Urol Res (1989) 17:295–297

Urological Research

Eosinophil cationic protein in urine in patients with urinary bladder tumors

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Accepted: September 15, 1988

Summary. Measurement of eosinophil cationic protein (ECP) in urine constitutes a new biochemical method for assessment of local eosinophil activity in the bladder. ECP in urine was measured in 18 patients previously treated for various types of urinary bladder tumors and a comparable control group of 18 normals. The median concentration of ECP in urine from the patients was 46.5 arb. U/l versus 24.5 arb. U/l from normals. This difference was statistically highly significant ($p < 0.01$). This study suggests that eosinophils are involved in the host tumor relationship in patients with urothelial neoplasia. Measurement of ECP in urine may imply a new concept for assessment of urothelial tumors.

Key words: Eosinophil cationic protein – Urinary bladder – Urothelial tumors – Eosinophil leucocyte

Introduction

Eosinophil leucocytes are frequently observed in varying numbers in and around neoplastic tissue [2, 12–14, 20]. The exact function of these eosinophils remains unclear. However, the clinical observation that the presence of eosinophils in the stroma of carcinomas usually is associated with a good prognosis has lead to the concept that they may participitate in the host defence against tumor growth [12, 14]. Therefore, assessment of local activity of eosinophils may reflect at least partly the immunological competence of the host and thus, being a contributing predictor of the ultimate fate of cancer patients.

Assessment of local tissue activity of eosinophils by conventional histopathology or by counting peripheral eosinophils is unreliable [1, 3]. Therefore, measurement of biochemical markers of eosinophils merits attention since they seem to offer a more precise evaluation of eosinophil activity.

Eosinophil cationic protein (ECP) has been isolated from human eosinophils and constitutes 30% of the protein of the eosinophil granules [19]. ECP has been proven toxic against many cells and tissues (see [16]). Since ECP is unable to pass beyond the kidney (Dr. P. Venge, personal communication) it is suggested that the concentration in urine reflects local eosinophil activity in the urinary tract. Consequently, measurement of ECP in urine may be of value in the diagnosis of urothelial neoplasia, in the prediction of prognosis and in following response to therapy. This concept initiated this pilot study to determine ECP concentration in the urine from a group of patients with tumors of the urinary bladder and also from a control group.

Materials and methods

In a random group of 18 patients admitted for follow-up cystoscopy after previous treatment for bladder tumors, urine was collected for 24 h prior to the cystoscopy, while the patients were ambulatory. There were 7 men and 11 women with a median age of 72 years (range 50–93 years). In 7 of the patients the primary tumor or a recurrence had been invasive; 2 had a non-invasive and one an invasive recurrence at the time of investigation. In 10 patients the primary tumor and subsequent recurrences were non-invasive and 5 had a non-invasive recurrence at the time of investigation. One patient had carcinoma in situ. Six patients had previously been submitted to high voltage external irradiation and 6 patients to intravesical chemotherapy.

Four patients had a slight elevation of serum creatinine (0.14–0.19 mmol/l) and there patients had significant growth of bacteria in the urine ($> 10^5$).

The urine was collected over 24 h and a sample of 10 ml from each patient was frozen for subsequent analysis. ECP was measured with an enzyme immunoassay as described previously [5]. The antigen used to raise the antibody was isolated from urine from patients with interstitial cystitis. The antibody employed was sheep antihuman ECP. All patients were followed for at least 1 year. The control group consisted of 18 healthy (10 women and 8 men) volunteers, with a median age of 62 years (range 35–82 years).

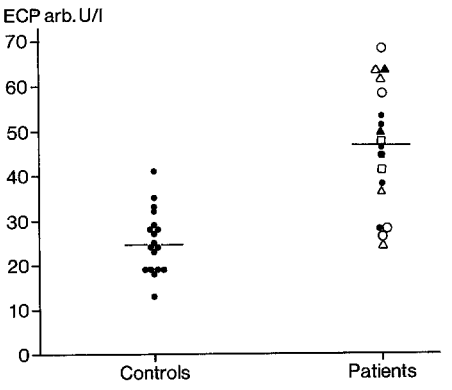

Fig. 1. Urinary concentration of eosinophil cationic protein in 18 patients with urothelial tumor disease and 18 controls. The median values, represented by the horizontal bars, are significantly different ($p<0.01$). In the patient group filled symbols indicate that tumor was present at the time of ECP measurement. ■/□ = urinary tract infection; ▲/△ = previous radiation

Statistical analyses were performed with the Mann-Whitney rank sum test. Probabilities less than 0.05 were considered significant.

Results

The median concentration of ECP in urine from patients with bladder tumor disease was 46.5 arb. U/l (range 24.0–68.0 arb. U/l), while in the control group the median concentration was 24.5 arb. U/l (range 13.0–41.0 arb. U/l) (Fig. 1). This difference in median concentration of ECP between patients with bladder tumor disease and normals was highly significant ($p<0.01$).

During the follow up period 12 patients had tumor recurrence and 4 patients died.

An analysis based on tumor grade, tumor invasivenes, recurrences and urine cultures produced groups too small for statistical evaluation.

Discussion

Measurement of ECP in urine constitutes a new biochemical method for assessment of local eosinophil activity in the bladder.

This study demonstrated a significantly elevated concentration of ECP in urine in patients with urothelial tumor disease. To our knowledge this is the first report on this subject. Our observation suggested that eosinophils may be involved in the host-tumor relationship in patients with neoplastic urothelial tumors, which corroborated with histological studies [17].

The specific relationship between ECP and bladder tumor disease is unclear. Eosinophils, however, seem to possess the ability to destroy tumor cells [15]. The toxic effects of eosinophils on cells are due largely to the secretion of various granule proteins following stimulation [16]. Human eosinophils contain several basic proteins of which ECP is a potent cytotoxic agent [19]. Eosinophil peroxydase (EPO) has also been shown to be cytotoxic to mouse ascites lymphoma cells [8].

There are several explanations for the tumor-associated eosinophilia. The carcinoma cells themselves may produce an eosinophilostatic substance [18] or eosinophils may be attracted by immunologic interactions of T-lymphocytes and/or mast cells with antigens of the carcinoma cells [2]. In this context it is interesting to note that mast cells are frequently observed around carcinomas of the uterine cervix which is the type of tumor in which the presence of eosinophils has particularly been described [6].

The assessment of local eosinophil-cell function is difficult. Quantitation of the circulating eosinophil-level does not necessarily parallel the magnitude of eosinophilic response in tissue [3] and semiquantitative histopathologic assessment is hampered by the fact that degenerative remains of many cell types will stain with eosin and degranulation of the eosinophil would result in loss of the cells characteristic staining [1]. Therefore, measurement of markers of eosinophils such as ECP in urine may offer a more precise evaluation of eosinophil activity in patients with urothelial tumors, and ECP in urine may appear to be of value as a tumor marker.

Various biochemical markers have been investigated in patients with bladder tumors [7, 9, 10]. The clinical relevance of these markers, however, remains to be established. The presence of a particular "marker" in the urine does not necessarily imply that it is a reflection of an integral process in the development or response to bladder tumor. Its presence may simply reflect increased permeability or breakdown of urine/basement membrane barriers, increased fragility of eosinophils in the bladder wall or inflammatory processes. Inflammation often coexists with malignant tumors and ECP in urine has been found to be elevated in patients with interstitial cystitis, which is a chronic abacterial inflammatory condition of the bladder [11]. Consequently, the question arises whether the elevated level of ECP is due to the presence of tumor per se or is a secondary phenomenon due to coexisting inflammation. The latter seems to be the case for some tumor markers such as the acute-phase proteins [10] and carcinoembryonic antigen in urine [4]. The majority of the patients tested in this study had previously been treated by radiation therapy or intravesical chemotherapy, each of which might have induced an inflammatory response.

This preliminary study represents only one measurement of ECP in urine from each patient with a disease that may have a most variable course in the individual patient. Longitudinal studies as well as studies of sensitivity and specificity will be necessary in order to elucidate the possible significance of ECP in urine as a marker of neoplastic bladder disease.

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