ORIGINAL ARTICLE

Taylor&Francis healthsciences

Reactive Oxygen Species Production by Blood Neutrophils of Patients with Laryngeal Carcinoma and Antioxidative Enzyme Activity in Their Blood

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Acta Oncologica Vol. 43, No. 3, pp. 252–258, 2004

Squamous cell carcinoma of the head and neck is a devastating illness with a severe impact on affected individuals. Several mechanisms may lead to oxidative stress in tumor-bearing patients, among others chronic inflammation. Inflammatory cells, especially macrophages and neutrophil leukocytes, may produce reactive oxygen species (ROS) which participate in carcinogenesis and tumor-associated immunosuppression. The aim of the study presented in this paper was to compare the production of reactive oxygen species (ROS)—superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2)—by neutrophils isolated from the blood of 16 patients with larynx carcinoma and 15 healthy controls. The serum activity of superoxide dismutase and catalase as well as the total peroxidase activity in serum have also been estimated. The production of ROS, especially spontaneous and phorbol 12-myristate 13-acetate (PMA)-induced O_2^-, was relatively higher in the patients with larynx carcinoma than in the healthy controls and increased parallel with the tumor stage (tumor, node, metastasis—TNM staging). The serum activity of catalase and peroxidase was also highest in the patients with stage T3 and T4 larynx carcinoma. After partial or total laryngectomy, a significant decrease in ROS production and the serum activity of catalase and peroxidase was observed. In contrast, the serum level of superoxide dismutase, which had been low prior to surgery, especially in the patients with advanced tumor stages (T3–T4), increased significantly afterwards. The results indicate the existence of oxidative stress in the blood of patients with larynx carcinoma and its significant decrease after partial or total laryngectomy.

Received 1 September 2003
Accepted 17 February 2004

Several mechanisms may lead to oxidative stress in cancer patients. Chronic inflammation occurring in patients with advanced tumors may be attributable to oxidative stress responsible for DNA damage and mutations of tumor suppressor genes, which are critical events in carcinogenesis (1) and can adversely affect the immune functions. For example, reactive oxygen species (ROS) produced by phagocytes are able to inhibit non-specific and tumor-specific cytotoxicity (2). Several lines of evidence indicate that neutrophil leukocytes act non-specifically against tumor cells. The correlations between tumor infiltration and the clinico-pathological features of tumors have not been estimated exactly (3). However, during cancer development, a marked increase in the ROS-generating activity of neutrophils in the circulating blood has been described (4) as the result of an increase in both the specific activity of leukocytes and the total number of neutrophils in the blood.

Oxidative stress is a disturbance in the balance between the production of ROS and antioxidant defenses. It occurs when an excessive production of ROS overwhelms the antioxidant defense system and when there is a significant decrease in or lack of antioxidant defense. Mammalian cells are protected against ROS by enzymatic and non-enzymatic antioxidant defenses. The primary antioxidant enzymes (AOE) are superoxide dismutase (SOD), which catalyzes the dismutation of superoxide anions (O_2^-) to hydrogen peroxide (H_2O_2), and catalase and glutathione peroxidase (GSH-Px), which catalyze the degradation of H_2O_2 to water and O_2. These enzymes are expressed in different tissues, among others in blood cells (5).

Our literature survey has revealed that there are a limited number of studies related to oxidative stress in laryngeal carcinoma. A significant growth in lipid peroxidation (6) and ROS generation in blood leukocytes as well as a

 ISSN 0284-186X
DOI: 10.1080/02841860410029708

significant reduction of AOE activity in the blood of patients with carcinoma of the larynx has been described (7). The data from the literature are somewhat conflicting, and other authors (8) have not detected any changes in antioxidant components such as GSH-Px and CuZn-SOD. Moreover, an elevated number of monocytes, accompanied by a decrease in their functions such as chemotaxis, random migration, or nitroblue tetrazolium reduction, has been described in patients with larynx carcinoma (9). Neutrophils of patients with larynx carcinoma are also characterized by a deficiency in the activity of some enzymes such as beta-glucuronidase and myeloperoxidase (10).

Therefore, the aim of our study was to examine the production of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) by the neutrophils isolated from the blood of 16 carefully selected, newly diagnosed patients with larynx carcinoma and 15 healthy persons (the control). Resting (spontaneous) production of ROS by neutrophils, as well as phorbol ester (PMA)-induced ROS production, was measured. Moreover, the serum activity of some antioxidative enzymes such as superoxide dismutase and catalase and the total peroxidase activity in serum were measured in order to estimate the level of oxidative stress present in the blood of patients with different stages of larynx carcinoma.

MATERIAL AND METHODS

Patients

Overall, 16 newly diagnosed patients with carcinoma of the larynx at Lublin District Hospital were included in the study. At enrolment, demographic data including age and gender were recorded. Tumor primary site and tumor, node, metastasis (TNM) staging were determined for each patient using the 'American Joint Committee cancer manual for staging cancer' (Philadelphia: J.B. Lippincott; 1977). Excluded from this study were patients who had had prior cancer treatment, had a coexisting disease such as diabetes or cirrhosis, or had a metastatic disease beyond the cervical lymph nodes. The mean age was 56.6 ± 12.1. Fifteen patients were male and 1 was female. The demographic data and tumor staging are presented in Table 1. Patients were interviewed to determine their smoking and alcohol consumption habits. All patients were long-term tobacco and alcohol users, i.e. they had smoked more than 10 cigarettes daily and drunk more than one drink daily for several years. After admission to hospital, all patients abstained from alcohol but about half of them continued smoking. Depending on the disease stage and other clinical factors, patients received definitive or partial surgery and (after the experiment) radiation therapy.

The healthy control patients (15 persons) were university workers, all of whom were smokers (they smoked 5–10 cigarettes daily) but none drank alcohol. The mean age was 44 ± 18 years. There were 14 men and 1 woman. Written informed consent approved by the Ethical Committee was obtained from each patient.

Table 1

Characteristics of patients with larynx carcinoma (group I) and controls (group II)

Characteristics	I	II
No. of patients	16	15
Median age (years)	56.6 ± 12.1	44 ± 18.4
Males	15	14
Females	1	1
Tumor	T1–2	8 (age 55.7 ± 9.2)
	T3–4	8 (age 57.0 ± 7.3)
N	012	
N	2	2
N	3	2
Surgery	Total 11	
	Partial 5	

On initial examination of the patients, samples were obtained from the larynx by biopsy and fixed in 10% buffered formaldehyde for pathological and immunohistochemical analysis.

Heparinized and unheparinized blood samples were taken from each patient twice: on the day of diagnosis (usually 4–6 days after admission to hospital) and two weeks after surgery, but before radiation therapy.

Granulocyte separation

Blood (8 ml) was collected in heparinized tubes (20 U/ml, Heparinum-Polfa). Granulocytes were separated according to the Sigma procedure: a gradient was formed by layering an equal volume of Histopaque-1077 over Histopaque-1119 (both from Sigma, St Louis, Mo, USA). Whole blood was carefully layered onto the upper Histopaque-1077. The tubes were then centrifuged at $700 \times g$ for 30 min. Granulocytes were isolated from the Histopaque-1077/1119 inter-phase and washed three times with HBSS (Hanks' Balanced Salt Solution), centrifuged ($350 \times g$ for 15 min) and resuspended in HBSS.

Measurement of superoxide anion (O_2^-) production by cytochrome-c reduction assay (11)

HBSS (176 µl), 12.5 µl of cytochrome-*c* solution in HBSS (final concentration 75 µM), 5 µl of either SOD solution (final concentration 60 U/ml) or 5 µl of distilled water, and 50 µl of neutrophil suspension (final density of 2.5×10^5 cells/well) were added into each well on a 96-well plate. After 3 min of incubation, the neutrophils were activated with PMA (final concentration 1 µg/ml) in 6.5 µl/well. The microplate was incubated at 37°C for 60 min and transferred to the microplate reader. The absorbance values at 550 nm (differences in OD between samples with and without SOD) were converted to nanomoles of O_2^- based on the extinction coefficient of cytochrome-*c*:

$\Delta E_{550} = 21 \times 10^3\ M^{-1}cm^{-1}$. The results were expressed as nanomoles of O_2^- per 1×10^6 cells per 60 min.

Measurement of hydrogen peroxide (H_2O_2) production (12)

The assay was based on horseradish-dependent peroxidation (HRPO) of phenol red by H_2O_2 leading to the formation of a compound that exhibits absorbance at 600 nm. Neutrophil suspension (4×10^6 cell/ml of HBSS) was distributed into wells (50 µl/well) on a 96-well microplate. The cells were covered with 50 µl/well of the assay solution, which was prepared on the day of the experiment and consisted of HBSS, phenol red (Sigma, final concentration 0.56 mM), HRPO (Serva, Heilderberg, Germany, final concentration 20 U/ml) and PMA (Sigma, final concentration 1 µg/ml). To a control well, assay solution without PMA was added. The plate was incubated for 60 min at 37°C and then the reaction was stopped by adding 10 µl/well of 1N NaOH. After 3 min of incubation, the plate was read at 600 nm in the microplate reader.

The results were expressed as nanomoles H_2O_2 per 10^6 cells per 60 min based on the phenol red extinction coefficient ($\Delta E_{600} = 19.8 \times 10^3\ M^{-1}cm^{-1}$).

Serum catalase (CAT) activity assay

Serum was separated by centrifugation of unheparinized blood and kept at −20°C before measuring the enzyme activity. The assay was performed according to the method described by Pifferi et al. (13) and modified by Nowak (14). Briefly, the reaction mixture prepared in the Eppendorf tube consisted of 500 µl of 0.05 M phosphate buffer pH 7.0, 300 µl of distilled water, 50 µl of 1.1 mM H_2O_2 in distilled water, and 50 µl of the serum sample (or 50 µl of distilled water as blank). After 5 min of incubation at 25°C, 100 µl of 50% trichloroacetic acid (TCA, Sigma) was added to each tube and the tubes were centrifuged ($1000 \times g$ for 5 min). Next, 10 µl of titanium (IV) reagent (24) was added to each tube and 200 µl of the supernatant was transferred into wells on a 96-well microplate. The absorbance was read at 405 nm in the microplate reader. The results were expressed as CAT activity in U/ml of serum, after comparison with the standard curve, prepared by plotting the absorbance (OD) at 405 nm (ordinate) as a function of standard CAT (Sigma) concentration (abscissa) between 0 to 33 U/ml. One unit of CAT decomposed 1.0 µmole of H_2O_2 per min at pH 7.0 and 25°C.

Serum peroxidase (PER) activity assay (15)

The reaction mixture contained 0.1 ml of 1% o-dianisidin (Sigma) in methanol with 9.9 ml of 0.003% H_2O_2 in 0.05 M phosphate buffer pH 7.0. The serum samples in a 96-well plate (10 µl/well) were mixed with 190 µl/well of the reaction mixture. The extinction was read in the microplate reader at 450 nm after 60 s incubation at 25°C. The results were expressed as whole peroxidase activity in U/ml of serum after comparison with the standard curve, prepared in the same way as described for CAT, in the value range of standard peroxidase activity (HRPO, Serva) between 0 and 2.5 U/ml of phosphate buffer. One unit of peroxidase was defined as enzymatic activity that catalyzed the decomposition of 1 µmol of H_2O_2 per minute at 25°C, pH 7.0.

Serum superoxide dismutase (SOD) activity assay

To measure the SOD activity, we modified the method described by Oberley and Spitz (16). Briefly, to each well on a 96-well microplate, was added 160 µl of a reaction mixture consisting of DETAPAC (diethylenetriamine pentaacetic acid, Sigma, final concentration 1 mM in 0.05 M phosphate buffer, pH 7.8), MTT (tiazolyl blue, Sigma, final concentration 1 mg/ml in phosphate buffer), and xantine (Sigma, 1.8 mM in buffer); 20 µl of serum or 20 µl of phosphate buffer (blank) was added to the wells. The reaction was started by adding 20 µl of xantine oxidase (final concentration 0.01 U/ml of phosphate buffer). The microplate was incubated at 25°C for 60 min and the absorbance of the samples and the blank was read at 570 nm in the microplate reader. The results were expressed in U of SOD/ml of serum after comparison with the standard curve, prepared by diluting standard SOD (Sigma) in the range from 0 to 0.935 U/ml of phosphate buffer. One unit of SOD inhibited by 50% the rate of MTT reduction generated by xantine/xantine oxidase system at 25°C, pH 7.8.

Data analysis

Data were expressed as mean ± SD. Statistical analysis was performed using a non-parametric Mann–Whitney U-test for comparing the results obtained in patients and control groups. A Wilcoxon non-parametric test was used to compare the differences within patient groups. The patients were divided into groups with early stages of tumor (T1 and T2) and those with stages T3 and T4. Statistical significance was set at $p < 0.05$. Spearman's correlation analysis was used to examine the relations between the data.

RESULTS

There was no statistical difference in age between the patients and the healthy controls or between T1–2 and T3–4 groups (see Table 1). When spontaneous (non-induced) ROS production by the neutrophils isolated from the blood of patients with larynx carcinoma was examined (Fig. 1, Table 2, total group A) and compared with the healthy controls, significantly higher amounts of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) were detected. PMA-induced O_2^- production was also higher than in the controls as was catalase and peroxidase activity detected in sera of the patients.

One exception to this tendency was superoxide dismutase activity, which was significantly lower in group A than in

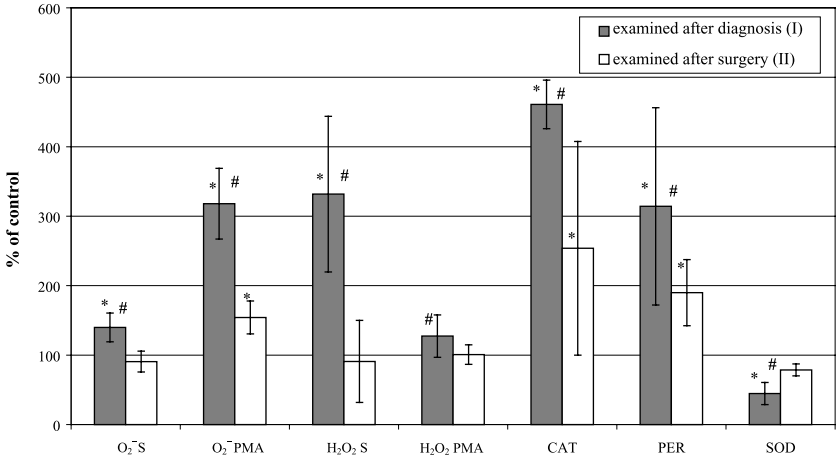

Fig. 1. Spontaneous (S) and PMA-induced (PMA) O_2^- and H_2O_2 production by neutrophil leukocytes of patients with laryngeal carcinoma and antioxidative enzyme activity in serum: catalase (CAT), peroxidase (PER), and superoxide dismutase (SOD). The results are expressed as a percentage of control (healthy subjects). * Statistically significant in comparison with control. # Statistically significant between blood sample taken before and after surgery.

the control group (group D). We suppose that the enhancement of the neutrophil 'oxidative burst' and the elevation of catalase and peroxidase activities accompanied by the decrease in superoxide dismutase activity were connected with larynx carcinoma development, as significant changes in all the examined parameters were observed after surgery (AI and AII in Table 2). This supposition was also confirmed when we analyzed group B patients with tumor stages T1+T2 separately and compared them with group C patients with tumor stages T3+T4. The comparison of those two groups revealed the relationships between the examined parameters and the tumor burden. The neutrophils of group C produced more ROS and had a lower superoxide dismutase activity in sera than in group B. In contrast with superoxide dismutase, catalase and peroxidase activity was higher in group C than in group D but those

Table 2

Parameters analyzed: percent of control (mean ± SD) and statistical significance among the groups

Group		O_2^- S	O_2^- PMA	H_2O_2 S	H_2O_2 PMA	CAT	PER	SOD
A (n = 16)	I	139.8 ± 20.7	318 ± 50.8	331.8 ± 112	127.4 ± 30.5	461.5 ± 34.9	314 ± 142	44.6 ± 15.9
	II	90.67 ± 15	154.2 ± 23.7	90.9 ± 59	100.8 ± 14.1	253.8 ± 153.8	190 ± 47.6	78.7 ± 8.5
B (n = 8)	I	124.3 ± 18	288 ± 47	277.2 ± 104	110.6 ± 30.9	611.1 ± 40.9	385.7 ± 180	59.5 ± 6.38
	II	87.56 ± 10.8	137.2 ± 9.49	128.7 ± 62	92 ± 6.63	242.6 ± 213	152.3 ± 319	86.1 ± 7.44
C (n = 8)	I	155.4 ± 8.8	350 ± 32	387.8 ± 96	143.8 ± 20.7	320 ± 97.2	247.6 ± 47.6	29.7 ± 6.3
	II	93.2 ± 16.5	169.4 ± 23.7	53 ± 13.6	110 ± 13.7	264.3 ± 72.7	233 ± 809	71.2 ± 5.3
D (n = 15)		100	100	100	100	100	100	100
Statistical analysis								
A–D	I	< 0.004	< 0.0005	< 0.00001	NS	< 0.0005	< 0.0003	< 0.024
A–D	II	NS	< 0.002	NS	NS	< 0.001	< 0.0008	NS
AI–II	–	< 0.0004	< 0.0004	< 0.0004	< 0.005	< 0.006	< 0.013	< 0.004
B–D	I	NS	< 0.00016	< 0.001	NS	< 0.04	< 0.001	< 0.039
B–D	II	NS	NS	NS	NS	NS	< 0.001	NS
BI–II	–	< 0.01	< 0.01	< 0.01	NS	< 0.017	< 0.017	< 0.01
C–D	I	< 0.001	< 0.0001	< 0.0001	< 0.047	< 0.0001	< 0.0001	< 0.001
C–D	II	NS	< 0.0003	NS	NS	< 0.001	< 0.001	< 0.03
CI–II	–	< 0.01	< 0.01	< 0.01	< 0.017	NS	NS	< 0.011
B–C	I	< 0.0027	< 0.004	< 0.027	< 0.046	NS	NS	< 0.0007
B–C	II	NS	< 0.007	< 0.01	< 0.01	NS	NS	< 0.002

Group A = larynx carcinoma (group B+C) I = examined after diagnosis.
B = stage T1+T2 II = examined after surgery.
C = stage T3+T4.
D = control.
O_2^- S = spontaneous O_2^- production.
O_2^- PMA = PMA-induced O_2^- production.
NS = not significant.

differences were not statistically significant. After surgery (all patients from group C had total laryngectomy, in group B there were 5 patients with partial and 3 patients with total laryngectomy), a significant decrease in ROS production and a small (insignificant) decrease in catalase and peroxidase activity were observed. In both groups, superoxide dismutase activity rose after the surgery. Spearman's analysis and correlations between the data are presented in Table 3 and indicate positive correlations between superoxide anion and hydrogen peroxide, catalase and peroxidase, and hydrogen peroxide and catalase; and negative ones between superoxide anion and superoxide dismutase activity, or superoxide anion and hydrogen peroxide.

DISCUSSION

This study was designed to investigate oxidative stress parameters in the blood of patients with laryngeal carcinoma. Fifteen males and 1 female participated in the study. The gender composition of the patient group was a reflection of the higher incidence of laryngeal carcinoma in Polish men than in Polish women. It is well known that apart from genetic factors, sex hormones, and viral infections, alcohol and tobacco consumption are the most important risk factors for laryngeal carcinoma, and there are more heavy smokers and drinkers among 40- to 60-year-old men in Poland than among women in the same age group (17–20).

The results of our study strongly indicate that the neutrophils of tumor-bearing patients produce more superoxide anion and more hydrogen peroxide than the neutrophils of healthy controls. Moreover, tumor dissection causes a significant drop in ROS production. The question arises, then, as to whether the group of patients is comparable to the controls. There were no statistically significant differences between the examined groups but the control subjects did not consume alcohol, while in the patient group all subjects were heavy drinkers. It should be stressed, however, that when blood samples were taken from the patients, they had abstained from alcohol for several days after admission to hospital. As mentioned above, alcohol consumption, especially accompanied by smoking, is an important risk factor for laryngeal carcinoma. It is known that though alcohol is not a carcinogen, it may act as co-carcinogen under special conditions. Ethanol metabolism leads to the generation of acetaldehyde and ROS. These highly reactive compounds bind rapidly to cell constituents, among others to DNA. This leads to chromosomal aberrations and a drop in the level of intracellular antioxidants. Induction of cytochrome P450E1 by ethanol, which is associated with enhanced activation of various procarcinogens present in tobacco smoke, is also considered as an important mechanism in carcinogenesis (17).

Table 3

Spearman's correlations of analyzed parameters

Group	r	p
Group A		
O_2^- S I/H_2O_2 S I	0.54	0.028
Group B		
Cat II/Per II	0.70	0.04
O_2^- S I/SOD I	−0.76	0.028
Group C		
O_2^- PMA I/H_2O_2 PMA I	−0.84	0.008
H_2O_2 S II/Cat II	0.81	0.014

For legend see Table 2.

Among the mechanisms involved in oxidative stress in patients with laryngeal carcinoma, two seem to be of importance. The first is an altered energy metabolism resulting from malnutrition and vitamin deficiency, leading to ROS accumulation; the second mechanism is the non-specific chronic activation of the immune system. ROS produced by macrophages and neutrophils may participate in carcinogenesis and are able to inhibit non-specific and tumor-specific cytotoxicity (2).

Neutrophils constitute about 60% of the circulating leukocytes (21). Several lines of evidence indicate that neutrophils act non-specifically against tumor cells. A marked increase in the ROS-generating activity of neutrophils in the circulating blood during the development of the cancer has been described (3, 4) as the result of an increase in both the specific activity of leukocytes and the total number of neutrophils in the blood. This increase is accompanied by decreased monocyte functions such as chemotaxis, random migration, and nitroblue tetrazolium reduction (9). Neutrophils of patients with larynx carcinoma are also characterized by a deficiency in the activity of such enzymes as beta-glucuronidase and myeloperoxidase (10).

In contrast with the above-mentioned results of other authors, in our experiments neutrophils isolated from the blood of patients with larynx carcinoma produced more ROS than the neutrophils of the healthy controls. Our results can, at least partially, explain the reasons for lipid peroxidation in the blood of patients with larynx carcinoma (6), but we do not know how to account for the fact that neutrophils in the experiments of other authors are deficient in phagocytosis while they are able to produce ROS in our study. A possibility exists that signal transduction pathways differentially regulate different functions of neutrophils such as phagocytosis, degranulation, and ROS production (22).

Bearing in mind the factors that can activate neutrophils in patients with larynx carcinoma one may suppose that tumor-associated antigens that are known to be cytokine inducers in blood cells (23) play such a role. Also, immune complexes of tumor antigens with specific antibodies that were detected in the blood of the patients could be

activators of neutrophils (24). Moreover, in patients with larynx carcinoma, increased levels of IL-6 and TNF were detected, which are responsible for weight loss and the induction of serum acute phase response. GM-CSF, IL-4, and IL-6 but also IL-10 and TGF-β were extracted from tumors derived from infiltrating leukocytes rather than tumor cells. It seems likely that those cytokines can be not only stimulators (IL-6, TNF, GM-CSF) but also inhibitors (IL-10 and TGF-β) of neutrophil activity. Neutrophils, when activated, produce ROS and participate in oxidative stress. ROS are highly reactive substances that can cause a wide spectrum of cell damage processes, including lipid peroxidation, enzyme inactivation, and DNA damage. Normally, ROS are neutralized by natural antioxidants such as vitamins C or E; however, the level of vitamin E in the blood of patients with larynx carcinoma is low (25–28).

Mammalian cells are protected against ROS not only by non-enzymatic but also by enzymatic antioxidant enzymes (AOE) such as superoxide dismutase, which catalyzes the dismutation of superoxide anions to hydrogen peroxide, catalase, and glutathione peroxidase, which catalyze degradation of H_2O_2 to water and O_2. These enzymes are expressed in different tissues, among others in blood cells (5).

In our experiments, we detected an increased activity of catalase and peroxidase in sera of patients with larynx carcinoma correlated with the tumor stage. Our results are, however, inconsistent with the results of other authors who detected a significant reduction of AOE activity (7) or (8) did not detect any changes in antioxidant components such as GSH-Px and CuZnSOD. One possible explanation for those differences is that the authors mentioned above examined AOE activity in blood cells while we examined serum AOE activity.

It is well known that the plasma levels of AOE, though low, are very important in the regulation of the plasma levels of ROS and in the protection of plasma compounds against ROS.

Several types of GSH-Px have been identified in blood: two are classical cellular enzymes found in red blood cells and one an extracellular enzyme (eGSH-Px) found in the plasma. Plasma eGSH-Px differs distinctly from cellular GSH-Px in substrate specificity, gene structure, and gene localization. This enzyme is actively secreted into the plasma by kidney proximal tubules, and by the liver, heart, lungs, and skeletal muscles and plays an important role in the protection of extracellular fluid components against peroxide-mediated damage (29). Since o-dianisidine has been shown to be able to detect several glutathione peroxidase activities (15), we used it to detect total peroxidase (PER) activity present in serum.

We also examined the serum activity of SOD, which, as is known from the literature, is connected with the expression and release of extracellular SOD 3 (30) (which differs from cytosolic SOD1 and mitochondrial SOD2), and detected that its activity was low in sera of patients with larynx carcinoma. Our results are in agreement with other authors (7) who detected decreased superoxide dismutase activity in blood cells of larynx carcinoma patients.

The results of this study preclude the possibility that observed changes in AOE activity in sera of patients with laryngeal tumors may be caused by alcohol consumption. Data obtained from experiments on rats and also human studies strongly indicate a quite different pattern of alcohol-induced disturbances in serum AOE activity. Usually, chronic alcohol consumption significantly decreased the serum activity of catalase, peroxidase, and superoxide dismutase (31), while in our study a significant increase in catalase and peroxidase activity was observed in tumor-bearing patients.

Oxidative stress can be defined as a disturbance in the prooxidant-antioxidant balance in favor of the former. The observation that the production of ROS, spontaneous and induced by PMA, is relatively higher in patients with larynx carcinoma than in the healthy control, and that the activity of SOD is lower, suggests that an excessive production of superoxide anion may induce oxidative stress in the blood of patients with larynx carcinoma. Total or partial laryngectomy significantly decreases oxidative stress, because a decrease in ROS production is accompanied by a growth in serum SOD activity.

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