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EFFECT OF IMMUNIZATION WITH PORCINE κ -2-ELASTIN ON ELASTASE-LIKE ACTIVITY IN RABBIT AORTA

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Presented at 25th Meeting of the Polish Biochemical Society; September, 1989

In the aorta of rabbits immunized with porcine κ -2-elastin, a statistically significant increase in the elastase-like activity was observed.

Elastin is one of the major structural components of arterial wall and it contributes significantly to the elastic properties of the arteries [1]. It is generally accepted that content of elastin in the blood vessels is diminished in certain arterial diseases such as atherosclerosis and hypertension [2]. Recent identification of elastase-like activity in the cell membranes of vascular smooth muscles has evoked considerable interest in exploring the relationship between the vascular elastin content and the elastase-like activity in atherosclerosis [3].

Elastin peptides, like κ -2-elastin, obtained by enzymatic or chemical hydrolysis of insoluble elastin, induce typical atherosclerotic lesions in the aorta and other blood vessels when injected into rabbits [4].

The aim of the present work was to investigate elastase-like activity in aorta of rabbits immunized with porcine κ -2-elastin.

MATERIALS AND METHODS

Forty male New Zealand rabbits 12 weeks old, weighting 2200 ± 50 g, were used in the experiment. The animals were kept on standard diet with water *ad libitum*.

The animals were divided into two equal groups. The experimental group received injections of 1.5 mg of porcine κ -2-elastin dissolved in 0.5 ml of 0.15 M NaCl mixed with 0.5 ml of complete Freund's adjuvant (CFA). The animals from the control group received injections of 0.5 ml of 0.15 M NaCl + 0.5 ml CFA. The rabbits received multiple subcutaneous injections on the back. The

immunization was carried out for 12 weeks: three times a week during the first 6 weeks and once a week during the following period of time.

Insoluble porcine elastin from aorta was obtained by the method of Robert & Hornebeck [5], and soluble κ -2-elastin was extracted by the method of Robert & Poullain [6], as modified by Moschetto *et al.* [7].

The animals were killed in 2 series: after 6 and 12 weeks of experiment. The part of aorta ranging from the heart to the iliac bifurcation was isolated after thoracotomy. Aorta was rapidly removed and placed in ice-cold sucrose solution (250 mM) buffered with 10 mM imidazole at pH 7.4. The endothelium and adventitia were separated from the aortic medial layers. Aortic muscle strips were then minced and homogenized in the same sucrose buffer using an all-glass homogenizer. The homogenates were centrifuged at $1000 \times g$ for 10 min and the supernatant was used for biochemical analysis. Elastase-like activity was determined spectrophotometrically according to Ito *et al.* [3], the colour formed with succinyl trialanine *p*-nitro-anilide (Sigma, St. Louis) being measured at 410 nm and 37°C, after bringing the pH up to 8.0.

RESULTS

The effect of the immunization with porcine κ -2-elastin on the elastase-like activity in rabbit aorta is presented in Table 1. As it can be seen, there was a statistically significant increase in elastase-like activity after 6 weeks of immunization with the elastin peptide. Similar results were obtained when rabbits were immunized for 12 weeks.

DISCUSSION

It has been demonstrated that elastase-like activity in the aorta is closely associated with vascular muscle plasma membranes. This localization may provide a reasonable explanation for the extracellular connective tissue metabolism in the vascular wall [3].

Table 1
Effect of immunization with porcine κ -2-elastin on elastase-like activity in aorta of rabbits

Elastase-like activity is expressed as the change of absorbance (A) at 410 nm over a reaction period of 24 h at 37°C per 1 g of wet tissue. Both for the control and experimental groups $n=10$; mean values + S.D. are given

Weeks of experiment	Control group	Experimental group
6	0.89 + 0.12	1.07 + 0.17*
12	0.94 + 0.13	1.22 + 0.20**

* $P < 0.02$ ** $P < 0.01$.

The elastolytic activity increases linearly with the degree of the atherosclerotic lesion and potentially with the age of the patients. These results suggest that atherosclerosis and age are two independent and cumulative factors determining the level of aortic elastase-like activity [8].

Yamada *et al.* [9] also reported that the elastase-like activity was elevated in the aorta of spontaneously hypertensive rats. Similar results were obtained by Kwan *et al.* [10] in the aortic extracts prepared from diabetic rats. In both cases an increase in the elastase-like activity was accompanied by the reduction in aortic elastin concentration.

Elastin peptides exert chemotactic effects on human monocytes and fibroblasts [11]. It was found that κ -2-elastin stimulates the increase in cytosolic free calcium and the release of intralysosomal enzymes (elastase) from monocyte monolayers [12]. Furthermore, κ -2-elastin stimulates the Ca^{2+} uptake by, and inhibits the Ca^{2+} efflux from human monocyte monolayers [13].

The results obtained in our experiment suggest that biological effects induced by immunization with κ -2-elastin may also be observed in smooth muscle cells of the arterial wall.

Increased activity of elastase-like enzymes may be responsible for morphological and biochemical changes in the aorta of rabbits immunized with κ -2-elastin, which were found in our previous investigation [14].

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