Diabetologia (1991) 34: 198–200
0012186X9100052F

Diabetologia

Rapid communication

Glucose enhances type IV collagen production in cultured rat glomerular mesangial cells

M. Haneda[1], R. Kikkawa[1], N. Horide[1], M. Togawa[1], D. Koya[1], N. Kajiwara[1], A. Ooshima[2] and Y. Shigeta[1]

[1] The Third Department of Medicine, Shiga University of Medical Science, Shiga
[2] The First Department of Pathology, Wakayama Medical College, Wakayama, Japan

Summary. Type IV collagen production by cultured glomerular mesangial cells and the effect of glucose on it were evaluated in order to explore the possible contribution of mesangial cells to the accumulation of type IV collagen in mesangial matrix typically seen in diabetes. Type IV collagen was measured quantitatively by enzyme-linked immunosorbent assay. The majority of type IV collagen was secreted into culture media and secreted-type IV collagen increased with cell growth in early log phase and decreased in late log phase and after confluency. By exposing the cells to high concentrations of glucose (27.8 mmol/l), both secreted- and cell-associated-type IV collagens increased significantly compared with the cells cultured under normal glucose concentrations (5.6 mmol/l) or under equivalent concentrations of mannitol, resulting in a significant increase in total type IV collagen accumulation from 32.1 ± 6.4 (under 5.6 mmol/l glucose) to 51.0 ± 4.6 μg/dish (mean ± SD, $n = 4$) on day 4, from 113.6 ± 6.6 to 156.8 ± 7.1 on day 6, from 248.5 ± 15.2 to 310.0 ± 12.6 on day 8 and from 372.4 ± 14.8 to 507.9 ± 17.2 on day 12. These results indicate the importance of glucose-induced alteration of mesangial cell function in the development of diabetic mesangial expansion.

Key words: Glomerular mesangial cells, type IV collagen, effects of high glucose, mesangial expansion, diabetic nephropathy.

Expansion of the glomerular mesangium has been considered to be one of the major histological characteristics in diabetic nephropathy [1]. The degree of mesangial expansion has been reported to be strongly related to the clinical manifestations of diabetic nephropathy, such as albuminuria and decrease in glomerular filtration rates [1]. It is, therefore, important to elucidate the mechanism underlying mesangial expansion. Since the mesangial matrix is normally composed of various macromolecules including type IV collagen which has been reported to increase in diabetic mesangium [2] and since mesangial cells have been considered to be responsible for the synthesis and metabolism of type IV collagen in mesangial matrix, increased synthesis and/or decreased degradation of type IV collagen by mesangial cells could result in the accumulation of matrix, leading to mesangial expansion.

Although mesangial cells in culture have been reported to synthesize various collagens including type IV collagen [3, 4], effect of glucose on the synthesis of type IV collagen has not been reported yet. We, therefore, examined the ability of mesangial cells to produce type IV collagen under high concentrations of glucose by measuring type IV collagen quantitatively with enzyme-linked immunosorbent assay (ELISA).

Materials and methods

Mesangial cell culture

Glomeruli isolated from Sprague-Dawley rats weighing 50 to 100 g by the sieving method were cultured in RPMI 1640 medium containing 20% fetal calf serum (FCS, Gibco, Grand Island, NY, USA) and antibiotics. Cultured cells were identified as mesangial cells by their morphological and biochemical characteristics as described previously [5].

For the experiment, mesangial cells were plated on 60 mm culture dish in a density of 77,000 cells/plate. On the next day, media were changed to the experimental media as follows; 1.) RPMI 1640 with 5.6 mmol/l glucose (control), 2.) RPMI 1640 with 27.8 mmol/l glucose (high glucose) and 3.) RPMI 1640 with 5.6 mmol/l glucose and 22.2 mmol/l mannitol (mannitol); each medium contains 20% FCS, 50 μg/ml ascorbic acid, 50 μg/ml β-aminopropionitrile and antibiotics. Medium was changed on days 4, 6, 8, and 12, and stored at −20°C until the assay. In order to solubilize the cell-associated collagen, cells were washed twice with phosphate-buffered saline (PBS), incubated with PBS containing 0.05% Triton X-100 at 4°C overnight, sonicated, and stored at −20°C.

Isolation of type IV collagen and preparation of antibody

Type IV collagen was isolated from the lung and kidney of 20 Sprague-Dawley rats using pepsin digestion and differential salt precipitation method as previously reported [6]. The purity of type IV

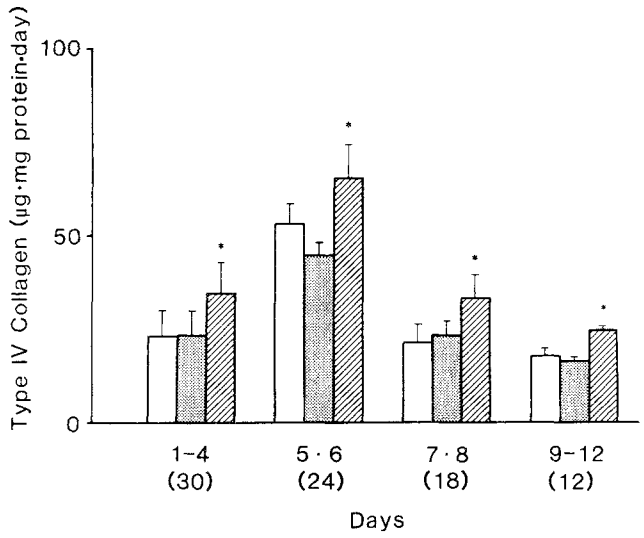

Fig. 1. Type IV collagen secreted from glomerular mesangial cells cultured under normal glucose (control, ▭), normal glucose plus mannitol (mannitol, ▭), or high glucose (▭) condition. Type IV collagen secreted into culture media was measured at the day indicated on the horizontal axis and divided by cellular protein and days. Thus, values shown are μg/mg protein/day (mean ± SD). Numbers are shown in parentheses. *$p < 0.01$ vs control and mannitol

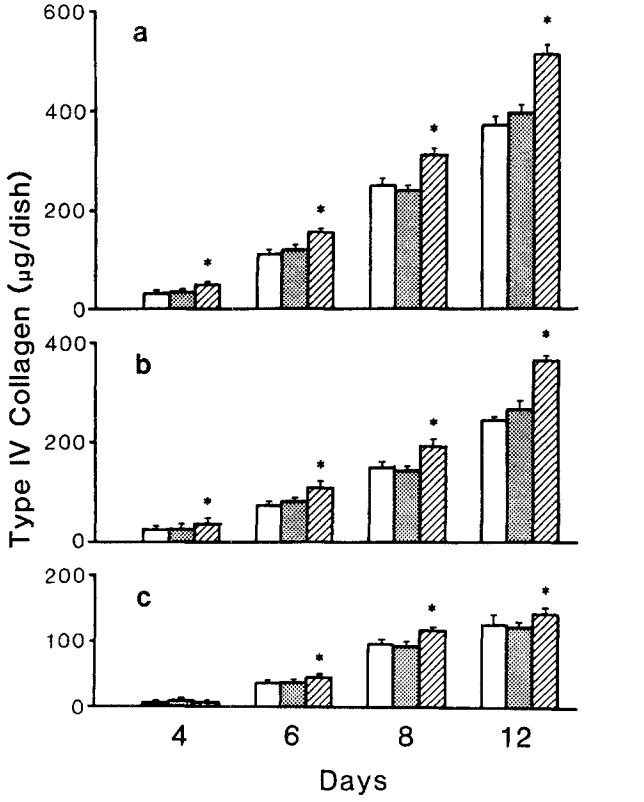

Fig. 2. Accumulated amount of total (**a**), secreted (**b**), and cell-associated (**c**) type IV collagen in glomerular mesangial cells cultured under normal glucose (control, ▭), normal glucose plus mannitol (mannitol, ▭), or high glucose (▭) condition. Values are mean ± SD ($n = 4$). *$p < 0.01$ vs control and mannitol

collagen was confirmed by SDS-polyacrylamide gel electrophoresis. Purified type IV collagen was dissolved in 0.5 mol/l acetic acid, dialyzed extensively against 0.5 mol/l acetic acid, and lyophilized.

Rabbit antisera to rat type IV collagen was raised by intradermal injection of 1 mg purified type IV collagen mixed with complete Freund's adjuvant as previously described [6]. Immunization was repeated every two weeks, four to six times in total. Sera were checked by enzyme-linked immunosorbent assay as described below using multi-well plate coated with type I, III, IV, V, or VI collagen, laminin, or fibronectin. IgG fraction was then prepared by precipitation with sodium sulfate, dialysis, and passing through diethylaminoethyl (DEAE) cellulose column, and concentrated.

Enzyme-linked immunosorbent assay of type IV collagen

Type IV collagen was measured by enzyme-linked immunosorbent assay reported by Rennard et al. [7] with slight modification. In brief, 50 μl type IV collagen standard or sample was incubated with the same volume of anti-type IV collagen IgG diluted with PBS containing 0.5% bovine serum albumin at 4°C for 16 h. Ninety μl reaction mixture was then transferred to the well of 96 multi-well plate (Falcon, Becton Dickinson and Co., Lincoln Park, NJ, USA) which was precoated with type IV collagen (1 μg type IV collagen/well in 0.02 mol/l carbonate buffer, pH 9.6 at 4°C overnight) and the plate was placed at 37°C for 60 min. After rinsing each well three times with a washing buffer (PBS with 0.1% Tween 20), 100 μl anti-rabbit IgG conjugated with peroxidase (Tago, Inc., Burlingame, Calif., USA) was added to the well, incubated at 37°C for 60 min and rinsed three times. Final reaction mixture (0.04 mg/dl o-phenyl enediamine dihydrochloride and 0.06% H_2O_2 in 0.05 mol/l citrate buffer, pH 5.0) was then added and incubated at room temperature for 2 min. The reaction was stopped by adding 50μl H_2SO_4 and absorbance at 490 nm was measured.

Results

Antibody against type IV collagen did not cross-react with type I, III, V, or VI collagen, laminin, or fibronectin, and type IV collagen could be measured quantitatively in the concentrations between 1 and 50 μg/ml. Intra-assay and inter-assay coefficient of variation was 2.9% and 4.1%, respectively.

Mesangial cells cultured under this experimental condition (containing 20% FCS) reached confluency by day 8 and, as shown in Figure 1, secreted type IV collagens increased with cell growth in early log phase (1–6 days) and decreased in late log phase and after confluency (7–12 days). Mesangial cells cultured under high glucose condition secreted a significantly greater amount of type IV collagen than the cells of control and mannitol groups.

When total amounts of type IV collagen accumulated during experimental periods were calculated, gradual increase of accumulation was observed in each group (Fig. 2a). Accumulation of type IV collagen secreted from the cells was almost three times greater than that associated with the cells (Fig. 2b, c). Among three groups, mesangial cells cultured under the high glucose condition showed the greatest accumulation of type IV collagen (Fig. 2a). This increase in total type IV collagen accumulation in the cells cultured under high concentrations of glucose was largely due to an increase in the accumulation of secreted type IV collagen (Fig. 2b), although type IV collagen associated with the cells cultured under the high glucose condition was also significantly greater than that in control and mannitol cells (Fig. 2c). Because the growth rate of the cells were not different among three groups in this experimental condition, similar results were obtained when the accumulation of type IV collagen was expressed as μg per mg protein or per μg DNA. Thus, final total

accumulation of type IV collagen (by day 12) in control, mannitol and high glucose group was 392.1 ± 46.1, 414.3 ± 45.2 and 533.6 ± 30.2 μg/mg protein or 2.06 ± 0.3, 2.11 ± 0.38 and 2.92 ± 0.50 μg/μg DNA (mean ± SD, $n = 4$), respectively.

Discussion

In this study, we clearly demonstrated that type IV collagen secreted from and associated with glomerular mesangial cells increased significantly when cultured under high concentrations of glucose.

Mesangial cells, in the present study, secreted almost three times more type IV collagen into culture media than that associated with the cells. This result is in concordance with the report of Haralson et al. [3], who measured various collagens in cultured rat mesangial cells and found that almost all type IV collagen was identified in culture media. On the contrary, Ardaillou et al. estimated the synthesis of collagens in cultured human mesangial cells and found only 30% of type IV collagen was in the secreted fraction [4]. Although the reason for this discrepancy is unclear at present, these results suggest that mesangial cells are able to secrete considerable amounts of type IV collagen, which might accumulate in mesangial matrix.

Type IV collagen secreted from mesangial cells increased with cell growth in early-log phase and decreased in late-log phase and after confluency. Similar phenomena have been observed in different types of cells. In rabbit smooth muscle cells in culture, collagen synthesis has been reported to decline as cells in culture attain confluence [8]. It would be thus possible that there is some relationship between the synthesis of collagen and proliferative activity or population density of the cells, although the mechanism is unclear.

The most important finding of this study is enhancement of accumulation of type IV collagen in mesangial cells by glucose. Total accumulation of type IV collagen in mesangial cells cultured under high concentrations of glucose increased throughout the study period, predominantly due to increase in accumulation of secreted type IV collagen. Although the present study cannot differentiate an increase in biosynthesis from a decrease in degradation of type IV collagen, recent reports in other types of cells favour the former hypothesis. Glucose has been reported to increase the expression of type IV collagen mRNA in cultured human endothelial cells [9]. Moreover, specific increase in steady state type IV collagen mRNA levels has been reported in kidney cortex of diabetic mice, KKAy [10]. It is, therefore, possible that glucose modulates transcription of type IV collagen gene and thus increases mRNA of type IV collagen in the cells in which biosynthesis of type IV collagen is one of the major functions. Furthermore, since we have found that glucose does induce metabolic derangements such as an increase in the activity of the polyol pathway in cultured mesangial cells [5], these metabolic derangements might be possible to mediate the effect of glucose on type IV collagen synthesis.

Because mesangial expansion has been considered to be a major abnormality of diabetic glomeruli, enhancement of type IV collagen production in glomerular mesangial cells by glucose could have a significant implication on the pathogenesis of diabetic nephropathy. Quantitative estimation of type IV collagen production could also be used in developing therapeutic intervention of diabetic nephropathy.

References

1. Mauer SM, Steffes MW, Ellis EN, Sutherland DER, Brown DM, Goetz FC (1984) Structural-functional relationships in diabetic nephropathy. J Clin Invest 74: 1143–1155
2. Abrass CK, Peterson CV, Raugi GJ (1988) Phenotypic expression of collagen types in mesangial matrix of diabetic and nondiabetic rats. Diabetes 37: 1695–1702
3. Haralson MA, Jacobson HR, Hoover RL (1987) Collagen polymorphism in cultured rat kidney mesangial cells. Lab Invest 57: 513–523
4. Ardaillou N, Bellon G, Nivez M-P, Rakotoarison S, Ardaillou R (1989) Quantification of collagen synthesis by cultured human glomerular cells. Biochim Biophys Acta 991: 445–452
5. Kikkawa R, Umemura K, Haneda M, Arimura T, Ebata K, Shigeta Y (1987) Evidence for existence of polyol pathway in cultured rat mesangial cells. Diabetes 36: 240–243
6. Matsumoto E, Muragaki Y, Ooshima A (1989) Increased serum type IV collagen peptide in carbon tetrachloride-treated rats. Acta Pathol Jpn 39: 23–29
7. Rennard SI, Berg R, Martin GR, Foidart JM, Robey PG (1980) Enzyme-linked immunoassay (ELISA) for connective tissue components. Anal Biochem 104: 205–214
8. Holderbaum D, Ehrhart LA (1984) Modulation of type I and III procollagen synthesis at various stages of arterial smooth muscle cell growth in vitro. Exp Cell Res 153: 16–24
9. Cagliero E, Maiello M, Boeri D, Roy S, Lorenzi M (1988) Increased expression of basement membrane components in human endothelial cells cultured in high glucose. J Clin Invest 82: 735–738
10. Ledbetter S, Copeland EJ, Noonan D, Vogeli G, Hassell JR (1990) Altered steady-state mRNA levels of basement membrane proteins in diabetic mouse kidneys and thromboxane synthase inhibition. Diabetes 39: 196–203

Received: 27 August 1990

M. Haneda, M.D.
Third Department of Medicine
Shiga University of Medical Science
Seta, Otsu
Shiga 520-21
Japan