Characteristic Change of Urinary Elastin Peptides and Desmosine in the Aortic Aneurysm

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To estimate elastin metabolism in aneurysm, urinary levels of desmosine and elastin peptide in patients ($n=23$, range 54 to 85 years old) with aneurysm were measured by ELISA and compared between two control groups divided by age (<10 years old and >20 years old). The amounts of urinary desmosine and elastin peptide in the aneurysm group were significantly increased compared with those in the older control group (>20 years old). There was a correlation between urinary desmosine and elastin peptide in the young group. On the other hand, no such correlation was observed in the aneurysm group and the older control group. The distribution of the ratio (desmosine/elastin peptide) in the aneurysm group was different from that of the young control group. We conclude that assay of elastin peptide and desmosine in urine are useful in characterizing elastin degradation in a patient with aneurysm.

Key words elastin peptide; desmosine; aneurysm

Arterial elastic fibers composed of elastin are a specific connective tissue protein. The solubilizing elastin peptide obtained by processing insoluble elastin in oxalic acid is called α -elastin. Elastin breakdown has previously been investigated by immunological determination of urinary elastin peptide or desmosine levels using anti- α -elastin or anti-desmosine antibodies^{1,2)} Desmosine is a specific cross-linked component which connects elastin molecules.

We reported previously that elastin is maintained in its native form by intra (desmosine) and inter (cystine, histidinoalanine) molecular cross-linkages and that these were readily denatured in various diseases (Marfan's syndrome, dissecting aneurysm and aortic aneurysm).³⁾ It has been reported that the aortic wall of an aneurysm is weaker than the normal aortic wall and there is an inverse correlation between elastin content and the elastolytic activity of aortic media homogenates.⁴⁾ The weak aortic aneurysm wall might be due to elastin degradation. We also reported that the arterial desmosine content decreased with progression of the aneurysm, and that the elastin decomposition activity rose in an experimental rat artery model.⁵⁾ These results suggested that the urinary excretion of desmosine-containing peptides was probably elevated due to aortic elastin degradation. The present assay methods for urinary elastin peptide and desmosine were used to compare the difference between aneurysm and control (young and older group).

MATERIALS AND METHODS

Urine samples of patients with non-arterial disease were provided by the Chigasaki Municipal Hospital, and were divided into 2 groups; a young group ($n=30$, range from 0 to 10, 4 ± 3 years old), and an older group ($n=33$, range from 20 to 99, 61 ± 24 years old). Urine samples of patients with aortic aneurysm were provided by the Tohoku University Attachment Hospital ($n=23$, range from 54 to 85, 66 ± 6 years old). Samples were obtained both pre- and postoperatively in

5 cases. α -Elastin peptides were prepared from human aortic insoluble elastin using the method described previously,⁶⁾ and anti- α -elastin antibody was produced as outlined in a previous report.¹⁾ Urinary elastin peptide and desmosine levels were measured by ELISA as previously reported.^{1,2)} Prior to the measurement of urinary desmosine, 1.0 ml of urine was added to 1.0 ml of 12N HCl, and hydrolyzed for 24 h at 110 °C. The hydrolysate sample was purified using CF1 cellulose mini-columns based on Skinner's method.⁷⁾ The solvent was removed by centrifugation and evaporation. The residue was freeze-dried, and then dissolved in buffer for ELISA of desmosine. Urinary creatinine level was measured using a Creatinine-Test kit (Wako). The amounts of urinary desmosine and elastin peptide relative to creatinine were calculated. Student's *t*-test was used to calculate the significance of differences.

RESULTS AND DISCUSSION

Elastin degradation is observed in lung diseases such as emphysema and chronic obstructive pulmonary disease,⁸⁾ and in the aortic diseases aneurysm and atherosclerosis.⁴⁾ Urinary excretion of desmosine and elastin peptide were shown to be increased in lung diseases,⁹⁾ while like changes were not reported in aortic diseases such as aneurysm. In our previous study,³⁾ the elastin cross-linkage component (isodesmosine or desmosine) in the aorta from a patient with aneurysm was greatly reduced in comparison with control (aortic regurgitation). We also confirmed that the denatured elastin, the decreased cross-linkage, caused on increase in solubilization by elastase treatment, consequently causing serum elastase so that the elastase-like activity tended to increase in aneurysm more than that in control. Therefore, it can be suggested that higher urinary elastin peptide and desmosine levels might reflect degradation of elastin in aneurysm. In the present study, the amounts of urinary elastin peptide and desmosine in the aneurysm group (66 ± 6 years old) were compared to those in

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the young (4 ± 3 years old) and older control groups (61 ± 24 years old). The amount of urinary elastin peptide was highest in the aneurysm group (486 ± 416 ng/mg creatinine; $n=23$), falling to 410 ± 455 ng/mg creatinine ($n=30$) in the young group and 236 ± 212 ng/mg creatinine ($n=33$) in the older group (Table 1). The amount of urinary desmosine was highest in the young group (500 ± 425 ng/mg creatinine; $n=30$), 313 ± 286 ng/mg creatinine ($n=23$) in the aneurysm group and 162 ± 162 ng/mg creatinine ($n=33$) in the older group (Table 1). In the previous report,¹⁰ the highest amounts of urinary desmosines were found in the early development period, and the amount declined during the pubertal and the young adult period and was constant thereafter. The amount of urinary elastin peptide and desmosine in the aneurysm group were similar to that in the young group. These results indicated that elevated urinary elastin peptide and desmosine caused the accelerated elastin turnover, similar to those seen

in chronic obstructive pulmonary lung disease. We previously confirmed that there was a correlation between desmosine and elastin peptides in the human aorta.²⁾ There may also be a correlation between changes in desmosine and elastin peptide level in human urine with age. We found that urinary elastin peptide level paralleled urinary desmosine content in the young group (Fig. 1: $r=0.430$, $p<0.05$), while there was no such correlation in urine from patients with aneurysm or in the older group (Fig. 1). Moreover, urinary elastin peptide and desmosine contents were found to be highly elevated in the aneurysm group as compared to the older control group (Table 1). Thus, degradation of elastin in the aneurysm group differed from that in both the young and older control groups. Next, we estimated the qualitative changes in urinary desmosine-containing elastin peptide in the same patients by analysis of samples obtained both pre- and postoperatively. No correlation was found between urinary desmosine and elastin peptide content in patients with aneurysm prior to the operation (Fig. 2); however, such correlation did tend to be present in these levels in the aneurysm group postoperatively, and this correlation was similar to that seen in the young control group (Fig. 2). These results may suggest that the pattern of elastin degradation postoperatively was similar to that in the young group.

In addition, there was an inverse correlation between elastin content and elastolytic activity in aneurysm aorta.⁴⁾ An inverse correlation was also reported between desmosine content and elastolytic activity in an experimental model.⁵⁾

Table 1. Urinary Desmosine and Elastin Peptide in the Young, Older and Aneurysm Groups

Group	Age	Desmosine /creatinine (ng/mg)	Elastin peptide /creatinine (ng/mg)
Old (control)	61 ± 24	162 ± 162	236 ± 212
Young	4 ± 3^a	500 ± 425^a	410 ± 455
Aneurysm	66 ± 6	313 ± 286^a	486 ± 416^a

Values are mean $\pm$ S.D. a) Statistically significant compared with control data ($p < 0.01$).

Fig. 1. Correlation between Desmosine and Elastin Peptide in the Young, Older and Aneurysm Groups

Fig. 2. Correlation between Pre- and Postoperative Urinary Desmosine and Elastin Peptide Levels in Patients with Aneurysm

Fig. 3. Relationship between the Desmosine/Elastin Peptide Ratio and the Frequency among Subjects in the Young, Older and Aneurysm Groups

These results suggested that elastin containing few cross-linkages was more easily degraded by elastolytic enzymes than the native. They also suggested that this low level of cross-linkage in elastin resulted in an increase in degradation of aortic elastin by elastase, elastase-like enzymes and matrix metalloprotease.

It appears that different immunoreactive desmosine containing elastin peptide might be excreted in urine with aneurysm. Calculation and comparisons of the ratio of desmosine to elastin peptide in aneurysm with the young and older control groups showed that content in the former had a

significant correlation with elastin peptide. The mean desmosine/elastin peptide ratio was 1.6, which was different from those in the older and aneurysm groups. We found that the relationships between the desmosine/elastin peptide ratio and the frequency in those two groups were different from that in the young group: distribution of the ratio in the young group was under (<1.6) 52%, upper (>1.6) 48%, while that in aneurysm and older group was mainly under (<1.6) 74% or 76%, respectively (Fig. 3). These results confirmed the quantitative or qualitative changes in desmosine-containing elastin peptide excretion in the urine from patients with

aneurysm. The distribution of the ratio of desmosine to elastin peptide in aneurysm was one feature of aneurysm. We conclude that assay of elastin peptide and desmosine in urine is useful in characterizing elastin turnover in patients with aneurysm.

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