EVALUATION OF METABOLISM OF CONNECTIVE TISSUE PROTEINS IN PATIENTS WITH LUNG CANCER

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The metabolism of connective tissue proteins has been found to be altered in the course of various diseases. The problem of interaction of neoplastic cells with the connective tissue interstice seems to be essential for understanding of the mechanisms regulating the processes of proliferation, infiltration and metastases of neoplasma [1].

The lung is an organ built in about 25% of connective tissue. Collagens of all types occur in the lung, with collagens of type I and II being most abundant. The lung is also rich in elastic fibres which contain elastin. Simultaneous occurrence of collagen and elastic fibres determines the properties of connective tissue which are especially important for the proper efficiency of the lung [2].

Enzymatic degradation of the connective tissue components in the course of neoplastic processes is of great importance since determination of metabolites of this tissue proteins in body fluids of patients with cancer could be helpful in making the clinical diagnosis more accurate and in therapy monitoring.
In the literature there are practically no data on elastin metabolism in cancer patients. A few communications related to small groups of patients with lung cancer point to increased urinary excretion of hydroxyproline and hydroxylysine, two highly representative collagen metabolites.

The present studies involved 96 patients with lung cancer (86 men and 14 women, average age 55.7 years), 10 patients with benign lung tumours (8 men and 2 women, average age 44 years), and a control group of healthy individuals (30 men and 10 women, average age 50 years). The diagnosis of lung cancer was based on histopathological examination. Determinations were performed on blood and urine of patients, who so far have not been subjected to irradiation or treated with cytostatic drugs or with kidney cortex steroids, prior to surgery; the patients with clinical symptoms of inflammatory changes in lungs or atherosclerosis were excluded in advance.

The following lung cancers were diagnosed: small-cell lung cancer (n = 20), squamous cell lung cancer (n = 49), adenocarcinoma (n= 11) and in 16 cases the histological type of neoplasma has not been identified. The stadium of the neoplastic process was evaluated according to the TNM classification elaborated by the International Anti-Cancer Union.

Blood was withdrawn from the elbow vein 12 h after the last meal and a 24 h period on a diet poor in collagen. Urine was taken from a single portion excreted after an over-night fast, following full emptying of the bladder. The determinations were made in duplicate. Total hydroxyproline and hydroxylysine were determined in blood plasma and urine by the method of Stegemann [3] and Blumenkrantz & Asboe-Hansen [4], respectively. The plasma elastolytic activity was determined according to Hornebeck et al. [5] with the synthetic substrate succinyl trialanine-p-nitroanilide (Suc-(Ala)3-pNA, Fluka Chemie AG, Buchs). A calibration plot was prepared with the use of highly purified porcine pancreatic elastase (120 U/mg, Serva, Heidelberg). The peptides derived from elastin were determined by the immunoenzymatic method of Kucich et al. [6]. Antibodies against elastin were assayed by the DOT-immunobinding method [7] and expressed as the titre following preparation of serial dilutions of the plasma studied. The results obtained were analyzed by Student's t test for unrelated samples (Table 1).

The concentration of total hydroxyproline in plasma, as well as its excretion in urine in the case of patients with lung cancer showed a statistically significant increase as compared both with the control group (p < 0.005), and with the patients having benign lung tumours (p < 0.005).
Table 1

**Markers of collagen and elastin metabolism in patients with lung cancer**

The excretion of collagen metabolites in urine is expressed as hydroxyproline/hydroxylysine - creatinine coefficients [8]. Elastolytic activity is expressed as nanograms of the elastase equivalent per 1 ml of plasma. The results for elastin-derived peptides are expressed as nanograms of kappa-elastin per 1 ml of plasma.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group (n = 40)</th>
<th>Lung cancer group (n = 96)</th>
<th>Benign tumour group (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH-Proline, plasma (μmol/l)</td>
<td>167.9 ± 44.9</td>
<td>250.3 ± 76.1</td>
<td>190.8 ± 49.9</td>
</tr>
<tr>
<td>OH-Proline, urine (μmol/mmol creatinine)</td>
<td>23.6 ± 6.1</td>
<td>34.1 ± 9.9</td>
<td>25.6 ± 6.6</td>
</tr>
<tr>
<td>OH-Lysine, plasma (μmol/l)</td>
<td>2.49 ± 0.52</td>
<td>3.25 ± 0.88</td>
<td>2.71 ± 0.68</td>
</tr>
<tr>
<td>OH-Lysine, urine (μmol/mmol creatinine)</td>
<td>2.03 ± 0.45</td>
<td>4.09 ± 1.25</td>
<td>2.94 ± 1.31</td>
</tr>
<tr>
<td>Elastolytic activity in plasma (ng eq. elastase/ml)</td>
<td>161.2 ± 51.1</td>
<td>153.4 ± 42.5</td>
<td>134.4 ± 35.2</td>
</tr>
<tr>
<td>Elastin-derived peptides in plasma (ng/ml)</td>
<td>118.4 ± 37.0</td>
<td>156.9 ± 62.8</td>
<td>128.9 ± 50.9</td>
</tr>
</tbody>
</table>

A similar dependence was found for hydroxylysine concentration in plasma and its urinary excretion in patients with lung cancer as compared with controls (p < 0.005) and with patients having benign lung tumours (p < 0.02). A relationship was also found between the values of the above parameters and cancer development: at stage II these values were statistically significantly higher, and at stages III and IV, highly significantly higher in comparison with the group of healthy controls.

A few observations published, made on small groups of patients point to an increase of urinary excretion of hydroxyproline in about 20 - 50% of the patients [9, 10]. The data concerning hydroxylysine in serum and urine are still more fragmentary. Among various histopathological forms of cancer, the highest concentration of those amino acids in plasma and their highest urinary excretion were found in cases of small-cell lung cancer and adenocarcinoma.
No significant difference was found in the elastolytic activity of plasma between the lung cancer group and the two other groups of patients. No significant differences were found, either, between the patients at different stages of cancer development, or between the histopathological subgroups.

The concentration of the elastin-derived peptides in patients with lung cancer was highly significantly higher as compared with the control group (\( p < 0.005 \)) but showed no statistical differences with the results for the benign lung tumour group. A positive correlation was observed between concentration of the elastin-derived peptides and the degree of clinical development of cancer. The highest concentration of these peptides was found in patients with adenocarcinoma and a concentration slightly lower in small-cell lung cancer and in not-identified lung cancer.

Studies on markers of elastin metabolism in various pathological states in man have been started but recently. The ability to hydrolyze elastin is characteristic of a group of enzymes termed elastases [11]. The elastolytic activity of plasma is the resultant of liberation of elastases from various tissues into the circulation, and the activity of specific inhibitors of these enzymes [12]. Neoplastic cells can also act as a source of elastolytic enzymes [13]. The results obtained on the elastolytic activity in plasma together with the observed increases in concentration of the elastin-derived peptides could point to the action of elastases \textit{in situ}, without any increase in the total elastolytic activity in plasma. The elastin-derived peptides formed in the course of elastin degradation act on specific receptors in the cytoplasmic membrane of several types of cells, among them also the neoplastic ones, induce various metabolic effects, i.e. chemotaxis, respiratory burst, excretion of proteolytic enzymes [14].

The elastin-derived peptides possess also immunogenic properties and, on penetrating into the circulation, induce formation of specific antibodies. The significance of these antibodies so far has been studied in experimental and spontaneous atherosclerosis [15]. The occurrence of antibodies against elastin in blood plasma can, on the one hand, reflect elastin metabolism and, on the other, immunological reactivity. Figure 1 presents the results of studies on the occurrence of anti-elastin antibodies in the analyzed groups. The antibodies were found to be present in two individuals of the control group, but were not found in any patient with the benign lung tumour. In the group of 96 patients with lung cancer, in 21 patients anti-elastin
Fig. 1. Anti-elastin antibodies in patients with lung cancer. Left panel: distribution according to the histological type of cancer; A - small-cell lung cancer, B - squamous-cell lung cancer, C - adenocarcinoma, D - not-identified. Right panel: distribution according to the stage of cancer development; A - stage II, B - stage III A, C - stage III B, D - stage III, E - stage IV. The number of patients is given on the ordinate. The results are expressed as the titre of antibodies.
antibodies were detected in varying amounts. The highest frequency of their appearance (45%) was found in patients with small-cell lung cancer.

The presented results on markers of collagen and elastin metabolism give no information about the exact site of pathological disturbances but reflect the general degree of those disturbances which are dependent on some properties of the cancer, especially on its histological structure. These markers can give valuable information in the case of difficulties in diagnosis and may help to distinguish malignant tumours from other, non-malignant processes. They also reflect indirectly the invasiveness and dynamics of cancer development. Full evaluation of the usefulness of these markers in monitoring of lung neoplasma requires further studies to be carried out during observations and therapy lasting for many months.

REFERENCES