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Manuscript received February 09, 2011; revised April 14, 2011

Lipid Peroxidation and Enzymatic Antioxidants in Ulcerative Colitis

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Abstract

Increased oxidative stress has been previously demonstrated in patients with inflammatory bowel disease. But this phenomenon has not been analyzed in the course of ulcerative colitis (UC). In this study we evaluated levels of malondialdehyde (the main product of lipid peroxidation), superoxide dismutase and catalase erythrocyte activities in 62 patients with active UC, in 22 patients after achievement of complete, endoscopic remission and in 52 control subjects. Significant increase of malondialdehyde in patients with active disease in comparison with control subjects, demonstrated in this study, suggests the presence of enhanced oxidative stress in active UC. Activation of enzymatic antioxidant system is characteristic of active UC, which is confirmed by an increase in superoxide dismutase and catalase erythrocyte activities in patients with active disease in comparison with control group. There is no significant difference in malondialdehyde and catalase erythrocyte activity between patients in remission of UC and the control subjects. The increase of conditional adaptive index in patients with active UC confirms large adaptive possibilities of enzymatic antioxidant system. The normal levels of malondialdehyde and catalase can be proposed as markers of complete disease remission in UC.

Key words: ulcerative colitis, oxidative stress, lipid peroxidation, malondialdehyde, superoxide dismutase, catalase, adaptive index.

Перекисное окисление липидов и ферментные антиоксиданты при неспецифическом язвенном колите

В ряде научных исследований доказано наличие оксидативного стресса при неспецифическом язвенном колите (НЯК). Однако, этот феномен не был изучен в динамике заболевания. В настоящей работе оцениваются уровни малонового диальдегида (основного продукта перекисного окисления липидов), супероксиддисмутазной и каталазной активности эритроцитов у 62-х больных в период обострения НЯК, у 22-х больных после достижения полной, эндоскопической ремиссии и у 52-х человек из группы контроля. Существенное увеличение концентрации малонового диальдегида у пациентов в период обострения заболевания по сравнению с контрольной группой, выявленное в данном исследовании, указывает на наличие выраженного оксидативного стресса при активном НЯК. Для обострения НЯК характерна активация ферментной антиоксидантной системы, что подтверждается значительным увеличением супероксиддисмутазной и каталазной активности эритроцитов у пациентов с активными формами заболевания по сравнению с контрольной группой. Статистически значимых различий уровней малонового диальдегида и каталазной активности не было выявлено между группой контроля и пациентами в период ремиссии. В период обострения заболевания установлено увеличение условного адаптационного индекса, что свидетельствует о широких адаптивных возможностях ферментной антиоксидантной системы. Нормальные уровни малонового диальдегида и каталазы могут быть рекомендованы как маркеры полной ремиссии заболевания при НЯК.

Ключевые слова: неспецифический язвенный колит, оксидативный стресс, перекисное окисление липидов, малоновый диальдегид, супероксиддисмутаза, каталаза, адаптационный индекс.

Introduction

Ulcerative colitis (UC) and Crohn's disease, known as inflammatory bowel disease (IBD), are chronic relapsing conditions of uncertain etiology and pathogenesis. IBD is thought to result from abnormal and ongoing activation of the mucosal immune system driven by the presence of normal luminal flora. This pathological response is most likely facilitated by defects in both the barrier function of the intestinal epithelium and the mucosal immune system. However, the specific pathways leading to tissue damage are not completely understood. Oxidative stress (OS) is a potential pathogenetic factor for IBD. The damaging action of reactive oxygen molecules (ROM) have been well demonstrated in the inflammation process [1]. The cascade of ROM production is initiated with the formation of the superoxide anion (O_2^-) by a single electron reduction of oxygen. O_2^- itself is a relatively unharmful oxidant, but it is dismutated spontaneously or enzymatically by superoxide dismutase (SOD) to yield the more reactive metabolite hydrogen peroxide (H_2O_2). The easily diffusible and rather long-living H_2O_2 is intracellularly detoxified to water by the enzymes catalase or glutathione peroxidase. However, H_2O_2 can also be metabolized to the secondary ROMs hydroxyl radical and hypochlorous acid (HOCl). Hydroxyl radical is extremely reactive with virtually every molecule it encounters. HOCl is a powerful ROM that is known to inactivate protease inhibitors (e. g. α_1-antitripsin), which disturbs the proteinase-antiproteinase balance and leads to propagation of extracellular matrix degradation and mucosal tissue damage [2].

Even though a lot of experimental research has demonstrated the role of oxidative stress in inflammatory models [3], the previous clinical studies have shown contradictory results. Several studies have established no difference in the levels of lipid peroxides and/or antioxidants between IBD patients and healthy persons [4, 5]. Others have made a conclusion that OS is of great importance in IBD [6] and have supposed that OS has an etiologic role in IBD [7].

In this study we aimed to determine the levels of lipid peroxidation by measuring malondialdehyde (MDA) and the activity of antioxidant system by measuring SOD and catalase activity in patients with UC and to evaluate the potential diagnostic value of these investigations.

Material and methods

Study groups

Sixty-two patients with active ulcerative colitis, treated from 2006 to 2009 in Gastroenterological department of Republican Clinical Hospital from Moldova were included in this study. The diagnosis of IBD was based on typical endoscopic, histological, and radiological findings. Age at diagnosis, type and extent of disease, and current therapy were recorded. The extent of disease was defined as proctitis/proctosigmoiditis, left-sided or extensive colitis. Disease activity was estimated according to modified Truelove&Witts [8] and Mayo scores. Biochemical parameters such as erythrocyte sedimentation rate, hemoglobin and C-reactive protein were also evaluated for each patient. The exclusion criteria were surgery, chronic active or acute infections and inflammatory diseases other than IBD, and the use of medications for at least one month prior to the examination (with the exception of UC medicine). All patients were followed for a long period of time (mean duration 2.7 years, range 1-5) or until complete remission. Complete remission was defined as the resolution of clinical symptoms, disease activity indices below 3 in the course of at least 3 months and endoscopic mucosal healing. Complete remission (without surgery and immunomodulators) was achieved in 22 cases. Fifty-two healthy persons formed the control group. Demographic and clinical characteristics of UC patients and demographic characteristics of persons from the control group are shown in table 1.

Lipid peroxidation and enzymatic antioxidants assay

Lipid peroxidation was estimated by colorimetric analysis of malondialdehyde – the main product of lipid peroxidation. MDA was evaluated in plasma using thiobarbituric acid-reactive substances reaction. Catalase and SOD - the main enzymatic antioxidants - were determined for analyses of antioxidant status. A spectrophotometric method for determination of catalase and SOD erythrocyte activity was performed by respective assay kits. For estimation of oxidative

Table 1

Demographic and Clinical Characteristics

	Ulcerative Colitis		Control group
	Active disease	Remission	
Number	62	22	52
Gender (F/M)	32/30	13/9	29/23
Mean age (yr)	44.5 ± 13.4	45.3 ± 12.7	37.2 ± 9.7
Age at diagnosis (yr)	39.8 ± 13.8	39.9 ± 13.2	
Extent			
Proctosigmoiditis	29 (46.8%)		
Left-sided colitis	19 (30.6%)		
Extensive	14 (22.6%)		
Disease activity			
Truelove and Witts	7.5 ± 2.6	1.5 ± 0.5	
Mayo	7.6 ± 2.3	1.5 ± 0.5	
Therapy			
5-ASA	48 (77.4%)	11 (50.0%)	
Steroids	15 (24.2%)	4 (18.2%)	
No therapy	14 (22.6%)	7 (31.8%)	

stress and antioxidant's activity a conditional adaptive index ratio was calculated: AI = catalase x SOD/MDA x 100.

Statistical analysis

Data are expressed as mean ± standard deviation of the mean values. Comparisons between the control subjects and UC patients were made by Student's *t* test, and between UC patients in active disease and in remission – by Student's paired *t* test. Correlation coefficients were calculated for estimation of interrelation between MDA, SOD, catalase and the severity of disease activity (points of Truelove&Witts and Mayo scores). Statistical analysis was performed by standard Excel programs.

Results

The levels of MDA differed significantly between the control subjects and patients with active UC (4.5 ± 0.3 μmol/ml *vs* 6.2 ± 0.6 μmol/ml respectively, $p < 0.001$) (tab. 1). The levels of MDA in remission of UC were significantly lower than in active disease (4.6 ± 0.3 μmol/ml *vs* 6.2 ± 0.6 μmol/ml, $p < 0.001$). No statistical differences were found between the control subjects and patients in remission of UC.

The correlation coefficients between the levels of MDA and indices of disease activity according to modified Truelove&Witts and Mayo scores were 0.76 and 0.78 respectively.

Significant increase was observed in enzymatic antioxidant SOD and catalase of erythrocytes in active phase of UC in comparison with the control subjects ($p < 0.001$) and with the patients in remission of disease. The level of catalase didn't differ in remission of disease and in control group. SOD activity also reduced in remission as compared to the active disease group however SOD levels remained significantly higher than in the control group ($p < 0.01$). The correlation coefficients between the enzymatic antioxidants and indices of disease activity according to Truelove&Witts and Mayo scores were: 0.72 and 0.71 respectively for SOD, and 0.62 and 0.64 – for catalase. Conditional AI was significantly higher

Table 2

Plasma malondialdehyde, erythrocytes superoxide dismutase and catalase levels in studied groups

	Control group (I) n = 52	Active disease (II) n = 62	Remission (III) n = 22	p		
				I/II	I/III	II/III
Malondialdehyde (μmol/ml)	4.5 ± 0.3	6.2 ± 0.6	4.6 ± 0.3	0.000	0.197	0.000
Superoxide dismutase erythrocyte activity (Un/mg Hb)	31.2 ± 3.8	47.0 ± 5.8	34.0 ± 3.9	0.000	0.006	0.000
Catalase erythrocyte activity (Un/mg Hb)	332.1 ± 29.4	452.7 ± 60.1	337.8 ± 33.4	0.000	0.470	0.000
Adaptive index (catalase x SOD/ MDA x 100)	23.0 ± 2.5	34.4 ± 5.4	25.1 ± 3.8	0.000	0.007	0.000

in active disease in comparison with the control subjects ($p < 0.001$) and with the patients in remission ($p < 0.001$).

No statistical differences were found between the levels of MDA, SOD, catalase and AI at patients with various grade of disease activity (tab. 3). The levels of catalase and SOD activity in some patients with severe UC were lower or comparable with the levels in control group. This finding is probably related to the depletion of reserve mechanisms.

Table 3

Plasma malondialdehyde, erythrocytes superoxide dismutase and catalase levels in groups of UC patients with various grade of disease activity

	Mild (A)	Moderate (B)	Severe (C)	p-value		
	n = 11	n = 42	n = 9	A/B	A/C	B/C
Malondialdehyde (μmol/ml)	5.7 ± 0.9	6.2 ± 0.5	6.6 ± 0.5	0.017	0.016	0.034
Superoxide dismutase erythrocyte activity (Un/mg Hb)	43.3 ± 7.1	47.2 ± 5.1	50.8 ± 4.9	0.043	0.015	0.059
Catalase erythrocyte activity (Un/mg Hb)	436.4 ± 76.6	449.2 ± 54.5	488.7 ± 56.1	0.528	0.105	0.055
Adaptive index (catalase x SOD / MDA x 100)	33.2 ± 6.6	34.1 ± 5.2	37.1 ± 4.7	0.631	0.154	0.117

Discussion

Oxidative stress has an important role in mechanisms of the inflammatory process of lessioned tissues including lesions of mucous membrane [1]. Among the immunoregulatory factors, reactive oxygen species are produced in abnormally high levels in IBD [7]. Although some conflicting results exist (caused, probably, by imprecise techniques or small sample size), the majority of experimental and clinical studies demonstrate the presence of excessive reactive oxygen metabolites in plasma and tissue specimens of IBD patients

[3, 6, 9]. The present study confirms significant increase of MDA – the metabolite of lipid peroxidation, in active UC.

To protect against adverse effects of free radicals and theirs derivatives there is an antioxidant system which includes enzymatic and non-enzymatic substances [10]. The main enzymatic antioxidants are superoxide dismutase, catalase and glutathione peroxidase and glutathione reductase. Non-enzymatic antioxidants include dietary compounds, such as vitamins (C and E) and minerals (selenium and zinc) and also glutathione, uric acid, polyphenols and others [11]. In the whole, studies have demonstrated a reduction of non-enzymatic activity in active IBD: decrease of plasma antioxidant vitamins [12], of glutathione [13] and of minerals [14]. Persistent oxidative stress, likely depletes non-enzymatic antioxidant resources even in mild and moderate IBD. The situation is different with enzymatic antioxidants. It seems that resources of the enzymatic antioxidant system are greater, and intensification of serum activity of glutathione peroxidase [15], of SOD and catalase was demonstrated [7] in active IBD. In the present study the significant increase of SOD and catalase erythrocyte activities was observed in patients with active UC in comparison with patients in remission and control subjects. Only in a few of the patients with severe UC were the levels of catalase and SOD activity were lower or comparable with the levels in control group and, probably, only in these patients are the resources of the antioxidant system depleted. The increase in conditional AI in patients with active UC confirms large adaptive possibilities of the enzymatic antioxidant system.

Although there are a lot of studies dedicated to oxidative stress in IBD we found only one article in Medline sources where the markers of oxidative stress and antioxidant activity were evaluated in the evolution of Crohn's disease [15], and none – in the evolution of UC. The results obtained in the study above demonstrated an enhanced oxidative stress in patients with active disease with decrease down to normal level of lipid peroxidation markers in remission. In our study we confirm this finding for UC: MDA and catalase are significantly higher in the patients with active UC than in the control group with no significant difference in their activity between patients in remission and the control subjects. The normal levels of MDA and catalase can be proposed as markers of a stable, complete disease remission in UC.

Conclusions

1. Significant increase in the metabolite of lipid peroxidation, MDA, in patients with active disease in comparison with control subjects argues the presence of enhanced oxidative stress in active UC.

2. Activation of enzymatic antioxidant system is characterized for active UC, which is confirmed by increase of superoxide dismutase and catalase activity in patients with active disease in comparison with control group.

3. There is no significant difference in MDA and catalase activity between patients in remission of UC and the control subjects.

4. The normal levels of MDA and catalase can be proposed as markers of complete disease remission in UC.

Achnowledgements

The authors achnowledge Vera Gratii, dr. biol. for the performing of biochemical tests.

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Manuscript received January 30, 2011; revised April 14, 2011