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Surgical stress induced alterations of antioxidative and immune system parameters

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The aim of the research was to explore whether the oxidative stress reflected by alterations of antioxidative system parameters might influence production of interleukin 18 (IL-18) playing an important role in the regulation of the immune response and is possibly involved in cancer pathology. The antioxidative system parameters and IL-18 levels in the blood of 49 kidney cancer patients were investigated before and after the surgical treatment. The amount of the lipid peroxidation marker malondialdehyde (MDA) was found to decrease in the post-surgical period, where as the catalytic activity of the primary antioxidant enzyme superoxide dismutase (SOD) was increased, both indicating a relative lowering of oxidative stress. The average change of reduced glutathione (GSH) and IL-18 was insufficient. Alterations of SOD, GSH and IL-18 were also analyzed with respect to MDA changes after surgical treatment. It was shown that the level of IL-18 was sufficiently higher after the treatment if MDA content was also increased (in 25% cases of the research), although neither SOD activity nor GSH concentration showed reliable differences. The high blood IL-18 level may be suggested to be a prognostic factor of cancerogenesis progression, also provided by a stable oxidative stress. A higher level of IL-18 may also indicate possible post-operative complications. In contrast, SOD activity was enhanced with a drop of MDA level after surgery (in 75% cases) when GSH concentration was stable and IL-18 level increased moderately. The lowered oxidative stress should be suggested to modulate the increase of IL-18 level.

Key words: kidney cancer, oxidative stress, antioxidative system, interleukin 18

INTRODUCTION

Surgical intervention may disturb or impair the antioxidative and immune systems potency of the organism [1]. The oxidant–antioxidant balance is very important for different cell functions. Oxidative stress may be a reason for alterations in intracellular signaling and the production of interleukins, chemokines, cytokines, etc. Interleukins are known to play a critical role in the regulation of the immune response and therefore may influence cancer pathogenesis [2].

Malondialdehyde (MDA) is known as the main parameter showing the level of lipid peroxidation process induced by oxidative stress. The primary antioxidative enzyme, superoxide dismutase (SOD), and reduced glutathione (GSH), a powerful antioxidant, are among the most important antioxidative system parameters of the organism [3]. Oxidative stress has been noted to be among the numerous factors determining the tumour progression and to contribute to a worse tumourogenesis prognosis after surgical treatment. Consequently, all the parameters mentioned are crucial for evaluating the antioxidative system state of cancer patients [4].

Jablonska et al. [5] have showed that a high production of angiogenic factor (VEGF) was associated with a low production of IL-18 in patients with oral cavity cancer. It has been reported also that patients with IL-18 levels > or = 310 pg/ml showed a significantly lower survival rate compared with patients of IL-18 levels < 310 pg/ml after surgery [6]. IL-18 is produced not only by various immune cells but also by non-immune ones. Therefore the concentration of IL-18 in cancer patients' blood after surgical stress may contribute to our understanding of the interrelation between the immune and antioxidative systems in the cancerogenesis process and the effect of surgical treatment.

Data on the relation of IL-18 level and of MDA, SOD and GSH values as the indices of antioxidative system

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status for cancer patients are lacking. Moreover, the influence of surgical stress on the alterations of these parameters is also studied inadequately.

The subject of the research was to investigate alterations of the MDA, SOD, GSH and IL-18 levees in cancer patients prior to and after the surgical treatment.

MATERIALS AND METHODS

The antioxidative and immune system parameters of 49 kidney cancer patients were studied.

Reduced glutathione (GSH), Ellman's Reagent (DTNB), NADPH, NADH, glutathione reductase (GR), 5-sulfosalicylic acid (SSA), EDTA, KH_2PO_4, Na_2HPO_4, KOH, HCl, CH_3COOH (ice), thiobarbituric acid (TBA), 2-propanol, N-methylphenazomium methyl sulfate (PMS), nitro blue tetrazolium were from Sigma.

The antioxidant status was evaluated by measuring the level of the lipid peroxidation product malondialdehyde (MDA), the activity of superoxide dismutase (SOD) and the amount of reduced glutathione (GSH) in blood plasma and erythrocyte hemolysate. The level of MDA was measured in blood plasma by the thiobarbituric acid (TBA) assay (Sigma, Germany) based on TBA reaction with MDA resulting in the release of spectrophotometrically detectable color complexes. The reaction mixture containing 0.2 ml blood plasma, 0.8 ml H_2O distilled and 1 ml TBA (0.6% (w/v) solution in ice acetic acid) was heated in a covered test tube at 100 ^oC for 30 min. The mixture was cooled and 1 ml 5 N KOH and 2 ml 2-propanol was added. The final content was centrifuged for 20 min at 8000 and the supernatant was analyzed spectrophotometrically for absorbance at 532 nm. The blank sample contained 0.2 ml distilled H_2O instead of the blood sample. GSH amount was determined spectrophotometrically in plasma using the GSH recycling system from Ellman's Reagent (DTNB) and glutathione reductase (GR) [7]. SOD catalytic activity was evaluated measuring the rate of inhibition of the reduction of nitro blue tetrazolium (Sigma, Germany) in erythrocyte hemolysate. The incubation mixture (consisting of 37 mg EDTA, 330 mg nitro blue tetrazolium, 55 mg PMS and 0.15 M phosphate buffer, pH 7.8) was kept overnight and filtrated. 2 mM NADH solution was prepared in Tris-EDTA buffer, pH 8.0. The reaction mixture containing 0.2 ml erythrocyte hemolysate, 3 ml incubation mixture and 0.1 ml NADH solution (blank sample contained 0.2 ml distilled H_2O instead of the hemolysate) was incubated at 20 ^oC for 10 min in the dark and then was analysed spectrophotometrically for absorbance at 540 nm.

The serum IL-18 level was measured by enzyme-linked immunosorbent assay ELISA (Immunoassay kit, Biosourse, Belgium). The incubation of the blood serum sample and the substrate mixture in the wells resulted in the production of a color complex in proportion to the IL-18 concentration in the sample. The absorbance of each well was detected with a microtiter reader (Multiskan EX, Labsystem Oy) at a wave length of 450 nm.

Statistical analysis was performed using the statistical software SAS version 8.2. The differences of comparative values were estimated as reliable at $p \le 0.05$ and tendencies to differ in the corresponding indices at $0.05 < p \le 0.1$.

RESULTS AND DISCUSSION

Blood samples of 49 kidney cancer patients were collected and analyzed to determine the level of lipid peroxidation, the status of antioxidative system activity and the level of IL-18. The blood was tested prior to the surgical treatment and at least one month after the intervention in order to avoid the fluctuation of the parameters due to the surgery-induced stress. MDA was determined as the main parameter indicating the degree of lipid peroxidation caused by oxidative stress. SOD and GSH were evaluated as belonging to the most important indices of antioxidative system. IL-18 was chosen for definition as a tumour progression prognostic factor, also known as a possible post-surgical survival rate determining factor [6, 8].

Quantitative changes of all parameters mentioned before and after surgery are summarized in Table 1. The number of analytes for each parameter differed due to technical circumstances. It is evident that two of the four parameters, MDA concentration and SOD catalytic activity, showed reliable changes in the post-surgical period. The average drop of MDA and a rise of SOD values could be the markers of a relative lowering of the oxidative stress [9]. The average increase of GSH and IL-18 concentration was found to be not reliable. The main reason why the GSH parameter was changed insufficiently should be numerous other functions of the tripeptide beside its role as a powerful antioxidant. The modulation of immune system activity is known as one of those [10]. On the other hand, the change of the IL-18 parameter was also not reliable, most probably because of the interaction with other cytokines or immune cells reflecting the effect of the surgical treatment on immune system functions.

SOD and GSH parameters as well as IL-18 were analyzed with respect to MDA changes after surgery.

Table 1. **Antioxidative and immune system parameters of patients in pre- and post-surgical periods**

Patient group	n	MDA nmol/ml	n	SOD $Ux10^4$/ml	n	GSH nmol/ml	n	IL-18 pg/ml
Prior to surgery	49	17.2 ± 0.61	47	1.4 ± 0.07	33	0.3 ± 0.04	37	280 ± 21.4
Post surgery	49	14.2 ± 0.59	47	2.0 ± 0.08	33	0.4 ± 0.10	37	312 ± 27.9
p		0.001		0.001		0.723		0.168

Table 2. **Antioxidative and immune system parameters of patients in pre- and post-surgical periods with respect to MDA concentration**

a) **increased MDA after surgery**

Patient group	n	MDA nmol/ml	n	SOD $Ux10^4$/ml	n	GSH nmol/ml	n	IL-18 pg/ml
Prior to surgery	12	15.6 ± 1.08	10	1.7 ± 0.19	10	0.4 ± 0.07	10	263 ± 28.7
Post surgery	12	18.3 ± 1.44	10	2.0 ± 0.16	10	0.5 ± 0.28	10	364 ± 38.1
p		0.003		0.235		0.486		0.040

b) **decreased MDA after surgery**

Patient group	n	MDA nmol/ml	n	SOD $Ux10^4$/ml	n	GSH nmol/ml	n	IL-18 pg/ml
Prior to surgery	36	17.9 ± 0.71	36	1.3 ± 0.07	22	0.3 ± 0.05	24	276 ± 29.1
Post surgery	36	12.9 ± 0.47	36	2.0 ± 0.09	22	0.3 ± 0.05	24	301 ± 40.0
p		0.001		0.001		0.662		0.460

Two groups of analytes were separated in the order of increased (in 25% cases) or decreased (in 75% cases) MDA concentrations in the post-surgical period. The results are presented in Table 2. It should be noted that the concentration of IL-18 was sufficiently higher after surgery if the level of MDA was also increased, although neither SOD activity nor GSH concentration showed reliable differences. It could be suggested that the parameters indicating antioxidative system status are either not dependent on MDA increase or that the higher levels of the dialdehyde could tend to provide a weaker antioxidative defense [11]. In contrast, changes of the concentration of IL-18 seemed to be related to a higher MDA concentration in the post-surgical period. It may be suggested that the oxidative stress influences the production of IL-18. The higher levels of IL-18 may represent the possible post-operative complications [12]. Moreover, an increase of the lipid peroxidation parameter after surgery should be regarded as a marker of a stabile oxidative stress [9].

Different data were obtained while comparing the other three parameters with a drop of MDA (Table 2). SOD catalytic activity was reliably enhanced in this case. There was change in the GSH concentration after surgical treatment and the level of IL-18 was increased, but not signiti cantly. The decrease of MDA level and the increase of SOD activity could indicate the tendency of a lower oxidative stress in the post-surgical period [9, 13].

In principle, IL-18 enhances the IL-12-driven Th1 immune response, but it can also stimulate Th2 immune responses in the absence of IL-12. Therefore the functions of IL-18 *in vivo* are very heterogeneous and complicated [14]. But it remains unclear what physiological conditions or stimuli induce IL-18 production.

It could be concluded that the higher IL-18 level in patient's serum after surgical treatment was related to the increased MDA level. As shown previously [15], the high level of IL-18 and of MDA may be regarded as a worse prognostic factor of post-surgical survival. A more detailed investigation of the correlation of the other parameters of both systems is necessary in perspective for providing a more effective treatment and determining the prognostic factors of the disease.

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OPERACINIS STRESAS IR ANTIOKSIDACINĖS BEI IMUNINĖS SISTEMŲ RODIKLIŲ POKYČIAI

Santrauka

Tyrimo tikslas – nustatyti, ar oksidacinis stresas, atspindimas antioksidacinės sistemos rodiklių pokyčių, turi įtakos interleukino 18 (IL-18) koncentracijai pacientų kraujo serume. Antioksidacinės sistemos rodikliai ir IL-18 kiekis 49 inkstų vėžiu sergančių pacientų kraujyje nustatyti prieš ir po operacinio gydymo. Tyrimų rezultatai rodo, kad lipidų peroksidacijos žymens – malono dialdehido (MDA) – kiekis po operacijos mažėja, o pirminio antioksidacinės sistemos fermento – superoksiddismutazės (SOD) – katalizinis aktyvumas didėja, ir tai santykinai iliustruoja ir oksidacinio streso mažėjimą. Redukuoto glutationo (GSH) ir IL-18 kiekiai nebuvo statistiškai reikšmingai pakitę. Atlikta SOD, GSH ir IL-18 pokyčių analizė MDA sumažėjimo arba padidėjimo po operacijos atžvilgiu. Nustatyta, kad ir IL-18 kiekis po operacijos reikšmingai padidėjo, padidėjus MDA kiekiui (25% visų tirtų atvejų), kai nei SOD aktyvumo, nei GSH koncentracijos padidėjimo reikšmingų skirtumų nenustatyta. Literatūros duomenimis, IL-18 gali būti piktybinio proceso progresavimo ir blogesnio išgyvenimo rodiklis. SOD aktyvumas reikšmingai padidėjo po operacijos, sumažėjus MDA kiekiui (75% visų tirtų atvejų), nors GSH koncentracija nepakito, o IL-18 kiekis padidėjo nedaug. Taigi galima daryti prielaidą, kad sumažėjęs oksidacinis stresas koreliuoja su IL-18 kiekio pokyčiu.