

ACTIVITY AND CONTENT OF ANTIOXIDANT ENZYMES IN PROSTATE TUMORS

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Aim: To investigate the antioxidant enzyme system in blood of men with benign hyperplasia of prostate (BHP) and with prostate adenocarcinoma (CaP). **Methods:** The spectrophotometrical methods were applied to study content and activity changes of superoxide-dismutase (SOD), catalase (CAT), ceruloplasmin (Cp), tripeptide glutathione (GSH), glutathione-peroxidase (GSH-Px), and glutathione-reductase (GR). Lipid peroxidation was evaluated using thiobarbituric acid (TBA)-test. Blood plasma and erythrocytes of men with prostate tumors served as the material for the studies; n = 15 for each group. **Results:** SOD activity was increased in BHP (24.65 ± 1.20 U/ μ l) and decreased in CaP (11.45 ± 0.89 U/ μ l), CAT activity remained unaltered in BHP (12.41 ± 0.85 mcat/ml) and was slightly declined in CaP (9.52 ± 0.56 mcat/ml). Cp was increased in both kind of tumors, especially in CaP (54.27 ± 7.22 mg%), as well as GSH (0.736 ± 0.07 μ M/l) and GR (0.031 ± 0.002 μ M/g.Hemogl/min). GSH-Px was sharply increased in BHP (0.67 ± 0.05 μ M/g.Hemogl/min) and reduced in CaP (0.16 ± 0.01 μ M/g.Hemogl/min). **Conclusion:** The development of BHP reflects relatively weakly on blood system as activity and content of antioxidant enzymes are not revealing marked changes in this disease. The significant changes are revealed in case of CaP, showing the reduced functional state of blood antioxidant enzyme system.

Key Words: benign prostate hyperplasia, prostate adenocarcinoma, oxidative stress, antioxidant enzymes.

The oxidative stress is an accompanying process in a number of pathologies [1, 2]. This event is elicited by increased concentration of the reactive oxygen species (ROS), which induce damage of DNA, protein oxidation and lipids peroxidation. The cause-result relation between oxidative stress and specific disease is not elucidated so far. It is unclear if excess production of ROS represents one of the factors of pathology development, or, contrariwise, a disease elicits oxidative stress, as one of destructive processes accompanying a pathophysiological condition. In the last years the protective role of ROS became actual [3]. However, oxidative stress accompanied with lipid peroxidation are still viewed as a precursor of many kinds of pathology — atherosclerosis, diabetes, neurodegenerative diseases and, finally, carcinogenesis. In the latter case DNA interaction with ROS might play a critical role in malignant transformation of the cells. An oxidative stress occurs in different kinds of tumors, and is considered among the causes of carcinogenesis [4].

An oxidative stress occurring during tumor growth is reflected on content and activity of the antioxidants, including blood antioxidant enzymes: SOD [5], CAT [6], Cp [7, 8], GSH and GSH-dependent system, which encompasses GR, GSH-Px and GT [9]. The alteration displayed by the antioxidant enzymes depend on type and differentiation grade of tumor [10].

The aim of our investigation was to study the state of blood AOS in patients with BHP and CaP.

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Abbreviations used: BHP — benign prostate hyperplasia; CaP — prostate adenocarcinoma; ROS — reactive oxygen species; AOS — antioxidant system; SOD — superoxide-dismutase; CAT — catalase; Cp — ceruloplasmin; GSH — reduced glutathione; GSH-Px — glutathione-peroxidase; GR — glutathione-reductase; GT — glutathione-transferase; MDA — malondialdehyde.

MATERIALS AND METHODS

Erythrocytes isolation. The blood plasma and erythrocytes of the patients (before therapy) with BHP (n = 15) and CaP (n = 15) were studied. The patients were 60–75 years old, with primary tumors. The control group consisted from 15 healthy males of similar age. The material was obtained and diagnosis was estimated by the Georgian National Center of Urology by means of rectal, histological, and echographic examination of the prostate. The erythrocytes and plasma were isolated from 10 ml of vein blood by routine procedure [10]. The study was approved by the Ethical committee of the institute and informed consent was obtained from each patient.

Enzymes activity and content evaluation. Analysis of SOD activity was performed routinely as described in [11] and expressed in U/ μ l. The standard samples for all given enzymes (except Cp) were obtained from Sigma Chemical Co. CAT activity was analyzed with the use of hydrogen peroxide (AO “CHIMPROM”) and expressed in mcat/ml [12].

For evaluation of Cp plasma content modified Ravin method was used [13]. It implies Cp-induced oxidation of the *p*-phenylenediamine dihydrochloride (*p*-PDA, Lancaster). Cp content is expressed in mg/% [13]. GSH content was evaluated by standard approach using dithionitrobenzoic acid (DTNB), and expressed in μ M/l [14]. GSH-Px activity was evaluated as described [10] and expressed in micromols/1 g of hemoglobin/minute.

Activity of GR was assessed by routine technique [10] and expressed in micromols/1g hemoglobin/minute. Lipid peroxidation was evaluated using thiobarbituric acid (TBA-test) and expressed as amount of malondialdehyde (MDA) (μ M/L plasma) [15].

Statistical analysis. Experimental data were processed by means of standard variation statistics MINITAB (Basic statistic), $P \leq 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Investigation have shown that in case of BHP intensification of lipid peroxidation occurred as evidenced by increased MDA content by ~ 1.5-times and in CaP — by ~ 2.6-times (Table 1), compared with control group, pointing the intensified free radical processes.

Table 1. Alterations of lipid peroxidation and antioxidant enzymes: superoxide-dismutase (SOD), catalase (CAT) and ceruloplasmin (Cp) levels in blood of patients with prostate tumors

	SOD	CAT	Cp	MDA
	In erythrocytes U/ μ l	In erythrocytes mcat/ml	In plasma mg%	In plasma μ M/L
Control group	18.73 \pm 1.05	12.98 \pm 1.02	25.55 \pm 1.22	0.80 \pm 0.07
BHP	24.65 \pm 1.20	12.41 \pm 0.85	32.09 \pm 1.61	1.21 \pm 0.02
	$P < 0.001$	$P > 0.05$	$P < 0.01$	$P < 0.0001$
CaP	11.45 \pm 0.89	9.52 \pm 0.56	54.27 \pm 7.22	2.09 \pm 0.01
	$P < 0.0001$	$P < 0.01$	$P < 0.001$	$P < 0.0001$

Notes: values expressed as means \pm SEM; P value is given vs control group; n = 15 of patients in each group.

The studies of the SOD activity in the blood erythrocytes have shown that increase of SOD activity was sharply manifested in case of BHP (~ 1.3-times, $P < 0.001$), compared with the control group (see Table 1), while in CaP, activity of the enzyme decreases versus control group (~ 1.6-times, $P < 0.0001$) and BHP (~ 2.2-times, $P < 0.0001$). The CAT activity in the blood erythrocytes remained practically unaltered in BHP group (see Table 1), while in the erythrocytes of the patients with CaP, well manifested decrease of its activity occurred compared with the control group (~ 1.4-times, $P < 0.01$; see Table 1). Our studies have also shown that in conditions of BHP, in 70% of the patients, concentration of Cp in the blood plasma remains within normal range, while the mean index of Cp approaches the upper limit of normal level and is 32.09 \pm 1.61 mg/% ($P < 0,01$, see Table 1). In the CaP group, two-fold increase of the Cp concentration occurred versus normal level — 54.27 \pm 7.22 mg/% ($P < 0.001$).

Investigation of the GSH-dependent system revealed that the amount of the GSH in erythrocytes of the patients with prostate tumors, sharply increased in both, BHP (~ 1.8-times, $P < 0.01$) and CaP (~ 4-times, $P < 0.0001$) groups, as compared with the control group (Table 2). The GSH-Px activity sharply increased in BHP, as compared with the erythrocytes in both the control group ($P < 0.0001$) and CaP (see Table 2). In contrary, in a case of CaP activity of the enzyme decreased twice versus the control group ($P < 0.0001$) and ~ 4-times versus BHP group. Investigation of the GR activity in the blood of prostate tumor patients showed that it tends to elevate in parallel with aggravation of the disease ($P = 0.0001$ for BHP, $P < 0.0001$ for CaP, see Table 2).

Increase of SOD activity during BHP points at mobilization of AOS, resulted in increased production of the enzyme. Sharp decrease of SOD activity during CaP shows the contrary event — antioxidant defence

is evidently weakened during malignant transformation of prostate. A direct cause of decreased activity of SOD in the erythrocytes may be the excess of the superoxide radicals, resulted in SOD consumption, or the accumulation of hydrogen peroxide, inducing the inhibition of the enzyme.

Table 2. Alterations of glutathione-dependent enzymes (GSH-Px and GR) activity, and reduced glutathione (GSH) content in erythrocytes of patients with prostate tumors

	GSH-Px	GR	GSH
	μ M/g.Hemogl/min	μ M/g.Hemogl/min	μ M/l
Control group	0.32 \pm 0.03	0.008 \pm 0.002	0.184 \pm 0.02
BHP	0.67 \pm 0.05	0.019 \pm 0.001	0.336 \pm 0.05
	$P < 0.0001$	$P = 0.0001$	$P < 0.01$
CaP	0.16 \pm 0.01	0.031 \pm 0.002	0.736 \pm 0.07
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Notes: values expressed as means \pm SEM; P value is given vs control group; n = 15 of patients in each group.

An unequivocal increase of SOD without CAT increase in BHP group, may be a reason for accumulation of the hydrogen peroxide, and thus for aggravation of the oxidating stress (see Table 1). Respectively, it may serve as one of the causes for benign tumor transformation into the malignant one. Alteration of CAT, in case of hormone-dependent tumors, are unpredictable [16]. Decrease of CAT activity in erythrocytes during CaP, points at functional decrease of AOS as well as in case of SOD and it should be due to increased volume of the hydrogen peroxide during the clear intensification of lipids peroxidation. Another possible mechanisms of CAT activity attenuation during CaP, may be the increase of the lysosomal acidic phosphatase in blood [17], because the lysosomal enzymes, especially the acidic phosphatase, are able to influence the CAT activity and induce its inhibition.

Despite the elevation of the oxidative processes Cp content is increased in parallel with aggravation of disease (see Table 1), that arises from the fact that Cp belongs to the acute-phase proteins [18]. Biosynthesis of Cp represents a compensatory-adaptive response of an organism challenged by pathology. Therefore, exclusively high content of the Cp in the blood during the CaP should be caused by especially increased demand for Cp as for the main blood antioxidant. Furthermore, the hormonal shifts occurring in prostate tumors, may represent one of the possible reasons of Cp content changes. Influence of estradiol results in increased biosynthesis of Cp in the liver at the transcription level, therefore elevated level of estradiol which takes place in prostate tumors, as hormone-dependent tumors may induce increase of Cp amount in the blood.

Being a major intracellular antioxidant GSH acts as a strong antitoxicant conjugating toxic compounds, cytostatics, carcinogens, mutagens, drugs and etc; [9]. Therefore, the increase of GSH along with disease aggravation, is the defence reaction directed to the cell protection from oxidizing stress and impact of toxic substances. One of additional causes of GSH concentration growth during prostate tumors might be increased proliferation of the tumor cells, since glutathione system controls the cell cycle and its concentration increases in parallel with cellular prolifera-

tion [9]. The steroid hormones, especially estrogens, promote the growth of GSH content and GR activity as well [19].

GSH-Px fulfills detoxication of hydrogen peroxide and lipid hydroperoxides together with GSH and GR, that increases stability of erythrocytes against hemolysis [20, 21]. In BHP group the sharp increase of GSH-Px activity indicates the counteraction of AOS against developed benign tumor. In case of CaP reduced activity of the enzyme precisely shows a state of AOS. As to GR, its increased activity in both types of tumors must be due to increased demand for reduced glutathione, i. e. must represent a compensatory reaction of an organism, which is important in maintenance of resistance of erythrocytes against oxidizing stress and hemolysis.

Alteration of the antioxidant enzymes level has the common trend: in conditions of benign tumor their variability is not manifested markedly and is directed to recover the normal defence, while in case of malignization sharp and often incurable changes are observed almost for all enzymes. The development of BHP reflects relatively weakly on an organism blood system and the mechanisms of the antioxidant defence still show coordinated, balanced interaction. The significant changes revealed in case of CaP indicate the reduced functional state of blood AOS. Decreased activity of SOD and GSH-Px reflects destructive processes occurring in blood during malignant transformation when the blood AOS can't fulfill effective defence, while increased activity of Cp, GSH and GR represents a compensatory response against the aggravation of pathology.

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СОДЕРЖАНИЕ И АКТИВНОСТЬ АНТИОКСИДАНТНЫХ ФЕРМЕНТОВ У ПАЦИЕНТОВ С ОПУХОЛЯМИ ПРЕДСТАТЕЛЬНОЙ ЖЕЛЕЗЫ

Цель: исследование системы антиоксидантных ферментов в крови больных с доброкачественной гиперплазией простаты (ДГП) и аденокарциномой простаты (АКП). **Методы:** изменения содержания и активности супероксид-дисмутазы (СОД), каталазы (КАТ), церулоплазмينا (ЦП), трипептида глутатиона (ВГ), глутатион-пероксидазы (ГП) и глутатион-редуктазы (ГР) были изучены спектрофотометрическими методами. Перекисное окисление липидов оценивали с помощью тиобарбитуровой кислоты (ТБК-тест). Материалом для исследования служили плазма крови и эритроциты больных с опухолями простаты, $n = 15$ для каждой группы. **Результаты:** активность СОД повышалась в ДГП ($24,65 \pm 1,20$ ед./мкл) и снижалась в АКП ($11,45 \pm 0,89$ ед./мкл). Активность КАТ оставалась неизменной в ДГП ($12,41 \pm 0,85$ мкат/мл) и слегка снижалась в АКП ($9,52 \pm 0,56$ мкат/мл). Содержание ЦП повышалось в обоих типах опухолей, в особенности в АКП ($54,27 \pm 7,22$ мг%), так же как ВГ ($0,736 \pm 0,07$ мкМ/л) и ГР ($0,031 \pm 0,002$ мкМ/г.гемогл/мин). Активность ГП резко возрастала в ДГП ($0,67 \pm 0,05$ мкМ/г.гемогл/мин) и снижалась в АКП ($0,16 \pm 0,01$ мкМ/г.гемогл/мин). **Выводы:** развитие ДГП сравнительно слабо отражается на системе крови, так как содержание и активность антиоксидантных ферментов не показывают заметных изменений при этой болезни. Значительные изменения были выявлены в группе больных с АКП, что указывало на подавление функционального состояния системы антиоксидантных ферментов крови.

Ключевые слова: доброкачественная гиперплазия предстательной железы, аденокарцинома предстательной железы, оксидативный стресс, антиоксидантные ферменты.