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TOTAL SCAVENGER CAPACITY OF ERYTHROCYTES AND PLASMA IS A GOOD PREDICTIVE FACTOR IN INFLAMMATORY BOWEL DISEASES

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Free radical reactions are involved in immuno-inflammatory processes e.g. respiratory burst of tissue macrophages, enteroendocrine biochemical pathway, arachidonic acid cascade and the P450 system of the intestinal tract. However nutrients can also contain free radical precursor molecules.

The total scavenger capacity is a result of multifactorial defence mechanisms against harmful free radicals, both of internal and external origin. Tissues, cells and subcellular particles exhibit different specific defence activity in pathological processes, which involve free radical formation. In this study the antioxidant defence system was measured in erythrocytes and plasma of patients with Crohn's disease and ulcerative colitis. A chemiluminesce method was developed using a Berthold type luminometer for screening.

22 patients (13 males, aged 34.1 ± 11.1 and 9 females, aged 41.4 ± 10.4) with different forms of Crohn's disease (7 patients inactive, 10 moderate and 5 severe) were investigated. Another cohort of 22 patients (10 males, aged 35.9 ± 11.1 and 12 females, aged 40.1 ± 15.9) with ulcerative colitis were also assessed into three groups according to the severity of the disease: 4 inactive, 10 moderate and 8 severe. 26 healthy individuals (10 males, aged 26.6 ± 11.3 and 16 females, aged 34.24 ± 13.62) served as control.

In Crohn's disease we did not find correlation between the low scavenger capacity of erythrocytes and the severity of the disease, while scavenger capacity of the plasma showed significant alterations. In ulcerative colitis the total scavenger capacity of erythrocytes showed a non-significant increase in the inactive and moderate stages. It is assumed that in Crohn's disease the defensive antioxidant capacity of the erythrocytes is exhausted due to chronic inflammation, a long life-cycle or a possible bone marrow defect. Evaluation of chemiluminescent studies was combined with assessment of clinical data, vitamin A and E levels, and determination of the erythrocyte catalase and glutathione peroxidase activities.

INTRODUCTION

In terms of genetic background and neuroendocrine regulation of immune reactivity to the antigens of alimentary and bacterial origin, the inflammatory intestinal diseases can be considered as multifactorial (Nielsen & Rask-Madsen, 1996; Stread, Perdue, Cooke, Powell & Barrett, 1992). The expression of genes encoding cytokines (IL-1 beta, IL-6, IL-8, tumor necrosis factor alpha products), the role of circulating antiinflammatory cytokine IL-10, and the regulation of prostaglandin and leucotriene pathways by ω-3 fatty acids occurring in food are well known. Furthermore, the role of free radical reactions and antioxidant defensive mechanisms of the intestine is also well documented in the literature (Sher, D'Angelo, Stein, Bailey, Burns & Wise, 1995; Kucharzik, Stoll, Lugering & Domschke, 1995; Holtkamp, Stollberg & Reis, 1995; Hommes, Melman, de Haas, Kate, von dem Borne, Tytgat & Deventer, 1996; Pereira, Cassell, Engelman, Sladen, Murphy & Dowling, 1996). Modern surgical interventions and therapy make permanent remission possible. Erroneous nutritive habits, lipid rich diet, and a concomitant consumption of animal fatty acids and cholesterol together with retinol, as well as a high intake of carbohydrates significantly increase the relative risk both in ulcerative colitis and in Crohn's disease (Tragnone, Valpiani, Miglio, Elmi, Bazzocchi, Pipitone & Lafranchi, 1995; French, Parrott, Kielo, Rajotte, Wang, Thomson & Clandinin, 1997; Lorenz-Meyer, Bauer, Nicolay, Schulz, Purrmann, Fleig, Scheurlen, Koop, Pudel & Carr,

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1996; Reif, Klein, Lubin, Farbstein, Hallak & Gilat, 1997).

Aminosalicylates such as 5-aminosalicilic acid (5-ASA), sulphasalazine, 4-ASA and olsalazin used in therapy exhibit superoxide scavenger properties; furthermore, 5-ASA and benzalasin also scavenge hydroxyl radicals (Pearson, Jourd'heuil & Meddings, 1996; Allgayer, Hofer, Schmidt, Bohne, Kruis & Gugler, 1996). This medication can facilitate the depleted mucosal antioxidant protective function, which develops in inflammatory bowel diseases (IBD). Biopsies obtained from inflammatory parts of mucosa both in Crohn's disease and in ulcerative colitis show lower levels of urate, glutathione and ubiquinol-10. Ascorbic acid concentration was also decreased in samples obtained from inflammatory bowel in the IBD patients. Furthermore, reduction of dehydroascorbic acid by GSH/NADPH dependent dehydroascorbic acid reductase significantly decreased in inflamed mucosa, whereas alpha tocopherol content remained unchanged compared to controls (Hoffenberg, Deutsch, Smith & Sokol, 1987; Buffinton & Doe, 1995a, 1995b; Bhaskar, Ramakrishna & Balasubramanian, 1995). A scavenger isoenzyme glutathione-S-transferase μ, is dominantly inherited and is expressed in approximately half of the population. Lower incidence of GST expression was detected in patients with ulcerative colitis and Crohn's disease (Hertervig, Nilsson & Seidegard, 1994). This finding is associated with a more severe clinical course leading to colectomy. Superoxide dismutase and catalase enzyme activities are also decreased in inflammatory bowel.

Malnutrition is a characteristic symptom in IBD, which affects the free radical/antioxidant balance of the organism (Bhaskar *et al.*, 1995; McKenzie, Baker, Buffinton & Doe, 1996).

Therefore a simple method of luminol-chemiluminescence was used to study the antioxidant protective mechanisms in patients with IBD. Chemiluminescence is not a new method; it has been introduced into clinical practice about two decades ago (van Dyke, 1985; Maxwell, Wiklund & Bondjers, 1994; Horváth & Blázovics, 1993; Okabe, Maeda, Okada, Ueki, Sakurai, Matsui & Yao, 1991).

Luminol-dependent chemiluminescence, as an indicator of a free radical reaction is suitable for detection of antioxidant status of the tissues. The H_2O_2/$^{\cdot}OH$-microperoxidase-luminol system can be used to measure the total scavenger capacity of the plasma and erythrocytes. We have also determined the vitamin A and E concentrations in the serum. Glutathione peroxidase and catalase activities were measured in the erythrocytes.

MATERIALS AND METHODS

Materials

Cumene hydroperoxide, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), luminol, and microperoxidase were obtained from SIGMA (St. Louis); serum bovine albumin from CALBIOCHEM (La Jolla). All other reagents were purchased from Reanal (Budapest).

Patients

26 Caucasian volunteers (10 males aged 26.6 ± 11.3 and 16 females aged 34.2 ± 13.6) served as a control group. Patients with both diseases were divided into three groups of severity using the CDAI activity index: inactive, moderate and severe. The CU activity index was also calculated (Best, Becktel, Singleton & Kern, 1976).

22 patients had ulcerative colitis of different severity: inactive – 1 male aged 19, and 3 females aged 48.0 ± 23.0; moderate – 3 males aged 36.3 ± 16.5, and 7 females aged 39.5 ± 14.7; severe – 4 males aged 37.7 ± 6.8, and 2 females aged 30.0 ± 5.6; 2 other male patients had extremely severe form of the disease (54.0 ± 35.3). 22 other patients had Crohn's disease: inactive – 4 males aged 33.5 ± 13.0, and 3 females aged 36.5 ± 9.2; moderate – 7 males aged 33.4 ± 11.3, and 3 females aged 50.3 ± 6.8); severe – 2 males aged 38.0 ± 12.7, and 3 females aged 33.3 ± 8.7.

Permission number: TUKEB 24/1996.

Therapy

All patients received same standard therapy recommended by WHO.

Patients with inactive ulcerative colitis and Crohn's disease were treated with 5-ASA and 5-ASA + immunosuppressor Imuran respectively. The therapy of patients with moderate ulcerative colitis and Crohn's disease consisted of 5-ASA + local steroid and 5-ASA + Imuran + local antibiotic Klion. The therapy of patients with severe ulcerative colitis or Crohn's disease was: 5-ASA + local steroid or 5-ASA + systemic steroid, and steroid + antibiotics (Klion or Cyprobay) + elementary diet.

Separation of plasma, sera and erythrocytes

All blood samples were obtained by venipuncture. Blood was collected into vacutaine tubes containing citrate for the plasma (Vacuette/Greiner

9NC Coagulation) and into native tubes for the sera (Vacuette/Greiner Serum Beads). The plasma and sera were centrifuged immediately at 4°C at 3000 rpm for 10 min using the standard method. The erythrocytes (RBC) were separated and washed three times with isotonic (0.9%) NaCl solution. After washing and centrifugation procedures (10 min at 3000 rpm) the hemoglobin content of RBC was determined by Haemisol Reagent Kit (HUMAN Vaccine Producing and Research Institute, Gödöllõ).

The haemoglobin content was adjusted to 1 g% uniformly.

Chemiluminescence study

A recently developed chemiluminescence assay adapted to a Berthold Lumat 9501 instrument was applied for determination of the total scavenger capacity of the plasma and RBC to assess the antioxidant deficiency in patients with intestinal diseases (Horváth & Blázovics, 1993; Blázovics, György, Zsinka, Biacs, Földiák & Fehér, 1989).

Procedure was carried out by the method of Blázovics, Kovács, Lugasi, Hagymási, Bíró & Fehér (1999). The first trigger solution of H_2O_2 (0.30 ml, 10^4 dilution) is sucked from the tank and kept before the measuring chamber in the first position, similarly, the second trigger solution of microperoxidase (0.30 ml, 1 mmol/l) as a catalyst is sucked and kept in the second position. The light emission is started by adding the alkaline luminol solution (pH 9.8) (in 0.050 ml of 7×10^{-5} mol/l) into a polystyrol tube and the mixing is started just in front of the photomultiplier, after pressing the button of the dispenser arm. Photon output is accumulated during 30 sec and expressed in RLU. (RLU is a relative light unit corresponding to the count recorded at the anode of photomultiplier, divided by ten and multiplied by the RLU factor, which allows compensation for inevitable individual fluctuations of the cathode sensitivity of the photomultiplier as determined by the Berthold Company.) All reaction components were prepared in doubly distilled water. The solution of the reaction system must be protected from direct light, and the system is stable at room temperature (22–25°C) for at least 1 hour. The volumes of plasma and erythrocyte samples were 0.15 and 0.05 ml respectively. The biological samples were added to luminol solution and mixed with vortex (10 sec) before fixing the tube in a holder. Plasma and erythrocytes of patients were separated with centrifuge (see below).

Routine laboratory data

The following laboratory parameters were used to calculate the BEST index and the CU activity index: total protein, albumin, γ-glutamyltransferase, SGOT, SGPT, alkaline phosphatase, glucose, creatinin, total bilirubin, direct bilirubin, WBC, lymphocytes granulocytes, RBC, haemoglobin, MCV, MCH, MCHC hematocrit, platelets, Ca, Na, K (Diagnosticum Rt, Budapest). Laboratory studies were performed on a HITACHI 717 Analyser.

Enzyme measurements

Glutatione peroxidase activity was determined according to Sedlak and Lindsay (1968), and Chin, Stults & Tappel (1976). The assay is based on the oxidation of reduced glutathione by glutathione peroxidase using cumene hydroperoxide as oxidizing agent. The remnant reduced glutathione was measured with Ellmann's reagent spectrophotometrically at λ = 412 nm. Enzyme activity is given as μmol of reduced glutathione consumed in one minute/mg protein.

Spectrophotometric determination of catalase activity (λ = 230 nm) was based on decomposition of hydrogen peroxide (Beers & Sizer, 1952). The enzyme activity expressed in Bergmeyer units [BU] is defined as amount of hydrogen peroxide (in grams) consumed in one minute/gram protein sample.

Protein content of sera and plasma was measured by the method of Lowry, Rosebrought, Farr & Randall (1951), using bovine albumin as a standard.

Determination of vitamin A and vitamin E

Vitamin A and vitamin E content in the sera was determined by HPLC methods using a GILSON MODULAR and a UV-115 type detector. Simultaneous determination of vitamin A and E was carried out by method of Rudy, Ibarra, Zeigler, Howard & Argyle (1989). 0.2 ml of serum samples were extracted with hexane, the extracts were separated with ethanol : water (200 : 20) and 0.2 ml of the sample was injected in a BSP C18 column.

Statistical analysis

One-way ANOVA statistical analysis was applied to evaluate the significance between patient's groups.

For two-variable statistical analysis linear regression ($y = a + bx$, R^2), exponential regression ($y = \ln a + bx$, R^2), and logarithmic regression ($y = a + b\ln x$, R^2) were used.

All the tables present means ± standard deviation (S.D.)

Each measuring point represents five parallel data in luminol-dependent chemiluminescence experiments. The figures show the means when c.v.% was under 5.00%. Significance level was determined at $P < 0.05$.

RESULTS

The four following diagrams show the parameters of the applied methods for detection of total tissue scavenger capacity by luminometry.

Fig. 1 shows the concentration dependence of luminol chemiluminescence in RLU (relative light unit) as explained above. Mathematical analysis of luminol dependent chemiluminescence according to log-log transformation between luminol concentration and chemiluminescence intensity showed a linear correlation:

$$y = 1E+06x + 3E+06;\ R^2 = 0.9737;\ P < 0.05$$

Fig. 2 informs about the role of microperoxidase catalysed the H_2O_2/$^{\cdot}OH$ reaction in this system. Linear regression analysis showed:

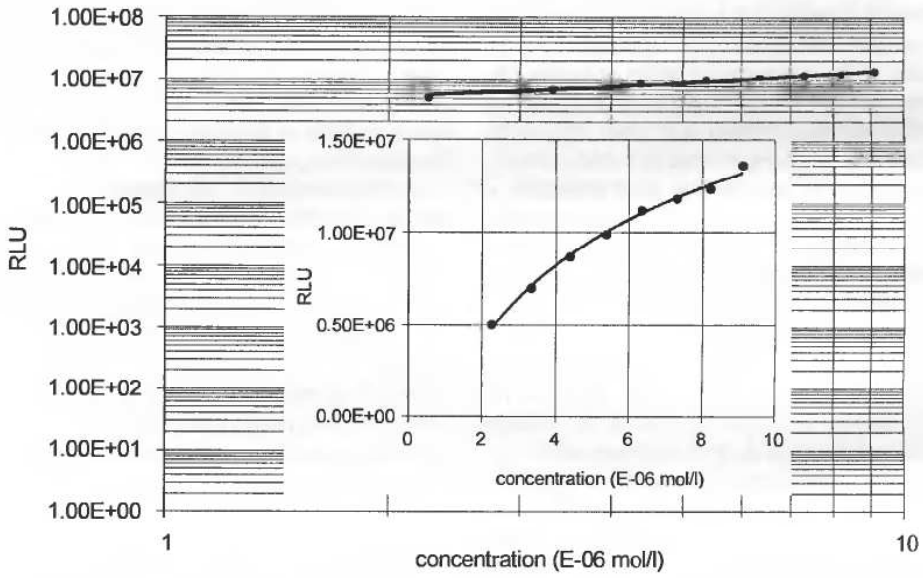

Fig. 1. Dependence of chemiluminecence on the luminol concentration. The reaction mixture contained an equivalent concentration of H_2O_2 (10^4 dilution) in a 300 µl volume, equivalent concentration of microperoxidase (1 mmol/l) in 300 µl and different concentrations of luminol (plotted on the x-axis) in a total volume of 650 µl. The reaction was performed at room temperature. Measurement time 30 sec

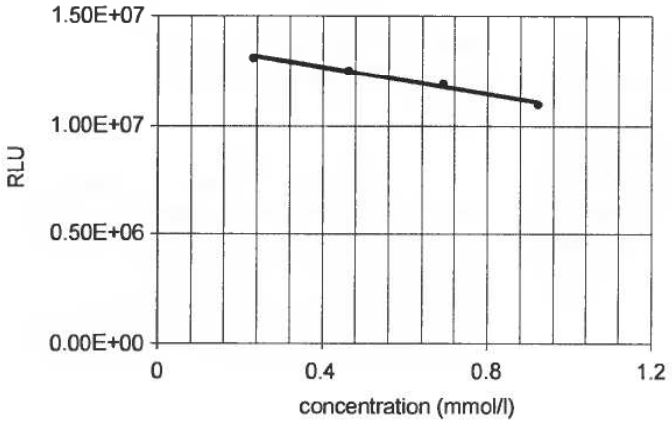

Fig. 2. Dependence of chemiluminecence on the microperoxidase concentration. Reaction mixture contained an equivalent concentration of H_2O_2 (10^4 dilution) in 300 µl, equivalent concentration of luminol (7×10^{-5} mol/l) in a 50 µl volume and different concentrations of microperoxidase (plotted on the x-axis) in a total volume of 650 µl. The reaction was performed at room temperature. Measurement time 30 sec

$y = -3E+06x + 1E+07$; $R^2 = 0.9831$; $P < 0.05$

$y = 75198x + 7E+06$; $R^2 = 0.8661$; $P < 0.05$

Hydrogen peroxide concentrations significantly affect the chemiluminescent light in the applied concentration range ($10^5 \rightarrow 10^3$ dilutions) (Fig. 3).

After a log-log transformation linear connection could be observed:

The diagram in Fig. 4 shows time dependence of chemiluminescent reaction at room temperature with standard concentration of luminol, microperoxidase and hydrogen peroxide. Mathematical analysis showed:

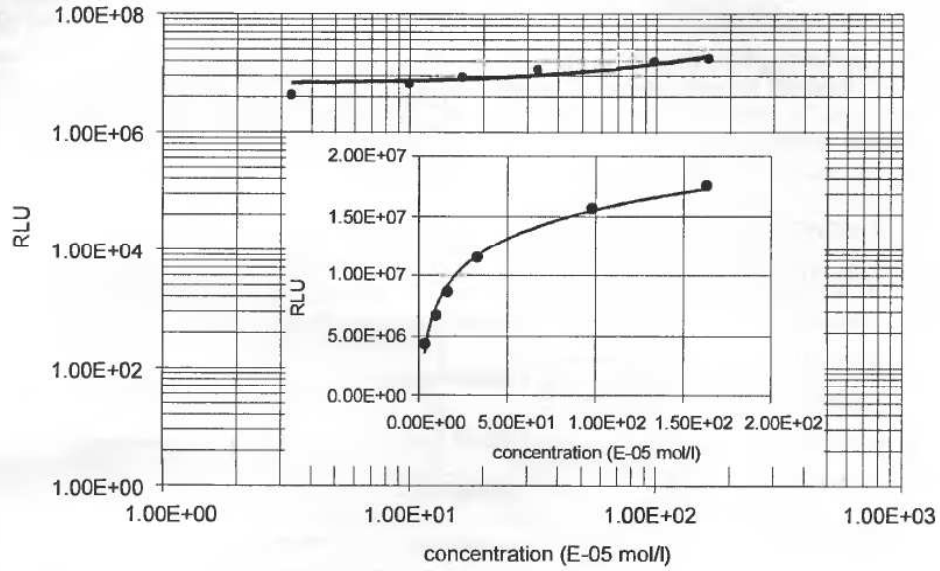

Fig. 3. Dependence of chemiluminecence on H_2O_2 concentration. Reaction mixture contained equivalent concentration of luminol (7×10^{-5} mol/l) in a 50 µl volume, equivalent concentration of microperoxidase (1 mmol/l) in a 300 µl volume and different concentrations of H_2O_2 (plotted on the x-axis) in a total volume of 650 µl at room temperature. Measuring time 30 sec.

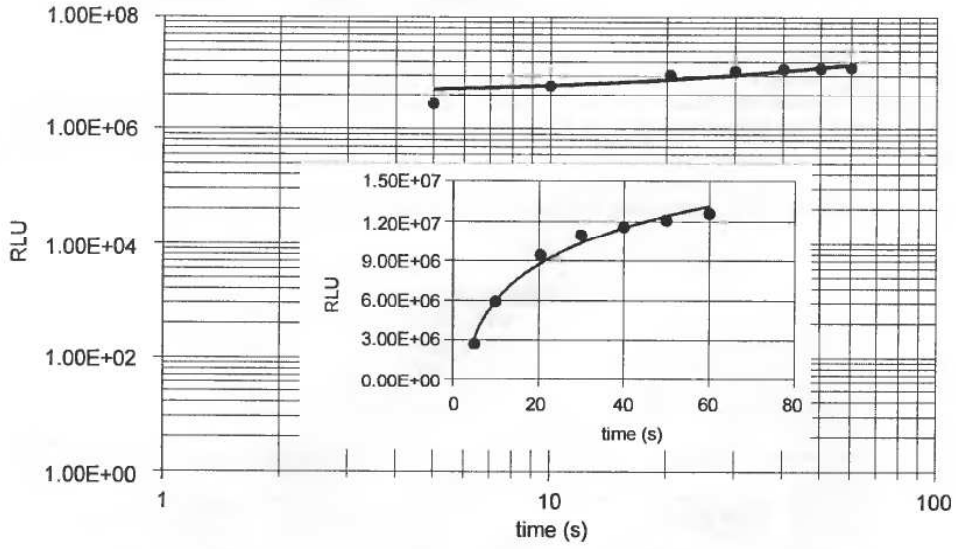

Fig. 4. Time dependence of chemiluminescence. Reaction mixture contained equivalent concentration of luminol (7×10^{-5} mol/l) in a 50 µl volume, standard concentration of H_2O_2 (10^4 dilution) in a 300 µl volume and standard concentration of microperoxidase (1 mmol/l) in a 300 µl volume in a total volume of 650 µl. The reaction was performed at room temperature. Measuring time is plotted on the x-axis

$y = 161900x + 4E+06$; $R^2 = 0.8135$; $P < 0.05$

The standardised chemiluminescence was decreased by standard solutions of bovine serum albumin (Fig. 5), which indicates that the system is suitable for determination of the total scavenger capacity. Regression analysis between scavenger solution and remnant chemiluminescence light intensity showed an exponential function:

$y = 2E+07e^{-0.4256x}$; $R^2 = 0.9238$; $P < 0.05$

All laboratory examinations of the patients were carried out under adequate therapy.

Neither erythrocyte catalase, nor GSH-Px activities in ulcerative colitis and Crohn's disease did differ from those in the control patients (Table 1).

Although the concentrations of vitamin A and vitamin E in sera were lower in Crohn's disease than in ulcerative colitis, we failed to find significant differences, compared to the control (Table 2).

The total scavenger capacity of the erythrocytes indicated serious disease even despite antioxidant therapy (5-ASA or adequate medicine) in all

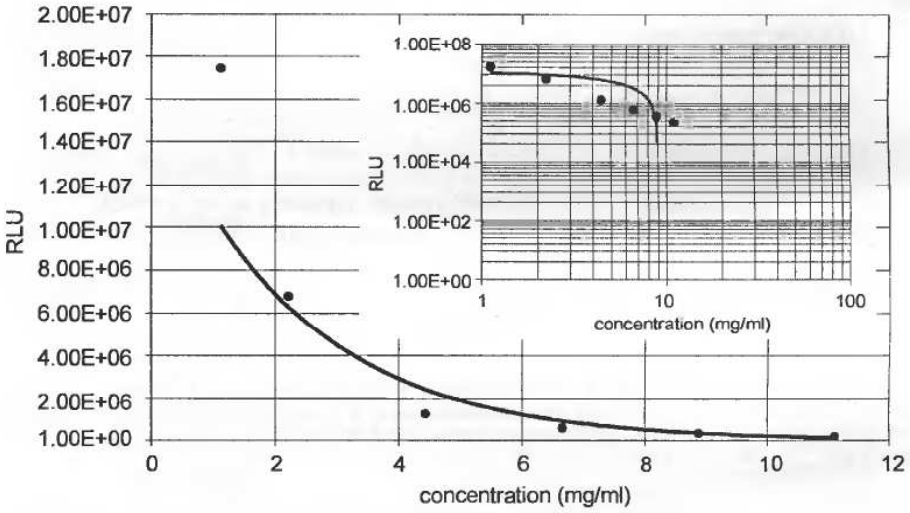

Fig. 5. Effect of bovine serum albumin on chemiluminscence. The volume of the reaction mixture was increased by 150 µl, yielding a total volume of 800 µl. The reaction mixture contained equivalent concentration of H_2O_2 (10^4 dilution) in 300 µl, equivalent concentration of microperoxidase (1 mmol/l) in 300 µl, equivalent concentration of luminol (7×10^{-5} mol/l) in 50 µl and different concentrations of serum bovine albumin. The measurement was carried out at room temperature in 30 sec

Table 1. Activities of H_2O_2-decomposing enzymes in erythrocytes of patients with inflammatory bowel diseases

Samples	Erythrocyte parameters	
	Catalase (BU/g prot.)	GSH-Px (U/mg prot.)
Control (n = 13)	6.44 ± 0.87	17.58 ± 1.64
Ulcerative colitis (n = 22)	6.15 ± 1.25	17.00 ± 2.65
Crohn's disease (n = 22)	6.23 ± 0.88	17.45 ± 3.00
Significance: (P < 0.05)	n.s.	n.s.

Table 2. Concentration of vitamins A and E in blood plasma of patients with inflammatory bowel diseases

Samples	Serum parameters	
	Vitamin A (µmol/l)	Vitamin E (µmol/l)
Control (n = 13)	1.86 ± 0.66	23.91 ± 10.45
Ulcerative colitis (n = 22)	1.96 ± 0.63	32.07 ± 7.36
Crohn's disease (n = 22)	1.31 ± 0.37	27.75 ± 8.58
Significance: (P < 0.05)	n.s.	n.s.

phases of Crohn's disease and in severe ulcerative colitis. After therapy there was a significant decrease of chemiluminescent light even in a moderate form of ulcerative colitis indicating a better total scavenger capacity, compared to the severe phase. In the inactive form of ulcerative colitis the measured TSC expressed in RLU% approached the control value (Table 3). Similar chemiluminescent phenomena were observed in total scavenger capacity of the plasma in the severe forms of both types of IBD (Fig. 6 and 7). The RLU % values plotted on the y-axis showed a progressive impairement in the total scavenger capacity in the severe forms of both diseases. Two patients with ulcerative colitis had extremely high chemiluminescence intensity values in the plasma (RLU% ~70) in the applied medium (H_2O_2/˙OH-luminol-microperoxidase) (Fig. 7).

The above finding might be explained that these two patients received therapy only few days before checking their plasma samples.

DISCUSSION

Activities or concentrations of individual antioxidants *per se* (enzymes, vitamins or function groups) do not represent the total antioxidant status of the specimens. It has been found that in the colonic mucosal biopsy of the patients with ulcerative colitis only glutathione transferase activity was significantly decreased, whereas catalase, glutathione peroxidase and glutathione reductase activities remained unchanged, compared to the normal specimens (Buffinton & Doe, 1995a; Bhaskar *et al.*, 1995; McKenzie *et al.*, 1996). In

Table 3. Effect of erythrocyte on chemiluminescence intensity in hydrogen peroxide/hydroxyl-luminol-microperoxidase system (RLU%)

	Crohn's disease		
	Inactive (n = 7)	Moderate (n = 10)	Severe (n = 5)
	107.73 ± 14.01	95.22 ± 7.47	110.95 ± 20.43
		Significance: (P < 0.05)	control vs. inactive
			vs. moderate
Control (n = 26)			vs. severe
31.19 ± 6.08	Ulcerative colitis		
	Inactive (n = 4)	Moderate (n = 10)	Severe (n = 18)
	40.49 ± 6.25	61.33 ± 6.62	110.68 ± 25.62
		Significance: (P < 0.05)	control vs. moderate
			vs. severe

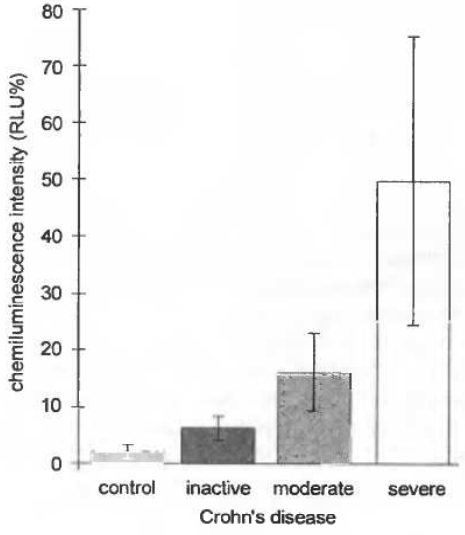

Fig. 6. Effect of blood plasma on the chemiluminescence. Control vs. moderate form of the disease (P < 0.10)

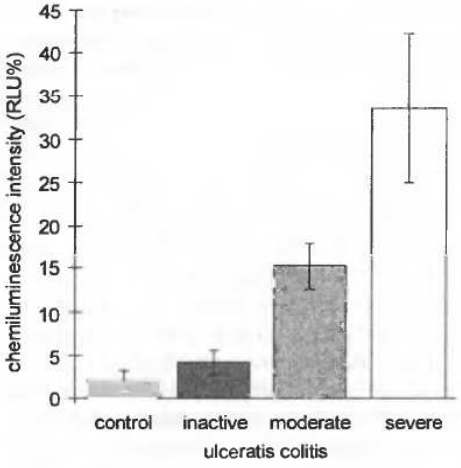

Fig. 7. Effect of blood plasma on the chemiluminescence. Control vs. moderate form vs. severe form (P < 0.05)

the rectal tissue myeloperoxidase activity was increased due to neutrophil infiltration, but malondialdehyde concentration was unchanged (van de Wal, van der Sluys Veer, Verspaget, Mulder, Griffioen, van Tol, Pena & Lamers, 1993). In the inflammatory intestinal diseases the α-tocopherol level also remained unchanged, while in Crohn's disease the total peroxyl radical scavenging capacity and the urate concentration were lowered; in mucosal samples of patients with ulcerative colitis the total glutathione level was reduced (Buffinton & Doe, 1995a). Metallothionein content in mucosa was also lower in the active state of IBD. At the same time tissue SOD activity was not changed in the mucosa of patients with ulcerative colitis, compared to the control samples (Mulder, van der Sluys Veer, Verspaget, Griffioen, Pena, Janssens & Lamers, 1994). Selenium status was also lower in patients with Crohn's disease compared to the control group (Rannem, Ladefoged, Hylander, Hegnhoj &, Jarnum, 1992).

The healthy plasma and erythrocytes are rich in antioxidants, their major types, however, are different. Therefore a variety of studies is required to evaluate the redox state of the biological samples. The assays are very expensive and time consuming, furthermore the results are sometimes contradictory. The present chemiluminescent method has been developed to determine the total scavenger capacity (TSC) in a minute volume of plasma or erythrocyte suspension. This simple routine measurement for rapid tests is performed at room temperature in 30 sec. The method is based on luminol chemiluminescence, when the reaction of hydrogen peroxide with luminol is catalysed by microperoxidase. In a Fenton-type reaction the $^{\cdot}OH$ radicals react with luminol yielding an aminophthalate anion and light quantum, which can be detected in the course of the reaction. The biological samples can scavenge free radicals with a moderate light emission in a concentration-dependent manner. The linearity can be observed after a log/log transformation.

The scavenger capacity of the samples obtained from healthy individuals and patients should be expressed in RLU% (relative light unit %) of the standard (basic chemical reaction).

The above method is suitable for assessment of free radicals and antioxidant balance in patients with IBD. Glutathione peroxidase and catalase activities determined in the RBC of the patients did not differ from those of the healthy subjects.

The scavenging function of erythrocyte suspension was significantly lower in the severe and moderate phases of Crohn's disease and slightly lower in the inactive stage. Similar to the control, erythrocytes of the patients with inactive form of ulcerous colitis had a better total scavenger capacity. Exhaustion of the scavenger capacity of the organism despite the therapy observed in Crohn's disease allows to assume that inflammatory processes affect the total antioxidant status of erythrocytes. Plasma chemiluminescence studies reflect rather the momentary situation (momentary improvement) in the course of therapy of Crohn's disease. This chemiluminescent method is suitable for evaluation of homeostasis with or without antioxidant or streroid therapy, furthermore relapses can be distinguished from inactive periods. These data are in concordance with the literature reports (Pearson *et al.*, 1996; Allgayer *et al.*, 1996; Eliakim, Karmeli, Ohon & Rachmilewitz, 1993). Due to their function erythrocytes protect themselves against free radicals of external or internal origin, therefore intact erythrocytes play an important role in normal homeostasis.

Erythrocytes have superoxide dismutase and catalase activities and contain Se, Zn and vitamins E and A. In chronic diseases, such as inflammatory bowel diseases, both maturation and function of erythrocytes are impaired. These processes depend on the incidence of bleeding. Decreased erythrocyte membrane fluidity in the lipid bilayer was demonstrated justified by electron-spin resonance spectroscopy. Significant alterations in the lipid compounds were also found in the Crohn's disease, although the cholesterol/phospholipid molar ratio was not changed (Aozaki, 1989). A lower level of the lipid compounds of plasma lipoproteins, especially of polyunsaturated fatty acids was also reported. Lipid alterations in the plasma and RBC membrane persisted even in the inactive phase of the disease (Belluzzi, Brignola, Campieri, Gionchetti, Rizzello, Boschi, Cunanne, Miglioli & Barbara, 1994). The low level of the folic acid in the erythrocytes contributed to the impairment of lipid metabolism (Tominaga, Iida, Aoyagi, Kohrogi, Matsui & Fujishima, 1989). Zn status of the erythrocytes showed no significant changes both in Crohn's disease and ulcerative colitis, while certain minor changes were observed in the plasma Zn levels in both diseases (Ainley, Cason, Carlsson, Slavin & Thompson, 1988). In Crohn's disease selenium deficiency was also observed in the plasma and erythrocytes (Jacobson & Plantin, 1985).

CONCLUSIONS

A non-invasive determination of the total scavenger capacity in plasma and erythrocytes do not bother the patients. This method can be useful in staging of the diseases and therapy planning in patients with inflammatory intestinal diseases.

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