Increased decorin mRNA in diabetic mouse kidney and in mesangial and tubular cells cultured in high glucose

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Mogyorosi, Andras, and Fuad N. Ziyadeh. Increased decorin mRNA in diabetic mouse kidney and in mesangial and tubular cells cultured in high glucose. Am. J. Physiol. 275 (Renal Physiol. 44): F827–F832, 1998.—The core protein of the proteoglycan decorin binds and neutralizes transforming growth factor-β (TGF-β). Activation of TGF-β is crucial to tissue injury in diabetic nephropathy, but it is not currently known whether decorin plays a role in this disease. Mouse kidney cortex demonstrates more than a twofold increase in decorin mRNA after 1, 2, 3, and 6 wk of streptozotocin diabetes. Various mouse and rat renal cell types are studied in culture under normal or high-glucose conditions. Mouse glomerular mesangial and proximal tubular epithelial cells constitutively express decorin, and high glucose (450 mg/dl) increases decorin mRNA fourfold compared with 100 mg/dl glucose. Unlike rat mesangial cells, rat glomerular epithelial and endothelial cells do not constitutively express decorin, and no induction is observed in high glucose. When mouse mesangial and proximal tubular cells are exposed to TGF-β1 (1 ng/ml), decorin mRNA is significantly decreased. Our findings suggest that the increased decorin expression in the diabetic kidney may counteract the hypertrophic and profibrotic effects of increased TGF-β levels and that a negative feedback loop may exist between decorin and TGF-β.

EVIDENCE FOR A PATHOGENIC role of transforming growth factor-β (TGF-β) in mediating the pathological features of diabetic kidney disease has been demonstrated in experimental models using in vitro (31) and in vivo (21) approaches. Furthermore, kidney TGF-β mRNA and protein levels are upregulated in various human renal diseases, including diabetic nephropathy (8, 23, 30).

Decorin is a small extracellular proteoglycan (92.5 kDa) comprising a 40-kDa core protein and a single chondroitin sulfate side chain. The core protein binds and neutralizes extracellular TGF-β and antagonizes its prosclerotic effect (1). This property has been exploited for therapeutic purposes. In experimental glomerulonephritis in the rat, administration of decorin alleviates extracellular matrix accumulation and proteinuria (1). Glomerulonephritic rats transfected with decorin cDNA show significantly decreased glomerular TGF-β levels and amelioration of proteinuria and the increased extracellular matrix accumulation (7). Renal decorin mRNA and protein as well as active TGF-β levels are upregulated in experimental hydronephrosis in the rat (4). In human biopsy specimens of various chronic renal diseases, intrarenal decorin immunoperoxidase staining is significantly enhanced, and decorin proved to be the best predictor among matrix components of the severity of interstitial fibrosis and renal failure (25). However, it is not currently known whether decorin plays any role in noninflammatory kidney diseases such as diabetic nephropathy. Such information would be important before considering the potential therapeutic benefits of decorin in the future.

The aim of our present study was to evaluate the renal expression of decorin in streptozotocin (STZ)-diabetic mice and to examine the effect of high ambient glucose on the expression of decorin in glomerular mesangial, epithelial, and endothelial cells as well as in proximal tubular cells in culture. We were also interested in whether TGF-β, a cytokine that has a well-established role in the pathophysiology of diabetic nephropathy and is itself upregulated in many in vitro and in vivo models of renal diseases (15), can exert an effect on the expression of the decorin gene, perhaps as a component of a negative feedback loop.

MATERIALS AND METHODS

Induction of Diabetes and Experimental Protocols

C57Bl female mice were fed a standard pellet laboratory chow and were provided with water ad libitum. Diabetes was induced in weight-matched 8-wk old mice (~20 g) by two consecutive daily intraperitoneal injections of STZ (200 mg/kg; Sigma Chemical, St. Louis, MO) dissolved in 10 mM sodium citrate, pH 5.5; controls were injected with buffer alone. A relatively high dose of STZ was needed to induce diabetes, because mice, as opposed to rats, are relatively resistant to the diabetogenic effect of the drug. On the same day that glucosuria was first present, up to 0.5 U NPH insulin (Eli Lilly, Indianapolis, IN) was administered to maintain the blood glucose concentration in the moderately hyperglycemic range of 250–400 mg/dl and to prevent ketonuria. In an additional group of diabetic mice, the insulin dose was increased up to 1.0 U NPH daily to maintain the blood glucose concentration below 140 to 180 mg/dl. Groups of diabetic and control mice were killed after 1, 2, 3, and 6 wk following the detection of glucosuria. Kidney cortex was excised, immediately frozen in liquid nitrogen, and stored at –70°C for subsequent RNA extraction.

Cell Culture

Murinetransformed and untransformed glomerular mesangial cells. Murine mesangial cells (MMC) were isolated by differential sieving from kidneys harvested from 8-wk old naive SJ L/J (H-2b) mice (28). Cells were grown for 72 h in the presence of 50 mM D-valine (Sigma), replacing L-valine in the
medium to exclude fibroblasts. Untransformed murine mesangial cells were designated uMMC. Subconfluent cells were transformed with a nonreplicating, noncapsid-forming strain of SV40 to establish a permanent cell line (28, 31) which was here designated tMMC. The cells maintain a differentiated phenotype as evidenced by the typical spindle-like appearance, positive staining for vimentin and desmin, production of several matrix components including collagens I and IV, and contraction in response to angiotensin II stimulation.

Murine proximal tubular cells. The mouse cortical tubule cell line (MCT) was derived from microdissected proximal tubule segments of normal SJL mice and stabilized in long-term culture by SV40 transformation (6). The cells exhibit many phenotypic features of differentiated proximal tubular epithelial cells, including positive staining for cytokeratin, sodium-phosphate cotransport, and production of collagen IV and laminin.

Rat glomerular mesangial cells. Rat glomerular mesangial cells (RMC) were isolated from Sprague-Dawley rats by differential sieving and were characterized and grown in a manner similar to that described above for murine mesangial cells.

Rat glomerular endothelial cells. Rat glomerular endothelial cells (GEndC) were isolated and characterized as described elsewhere (29). The cells exhibit a cobblestone appearance when confluent in culture and stain positively with CD31 (PECAM-1), factor VIII, and lectin BSI (29).

Rat glomerular epithelial cells. Rat glomerular epithelial cells (GEpiC) are a gift of Dr. David J. Salant (Boston University) (16). The cells display cobblestone appearance when confluent and culture and stain positively with CD31 (PECAM-1), factor VIII, and lectin BSI (29).

Culture Media

The medium used for all cell types with the exception of GEpiC was DMEM (GIBCO-BRL, Gaithersburg, MD) supplemented with 100 µg/ml streptomycin, 100 U/ml penicillin, 2 mM glutamine, and 10% fetal calf serum (32). Cells were subcultured every 72 h and incubated in a humidified atmosphere of 5% CO₂-95% air at 37°C. GEpiC were grown in standard K1 medium consisting of 47.5% DMEM, 47.5% Ham’s F-10 (GIBCO), 4.9% NuSerum (Collaborative Research, Bedford, MA), and a 0.1% hormone mix described previously (5).

Northern Hybridization

Cells were rested for 24 h in serum-free media, then carried for different time periods (24, 48, 72, and 96 h) in 0.5% fetal calf serum/DMEM containing either 100 mg/dl (5.6 mM) or 450 mg/dl (25 mM) D-glucose. RNA extraction from harvested cells or kidneys was performed as previously described (22, 28). For Northern blots, 25 µg total RNA was electrophoresed through a 1.0% agarose gel with 2.2 M formaldehyde. The RNA was electroblotted onto GeneScreen Plus nylon membranes (NEN Research Products, Boston, MA) and ultraviolet cross-linked. Integrity and equal loading of RNA samples were assessed by methylene blue staining of the transferred RNA (22). The membranes were prehybridized for 4 h at 65°C in a buffer containing 5× SSPE (1× SSPE = 149 mM NaCl, 10 mM NaH₂PO₄, 1 mM EDTA), 5× Denhardt’s (50× Denhardt’s = 1% Ficoll, 1% polyvinylpyrrolidone, 1% bovine serum albumin), 0.1% SDS, 100 µg/ml denatured salmon sperm DNA, and 50% (vol/vol) formamide. cDNA inserts were separated from their plasmids in low-melt agarose and labeled with 5 µCi [³²P]dCTP (3,000 Ci/mmol; Amersham, Arlington Heights, IL) using hexamer primers. The decorin probe used was a 1,372-bp murine decorin cDNA segment (gift from Dr. Renato V. Iozzo, Thomas Jefferson University, Philadelphia, PA) (19). Blots were hybridized with 1 × 10⁶ cpm/ml probe in hybridization buffer (same as prehybridization buffer except that 2× Denhardt’s was used) for 16 h at 65°C. Membranes were washed for 5 min twice in 2× SSC (20× SSC = 3 M NaCl, 0.3 M sodium citrate, pH 7.0) at room temperature, then in 2× SSC with 0.1% SDS for 15 min at 65°C, followed by two 15-min high-stringency washes in 0.1 SSC and 0.1 SDS at 65°C. The membranes were then

![Fig. 1. Northern hybridization of RNA isolated from kidney cortex extracts of nondiabetic and streptozotocin (STZ)-induced diabetic mice using a cDNA probe for decorin. A: representative Northern blot showing increased decorin message in kidney cortex from STZ-induced diabetic mice (D) in mice after 1, 2, 3, and 6 wk of diabetes compared with nondiabetic control mice (N). Standardization was performed by Northern hybridization with mprL32. B: quantitative results (mean ± SE) of hybridizations demonstrating increased decorin mRNA/mprL32 ratio in kidney cortex of diabetic mice. Values in nondiabetic mice (normal; n = 8) were assigned a ratio of 1. Diabetic (Diab) mice were studied after 1 wk of diabetes (1w; n = 3), 2 wk (2w; n = 8), 3 wk (3w; n = 3), and 6 wk (6w; n = 3). A group of diabetic mice were treated daily with sufficient insulin to keep the blood glucose level below 180 mg/dl (Diab + Ins; n = 3). *P < 0.01 vs. normal.](http://ajprenal.physiology.org/)

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autoradiographed with intensifying screens at $-70^\circ C$ for 1–4 days. Blots were stripped and subsequently rehybridized with probes encoding the housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 18S ribosomal RNA, or mouse ribosomal protein L32 (mrpL32) (14) to account for small loading and transfer variations. Exposed films were scanned with a densitometer (Hoefer Scientific Instruments, San Francisco, CA), and relative RNA levels were calculated.

Statistical Analysis

Data are presented as the means ± SE. Comparison between two groups was performed by unpaired t-test. $P < 0.05$ was considered significant.

RESULTS

Diabetic Mice

To investigate the potential role of decorin in an in vivo model of diabetic kidney involvement, we evaluated decorin mRNA level in the kidney cortex of mice with STZ-induced diabetes. Figure 1A is a representative Northern blot demonstrating upregulation of the mRNA in the kidney cortex after 1–6 wk of established diabetes compared with nondiabetic control mice. Figure 1B summarizes the results of these studies. The stimulation of decorin mRNA in the diabetic kidney was rapid and sustained, with a twofold increase in the mRNA level after 1 wk of diabetes and up to 2.5-fold after 2, 3, and 6 wk. For instance, after 2 wk of diabetes, the relative decorin mRNA level was increased by $2.30 \pm 0.50$-fold ($n = 8$; $P < 0.01$ vs. control mice). Figure 1B also depicts the response to daily treatment of mice with a relatively high dose of insulin. With such a regimen, the blood glucose concentration was maintained below 180 mg/dl, and the decorin mRNA level was only $1.48 \pm 0.63$-fold that of control mice ($n = 3$, not significant). These findings are consistent with a stimulatory effect of the diabetic milieu on decorin expression rather than a direct effect of STZ.

Mouse Mesangial Cells

To further delineate the significance of increased decorin gene expression in the diabetic kidney, we investigated different types of cultured renal cells under basal and high-glucose conditions. Decorin mRNA was found to be constitutively expressed in uMMC (Fig. 2A) as well as in tMMC (Fig. 2B). The decorin mRNA level was significantly increased by culturing either uMMCs or tMMCs in high-glucose media (450 mg/dl) compared with normal glucose media (100 mg/dl) (Fig. 2A and B). For instance, stimulation by high-glucose media for 72 h caused a 4.09 ± 1.24-fold increase ($n = 6$) in the ratio of decorin mRNA level to that of GAPDH in tMMC (Fig. 2C).

When exogenous TGF-β1 at a concentration of 1 ng/ml was added in the last 24 h of culture to tMMC, the basal as well as the high-glucose-stimulated level of decorin mRNA relative to that of mrpL32 were reduced by more than 80% ($n = 3$) (Fig. 3).

Fig. 2. Northern hybridization of RNA isolated from mouse mesangial cells cultured under normal or high-glucose conditions. A: hybridization of untransformed mouse mesangial cells (uMMC) demonstrating increased decorin message in cells cultured for 72 h in high glucose (H; 450 mg/dl) compared with normal glucose (N; 100 mg/dl). Standardization was performed with glyceraldehyde-3-phosphate dehydrogenase (GAPDH). B: similar blots of transformed mouse mesangial cells (tMMC) showing increased decorin message in high glucose (H) vs. normal glucose (N) in four different pairs of experiments. C: quantitative analysis of hybridizations demonstrating increased decorin mRNA in tMMC cultured in high glucose for 72 h. Values are means ± SE; $n = 7$ per group. *$P < 0.01$. 
Rat Glomerular Cells

We next investigated whether decorin expression is confined to mesangial cells within the glomerular compartment. We therefore analyzed different rat glomerular cell types that were available to us. As in mesangial cells derived from mouse, decorin mRNA was constitutively expressed in RMC but not in rat GEpiC or GEndC cells (Fig. 4). When cultured in high ambient glucose, no inducible decorin mRNA expression was observed in either the glomerular epithelial or endothelial cells (Fig. 4). On the other hand, culturing rat mesangial cells in high glucose for up to 96 h caused a 3.0-fold increase in the ratio of decorin message to that of GAPDH as compared with normal glucose (Fig. 4).

Mouse Proximal Tubular Cells

We next investigated whether decorin expression is evident in nonglomerular cells in the kidney. MCT were examined because of the demonstrated increase in decorin mRNA in the kidney cortex of STZ-diabetic mice. When cultured under high-glucose conditions for 72 h, MCT cells exhibited increased decorin mRNA levels compared with normal glucose (Fig. 5); the ratio of decorin mRNA to that of mrpL32 was increased by 3.4-fold in high-glucose media (n = 3). As with mesangial cells, the addition of exogenous TGF-β1 (1 ng/ml) for the last 24 h of culture caused marked reduction in the decorin mRNA level under both normal and high-glucose conditions (Fig. 5).

DISCUSSION

Our study is the first to show that decorin mRNA levels are upregulated in the kidney cortex of an animal model with diabetic renal involvement. The only available evidence for a role of decorin in diabetic kidney disease comes from in vitro experiments where decorin mRNA expression and protein production were found to be increased in human mesangial cells cultured under high-glucose conditions (26). Our study expands on
these findings by demonstrating that rat and mouse mesangial cells also exhibit increased decorin mRNA levels when cultured under high-glucose conditions. High glucose also exerted a similar effect on mouse proximal tubular cells. We also show that neither glomerular epithelial nor endothelial cells constitutively express decorin mRNA as detected by Northern analysis, and there is no induction of the message by high-glucose media. In this regard these latter cells behave like bovine myocardial endothelial cells (11). Furthermore, our studies demonstrated that exogenous TGF-β1 exerts an inhibitory effect on decorin gene expression in cultured mouse mesangial and proximal tubular cells.

In a previous study we demonstrated that the development of renal hypertrophy in the STZ-diabetic mouse is characterized by increased mRNA levels for TGF-β1 and its primary signaling receptor, the type II receptor (21). Increased renal TGF-β1 mRNA level is also a feature of models of spontaneous type 1 diabetes mellitus such as the nonobese diabetic mouse and the BioBreeding rat (22). In fact, the TGF-β system appears to be implicated in the development of diabetic renal hypertrophy since neutralization of TGF-β using repeated injections of anti-TGF-β antibody results in attenuation of kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice (21). Increased decorin gene expression by high ambient glucose, as shown by our current study, may thus represent a mechanism to counteract the injury produced by high-glucose-stimulated TGF-β1 (18, 28).

Since hyperglycemia in vivo and high ambient glucose in vitro stimulate renal cell TGF-β1 gene expression (18, 28) and our present studies provide ample evidence that the same happens to decorin gene expression in renal cells under similar circumstances, we also examined the effect of TGF-β1 alone on decorin mRNA abundance in murine glomerular mesangial and proximal tubular cells. We found that addition of TGF-β1 downregulates decorin expression in these cells in culture. The mechanism of this downregulation of decorin by TGF-β1 needs to be further investigated. Nevertheless, this inhibitory effect of TGF-β1 on decorin gene expression suggests that the increased renal TGF-β1 level in the diabetic state may participate in a negative feedback loop involving decorin expression.

Regulation of decorin expression by TGF-β may vary according to the species and cell type being investigated. Our study demonstrating an inhibitory effect of TGF-β1 on decorin gene expression is not entirely in concordance with those of Border et al. (2) and Takeuchi et al. (24), who found upregulation of decorin by TGF-β1 in cultured rat mesangial cells and murine osteoblast-like MC3T3-E1 cells, respectively. Moreover, in other previous publications, TGF-β was not found to significantly change decorin expression in either cultured fetal bovine tendinous tissue (17) or monkey arterial smooth muscle cells (20). It is noteworthy that the study by Robbins et al. (17) actually found a slightly decreased decorin mRNA level in response to treatment with exogenous TGF-β1. However, in studies similar to ours, Kahari et al. (9, 10) described TGF-β1-induced downregulation of decorin mRNA and protein level in cultured human skin fibroblasts. Several other studies came to the same conclusion in experiments on fibroblast cell cultures (3, 13, 27).

There are some data available regarding the regulation of decorin gene expression. Mauviel et al. (12) demonstrated a 48-bp promoter segment that functions as a bimodal regulator of decorin gene expression in human dermal fibroblasts; tumor necrosis factor-α (TNF-α) downregulates and interleukin-1β upregulates decorin expression by interacting with this site. Although TGF-β1, like TNF-α, decreased decorin mRNA by 60%, these investigators did not find a TGF-β1 response element in their decorin promoter constructs (13). In the experiments by Kahari et al. (9), the downregulating effect of TGF-β on decorin was prevented by dexamethasone. Clearly, much work is needed to delineate the mechanism by which TGF-β modulates decorin expression in various cell types.

In the study by Wahab et al. (26) on human mesangial cells cultured under high-glucose conditions, decorin mRNA levels were essentially unchanged after 7 days, and the maximum upregulation of the decorin message was described after 21–28 days; TGF-β1 mRNA was maximally stimulated at 7 days and less elevated after 21–28 days (26). These findings are consistent with a downregulating effect of TGF-β on decorin expression and support our results in rat untransformed mesangial cells, where we found no increase in decorin mRNA after 48 h but significant increase after 96 h of high-glucose exposure. These results also argue for a TGF-β1-decorin counterregulatory mechanism.

We conclude that the diabetic state is not a deficiency state as far as the renal expression of decorin is concerned. The increased renal cortical expression of decorin in STZ-diabetic mice likely reflects an upregulation of decorin message in both glomerular mesangial as well as in proximal tubular epithelial cells. Hyperglycemia per se is a sufficient stimulus for the increase in the decorin mRNA level in these cell types. Given the known TGF-β binding properties of decorin and the role that TGF-β plays in mediating renal hypertrophy and matrix accumulation in diabetes, our current findings suggest that increased decorin production in models of diabetic renal disease may represent a mechanism by which renal cells counteract the injury that is produced by high-glucose-stimulated TGF-β1 levels. Moreover, TGF-β itself downregulates the decorin message in mesangial and proximal tubular cells, perhaps as a component of a negative feedback loop (see Fig. 6), and
this adds a further dimension of complexity to the multifaceted interplay of factors likely responsible for the cellular injury in diabetic nephropathy. Future studies will be needed to examine whether decorin knockout mice can develop more severe diabetic lesions in the kidney and whether supplementation of exogenous decorin will be of any benefit in the management of diabetic kidney disease.

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