases, and ulcerative colitis [11].

5-Aminosalicylic acid, an active metabolite of sulfasalazine used as a drug in ulcerative colitis, is an excellent sweep of oxygen reactive forms. It seems that therapeutic effects of sulfasalazine at least partially depend on inactivation of ROF that are produced by enterocytes and phagocytes. More extensive incidence of neoplasia in the large bowel that occurs in this disease can also be ascribed to ROF as a factor causing mutagenesis [11-13].

Table I. Comparison of malonic dialdehyde concentration in particular groups of analysed patients with inflammatory bowel diseases

Tabela I. *Porównanie stężenia dialdehydu malonowego w poszczególnych grupach badanych pacjentów z nieswoistymi zapaleniami jelit*

Group		Malonic dialdehyde			Normality		
		n	mean [µmol/g Hb]	SD	W	W_{kr}	normality
I		14	0.28	0.05	0.876	0.874	yes
II		13	0.33	0.12	0.831	0.866	no
III		68	0.29	0.05	–	–	yes
Kruskal-Wallis test (χ^2_{kr} = 9.49)	H		8.29				
	p		0.08 (*ns*)				

SD – standard deviation, n – number, ns – not significant

Table II. Comparison of anion radical peroxide concentrations in particular groups of analysed patients with inflammatory bowel diseases

Tabela II. *Porównanie stężeń anionorodnika ponadtlenkowego w poszczególnych grupach badanych pacjentów z nieswoistymi zapaleniami jelit*

Group		Anion radical			Normality		
		n	mean [µmol/g Hb]	SD	*W*	W_{kr}	normality
I		14	182.2	84.4	0.919	0.874	yes
II		13	231.8	156.8	0.905	0.866	yes
III		68	234.4	114.3	–	–	yes
Bartlett's test	χ^2		5.12				
(χ^2_{kr} = 9.49)	*p*		0.27 (*ns*)				
ANOVA test	*F*		0.74				
(F_{kr} = 2.44)	*p*		0.56 (*ns*)				

SD – standard deviation, n – number, ns – not significant

Table III. Comparison of nitric oxide concentration in particular groups of analysed patients with inflammatory bowel diseases

Tabela III. *Porównanie stężenia tlenku azotu w poszczególnych grupach badanych pacjentów z nieswoistymi zapaleniami jelit*

Group		Nitric oxide			Normality		
		n	mean [µmol/g Hb]	SD	*W*	W_{kr}	normality
I		12	1.39	0.76	0.940	0.859	yes
II		13	1.88	2.17	0.691	0.866	no
III		68	1.69	1.37	–	–	yes
Kruskal-Wallis test	*H*		2.35				
(χ^2_{kr} = 9.49)	*p*		0.67 (*ns*)				

SD – standard deviation, n – number, ns – not significant

Wojtysiak *et al*. [14] noted increased MDA concentration in the serum and erythrocytes in adult patients during the acute phase of ulcerative colitis in relation to the comparative group (healthy persons). During disease remission, serum MDA concentration was higher than in the comparative group, but lower than during the acute phase of the disease, while MDA concentration in erythrocytes was lower than in patients during the acute phase of the disease, but also lower than in the comparative group. Moreover, increased leucocytosis and increase of superoxide radical generation by granulocytes of peripheral blood in relation to the comparative group were observed during the acute phase of the disease. After treatment, during remission superoxide radical generation was lower than during the acute phase and also lower than in the comparative group. Also leucocytosis and granulocytosis in peripheral blood decreased.

Similar studies were performed by Dziki and Wojtysiak [15], who observed increased MDA concentration in the serum and in erythrocytes in patients during the active phase of ulcerative colitis in relation to the comparative group. Leucocytosis and granulocytosis in peripheral blood were increased. Also amounts of anion radical peroxide generated by neutrophils were increased. Concerning patients during remission, concentrations of analysed pro-oxidative factors did not differ statistically significantly from concentrations of these factors in the comparative group.

Ignyś *et al*. [10] revealed increased MDA concentrations in erythrocytes in children with ulcerative colitis before treatment with 5-ASA and gradual decrease of MDA concentrations after 3 and 6 months of treatment. MDA concentrations in the group of children suffering from Crohn's disease

and in the group of children with non-specific colitis were similar. The highest Malonic dialdehyde concentration before treatment was observed in the group of patients with non-specific colitis, but the lowest in the group of patients with ulcerative colitis. Malonic dialdehyde concentration decreased after treatment in every case, but it did not reach the concentration level of the comparative group (it was higher than in the comparative group).

Therefore it is possible to conclude that increased concentration of pro-oxidative indicators occurs in inflammatory bowel diseases. It was also revealed that this situation mainly appears during the acute phase of inflammatory bowel diseases, but concentrations of pro-oxidative factors decrease during treatment, reaching in some cases an equal or lower level than in the comparative group.

Our studies determined the concentration of several pro-oxidative factors, among all anion radical peroxide released by activated granulocytes, MDA in erythrocytes and NO in the serum of children suffering from ulcerative colitis in remission, and Crohn's disease in remission. There were no proven statistically significant differences among concentrations of these above mentioned pro-oxidative factors in all analysed groups in relation to the comparative group.

The results of our studies did not correlate with previous observations of other researchers.

It is difficult to explain the differences among the results of these studies. Higher mean MDA concentrations were indeed observed in group II in relation to the comparative group, but statistical analysis did not show significance for this difference. Perhaps it would be possible to demonstrate a statistically significant difference in concentration of the studied parameter by comparing larger analysed groups.

Conclusions

On the basis of obtained results, it seems that pro-oxidative factors do not play an essential role in pathogenesis of inflammatory bowel diseases in children and teenagers.

References

1. Olędzki R, Kędziora-Kornatowska K. Mechanizmy antyoksydacyjne w organizmie człowieka. Valetudinaria – Post Med Klin Wojsk 2006; 11: 15-20.
2. Postępski J, Opoka-Winiarska V, Tuszkiewicz-Misztal E. Znaczenie reakcji wolnorodnikowych w patogenezie wybranych schorzeń u dzieci. Pediatr Pol 2000; 75: 767-76.
3. Szelachowska M, Abdelrazek S, Zonenberg A, Kinalska I. Rola stresu oksydacyjnego w procesach chorobowych. Terapia 2002; 10: 19-22.
4. Liczmański AE. Toksyczność tlenu. Uszkodzenie żywych komórek. Post Biochem 1988; 34: 273-91.
5. Królikowska A, Kędziora-Kornatowska K, Kornatowski T, et al. Znaczenie melatoniny w procesie starzenia się i onkogenezy. Valetudinaria – Post Med Klin Wojsk 2005; 10: 17-20.
6. Olędzki R, Kędziora-Kornatowska K. Wpływ stresu oksydacyjnego na wybrane procesy fizjologiczne i patologiczne w organizmie człowieka. Valetudinaria – Post Med Klin Wojsk 2006; 11: 21-9.
7. Karasek M, Lewiński A, Russel J, Reiter RJ. Melatonina: znaczenie kliniczne i zastosowanie terapeutyczne. Endokrynol Pol 2001; 52: 81-100.
8. Bała G, Czerwionka-Szaflarska M, Chrobot AM. Aktywność enzymów antyoksydacyjnych w przebiegu zapalenia błony śluzowej żołądka i dwunastnicy u dzieci. Przegl Pediatr 2000; 30: 28-33.
9. Gaweł S, Wardas M, Niedworok E, et al. Dialdehyd malonowy (MDA) jako wskaźnik procesów peroksydacji lipidów w organizmie. Wiad Lek 2004; 57: 456-61.
10. Ignyś I, Krauss H, Malewski W, et al. Wpływ leczenia 5ASA na wykładniki stresu oksydacyjnego u dzieci z nieswoistymi zapaleniami jelita grubego. Nowa Pediatr 2003; 7: 99-103.
11. Granger DN, Hernandez LA, Grisham MB. Reactive oxygen metabolites: mediators of cell injury in the digestive system. Viewpoint Dig Dis 1989; 18: 13-6.
12. Czerwionka-Szaflarska M, Bała G, Drews G, et al. Rodniki tlenowe jako mediatory patofizjologii żołądkowo-jelitowej. Gastroenterol Pol 1997; 4: 303-6.
13. Yamada T, Grisham MB. Role of neutrophil-derived oxidant in the pathogenesis of intestinal inflammation. Klin Wochenschr 1991; 69: 988-94.
14. Wojtysiak J, Dziki A, Sibińska E, et al. Generowanie anionorodnika ponadtlenkowego (O_2^-) przez granulocyty krwi obwodowej oraz peroksydacja lipidów w osoczu i erytrocytach chorych na wrzodziejące zapalenie jelita grubego. Gastroenterol Pol 1996; 3: 55-9.
15. Dziki A, Wojtysiak J. Znaczenie wolnych rodników tlenowych w patogenezie wrzodziejącego zapalenia jelita grubego. Farmakol Pol 1997; 53: 675-82.