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TBARS, Carnitine, and Reduced Glutathione Levels in Human Bladder Carcinoma

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Abstract—In this study, we investigated tissue levels of reduced glutathione (GSH) and carnitine as well as thiobarbituric acid reactive substances (TBARS, as a marker of lipid peroxidation) levels in bladder carcinoma and control group of patients. The average GSH, carnitine and TBARS levels for tumor group were respectively 7.11 ± 3.3 μg/mg protein, 1.81 ± 0.39 nmol/mg protein, and 4.29 ± 3.2 μmol/mg protein, versus 14.45 ± 4.11 μg/mg protein, 2.14 ± 0.66 nmol/mg protein, and 2.3 ± 0.6 μmol/mg protein for normal bladder tissues. Thus, tissue reduced glutathione levels (GSH) were significantly lower in patients as compared with the control group ($p < 0.001$) whereas average TBARS levels in the tumor group were found to be higher than those in control group. The average tissue carnitine levels in the patient group were found to be lower compared with the control group but the difference was not statistically significant ($p > 0.05$).

Key words: TBARS, GSH, glutathione, carnitine, bladder carcinoma

Bladder cancer is the second most frequent tumor of the urogenital tract. Most bladder cancers are transitional cell carcinomas, 1.4% are adenocarcinoma (slightly higher in women, by 2%), and 0.2% are sarcomas [1, 2]. Bladder cancer grades are Gx (grade cannot be assessed), G1 (well differentiated), G2 (moderately differentiated), G3-G4 (poorly differentiated or undifferentiated) [1].

Free radicals are potentially dangerous byproducts of cellular metabolism that have direct effects on cell growth and development, as well as on cell survival, and play a significant role in the pathogenesis of atherosclerosis, cancer, aging, and several other conditions including inflammatory disease. Free radicals are generated in many metabolic reactions including ATP production in mitochondria. In the latter case, the leakage of electrons from the mitochondrial respiratory chain generates reactive oxygen species, namely superoxide anion and hydroxyl radicals. These species lead to production of hydrogen peroxide, from which further hydroxyl radicals can be easily formed in the presence of Fe^{2+} ions. Excessive amounts of reactive oxygen species (ROS) can start some lethal chain reactions that can inactivate vital enzymes and important subcellular elements and lead to cell death [3]. Moreover, ROS-induced lipid peroxidation has been implicated in malignant transformation [4-6]. Lipid peroxidation usually occurs when the hydroxyl radicals are generated close to or within membranes and attacks fatty acid side chains of membrane phospholipids resulting in accumulation of so-called thiobarbituric acid reactive substances, TBARS [7]. As a result of severe damage to cell structures by free radical generation, some chromosomal aberrations and carcinogenesis may develop [8].

These ROS-induced damaging processes within cells under normal conditions are controlled by the antioxidant defense system, including both enzymatic and nonenzymatic components, among the latter carnitine and glutathione (GSH) being the most important. GSH is an essential cellular thiol, which is present in concentrations up to 12 mM in mammalian cells. It has important antioxidant functions in detoxication of xenobiotics, in a number of isomerization reactions, and storage and transport of cysteine. In addition, GSH is essential for cell proliferation and maintaining the thiol redox potential in cells [9].

Carnitine is a naturally occurring compound in the body. This substance is an essential factor in the transport of long-chain fatty acids from the cytoplasm to the interior of the mitochondrion, where β-oxidation of fatty acids takes place, as well as in the removal of toxic compounds from cells [10]. L-Carnitine esters, for exam-

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ple, L-propionyl-carnitine, can increase the intracellular pool of L-carnitine. L-Propionyl-carnitine possesses a high affinity to carnitine acetyltransferase and thus is readily converted into propionyl-coenzyme A and free carnitine. L-Propionyl-carnitine might reduce the hydroxyl radical production in the Fenton system, by chelating the iron required for the generation of hydroxyl radicals [11]. Thus, levels of carnitine may indirectly reflect the intrinsic tissue defense against hydroxyl radicals.

There are only a few reports on oxidant–antioxidant profile in human bladder carcinoma. Therefore, we undertook the present study to characterize the tissue levels of TBARS (indicator for lipid peroxidation), carnitine, and GSH in patients with bladder carcinoma.

MATERIALS AND METHODS

Tumor samples were obtained from the Department of Pathology immediately after surgical intervention. Macroscopically homogenous pieces of the tumor were selected and samples were sent to the Department of Pathology for identification. Normal bladder tissues were obtained at autopsy. All tissues were stored at −70°C until analysis. According to the pathological analysis, tumor tissues were divided into two groups: 10 tumors with G1 and 5 tumors G2 (all tumor tissue were transitional cell carcinoma). Tumor and normal tissue samples were homogenized in phosphate buffer forming a 10% (w/v) homogenate. Homogenization was performed with a tissue grinder fitted with a Teflon pestle at a speed of 1000 rpm for 10 min.

Glutathione was measured by the Ellman procedure based on the development of a yellow color when 5,5′-dithiobis(2-nitro-benzoic acid) (DTNB) is added to compounds containing sulfhydryl groups [12]. Determination of carnitine was described elsewhere [13]. TBARS levels were measured spectrophotometrically [14]. Protein was assayed by the Lowry procedure [15].

The average tissue values of GSH, carnitine, and TBARS were analyzed using routine statistics (Student's *t*-test). Significance (p) values equal to or less than 0.05 were considered significant. All data were expressed as means ± standard deviation (M ± SD). For correlation analysis, Pearson correlation was used.

RESULTS

Results of determination of GSH, carnitine, and TBARS levels in tumor and control bladder tissues are presented in the table. The average GSH levels in tumors were significantly lower compared with the control group ($p < 0.001$). The average carnitine levels were also lower in patients compared to the control group, but the difference was not statistically significant ($p > 0.05$). The average TBARS levels in the group of patients were nearly twice higher than that in the control group but there was no statistically significant difference found between them ($p > 0.05$) because of high SD values. Also, we could not find any significant differences in GSH, TBARS, and carnitine values between G1 and G2 ($p > 0.05$). There was no correlation between carnitine, GSH, and TBARS levels in patients with bladder carcinoma (GSH/TBARS, $r = -0.1518$ ($p = 0.179$); carnitine/TBARS, $r = -0.1920$ ($p = 0.088$); and GSH/carnitine, $r = 0.1533$ ($p = 0.175$)).

DISCUSSION

Molecular oxygen and its reaction products, ROS, can cause serious injury to biological organisms through a variety of mechanisms. Carcinogenesis is though to be a multi-step process [16], and oxidative damage may be in the line of tumorigenesis through several mechanisms [17]. Free radical alterations of unsaturated lipids in cell membranes may result in loss of membrane fluidity and lead to cell lysis [16]. Also, the oxidative degradation of polyunsaturated fatty acids has been shown to create aldehydes such as TBARS, which can cause chaotic cross-linking between nucleic acid, protein, and lipid molecules [18].

Antioxidative defense mechanisms, which protect biological structures against oxidation, include enzymes (such as glutathione peroxidase, superoxide dismutase,

Tissue GSH, carnitine, and TBARS levels in bladder carcinoma and in control group

Group	*N*	GSH, μg/mg protein	Carnitine, nmol/mg protein	TBARS, μmol/mg protein
Patients	15	7.11 ± 3.3**	1.81 ± 0.39*	4.29 ± 3.2*
Control	10	14.45 ± 4.11	2.14 ± 0.66	2.3 ± 0.6

* $p > 0.05$ (not significant).
** $p < 0.001$.

catalase, etc.) and a number of low molecular weight free radical scavengers (carotenoids, ascorbic acid, glutathione, bilirubin, urate, and tocopherols) [10, 19]. Decreased levels of essential antioxidants in the circulation have been found to be associated with an increased risk of cancer [5].

Our findings indicate that TBARS levels in the tested cancerous tissues are higher than those in normal tissues. These findings are in agreement with recently published data [20, 21].

The glutathione system is very important for cellular defense against ROS. GSH both directly reacts with radicals in nonenzymatic reactions and serves as an electron donor in the reduction of peroxides catalyzed by cellular glutathione peroxidases, and cells are protected against injury by this redox cycle [9].

Many important experimental and clinical data concerning the physiological and pathophysiological role of carnitine have been accumulated in recent years [22-24]. It is now generally accepted that the major cellular function of L-carnitine in eucaryotic cells is the transport of acyl group from the cytosol to the mitochondrial matrix where these are further metabolized. Heart and skeletal muscles have the highest content of carnitine [25]. However, activity of palmitoyl-carnitine transferase in brain is comparable with that in kidney and skeletal muscles [25, 26]. In previous studies L-propionyl-carnitine was found to be able to scavenge superoxide anion, to inhibit lipoperoxidation of linoleic acid, and to protect Pbr 322 DNA from cleavage induced by UV-photolysis [11].

In our study, GSH levels in patients with bladder carcinoma were found to be significantly lower than that in the control group. The average carnitine level in the patient group was also lowered compared to that in the control group while the difference between patient and control group was not statistically significant ($p > 0.05$).

In conclusion, decrease in carnitine and glutathione levels (as a part of cellular antioxidant defense system) may be partially responsible for the weakness of ROS resistance of the cells under pathology studied and this situation may also cause higher TBARS levels in patients with bladder carcinoma compared to that in healthy individuals.

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