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ORIGINAL PAPER

Serum prolidase activity, oxidative stress, and nitric oxide levels in patients with bladder cancer

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Abstract
Objectives Prolidase is a member of the matrix metalloproteinase family. It plays a major role in collagen turnover, matrix remodeling and cell growth. Nitric oxide (NO) regulates many processes such as collagen synthesis and matrix remodeling. Thus, NO may augment angiogenesis, tumor invasion, and metastasis. The aim of this study was to investigate total antioxidant status (TAS), malondialdehyde (MDA) and NO levels in patients with bladder cancer and to determine their relationship with prolidase activity.
Design and methods Thirty-five patients with bladder cancer and 32 controls were enrolled. Serum TAS, MDA, prolidase activity and NO levels were determined.
Results Serum prolidase activity, NO levels and MDA levels were significantly higher in bladder cancer than controls (all, $P < 0.05$), while TAS levels were significantly lower ($P < 0.05$).
Conclusions Our results show that increased prolidase seems to be associated with increased NO levels and oxidative stress along with decreased antioxidant levels in bladder cancer.

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Keywords Bladder cancer · Nitric oxide · Total antioxidant status · Malondialdehyde · Prolidase activity

Introduction

Bladder cancer is a common tumor of the urinary tract (Macvicar 2000). It is the fourth most common type of cancer in men in the United States. The most common risk factors for bladder cancer are exposure to industrial carcinogens, cigarette smoking, male gender, and possibly diet (Wynder and Goldsmith 1977; Zeegers et al. 2004). Another major etiological factor is infestation by the parasite Schistosoma hematobium (Wynder and Goldsmith 1977).

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 suggested in 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 also has been reported that ROS can also augment tumor cell migration, increasing the risk of invasion and metastasis (Nishikawa 2008).

Nitric oxide (NO) is generated by the enzyme family of nitric oxide synthases (NOS). NO is expressed in a wide range of mammalian cells as macrophages, hepatocytes, and endothelial cells (Knowles and Moncada 1994). It is a short-lived free radical, a pleiotropic biomolecule, critical to several biological processes (Koshland 1993). NO has been suggested to play an important role in tumor biology with both facilitatory and inhibitory effects on tumor

growth (Wolf et al. 2000). Also, NO has many implications as a potential regulatory effector for prolidase and NO may regulate the activity of metalloproteinases (MMPs; Tsuruda et al. 2004). Although prolidase is a special type of MMP, it may be also regulated by NO because it catalyzes the terminal step in matrix breakdown (Tsuruda et al. 2004).

The extracellular matrix (ECM) consists of collagens, proteoglycans, and glycoproteins. During inflammation and cancer invasion, ECM is degraded by MMPs, resulting in the release of a large amount of peptides containing proline and hydroxyproline (Surazynski et al. 2008). Among the MMPs, prolidase is a manganese-dependent cytosolic exopeptidase, and it cleaving imidodipeptides and imidotripeptides with C-terminal proline or hydroxyproline (Myara et al. 1984). 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). However, to the best of our knowledge, serum prolidase enzyme activity has not been evaluated in patients with bladder cancer.

Therefore, the aim of this study was to investigate serum total antioxidant status (TAS), malondialdehyde (MDA) and NO levels in patients with bladder cancer and to determine their relationship with serum prolidase enzyme activity.

Methods

Subjects

Thirty-five (mean age of 63.54 ± 3.4) men patients with bladder cancer were enrolled in the present a cross-sectional study. All the patients were lifetime non-smokers and free of drug, alcohol, antioxidant supplement consumption, and any metabolic disease. None of the patients had any other significant disease or malignancies except bladder cancer and only the newly diagnosed patients with no prior chemotherapeutic treatment were included in this study.

Controls consisted of 32 healthy men of age 62.76 ± 4.8 randomly selected from a group of healthy non-smoking volunteers with no history of previous disease, drug, antioxidant agents or alcohol consumption.

The patient and control groups were of similar socioeconomic status. As for tumor staging, 28 patients were diagnosed with non-invasive tumor (Ta–T1) where as 7 patients with invasive tumor (T2–T4). Patients were classified into three groups with respect to tumor grading where 21 patients had well differentiated (G1), 9 had intermediate (G2), and 5 had poorly differentiated (G3) tumors. The superficial urothelial papillary tumors were graded according to the 2004 World Heath Organization (WHO) grading system into low-grade and high-grade papillary neoplasms.

The study protocol was carried out in accordance with the Helsinki Declaration as revised in 2000. All participants were informed about the study protocol and the written consent was obtained from each one.

Blood collection

Following 12 h of fasting period, blood samples were obtained in the morning. Blood samples were collected into empty tubes and immediately stored on ice at 4°C. The serum was then separated from the cells by centrifugation at 3,000 rpm for 10 min. Serum samples for the measurement of prolidase activity, TAS, NO and MDA levels were stored at −20°C until they were used.

Measurement of total antioxidant status

Serum TAS was determined using an automated measurement method, developed by Erel (2004). The results are expressed as mmol Trolox Equiv./l.

Measurement of serum lipid peroxidation

Serum lipid peroxidation was measured by estimating malondialdehyde (MDA) levels from serum as described by Yoshioka et al. The results are expressed as nmol/ml (Yoshioka et al. 1979).

Determination of prolidase activity

The serum prolidase activity was determined according to the method of Myara et al. (Myara et al. 1982), based on the measurement of proline by Chinard's reagent (Chinard 1952). The results are expressed as U/l.

Measurement of serum nitric oxide

Serum NO_2^-/NO_3^- concentrations were determined using the Griess reaction according to Tracey et al. (Tracey et al. 1995). The results were expressed as μmol/l.

Statistical analysis

All the results are expressed as means and standard deviation (mean ± SD). Non-parametric continuous variables were compared by Mann–Whitney *U* test. Parametric variables were compared using Student's *t* test. *P* value less than 0.05 was considered as statistically significant. Statistical evaluation was carried out with the SPSS 11.0.

Results

The demographic and clinical data of bladder cancer and control groups are shown in Table 1. There were no statistically significant differences between bladder cancer patients and controls in respect to age and BMI (all $P > 0.05$; Table 1).

Serum prolidase activity, NO levels, and MDA levels were significantly higher in bladder cancer than controls (all, $P < 0.05$), while TAS levels were significantly lower ($P < 0.05$; Table 2).

No correlation was observed between tumor grading, and serum prolidase activity, NO, MDA levels and TAS levels ($P > 0.05$).

Discussion

Collagen is major extracellular matrix component in various tissues, including kidney. It has been demonstrated that the human non-compliant bladder is characterized histologically by an increased deposition of ECM protein, especially type III collagen, in the muscle wall (Kaplan et al. 1997). Prolidase is a homodimeric iminodipeptidase that releases carboxy-terminal proline or hydroxyproline from oligopeptides. It plays a major role in collagen turnover, matrix remodeling and cell growth (Jackson et al. 1975). The primary biological function of prolidase enzyme in humans appears to involve the metabolism of collagen degradation products and the recycling of proline from dipeptides for collagen resynthesis (Surazynski et al. 2008). Moreover, it has been suggested that the prolidase enzyme activity may be a rate-limiting factor in the regulation of collagen biosynthesis (Surazynski et al. 2008).

Table 1 Demographic characteristics of the two groups in this study

Parameters	Controls ($n = 32$)	Patients ($n = 35$)	*P*
Age (year)	62.8 ± 4.8	63.5 ± 3.4	ns
Body mass index (kg/m^2)	23.42 ± 2.14	22.11 ± 1.16	ns

Values are mean ± SD

ns non-significant

Table 2 Prolidase activity, nitric oxide, oxidative and antioxidant levels in bladder cancer and controls

Parameters	Controls ($n = 32$)	Patients ($n = 35$)	*P*
TAS (mmol Trolox Equiv./l)	2.5 ± 0.2	1.1 ± 0.1	0.05
MDA (nmol/ml)	9.1 ± 0.4	16.8 ± 1.6	0.05
NO (μmol/l)	8.1 ± 0.8	17.1 ± 1.4	0.05
Prolidase (U/l)	25.4 ± 3.2	54.8 ± 4.3	0.05

Values are mean ± SD

TAS total antioxidant status, *NO* nitric oxide, *MDA* malondialdehyde

It is well known that the breakdown of tissue barriers is catalyzed by proteolytic enzymes released from the primary tumor (Duffy 1996). The ECM composed of collagens, proteoglycans, and glycoproteins is a major barrier against the invasion of tumor cells. Therefore, a tumor progression critically depends on the breakdown of collagen and other ECM proteins (Kleiner and Stetler-Stevenson 1999). MMPs are one of the most important enzymes for the breakdown of ECM proteins. Although serum prolidase activity is well documented in certain cancers, the exact cause is still unknown (Palka et al. 2002; Karna et al. 2000; Cechowska-Pasko et al. 2006; Arioz et al. 2009; Guszczyn and Sobolewski 2004; Camuzcuoglu et al. 2009). Some authors found that increased prolidase activities in cancer such as lung (Karna et al. 2000), breast (Cechowska-Pasko et al. 2006), endometrial cancer (Arioz et al. 2009), stomach cancer (Guszczyn and Sobolewski 2004), and ovarian cancer (Camuzcuoglu et al. 2009). In contrast, Palka et al. (2002) demonstrated that prolidase activity in pancreatic cancer was decreased. On the other hand, Yoshimura et al. (2004) investigated the urinary ECM measurement in urine from patients with bladder cancer. They have found that urinary ECM levels were higher in bladder cancer groups than normal bladder groups.

In the present study, we found that patients with bladder cancer had significantly higher serum prolidase activities than healthy controls. To the best of our knowledge, serum prolidase activity has not been evaluated in bladder cancer. This is the first report investigating serum prolidase activity in patients with bladder cancer.

Prolidase and the regulation of prolidase by NO may be important in the regulation of collagen turnover. Collagen, the most abundant protein in the body, constitutes more than a quarter of total body proteins. NO has been shown to play a role in regulating collagen metabolism. High NO concentrations are associated with increased collagen biosynthesis and modification (Mei et al. 2002). Surazynski et al. 2005 demonstrate that NO stimulates prolidase activity by increasing serine/threonine phosphorylation. High concentrations of NO can inhibit cell growth and

induce apoptosis in tumor cells (Cui et al. 1994). It has been suggested that NO plays an important role in tumor biology, with both tumor promoter and antitumor activity (Eijan et al. 1998). It has been reported that continuous NO production may be involved in the inflammatory process associated with the appearance of many human malignancies, including bladder cancer (O'Byrne and Dalgleish 2001). Furthermore, it has been reported that NO is elevated in the urine from patients with bladder cancer (Eijan et al. 2002). However, Bukan et al. (2003) did not observed any significant difference in serum total nitrite levels of the patients with bladder cancer and control subjects. In the present study, we found a significantly increased serum NO levels in patients with bladder cancer than in control subjects.

It is known that the harmful effects of ROS are controlled by various cellular defense systems consisting of enzymatic (catalase, glutathione peroxidase, superoxide dismutase etc.) and non-enzymatic (vitamins E, C, glutathione etc.) components (Mates et al. 1999). Epidemiological studies reveal that low levels of antioxidants are associated with an increased risk of cancer. Antioxidant depletion in the circulation may be due to the scavenging of lipid peroxides as well as sequestration by tumor cells (Sharma et al. 2007). However, if these systems are insufficient, severe metabolic malfunctions and oxidative damage to DNA may result, which, experimental studies in animals and in vitro have suggested, are an important factor in carcinogenesis (Marnett 2000). In the present study, we found a significantly decreased serum TAS levels in bladder cancer patients than in control subjects.

On the other hand, MDA, the major aldehyde end product of lipid peroxidation of membrane polyunsaturated fatty acids by free radicals, is an indicator of oxidative stress (Marnett 2000). In the present study, we found a significantly increased serum MDA levels in patients with bladder cancer than in control subjects. This is compatible with the results of two independent studies carried out by Yalcin et al. (2004) and Kaczmarek et al. (2001). Moreover, increased levels of lipid peroxidation have been reported in various malignant tumors including cervix (Beevi et al. 2007), prostate (Ozmen et al. 2006), breast (Tas et al. 2005) and lung (Gonenc et al. 2001).

Our results suggest that enhanced collagen turnover may occur in patients with bladder cancer. Increased prolidase seems to be associated with increased NO levels and oxidative stress along with decreased antioxidant levels in bladder cancer. Therefore, increased prolidase activity may, in part, play a role in the pathogenesis of bladder cancer. It is believed that the administration of antioxidant vitamins such as A, C, and E may be useful in preventing and treating bladder cancer.

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

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