J Membrane Biol (2017) 250:455–459
DOI 10.1007/s00232-017-9971-0

The Prolidase Activity, Oxidative Stress, and Nitric Oxide Levels of Bladder Tissues with or Without Tumor in Patients with Bladder Cancer

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Received: 30 May 2016 / Accepted: 18 July 2017 / Published online: 16 August 2017

Abstract This study was designed to evaluate the malondialdehyde (MDA), glutathione (GSH) and nitric oxide (NO) levels, and also prolidase, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) enzyme activities in malignant and benign cancers of bladder tissue. A total of 59 patients admitted to our clinic due to microscopic or macroscopic haematuria, were prospectively included in the study. Because of some reasons (no request to participate in the study, the inability to reach, other malignancies, alcohol consumption, metabolic disease), eight patients were excluded from study. Of the 51 patients, 25 were bladder tumor patients, and 26 were patients without cancers. The bladder tissue samples were obtained from all patients under anesthesia (spinal, epidural or general) for the measurement of MDA, GSH and NO levels, and prolidase, GSH-Px and SOD enzyme activities. Among the patients with bladder cancers, 7 patients were females and 18 patients were males, with an average age of 68.4 ± 2.49. Among patients without tumors, 6 patients were females and 20 patients were males, with an average age of 58 ± 2.05. In patients with bladder tumors, the oxidants (MDA, NO, prolidase) were higher, and the antioxidants (SOD, GSH, GSH-Px) were lower than those in patients without tumors. It was concluded that the oxygen free radicals play a role in the etiology of bladder cancers similar to many other tumors and inflammatory conditions. Therefore, we assume that antioxidants may provide benefits in the prevention and treatment of bladder cancer.

Keywords Cancer · Bladder · Status antioxidants · Superoxide dismutase · Nitric oxide · Oxidative stress

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Introduction

Reactive oxygen species (ROS) have been implicated in the pathogenesis of various diseases, including cancers (Templar et al. 1999). There is strong evidence linking oxidative stress and bladder cancer in literature (Yalcin et al. 2004). In previous studies, it has been demonstrated that ROS are directly involved in the oxidative damage of cellular macromolecules such as lipids, proteins, and nucleic acids in tissues (Batcioglu et al. 2006). Moreover, oxidative stress can lead to tumor angiogenesis. It has also been reported that ROS can augment tumor cell migration, increasing the risk of invasion and metastasis (Nishikawa 2008).

Nitric oxide (NO) is generated by the enzyme nitric oxide synthases (NOS). It is a short-lived free radical that is expressed in a wide range of mammalian cells as macrophages, hepatocytes, and endothelial cells (Knowles and Moncada 1994). NO has been suggested to play an important role in the biology of tumor growth (Wolf et al.

2000). The extracellular matrix (ECM) consists of collagens, proteoglycans, and glycoproteins. During inflammation and cancer invasion, ECM is degraded by metalloproteinases (MMPs), resulting in the release of a large amount of peptides containing proline and hydroxyproline (Surazynski et al. 2008). Prolidase is among the MMPs and its activity has been documented in erythrocytes, leukocytes, plasma, dermal fibroblasts, the kidney, brain, heart, thymus, and uterus (Liu et al. 2007). One of the consequences of neoplastic transformation is deregulation of tissue collagen metabolism. The final step of collagen degradation is mediated by prolidase (Surazynski et al. 2008; Palka et al. 2002). Prolidase activity has been investigated in various malignant tumors including pancreas cancer (Palka et al. 2002), lung adenocarcinoma (Karna et al. 2000), breast cancer (Cechowska-Pasko et al. 2006), endometrial cancer (Arioz et al. 2009), stomach cancer (Guszczyn and Sobolewski 2004), and ovarian cancer (Camuzcuoglu et al. 2009). It has been reported that the NO may regulate the activity of metalloproteinases (MMPs; Tsuruda et al. 2004). Although prolidase is a special type of metalloproteinases (metalloproteinases (MMPs) it may be also regulated by NO because it catalyzes the terminal step in matrix breakdown (Tsuruda et al. 2004).

Bladder cancer is the fourth most common type of cancer in men (Macvicar 2000). The most common risk factors for bladder cancer are cigarette smoking, exposure to industrial carcinogens, and possibly diet (Wynder and Goldsmith 1977; Zeegers et al. 2004). While smoking constitutes the major risk for bladder cancer, arsenic in drinking water, hair dyes, and food carcinogens are among the other etiological risk factors (Yalçın et al.2004; Pelucchi et al. 2006). It is known that the 4-aminobiphenyl and acrolein in cigarette smoke and free oxygen radicals cause the development of urothelial tumors. Smoking and environmental factors enhance the production of lipid peroxides and free oxygen radicals in the body. These harmful products are removed from the body through the intracorporeal antioxidants. Inefficient functioning of antioxidants or excess production of oxidants can cause tissue-injury leading to carcinogenesis. It has been shown that there is a relationship between oxidative stres and bladder tumors (Arikan et al. 2005; Ellidağ et al. 2013).

In our previous study (Gecit et al., 2012), we evaluated the serum prolidase activity, oxidative stress, and nitric oxide levels in patients with bladder cancer. However, this study was designed to evaluate the prolidase activity, oxidative stress, and nitric oxide levels of bladder tissues with or without tumor in patients with bladder cancer.

Materials and Methods

Subjects

After having obtained approval from the Local Ethics Committee (BADK22) and informed consent forms from the patiens, 59 patients who applied to the Urology Polyclinic of the Dursun Odabasi Medical Center at the Yuzuncu Yil University due to microscopic or macroscopic haematuria between January and September of 2013 were included in the study. Due to some reasons (no request to participate in the study, the inability to reach, other malignancies, alcohol consumption or metabolic disease), eight patients were excluded from the study. Of the remained 51 patients with haematuria, 25 patients had bladder tumors (tumor patients) and 26 patients did not have bladder tumors (non-tumor patients). The socio-demographical characteristics of the patients were similar. All patients were lifetime free of drug, alcohol, antioxidant supplement consumption, and any metabolic disease. None of the tumor patients had any other malignancies except bladder tumor. The non-tumor patients were diagnosed with bladder or ureteral stone, benign prostatic hyperplasia, ureterocele, microscopical haematuria, etc.

After completing the preoperative tests of all patients included in the study, operations were performed under anesthesia (spinal/dural/general) and lithotomy position in operating room conditions. The bladder was entered via a 20-french cystoscope (Storz, Germany) at a 30-degree angle. Punch biopsies were obtained with biopsy forceps from the bladder areas free from tumor (Group I, $n = 25$) and also bladder areas with tumor (Group II, $n = 25$) of tumor patients, and also bladder walls of non-tumor patients (Group III, $n = 26$). The obtained tissues were cleaned by washing the blood residue with saline (0.9% NaCl) and kept at -20 °C until the biochemical analyses. Before the biochemical studies, the tissues were homogenized in a 1.15% KCl-solution for 30 min under 14.000 RPMI. The homogenate was then centrifuged at 10.000g for 30 min and the obtained supernatant was used for biochemical measurements. Oxidants such as malondialdehyde (MDA) and NO levels, and prolidase activity, and also antioxidants such as glutathione (GSH) level, and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were determined by spectrophotometric methods in the Chemistry Laboratory of the Science Faculty at Yuzuncu Yil University, Van, Turkey.

Oxidant Measurements

The NO level was measured as total nitrite by the Griess method spectrophotometrically (Tracey et al., 1995).

Tissue protein analysis was performed using the Lowry method (Lowry et al., 1951) and the results were given as μmol/mg protein. The MDA was measured spectrophotometrically at 532 nm. Its levels were calculated by utilizing the molar extinction coefficient of the MDA-thiobarbituric acid complex and the unit was formulated as mMol/mL (Yoshioka et al., 1979). For prolidase measurement, the following methodology (Myara et al., 1982) was employed: 1 ml of glacial acetic acid and 1 ml of Chinard solution (glacial acetic acid—6 mol/L and orthophosphoric acid was combined at a volume ratio of 55/45% and supplemented with dissolved ninhydrin—3 g/dL) was added onto a clear supernatant of 0,5 ml. The mixture was then incubated for 20 min at 90 °C and cooled with ice. Immediately after cooling, the sample absorbances were read against the blind-sample without substrate at 515 nm in a spectrophotomer (Beckman Coulter DU530 UV/VIS Spectrophotometer, USA). The measured proline concentrations were calculated by comparing with the standart L-proline sample (5 mg/dL). The prolidase enzyme activity was determined as μmol/L of proline produced in 1 min by the enzyme-degradation of Gly-proline substrate to proline (the proline-absorbance coefficient at the ninhydrin reaction is 27.2).

Antioxidant Measurements

The GSH level was determined by the Beutler method (Beutler et al. 1975) using the principle of DTNB reduction by the reduced GSH. The GSH-Px enzyme activity was studied spectrophotometrically using the method modified by Paglia and Valentine (Paglia et al. 1967), in which t-buthyl-hydroperoxide was used as a substrate. The unit of GSH-Px was formulated as U/g and of GSH as mg/dL. The SOD enzyme activity was defined by the method modified by Sun et al. (1990). The principle of this method is based on the reduction of nitroblue tetrazolium (NBT) by the superoxide-producer xanthine–xanthine oxidase system. In our study, SOD activity was determined as Unit/milligram tissue protein.

Statistical Analysis

The definitive analyses on the studied parameters were presented as mean, standard deviation, and as minimum and maximum values. The one-way variance analysis (One-way ANOVA) was employed in the comparison of the group mean levels. The Pearson correlation coefficients were calculated to determine the inter-variance correlations. Furthermore, Receiver operating characteristics (ROC) analysis was performed to establish cut-off values for differentiating patient and control groups. The statistical significance level was set at 5%, and the SPSS (Version 9.2) statistical package software was used for the calculations.

Results

In patients with bladder tumors, the oxidants (MDA, NO, prolidase) were higher ($p < 0.001$), and the antioxidants (SOD, GSH, GSH-Px) were lower ($p < 0.001$) than those in patients without tumors. The results have been detailed in Figs. 1 and 2.

Discussion

This study was designed to evaluate the prolidase activity, oxidative stress, and nitric oxide levels of bladder tissues with or without tumor in patients with bladder cancer. It was found that in patients with bladder tumors, the oxidants

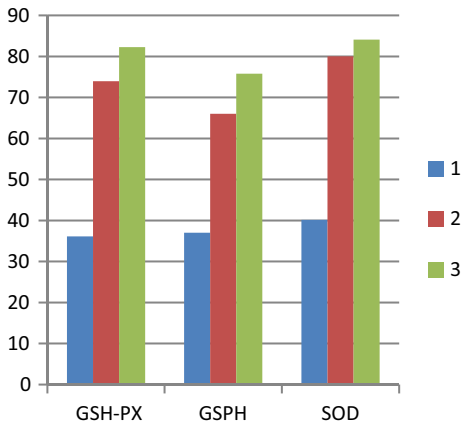

Fig. 1 The mean levels of antioxidants ($p < 0.001$)

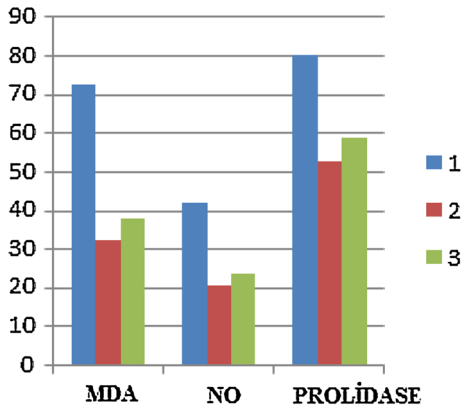

Fig. 2 The mean levels of oxidants ($p < 0.001$). 1. The levels of enzymes in tissues of tumors (n:25). 2. The levels of enzymes in benign tissues of patients with bladder tumor (n:25). 3. The levels of enzymes in tissues of patients without tumor (n:26)

(MDA, NO, prolidase) were higher, and the antioxidants (SOD, GSH, GSH-Px) were lower than those in patients without tumors. Peroxidation of membrane lipids occurs when the free radical levels exhaust the cellular antioxidant capacity (Guyton and Kensler 1993). Lipid peroxidation terminates by the conversion of lipid hydroperoxides to aldehydes and to other carbonyl products. On the other hand, the major aldehyde, MDA, is an indicator of oxidative stress, which is the end-product of membrane lipid peroxidation through the oxidation of polyunsaturated fatty acids by free radicals.

The ECM, composed of collagen, proteoglycan, and glycoproteins, constitutes a major barrier against tumor cell invasion. Thus, tumor progression critically depends on the degradation of collagen and other ECM proteins (Kleiner and Stetler-Stevenson 1999). The MMPs are the most important enzymes for the degradation of ECM proteins. Prolidase plays important roles in the collagen turnover, matrix regeneration, and cell proliferation (Surazynski et al. 2008). High concentrations of NO can block cell proliferation and trigger apoptotic cell death in tumor cells (Cui et al. 1994). It has been proposed that the NO plays an important role in tumor biology with both tumor-promoting and tumor-suppressive properties (Eijan et al. 1998).

In performed previously studies, the MDA levels were found to be higher in the serum of bladder cancer patients (Yalcin et al. 2004; Geçit et al.2012). We also found higher levels of MDA in the tumoral bladder tissue, which differs from the preceding studies by a direct analysis of the tumoral tissue, yet displaying parallelity to the serum results.

Some authors have found increased prolidase activity in malignancies such as lung (Karna et al. 2000), endometrium (Arioz et al. 2009), stomach (Guszczyn and Sobolewski 2004), and ovarian cancers (Camuzcuoglu et al. 2009). In contrary, Palka et al. (2002) demonstrated reduced prolidase activity in pancreas cancer. Yoshimura et al. (2004) investigated the ECM-components in the urine of bladder cancer patients and found these to be higher than in patients without malignancies. In our study, we found higher values of tissue prolidase activity at statistically significant levels both in comparison to the tissues obtained from patients without malignancies and also in comparison to the seemingly normal tissues from the bladders with tumors.

Higher NO levels were reported in stomach and esophagus cancers (Türkdoğan et al. 1998), yet Bukan et al. (2003) found no statistically significant difference in total serum nitrite levels in patients with bladder cancer. Nonetheless, there are studies reporting higher serum NO levels in patients with bladder cancer (Gecit et al. 2012). In our study, we found profoundly higher levels of NO in the tumors of bladder cancer patients in comparison to patients with no tumors.

The antioxidant system protecting the cells against the noxious effects of free radicals includes mainly the SOD and catalase (CAT) enzymes and additionally, glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rx), and sulfhydryl-containing molecules (Sun 1990). It is known that the detrimental effects of free radicals are controlled with defense systems formed by enzymatic (CAT, GSH-Px, SOD) and non-enzymatic (Vitamin E, Vitamin C, glutathione, etc.) components. Some researchers have found statistically significant lower levels of serum GSH-Rx and GSH-Px activities in bladder cancer patients, while these levels were found to be within normal limits in their control patients (Gecit et al. 2012; Arikan et al. 2005).

In our study, markedly reduced levels of antioxidants (GSH-Px, glutathione) were found in bladder cancer tissues, both in comparison with the bladder tissues of patients without tumors, and also in comparison with seemingly normal tissues of the bladders with tumors. Furthermore, distinct differences in SOD activities were found between normal and malignant cells in many cases. For instance, reductions of SOD and catalase were reported in hepatoma (Oberley et al. 1978) and in human lung cancer tissues (Jaruga et al. 1994). We also found lower levels of tissue SOD activity in tumors of the bladder cancer patients in comparison to benign tumors In another study lipid peroxidation in breast cancer tissues was significantly enhanced in conjunction with the significant increase in both enzymic and non-enzymic antioxidants when compared to the healthy controls (Wang et al. 2014).

It was concluded that free oxygen radicals are important in the etiology of bladder cancer and that antioxidants could provide benefits in prevention and treatment of bladder cancer. Measurement of oxidative stress will be an affective prognosis factor. Therefore, new therapeutic drugs may design for cancer treatment. We think that our study is important, since to the best of our knowledge, it is the first investigation of oxidants and antioxidants at the tumor tissue-level in bladder cancer. Nonetheless, we believe that further studies and more comprehensive approaches are necessary to obtain more accurate results regarding these relationships.

Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflict of interest.

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