HISTOCHEMICAL ANALYSIS OF RENAL FIBROSIS IN RATS WITH ADENINE-INDUCED CHRONIC RENAL FAILURE

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Long-term feeding of adenine to rats produces metabolic abnormalities resembling chronic renal failure in humans with renal fibrosis due to diffuse formation of foreign body granuloma with 2,8-dihydroxyadenine (DHOA) crystals. 1,2) In this animal model, the present study examined morphological changes in renal fibrosis and chemical assays of collagen fiber.

MATERIALS AND METHODS
Twenty-five male Wistar rats, 5 weeks old and weighing 150g, were used. The animals were fed on commercial food for 7 days after arrival. They were then fed on a control diet containing 0.75% adenine for 1,2,3,4,5,6,12,18,24,30, and 60 days (adenine-ingesting rats: 2 or 3 rats in each group). Adenine was added at a dose of 0.75g/100g of the control diet. Autopsied kidneys of 11 groups of the adenine-ingesting rats were embedded in paraffin and in Epon by routine procedures, and 3μm paraffin section and ultrathin Epon section were cut and employed for both light and electron microscopic examinations. In chemical assays of collagen fiber in the autopsied kidneys, hydroxyproline determination and discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were added.

RESULTS AND DISCUSSION
In histological changes in the kidneys in 25 rats (11 groups) in each feeding period (1 to 60 days) on an adenine diet, the following four findings were obtained.
1. The DHOA crystal was seen microscopically to appear as early as the first day in the proximal and distal tubulous lumina.
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2. Pericrystal infiltration by neutrophils and macrophages was observed from the third day. 3. Foreign body granuloma with the DHOA crystal was formed at the 5th day and increased gradually. 4. Collagen fiber in the foreign body granuloma appeared from about the 12th day and increased gradually (Fig.1), and the granuloma was replaced by a fibrous nodule at about the 30th day, which caused renal fibrosis. On the other hand, in chemical assays of collagen fiber in the kidney, as shown in Figure 2, the amount of hydroxyproline was enhanced rapidly to a level which is nine times the control at the 20th day, and later increased gradually. In addition, type III collagen content in pepsin-solubilized collagen from renal cortices was higher than that of the control rats, as judged by discontinuous SDS-PAGE. From these results, the diffuse increase in collagen fiber in the kidney of this animal model was regarded as a repair of the tissue which ensued from the formation of foreign body granuloma due to deposits of DHOA crystals.

Fig.1. Foreign body granuloma (PAM stain).

Fig.2. Relationship between feeding period on adenine diet and hydroxyproline in the kidney.

REFERENCES