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Cis 3 hydroxyproline reduces glomerular basement membrane thickness and collagen type IV synthesis in diabetic rats

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Summary. Glomerular basement membrane thickening is thought to be due to increased collagen synthesis and abnormal cross linking. Based upon the observation that the incorporation of distinct proline analogues leads to increased degradation of the newly abnormally formed collagen we administered cis 3 hydroxyproline orally to streptozotocin diabetic rats.

Measuring glomerular basement membrane thickness we found in the treated group significantly lower values. The solubility of collagen in the treated group was significantly increased, indicating the mechanism of action of the proline analogue. The collagen content of kidneys in the treated group was reduced as well correlating with the basement membrane thickness. Provided the absence of toxicity of cis 3 hydroxyproline its pharmaceutical use for the inhibition of basement membrane proliferation seems promising.

Keywords: Amino acids – glomerular basement membrane – Collagen – Hydroxyproline – Cis-3-hydroxyproline – Diabetes

Introduction

The streptozotocin diabetic rat is a useful animal model to study glomerular and mesangial changes. Basement membrane thickening occurs regularly and shortly after streptozotocin (STZ) administration.

Basement membrane thickening is thought to be due to increased collagen synthesis and abnormal cross linking (Brownlee and Spiro, 1979; Schnider and Kohn, 1981).

The vessel walls are thickening progressively until they are completely obstructed. This is in congruence with the pathogenesis of diabetic nephropathy.

We have selected this model of diabetic glomerular changes as sufficient data are available in literature to compare and evaluate our findings.

Cis 3 hydroxyproline (cis 3 OHP) is an analogue of proline, absent in the mammalian system. It exists in plants and primitive animal systems only (Häusler, 1981). This stereoisomer of 3 trans hydroxyproline, which is a main constituent of the collagen type IV, is incorporated into collagens instead of proline in significant amounts. Analogously to cis 4 hydroxyproline, which is in pharmacological experimental use for the treatment of excess collagen deposition in liver cirrhosis and lung fibrosis (Uitto et al., 1984; Riley et al., 1981; Prokop et al., 1979), the incorporation of cis 3 hydroxyproline prevents the triple helical conformation of collagens which is necessary for the structural conformation of the collagen network in the connective tissue (Jimenez and Rosenbloom, 1974). Nonhelical collagen in turn is highly susceptible to proteolytic cleavage which leads to its rapid degradation and subsequently to a net negative collagen synthesis.

We synthesized (Häusler, 1981) and used cis 3 hydroxyproline in order to reduce glomerular basement membrane collagen synthesis in the streptozotocin rat.

Animals

30 Sprague Dawley rats, white, female, 2 months old, were given streptozotocin in order to induce diabetes after a protocol given in a previous publication (Lubec et al., 1982). The animals had free access to rat cake (Altromin), the control animals to tap water. The experimental group was given 30–50 mg/kg/day cis 3 hydroxyproline orally in tap water for a period of three months. At the end of the experiment the animals were sacrificed and autopsied.

Kidneys were taken at autopsy and used for the following examinations:

Isolation of collagen was performed after the principle of Dixit (Dixit, 1979).

Collagen extracted from kidneys was characterized on SDS-polyacrylamide gel electrophoresis after the principle of Lämmli (1970) and silver stained with the commercially available kit supplied by Amersham.

Hydroxyproline determinations were performed after a standard colorimetric method.

At sacrification there was no significant difference between the groups in terms of body weight, food and fluid uptake, creatinine and urea levels and blood glucose.

Electron microscopy of kidney cortex was performed after a routine method and glomerular basement membrane thickness was measured after a method published by our group (Lubec et al., 1980).

For the evaluation of non-enzymatic glycosylation as glycemic control, the Fructosamine test R (Hoffman La Roche, Basle, Switzerland) was applied using rat sera.

Collagen solubility experiments were carried out as given in a previous publication (Lubec et al., 1990).

Statistical examinations were done using Wilcoxon's test and a standard assay for the correlation between the parameters basement membrane thickness and collagen content of kidneys.

Results

20 out of 30 animals survived the experimental period and showed clinically, and at autopsy, signs of the diabetic state. 11 animals were treated and 9 animals were not treated.

Isolation of collagen

As seen in Fig. 1, SDS-PAGE characterizing eluted collagens of both groups showed a comparable pattern typical for collagen type IV.

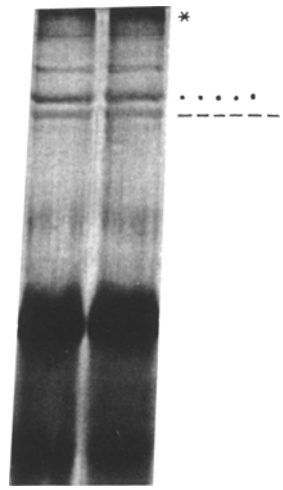

Fig. 1. The polyacrylamide gel electrophoretic pattern of eluted collagen type IV: left column: collagen eluted from nontreated animals; right column: collagen eluted from cis-3-OHP treated animals. No differences were observed in the patterns
Note for identification the collagen polymers (*) ..... the alpha-1 (IV)2 chain representing band and the ----- alpha-2 (IV) chain representing band

Hydroxyproline determinations

Collagen isolated from rat kidneys of the untreated group and based upon hydroxyproline determinations revealed 12,1 $\pm$ 3,2 mg collagen per 100 mg kidney weight versus 7,2 $\pm$ 1,2 mg collagen per 100 mg kidney weight in the cis 3 OHP treated panel. A statistically significant difference was calculated ($p < 0{,}001$).

Electron microscopical findings

On electron microscopy the group of untreated diabetic animals showed a mean basement membrane thickness of 3956 $\pm$ 1239A. In the cis 3 OHP treated group, a mean basement membrane width of 2255 $\pm$ 399A was observed.

Correlating basement membrane thickness with collagen content of the kidneys, we found a significant correlation $r = 0.69$ ($p < 0.02$).

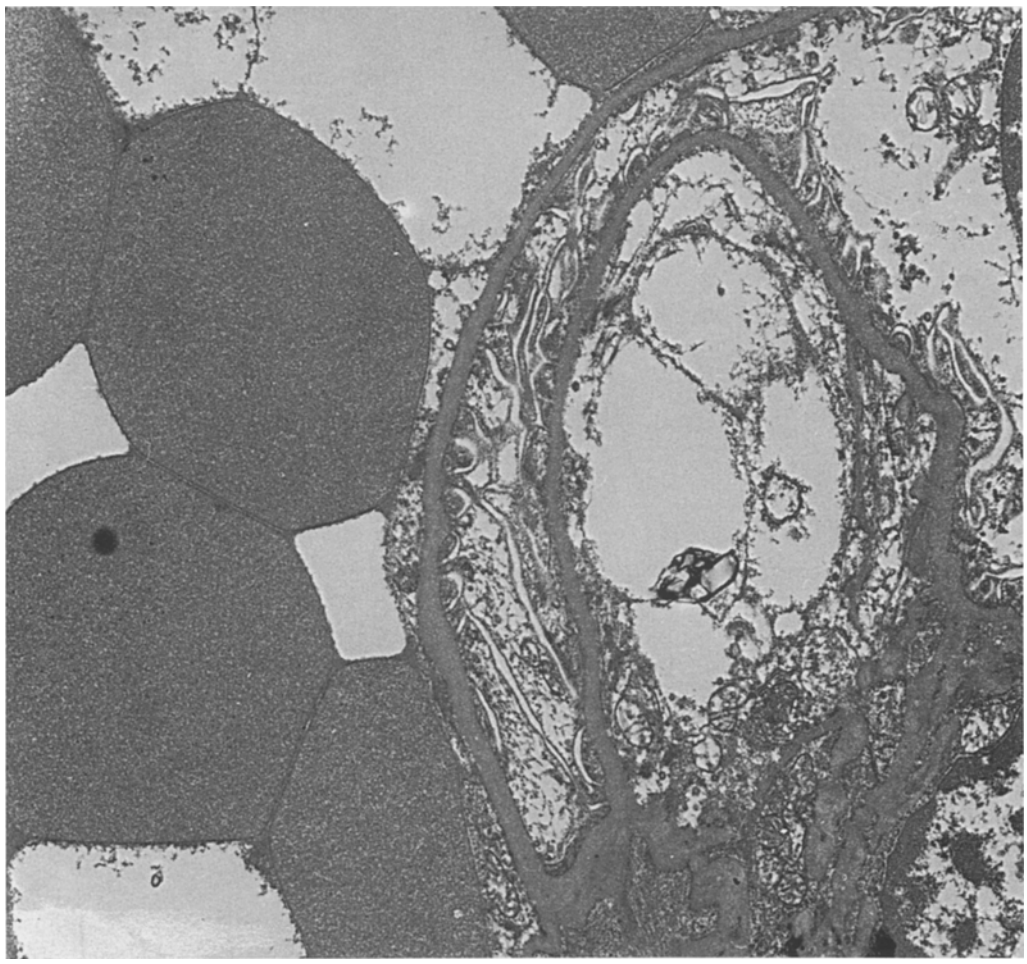

Fig. 2. Electron micrograph of the kidney section of an animal in the nontreated group (× 14400)

Glycemic control

The panel of nontreated rats showed mean values of 16.1 ± 2.5 μmol/l morpholinofructose in serum. The experimental group presented 15.4 ± 3.9 μmol/l. No significant differences could be shown using the cited statistical evaluations.

Solubility of collagen

Acid salt soluble collagen was eluted from homogenized kidneys. From the kidneys of treated animals 3.1 mg/100 mg kidney weight ±0.5 mg were eluted versus 1.9 ± 0.4 mg in the experimental group. The statistical comparison of groups showed significant differences in the solubility of collagen ($p < 0.0001$).

Discussion

As shown in the results, the cis 3 hydroxyproline treated group showed significantly reduced glomerular basement membrane thickness and a reduced col-

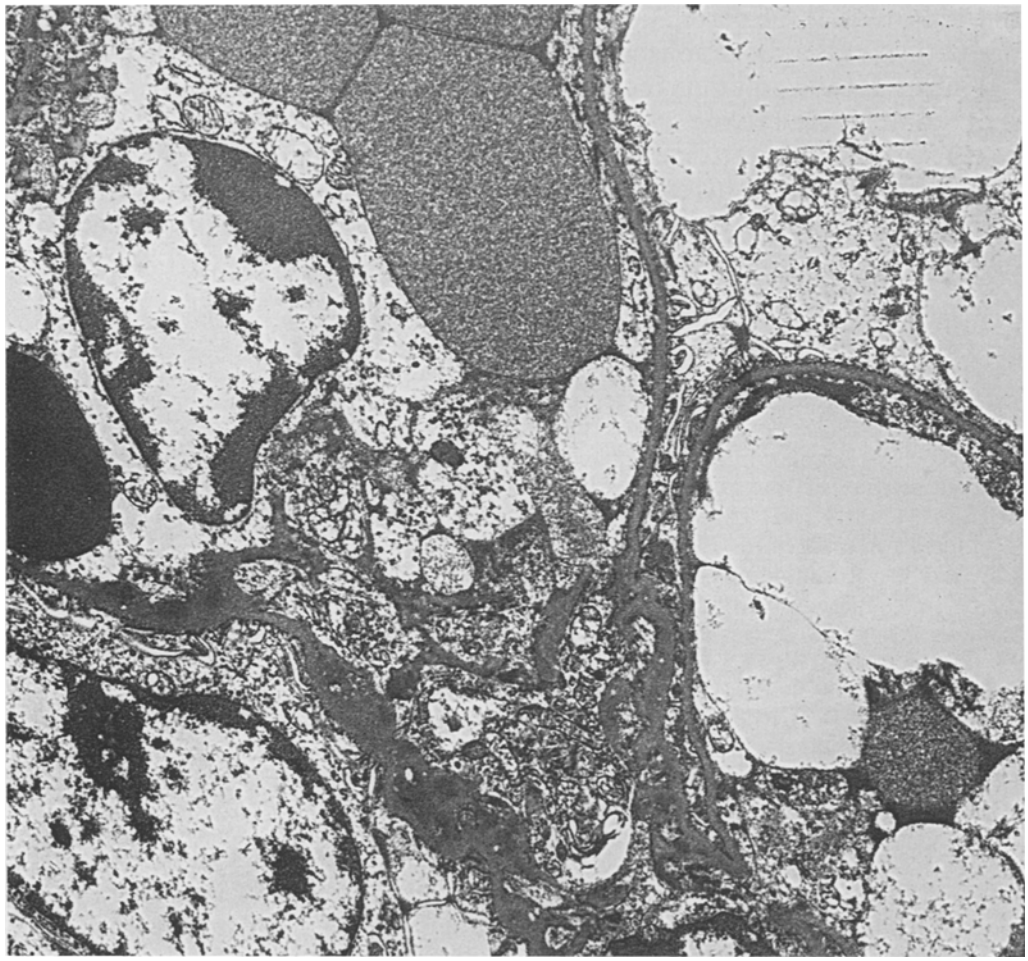

Fig. 3. Electron micrograph of a kidney section of an animal in the cis 3 hydroxyproline treated group (× 14400)

lagen content of kidneys. The differences in collagen content of kidneys cannot be due to differences in the severity of diabetes as the animals did not differ in their glycemic control, i.e. non-enyzmatic glycosylation expressed by fructosamine levels.

Solubility studies point to the expected reduced intramolecular cross linking of collagen, which is reflected by higher solubility resembling a lathyrogenic effect.

The mechanism of action resulting into the observed net negative collagen content was most probably the increased collagen degradation by the incorporation of cis 3 hydroxyproline. The incorporation of the analogue could be shown by the demonstration of cis 3 hydroxyproline in basement membrane collagen (Lubeç et al., 1991), although the possibility exists that cis 3 hydroxyproline might have interfered with proline synthesis, thus influencing collagen synthesis.

We cannot rule out that the reduction of other proteins of the basement membrane can be incriminated as well for the reduced basement membrane

thickness but, as the correlation between collagen content and basement membrane thickness was established, we suggest that collagen reduction played a significant role. In addition, the non-collagenous proteins of the basement do not show comparable proline contents.

Provided that no toxic effects of this amino acid analogue will be revealed, the administration of this compound could be considered as a promising experimental treatment of excess collagen deposition in vessel walls in diabetic and other glomerular diseases.

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