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ARTICLE

Redox homeostasis in gastrointestinal diseases

Anna Blázovics[1]*, Ágota Kovács[2], Ágnes Szilvás[3]

[1]II. Department of Medicine, Semmelweis University, Budapest, Hungary [2]Péterfy Sándor Hospital, Department of Gastroenterology, Budapest, Hungary [3]Saint John Hospital, Department of Gastroenterology, Budapest, Hungary

ABSTRACT A lot of information is spread all over the world by papers and by other media about oxidative stress and antioxidant defence as well as their connection to human health and diseases, although only a few examine redox homeostasis from this point of view, because of expenses. We offer a cost efficient simple methodological triad "DPPH radical scavenging ability, reducing power and induced chemiluminescent intensity" in plasma and red blood cells as a program to evaluate the individual requirements for correct self control. We are able to evaluate with these global methods the differences between redox homeostasis of inactive, moderate and severe phase of IBD patients, a circadian rhythm in seasons of patients, deviant food consumes and initial state of tumourous processes as well as post operative and metastatic states.
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KEY WORDS

DPPH radical scavenging ability
reducing power
induced chemiluminescent intensity
gastrointestinal diseases

Redox homeostasis can be considered as the cumulative action of free radicals and antioxidant defences, providing a suitable condition for life (Blázovics 2007). Moderate oxidative stress is important in signal transduction pathways and essential for proliferation and apoptosis. Oxidative stress can induce stress response genes, and moderate oxidative stress by down regulating the gene expression of several genes. DNA synthesis, selective gene expression, enzyme activation and modification of cell proliferation are involved in redoxi signal mechanisms. Moderate free radical production can modify the function of kinases or directly activate the transcription factors, thereby also influencing the gene regulation in the nucleus (Powis et al. 1997; Suzuki et al. 1997; Sebolt-Leopold et al. 1999; Straus et al. 2000; Haddad 2002; Horbinski and Chu 2005).

The presence and absence of some transition metal and non-metal elements significantly modify the signal transduction processes therefore, their optimal tissue concentrations are not doubtful (Pena et al. 1999; Kudrin 2000).

Redox homeostasis can be examined with a cost efficient simple methodological triad "DPPH radical scavenging ability, reducing power and induced chemiluminescent intensity" in plasma and red blood cells, to evaluate the individual requirements for correct self control. Applied with these methods differences of inactive, moderate and severe phases during applied therapy in IBD patients vs. healthy controls could be made. Erythrocyte scavenging function is significantly lower in the severe and moderate phases of Crohn's disease and slightly lower in the inactive stage. Similar to the control, the patients with inactive ulcerative colitis have a better redox status of red blood cells

(Blázovics et al. 1999). A circadian rhythm in the measured parameters in seasons of patients could be found. During summer months both the defence mechanism and the free radical activity differ from those of winter months (Blázovics et al. 2007). Deviant food consumers among patients also could be picked (Blázovics et al. 2004). The results were correlated with laboratory parameters and element concentrations of Caucasian IBD patients and healthy volunteers in both genders. The antioxidant defence system is partly related to element status via enzyme activity and uncontrolled free radical reactions (Pena et al. 1999; Szentmihályi et al. 2008).

The aim of this study was to examine the differences of redox homeostasis of healthy control, other patient control, IBD patients, discovered tumourous patients, treated tumourous patients and patients suffered from metastasis with these global methods and to make a correlation with other laboratory parameters, as well as to make an expense-reducing, methodical offer to survey their conditions.

Materials and Methods

1,1-diphenyl-2-picrylhydrasyl stable radical, luminol, hydrogen peroxide and microperoxidase were obtained from SIGMA (St. Louis). Tumour markers, CEA, CA 19-9, AFP kits (LIA-mAT immunoluminometry) were obtained from LIA-mAT (Budapest). CRP (CRP/AUT-000) was obtained from Diagnosticum Ltd. All other reagents in analytical stage were purchased from Reanal (Budapest).

Patients: Adult Caucasian (years: 25 - 60) volunteers from both genders, healthy controls (N = 10), patient controls (N =

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*Corresponding author. E-mail: blaz@bel2.sote.hu

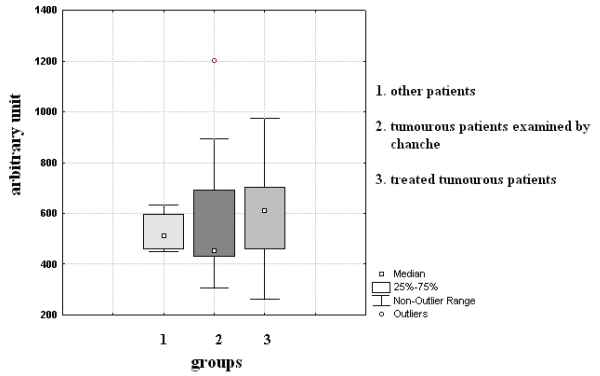

Figure 1. Glutathione peroxidase activity of red blood cells.

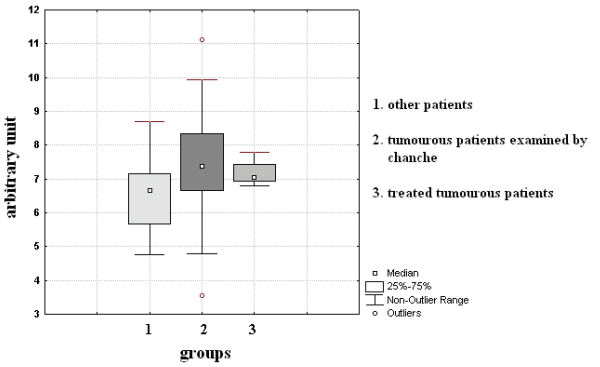

Figure 2. Superoxide dismutase activity of red blood cells.

22), discovered tumourous patients (N = 8), treated tumourous patients (N = 27), patients with metastasis (N = 6) and IBD patients (N = 113) including ulcerative colitis (34), Crohn's disease (79) were drawn into the study. They underwent routine examinations as well as 3D abdominal ultrasound examinations. Patients with positive routine or 3D ultrasound examinations underwent further examinations (endoscopy and

Table 1. Significant correlations in tumourous patients.

variable	Correlations of redox parameters of treated tumourous patients. Marked correlations are significant at p<0.05					
	RBCCL	TAS	Bile acid	SOD	GSHPx	PlasmaCL
RBCCL	1.00	0.14	-0.22	-0.26	-0.06	0.14
TAS	0.14	1.00	0.15	-0.13	0.09	-0.19
Bile acid	-0.22	0.15	1.00	-0.27	-0.24	-0.47
SOD	-0.26	-0,13	-0.27	1.00	0.47	0.38
GSHPx	-0.06	0.09	-0.24	0.47	1.00	0.29
PlasmaCL	0.14	-0.19	-0.47	0.38	0.19	1.00

RBCCL = red blood cell chemiluminescence; TAS = total antioxidant status; PlasmaCL = plasma chemiluminescence.

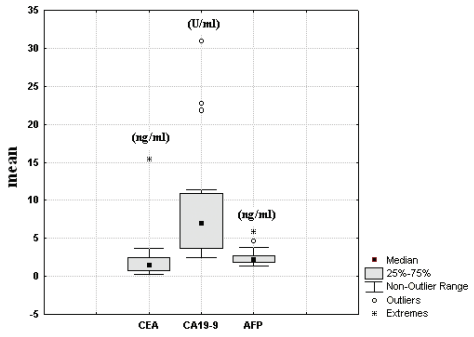

Figure 3. Tumour markers in other patients without tumours.

CT). Patients with Dukes B and C received the same recommended standard therapy after operation.

Permission number: TUKEB 167/1997; 15/2004 and IKEB 3944/2004.

Sera, plasma and red blood cells were separated using standard methods with centrifuge at 2500 rpm at 4°C. The haemoglobin content was adjusted to 10 g% uniformly for the measurements.

Routine laboratory parameters of sera were measured by Roche enzymatic in vitro assays. Tumour markers (CEA, CA 19-9, AFP / Berthold Lumat 9501 manual instrument), CRP, redox parameters were measured with routine laboratory parameters together. Plasma TAS: Randox® kit (Cat No. NX2332), superoxide dismutase RANSOD (SD125) and glutathione peroxidase RANSEL (RS505) were applied. (Randox laboratories, Ltd, Crumlin, UK).

Plasma hydrogen-donating ability (PHDA) was estimated in the presence of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the method of Hatano et al. (1988).

Oyaizu's method was adopted for the analysis of the reducing power of the plasma (PRP). The change in absorbance was measured, which accompanied Fe^{3+}-Fe^{2+} transformation at 700 nm, and the (PRP) was compared to that of ascorbic acid (Oyaizu 1986).

The chemiluminescence assay adapted to a Berthold Lumat 9501 instrument, which was applied for the determination of the total scavenger capacity of the plasma and red blood cells, to assess the antioxidant deficiency in patients with intestinal diseases. The scavenger capacity of the samples obtained from healthy individuals and patients were expressed in RLU (relative light unit) of the standard (basic chemical reaction; Blázovics et al. 1999).

Statistical analysis: All clinical tests were expressed as mean and standard deviation (SD). One-way ANOVA statistical analysis was applied to evaluate the significance between patient groups. Each measuring point represents five parallel data in luminol-dependent chemiluminescence experiments when c.v.% was under 5.00%. A value of P<0.05 was accepted as statistically significant.

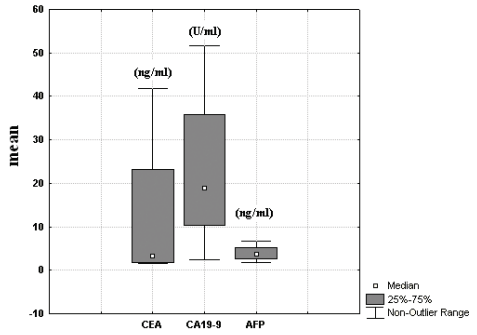

Figure 4. Tumour markers in colon tumourous patients examined by chance.

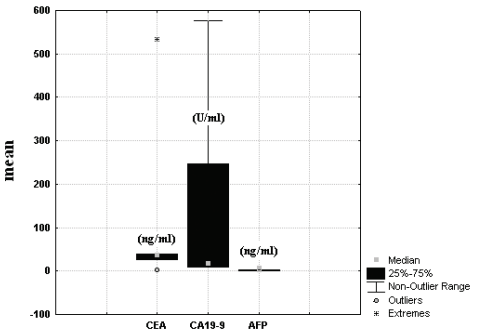

Figure 6. Tumour markers in colon metastases.

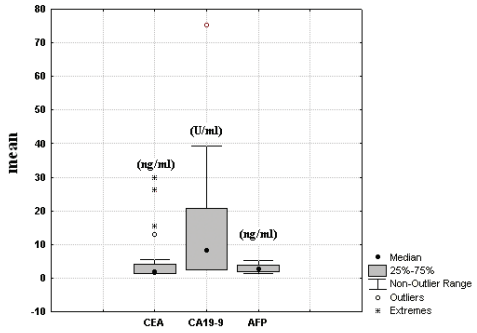

Figure 5. Tumour markers in treated colon tumourous patients.

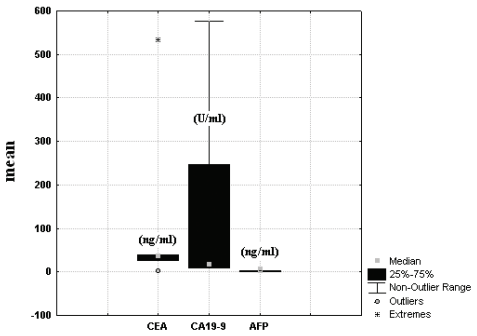

Figure 7. Stimulated chemiluminescence in red blood cells of patients with different gastrointestinal diseases.

Results and Discussion

Plasma-TAS, reducing power, H-donating ability and red blood cell GSHPx, SOD and chemiluminescence intensity (RLU) as well as serum tumour markers and laboratory parameters were determined.

Neither the activity of SOD nor GSHPx of red blood cells showed significant difference in different stages of tumourous patients, although variance was large (Figures 1. and 2.).

The results of tumour marker examinations strengthened that tumour markers showed large difference in different stages, although the highest in metastasis. Therefore tumour marker examinations are doubtful (Figures 3-6.).

We could not find any correlation between TAS or red blood cell RLU vs. tumour markers as well as between other redox parameters in cancer, whereas the correlations between redox parameters of female Crohn's patients were significant:

Table 2. Significant correlations in IBD.

positive correlation		negative correlation	
UA–HGB	(r = 0.7913; p = 0.004)	RBCCL–UA	(r = -0.7743; p = 0.009)
UA–HCT	(r = 0.8332; p = 0.001)	RBCCL – HGB	(r = -0.8214; p = 0.004)
UA–PHDA	(r = 0.6328; p = 0.020)	RBCCL – CRP	(r = -0.8987; $p < 10^{-4}$)
UA–PRP	(r = 0.6229; $p < 10^{-4}$)	RBCCL – PHDA	(r = -0.8281; p = 0.003)
PFSHG–Na	(r = 0.7472; p = 0.013)	RBCCL –PRP	(r = -0.8942; $p < 10^{-4}$)
PHDA– PRP	(r = 0.8780; $p < 10^{-4}$)	RBCCL –HTC	(r = -0.8131; p = 0.004)
RBC–HCT	(r = 0.6103; p = 0.046)	PHDA – TP	(r = -0.5783; p = 0.038)
PHDA-K	(r = 0.7040; p = 0.016)		

CRP = C-reactive protein; RBCCL = red blood cell chemiluminescence; HGB = haemoglobin; HTC = haematocrit; K = potassium; Na = sodium; PFSHG = plasma free SH group; PHDA = plazma H-donating ability; PRP = plasma reducing power; RBC = red blood cell; TP = total protein; UA = uric acid.

red blood cell SOD vs. GSHPx activities (r = 0,76), plasma RLU vs. H-donating ability (r = -0,73). In male Crohn's patients the correlation was weak: red blood cell SOD vs. plasma RLU (r = -0,51).

The correlations between parameters of plasma and red blood cells were the most weak in cancer cases. In the case of tumours in both genders, the results made us conclude that tumour growth and spreading damage components of the defence system seriously (Table 1.) and see Figure 7.

Reducing power and H-donating ability were significantly low in tumorous processes, and the reducing power was not changed significantly in different stage of IBD vs. healthy controls (174,54 ± 22,14 nmol AS). H-donating ability changed in control (60,62 ± 1,98%) vs. inactive (45,88 ± 3,0%) vs. severe (45,44 ± 3,00%) ulcerative colitis and in control (60,62 ± 1,98%) vs. severe (42,86 ± 4,52%) in Crohn's disease.

Plasma RLU vs. bile acid concentrations (r = -0,47) and red blood cell SOD vs. GSHPx (r = 0,47) activities (p<0,050) showed only weak correlations in cancerous patients.

The significant correlations in IBD can be seen in Table 2.

On the basis of results of red blood cell chemiluminescence examinations, significant difference could be established between patients with IBD and patients with colon cancer (Figure 7.). We could pick freshly discovered tumours and serious metastases.

Our previous results showed that in tumorous patients the protoporfirin IX – according to concentration – induces free radicals in small concentration and scavenges in higher concentration. At the same time, beside the high protoporphyrin concentration, HCHO (mobilized methyl group) concentration was significantly low in metastatic tumorous patients (Blázovics et al. 2008). (It was verified by Stryeer (1988), that arginine (38), methionine (65) and lysine (72) near the methionine (80) are methylated and coordinated towards the central iron of heme). Therefore it can be established, that in an early state of tumourous processes a low concentration of free protoporphyrin causes an extreme high free radical level, and the high concentration of protoporphyrin in metastasis causes a high antioxidant activity in our experimental system.

The examination of redox homeostasis will bring us closer to know more about tumorous inclinations.

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