

INHIBITORY EFFECT OF ELASTASE ON THE GLOMERULAR CAPILLARY BASEMENT MEMBRANE THICKENING OF THE EXPERIMENTAL CONGENITAL DIABETIC MICE (N.S.Y. MICE)

MASAO SHIBATA*¹, ATSUKO KAWANISHI*¹,
TSUNEKI KISHI*¹, KAIZO KOBAYASHI*¹,
TSUNEJI KUNO*², MAKOTO SASAKI*³ and
BUNJI YASUDA*⁴

¹Department of Medicine, Branch Hospital, Nagoya University School of Medicine, Nagoya, Japan. ²Electron Microscope Laboratory, Nagoya University School of Medicine, Nagoya, Japan. ³Department of 2nd Biochemistry, Nagoya City University Medical School, Nagoya, Japan. ⁴Hakuyokai Hospital, Nagoya, Japan.

Requests for reprints should be addressed to M. SHIBATA., Department of Medicine, Branch Hospital, Nagoya University School of Medicine, Diako-cho Higashi-ku, Nagoya, 461 Japan.

ABSTRACT

Elastase is an elastin lytic enzyme that inhibits the deposition of fat on arterial walls and suppresses fat buildup. The changes in the renal glomerular basement membrane were observed over 30 days of elastase administration in N. Y. S. mice (experimental congenital diabetic animals), and the treated group showed a lessening in the thickening of the basement membrane. The thickness of the basement membrane in treated animals was $2158.7 \pm 96.2 \text{ \AA}$ against $2743.8 \pm 168.6 \text{ \AA}$ in the control. Thus, a significant difference ($P < 0.01$) resulted between the groups of N. S. Y. mice, and elastase is presumed to act upon the metabolism of the renal glomerular basement membrane in them.

Key word: Elastase, Glomerular basement membrane, Diabetic mice.

INTRODUCTION

Discovered by Balo *et al.*¹⁾, elastase was extracted and purified from the pancreas as an enzyme by Banga²⁾. It breaks down elastin found in the connective tissue of blood vessels and the like. In recent years, the negligible activity of elastase has been measured using a synthetic substrate, so that its activity is also detectable in organs other than the pancreas, such as the spleen³⁾ or in cells (e.g., leukocytes)⁴⁾. It has also been reported that elastase can clinically control hyperlipemia and atherosclerosis.

The present investigators produced and reported on experimental congenital diabetic mice N. S. Y. (Nagoya, Shibata, Yasuda) as an animal model for diabetic angiopathy⁵⁾. These N. Y. S. mice have evidenced a thickening in the glomerular capillary basement membrane, and we investigated the possible effect of elastase on their basement membrane. The following

柴田昌雄・河西あつ子・岸 常規・小林快三・久野常治・佐々木実・安田文二

Received for Publication January 18, 1981

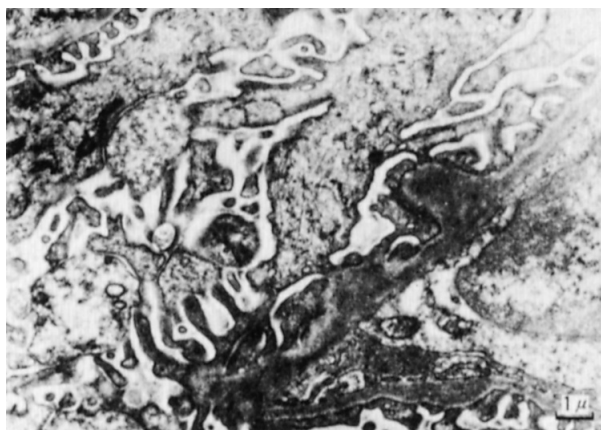
presents the striking results.

MATERIALS AND METHODS

N. S. Y. mice, bred in our laboratory, were employed as the experimental animals. Six-month-old F₁₂ littermates were used, and the mice were divided into two groups, the experimental group and the control. The experimental group is comprised of 4 mice and the control group is comprised of 3 mice. Elastase (340 unit/mg) was provided by Eisai Co., Ltd. and used in its purest form. An elastase physiological saline solution, adjusted to 5 mg/kg, was prepared for the test mice, which were injected daily for 30 days with 0.01 ml/g (mouse body weight) in the paralumbar muscle. Controls were administered 0.01 ml/g (body weight) saline solution for the same period. The glucose tolerance test (2 g/kg glucose administered intraperitoneally) was performed after the administration period was completed, both groups of mice were sacrificed, and the kidney was examined light and electron microscopically. Renal tissue for electron microscopy was fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer at pH 7.3 for four to six hours. The tissue was postfixed in an osmium tetroxide solution, then dehydrated through graded acetone and embedded in epoxy resin. Thin sections were examined with a Hitachi electron microscope. Using the method reported by Ireland⁶, measurements were performed with a magnifying micrometer at 10,000 magnifications to observe basement membrane thickening.

RESULTS

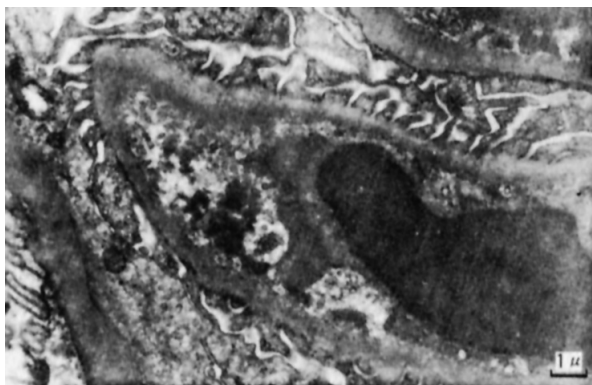
1. Body weight: Both experimental animals and controls were fed on Oriental Laboratory food. No difference was found in the weight of either group before and after elastase administration.
2. GTT: Glucose tolerance test results revealed no difference between groups.



(× 10,000)

Fig. 1. Electron micrograph of glomerulus from the control N. S. Y. mouse. The control N. S. Y. mice are six-month-old. This glomerulus show the thickness of the capillary basement membrane.

3. Electron microscopic findings (glomerular capillary): Findings in the control mice are shown in Fig. 1. Control showed the thickness of the capillary basement membrane and a marked increase in the basement membrane like material in the mesangium region. The experimental animals, on the other hand, exhibited the slight thickness of the capillary basement membrane (Fig. 2). However, the findings in the mesangium region displayed no noticeable change.



($\times 10,000$)

Fig. 2. Electron micrograph of glomerulus from the experimental N. S. Y. mouse. The experimental N. S. Y. mice are the same age. This glomerulus exhibited the slight thickness of the capillary basement membrane.

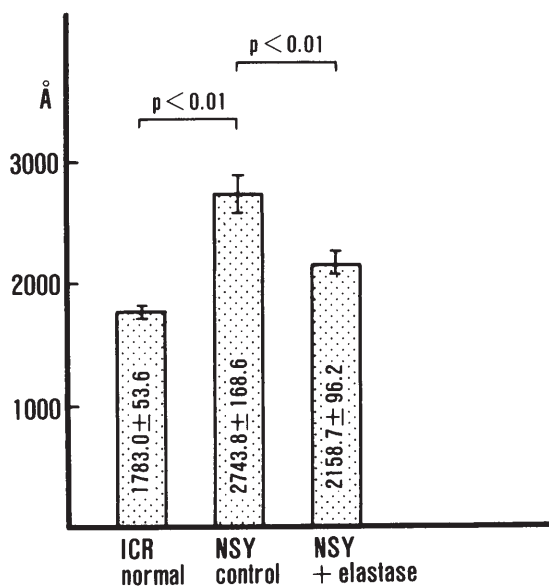


Fig. 3. Measurement of membrane thickness. ICR mice, control N. S. Y. mice and experimental N. S. Y. mice are the same age (Six-month-old).

4. Measurement of membrane thickness: The thickness of the glomerular capillary was measured in 100 different locations in each group, respectively. The thickness was found to be $2158.7 \pm 96.2 \text{ \AA}$ in the experimental animals, against $2743.8 \pm 168.6 \text{ \AA}$ in the controls. Thus, there was a significant difference ($P < 0.01$) between the two groups in the membrane thickness (Fig. 3).

DISCUSSION

Angiopathy is an important clinical factor in diabetes. Diabetic nephropathy, a form of diabetic microangiopathy, has a critical role in patient prognosis. Glomerular changes in diabetic renal disease indicates diabetic glomerulosclerosis. There are three types of the latter: diffuse lesion, nodular lesion, and exudative lesion. No special treatment exists currently for any of them.

On the other hand, various models for this glomerulosclerosis have been investigated to date. The present authors reported that the renal glomerulus in KK mice mentioned above were found to be suitable models for diabetic glomerulosclerosis⁷⁾. As compared with the spontaneously diabetic mice, the N. Y. S. mice possess an advantage in that the untreated I. C. R. mice can be used as the control. The basement membrane like material was increased in the glomerular mesangium region of N. S. Y. mice, and glomerular basement membranes were thickened. Thus, the present findings suggest that the renal glomerular changes evidenced in the N. S. Y. mice are a suitable model for human diabetic nephropathy⁸⁾.

Elastase is an elastin lytic enzyme. On the other hand, glomerular basement membrane consists of collagen proteins and noncollagen proteins. Kefalides⁹⁾ showed that this collagen was composed of 3 polypeptide chains and he named it type IV. Generally, collagen becomes stabilized by crosslinks.

Collagen is degraded by the sequence that its crosslinks are severed by the effect of neutral proteinase such as elastase, and then cut off by specific enzyme such as collagenase. In this study, it is considered that the effect of elastase on glomerular basement membrane has the same relation to the degradation mechanism of collagen in the glomerular basement membrane of N. S. Y. mice. The present findings of elastase effect are of interest in the elucidation of pathogenesis in diabetic glomerulosclerosis.

ACKNOWLEDGEMENT

We are most grateful to Eisai Co., Ltd. for supplying the purest elastase.

REFERENCES

- 1) Balo J. and Bang I.: The elastolytic activity of pancreatic extracts. *Biochem. J.*, **46**, 384—387, 1952.
- 2) Banga I.: Isolation and crystallization of elastase from the pancreas of cattle. *Acta Physiol. Acad. Sci. Hung.*, **3**, 317—324, 1952.
- 3) Starkey PM. and Barret AJ.: Neutral Proteinase of Human Spleen Purification and criteria for homogeneity of elastase and cathepsin G—. *Biochem. J.*, **155**, 255—263, 1976.
- 4) Dubin A., Koj A. and Chudzile J.: Isolation and Some Molecular Parameters of Elastase-Like Neutral Proteinase from Horse Blood Leucocytes. *Biochem. J.*, **153**, 389—396, 1976.
- 5) Shibata M. and Yasuda B.: New experimental congenital diabetic mice (N. S. Y. mice). *Tohoku J. Exp. Med.*, **130**, 139—142, 1980.

- 6) Ireland J.T.: Diagnostic criteria in the assessment of glomerular capillary basement membrane lesions in newly diagnosed juvenile diabetics. In: Camerini—Davalos R, Cole H.S. eds. New York: *Academic Press*, 273—278, 1970.
- 7) Shibata M., Yasuda B. and Kobayashi K.: Studies on the causes of diabetic angiopathy. Part I: The glomerular lesion of KK mice. *J. Japan Diab. Soc.* 5, 412—416, 1973.
- 8) Shibata M. and Yasuda B.: N. S. Y. mice: New congenital diabetic mice for diabetic research. In Abstracts, 10th congress of the international diabetes federation. Amsterdam: *Excerpta Medica.*, 213—214, 1979.
- 9) Kefalides N. A.: Biochemistry of vascular disease in diabetes mellitus. *Diabetes*. P.P. 575—587, Edited by Bajaj, J. S. *Excerpta Medica. Amsterdam*, 1977.