The relationship between antioxidant enzymes and bladder cancer*

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Carcinogenesis proceeds through at least three distinct stages – initiation, promotion and progression. Free radicals play an important role in the multistep complex course of carcinogenesis. Urinary bladder has been recognized as a target organ for many carcinogens, including benzidine, β-napthylamine, 2 napthylamine, 4-aminobiphenyl. Antioxidants have been shown to inhibit both initiation and promotion in carcinogenesis.

The aim of presented study was to determine and compare the oxidant and antioxidant status in different clinical stages of bladder cancer and of control groups.

Study was conducted in fifty-two (n=52) patients with transitional cell epithelial cancer of bladder and in twenty-four (n=24) healthy adults as plasma and erythrocyte controls.

Malondialdehyde levels (4.636±1.118, 2.853±0.576 / 262.112±61.772, 203.788±35.340) were significantly higher and erythrocyte glutathione levels (6.272±1.708, 7.523±1.346) were significantly lower in bladder cancer patients group than in control group. Erythrocyte glutathione reductase and glutathione peroxidase (3.935±1.155, 5.481±1.626 / 8.729±1.614, 12.362±1.707) activities were significantly lower in cancer patients. In the other hand, glutathione S-transferase activities (3.100±1.177, 1.071±0.471) were found significantly increases.

We suggest that the values of glutathione S-transferase enzyme activity can be used for a tumor detection approach and even as an indicator of the biological behavior of the bladder carcinoma.

Key words: bladder cancer, free radicals, malondialdehyde, glutathione S-transferase, glutathione peroxidase

Clinical and epidemiological studies, as well as investigations in experimental systems, have provided evidence supporting an important role for free radicals in the etiology of cancer [11]. Carcinogenesis proceeds through at least three distinct stages. Initiation is a persistent and heritable alteration of a cell. Initiated cells may undergo malignant transformation if promotion and progression follow. This stage appears to be irreversible and may result from a mutation, translocation, or amplification in the target cell. Promotion, involves the selection and clonal proliferation of cells initiated by a chemical compound or other factors. The final stage of carcinogenesis is the progression of bening lesion into a highly malignant, rapidly growing neoplasm. During this stage, a loss of growth control and an escape from host defense mechanisms become the predominant phenotypic characteristics [14].

Free radicals are found to be involved in both initiation and promotion of multistage carcinogenesis. One may expect that free radical scavengers shoud function as inhibitors in the neoplastic processes. Antioxidants have been shown to inhibit both initiation and promotion in carcinogenesis and counteract cell immortalization and transformation [1, 10]. The protection of cells aganist damage from oxygen and its metabolites can be accomplished trough enzymatic and nonenzymatic means. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are considered the primary antioxidant enzymes, since they are involved in the direct elimination of active oxygen species glutathione S-transferase (GST), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PD) are secondary antioxidant enzymes which help in the detoxification of reactive oxygen species by decreasing peroxide levels or by maintainnig a steady supply of metabolic intermediates like glutathione (GSH) and NADPH for the primary antioxidant enzymes [12].

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Urinary bladder has been recognized as a target organ for many carcinogens, including benzidine, β-naphthylamine, 2-naphthylamine, 4-aminobiphenyl. The first well established cause of bladder cancer was occupational exposure to a class of chemicals known as the arylamines, which include the now established bladder carcinogens, 2-naphthylamine, aniline, and 4-aminobiphenyl [7]. Cigarette smokers overall have an approximately four fold higher risk of bladder cancer than nonsmokers, and the risk is increasing by number of cigarettes regularly smoked [4]. An important unresolved issue is the mechanism by which cigarettes induce bladder cancer. Aromatic amines including 2-naphthylamine and 4 aminopiphenyl are present in tobacco smoke in small amounts and are the leading candidates as the specific etiological agents. Choronic cystitis, from any cause, increases the risk of developing squamous cell carcinoma of the bladder. Cystitis related to schistosomiasis is world wide the most common cause of this form of cancer. Bladder calculi may cause a chronic cystitis and over a long period may predispose to squamous cell carcinoma [18].

Material and methods

This study was contucted in fifty-two (n=52) patients with transtional cell epitelial cancer of bladder (W/M, 8/44; mean ±SD, 65.1±20.2 and 60.5±9.8, respectively) and in twenty-four (n=24) healthy adults as control. (W/M, 8/16; mean ±SD 63.5±3.5 and 61.2±6.4, respectively). Patients were divided in two group by tumor stage and tumor cells differentation. Thirty-one patient of superficial tumor, twenty-one patient of invasive tumor. Twenty-two patient of Grade 1 and thirty patient of Grade 2 and 3.

All blood samples were drawn into tubes containing Li-heparin. After the tubes had been cantrifuged for 10 min at 4000 rpm, plasma was seperated, the buffy coat discarded and packed erythrocytes were washed three times with sterile 0.9% sodium chloride (w/v). Preparation of erythrocytes was performed immediately after blood collection. Samples were stored at –80 °C prior to analysis.

Plasma malondialdehyde levels were measured according to the method of BUEGE and AUST [2]. Erythrocyte glutathione levels were measured according to the procedure of BEUTLER et al using metaphosphoric acid for protein precipitation [6]. Glutathione-S transferase activity using 1-chloro-2,4-dinitrobenzene (CNDB) as a substrate was determined by the method of HABIG et al [16]. Erythrocyte glutathione peroxidase activity was determined by the method of PAGLIA and VALENTINE [25]. Enzyme activity was proportional to the rate of NADPH oxidation in the presence of H_2O_2 as a substrate. Erythrocyte glutathione reductase activity was measured in the presence of the oxidized form of GSH by following the oxidation of NADPH spectrophotometrically [30]. Hemoglobin levels were assayed by the commercial cyanmethaemoglobin method [13].

All chemicals were purchased from Sigma Chemical Company. The Student's t-test was used for the statistical analysis of the results.

Results

The mean plasma and erythrocyte MDA levels were significantly higher and erythrocyte GSH levels were significantly lower in patient group than in control group. Erythrocyte GR and GPx activities were significantly lower in patients (Tab. 1).

In both superficial and invasive tumor group erythrocyte and plasma MDA levels were significantly higher and erythrocyte GSH levels were significantly lower than in control group, but MDA and GSH levels were not different between the groups (Tab. 2).

In high grade group and low grade group according to tumor cells differentiation erythrocyte and plasma MDA levels were significantly higher and GSH levels were significantly lower than in control group, but MDA and GSH levels were not different between the groups.

GST activities were significantly higher in patient of superficial and invasive tumor group but enzyme activities were not different between groups. High-grade tumor groups GST activities were significantly higher than low-grade tumor group ($p<0.001$) (Tab. 3).

GR and GPx activities were not different between superficial and invasive tumor group and neither according to tumor cell differentiation on high-grade tumor and low-grade tumor groups.

Discussion

Free radicals play an important role in the complex course of multi step carcinogenesis. This information led many investigators to consider the possibility that intra- or extracellular generation of free radicals and or an intracellular pro-oxidant state may be important in producing many types of cancer [5, 17].

Enzymatic and non-enzymatic antioxidants are the free radical scavengers, however, are shown to be anticarcinogens. Antioxidants have been shown to inhibit both initiation and promotion in carcinogenesis [24].

In our study, we have found that the plasma and erythrocyte lipid peroxide levels in bladder cancer group were significantly higher than in control group. Lipid peroxide levels were not different between superficial and invasive bladder cancer group and neither between high grade and low-grade tumor group. Many investigators found that the lipid peroxidation products were elevated in different tumor types [8, 20, 26].

When compared to their appropriate normal cell counterparts, tumor cells have abnormal activities of antioxidant enzymes. ZACHARA et al [31] shown that decreased selenium concentration and glutathione peroxidase activity in blood and increase of these parameters in tumor tissue of the lung

Table 1. The parameters to compare in patient and control groups

Parameters	Patients group (n=52)	Control group (n=24)	p
GSH (nmol/gHb)	6.272 ± 1.708	7.523 ± 1.346	0.001
Plasma-MDA (nmol/ml)	4.636 ± 1.118	2.853 ± 0.576	0.001
Erythrocyte-MDA (nmol/gHb)	262.112 ± 61.772	203.788 ± 35.340	0.001
GPx (U/gHb)	8.729 ± 1.614	12.362 ± 1.707	0.001
GR (U/gHb)	3.935 ± 1.155	5.481 ± 1.626	0.001
GST (U/gHb)	3.100 ± 1.177	1.071 ± 0.471	0.001

Table 2. The parameters to compare in superficial and invasive tumor groups

Parameters	Superficial tm (n=31)	Invasive tm (n=21)	p
GSH (nmol/gHb)	6.38 ± 1.77	6.11 ± 1.64	0.586
Plasma-MDA (nmol/ml)	4.68 ± 1.14	4.579 ± 1.12	0.764
Erythrocyte-MDA (nmol/gHb)	270.6 ± 59.1	249.6 ± 64.9	0.233
GPx (U/gHb)	8.67 ± 1.61	8.82 ± 1.66	0.746
GR (U/gHb)	3.96 ± 1.02	3.90 ± 1.36	0.854
GST (U/gHb)	2.93 ± 1.22	3.36 ± 1.09	0.195

Table 3. The parameters to compare in low and high tumor groups

Parameters	Low grade (n=22)	High grade (n=30)	p
GSH (nmol/gHb)	5.919 ± 1.792	6.530 ± 1.625	0.206
Plasma-MDA (nmol/ml)	4.671 ± 1.143	4.610 ± 1.118	0.848
Erythrocyte-MDA (nmol/gHb)	256.218 ± 59.319	266.433 ± 66.041	0.561
GPx (U/gHb)	8.448 ± 1.455	8.936 ± 1.716	0.285
GR (U/gHb)	3.765 ± 0.986	4.059 ± 1.267	0.368
GST (U/gHb)	2.405 ± 0.768	3.610 ± 1.173	0.001

cancer. SUBAPRIYA et al [29] shown that tumor tissue was accompanied by decreased activities of SOD and CAT with increase in GSH and GSH-dependent enzymes. In contrast, enhanced lipid peroxidation with decrease in antioxidants was observed in the venous blood of oral squamous cell carcinoma patients. KOROTKINA et al [21] suggested that antioxidant enzyme activity changed insignificantly in benign breast tumors, but significantly decreased in malignant tumors. The severity of changes in malignant tumors depended on the degree of malignancy. MANJU et al [23] observed that significantly elevated levels of plasma TBARS and significantly lowered levels of GSH, GPx, GST, SOD vitamin C and vitamin E were observed in cervical cancer patients when compared to controls. POPADIUK et al [27] shown that erythrocyte GPx activity has shown a significant decrease in children with malignant tumors. No differences were observed in SOD activity between sick and healthy children.

In our study, in bladder cancer superficial and invasive types of patients GSH and related enzymes GPx and GR were found significantly decreases. In other hand, GST activities were significantly increase. The availability of sufficient concentrations of toxic and carcinogenic compounds will also be an important factor in the initiation of chemical carcinogenesis, and these compounds are likely to reach high concentrations in urinary bladder transitional epithelium but not in urinary bladder non-transitional tissue.

Glutathione transferase isoenzymes GSTM1-1 and GSTT1-1 have been shown to be risk modifiers in a number of different cancer [6, 9, 28]. LAFUENTE et al suggested that glutathione S-transferase P1 can be used as a marker in urine for bladder cancer [22].

Polymorphic enzymes glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) that are involved in the detoxification of many xenobiotics are involved in the etiology of bladder cancer [15]. JOHNS et al [19] observed the interaction between GSTM1 and other polymorphisms on the risk of bladder cancer and their interaction with environmental risk factors.

We found that the erythrocyte GSH levels, the GPx which catalyzes the destruction of peroxides by the way of GSH oxidation and the GR enzyme activity which obtain the re-reduction of GSSG were reduced in our patients with bladder carcinoma. However, we found the increased activity of GST enzyme which catalyzes the transformation of some carcinogenic agents like aromatic amines and their reactive metabolites those known to be the most important factor in the development of bladder carcinoma to less reactive compounds by conjugation with GSH. In addition, the enzyme activity was significantly high in the poor differentiated tumor cells when comparing with the well differentiated one. Expecting more studies supporting our results, we suggest that the increment of GST enzyme activity can be used as a tumor detection approach and even as an indicator of the biological behavior of tumor for bladder carcinoma.

In conclusions, if the inactivation of genes for antioxidant enzymes is one of the causes of carcinogenesis, we suggested prevention of the malignant transformation by the addition of these enzymes in the early stages of carcinogenesis. If, however, the changes in these enzymes result from malignant cells and if they are required for the maintenance of the malignant states, then the recovery of decreased enzymes could help to reverse the malignancy.

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