Prostaglandin D$_2$ inhibits TGF-β$_1$-induced epithelial-to-mesenchymal transition in MDCK cells

Aihua Zhang, Zheng Dong, and Tianxin Yang

1Division of Nephrology, University of Utah and Veterans Affairs Medical Center, Salt Lake City, Utah; and 2Department of Cellular Biology and Anatomy, Medical College of Georgia; and 3Medical Research Service, Veterans Affairs Medical Center, Augusta, Georgia

Submitted 17 April 2006; accepted in final form 7 August 2006

Zhang, Aihua, Zheng Dong, and Tianxin Yang. Prostaglandin D$_2$ inhibits TGF-β$_1$-induced epithelial-to-mesenchymal transition in MDCK cells. Am J Physiol Renal Physiol 291: F1323–F1331, 2006). This finding prompted us to examine the roles of other prostanoids: PGD$_2$, PGF$_2$, PGI$_2$, and thromboxane A$_2$ (TXA$_2$). Treatment with 10 ng/ml TGF-β$_1$ for 3 days induced EMT as reflected by conversion to the spindle-like morphology, loss of E-cadherin, and activation of α-smooth muscle actin (α-SMA). Treatment with PGD$_2$ remarkably preserved the epithelial-like morphology, restored the expression of E-cadherin, and abolished the activation of α-SMA. In contrast, PGI$_2$, carbocyclic thromboxane A$_2$, PGF$_2$, and its stable analog beraprost were without an effect. MDCK cells expressed DP$_1$ and DP$_2$ receptors; however, the effect of PGD$_2$ was neither prevented by DP$_1$ antagonist BW-A868C or DP$_2$ antagonist BAY-u3405 nor was mimicked by DP$_1$ agonist BW-245C. cAMP-elevating agents forskolin and 8-Br-cAMP blocked EMT. However, cAMP blockers H89 and Rp-cAMP failed to block the effect of PGD$_2$. PGD$_2$ did not seem to act via its metabolites as 15-deoxy-Delta(12,14)-prostaglandin J$_2$ (15d-PGJ$_2$) levels in the medium following incubation with 3 μM PGD$_2$ were well below the values predicted from the cross activity of the assay. Exposure to TGF-β$_1$ induced a threefold increase in reactive oxygen species production that was completely abolished by PGD$_2$. We conclude that J) PGD$_2$, but not PGI$_2$, PGF$_2$, and TXA$_2$ inhibit EMT, 2) PGD$_2$ inhibits EMT independently of DP$_1$ and DP$_2$ receptors, and J) PGD$_2$ exhibits antioxidant property which may, in part, account for the antifibrotic action of this PG.

Reagents and antibodies. Recombinant human TGF-β$_1$ was purchased from Calbiochem (Cambridge, MA). cAMP-elevating regents, 8-Br-cAMP, forskolin, 3-isobutyl-1-methylxanthine (IBMX; a phosphodiesterase inhibitor), Rp-cAMP, N-[2-(p-bromocinnamylamino)-ethyl]-5-isquinolsulfonyamide (H89), mouse monoclonal anti-α-SMA (catalog number: A5228), and rat monoclonal anti-E-cadherin (catalog number: U3254) antibodies were purchased from Sigma (St. Louis, MO). PGD$_2$, carbocyclic thromboxane A$_2$ (CTA$_2$; a stable analog of thromboxane A$_2$), PGF$_2$, and beraprost, 15d-PGJ$_2$, rabbit polyclonal anti-DP$_1$ (catalog number: 101640), anti-DP$_2$ (catalog number: 10007002), and anti-murine COX-2 (catalog number: 10007002), and anti-murine COX-2 (catalog number: 10007002), and anti-murine COX-2 (catalog number: 10007002), and anti-murine COX-2 (catalog number: 10007002). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

F1332

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incubator. Cells were grown to 80% confluence, serum-starved for 24 h, and then treated with vehicle or human recombinant TGF-β1 in the presence or absence of prostanooids or their analogs for the appropriate periods of time. Microscopic examination was performed during each experiment to assess the morphological changes before sample analysis.

**Immunoblotting.** MDCK cells were lysed and subsequently sonicated in PBS containing 1% Triton X-100, 250 μM PMSF, 2 mM EDTA, and 5 mM DTT (pH 7.5). Protein concentration was determined by Coomassie reagent. Thirty micrograms of protein from whole cell lysates were denatured in boiling water for 10 min, separated by SDS-PAGE, and transferred onto nitrocellulose membranes. The blots were blocked overnight with 5% nonfat dry milk in Tris-buffered saline (TBS), followed by incubation for 1 h with mouse anti-α-SMA monoclonal antibody, or rabbit anti-DP1 or DP2 polyclonal antibodies at a dilution of 1:1,000. After being washed with TBS, blots were incubated with a goat anti-HRP-conjugated second-ary antibody (1:1,000 dilution) and visualized with ECL kits (Amer- sham).

**Immunofluorescence microscopy for E-cadherin and α-SMA.** Cells were grown on coverslips and stimulated with TGF-β1 in the presence or absence of prostaglandins. The medium was removed, and the cell layer was rinsed with PBS. Cells were fixed and permeabilized with acetone-methanol for 10 min at −20°C, then were rehydrated with PBS, blocked with 5% BSA in PBS for 1 h. Coverslips were sequentially incubated with rat monoclonal anti-E-cadherin (1:400 dilution) or mouse monoclonal anti-α-SMA (1:200 dilution) and FITC-labeled goat anti-rat or mouse antibodies (1:200 dilution) each for 60 min at room temperature. Cells were then visualized and photographed by fluorescence microscopy at ×400 magnification. Negative controls were performed using nonimmune serum or IgG instead of first antibodies.

**cAMP assay.** Serum-starved MDCK cells grown in six-well plates were pretreated with 10 μM IBMX for 30 min and then treated with...
2 μM PGD₂ in the presence or absence of DP₁ antagonist, BW-A868C, or DP₂ antagonist, BAY-u3405, or treated with DP₁ agonist BW-245C. After treatment, medium was removed and the cells were washed with PBS. Immediately after being washed, 0.3 ml of 0.1 M HCl was added. After 20-min incubation, the cells were scraped and transferred into a centrifuge tube and spun for 10 min at 1,000 g to pellet the cell debris. The cAMP enzyme-linked immunosorbent assay was performed according to manufacturer’s instructions (Cayman Chemicals). The results were normalized with protein concentration.

15d-PGJ₂ enzyme immunoassay. 15d-PGJ₂ in the culture media was measured with an enzyme immunoassay kit from Assay Designs (Ann Arbor, MI). The assay was performed according to the manufacturer’s instruction. Briefly, 100 μl of the medium, along with a serial dilution of 15d-PGJ₂ standard samples, were mixed with appropriate amounts of alkaline phosphatase-conjugated 15d-PGJ₂ and 15d-PGJ₂ antiserum, and incubated at room temperature for 2 h. After the wells were emptied and rinsed with wash buffer, 200 μl of substrate solution were added. The optical density was read at 405 nm.

DCFDA fluorescence measurement of ROS. The fluorogenic substrate 2',7'-dichlorofluorescein diacetate (DCFDA) is a cell-permeable dye that is oxidized to highly fluorescent 2',7'-dichlorofluorescein (DCF) by H₂O₂ and can therefore be used to monitor intracellular generation of ROS. For measurement of ROS, cells were grown onto glass cover slides. When the cells reached confluence, they were washed twice with PBS and incubated for 30 min with 50 μM...
DCFDA and PGD2. Then the cells were treated by TGF-β1 for 30 min. At the end of the incubation period, the cells were again washed twice with PBS and imaged by confocal laser microscopy. To quantitate ROS levels, cells were seeded to 96-well plates and were treated as abovementioned. Relative fluorescence was measured by a fluorescence plate reader (FLUOstar OPTIMA) at excitation and emission wavelengths of 485 and 528 nm, respectively, three times at 90-s intervals. Relative fluorescence units (RFU) was expressed as fold increase over untreated cells.

**Caspase activity assays.** Serum-starved MDCK cells grown in six-well plates were treated with TGF-β1 in the presence or absence of PGD2. After treatment, the cells were washed with PBS and collected in CasPASE lysis buffer. The caspase-3/7/10 assay was performed by using CasPASE-3/7/10 assay kit (GenoTechnology, St. Louis, MO) according to the manufacturer’s instructions. Caspase activity was monitored as optical absorbance at 405 nm in a time-dependent manner.

**Statistical analysis.** One-way ANOVA was performed to test for statistical significance of differences among the values observed in each treatment group followed by a Bonferroni posttest. A P value <0.05 was considered significant. Values shown represent means ± SE.

**RESULTS**

**Effects of various prostanoids on TGF-β1-induced EMT.** In an earlier study, we showed that PGE2 had a remarkable inhibitory effect on TGF-β1-induced EMT in cultured MDCK cells. This prompted us to examine the effects of other prostanoids: PGD2, PGF2α, PGI2, and TXA2. To evaluate EMT, we used three independent parameters: cell morphology, the level of E-cadherin, and expression of α-SMA, assessed by phase-contrast microscopy, immunostaining, and immunoblotting, respectively. We first monitored the morphological changes in MDCK cells treated by TGF-β1 alone or in combination with individual prostanoids or their analogs. Under basal state, MDCK cells exhibited typical epithelial-like morphology (Fig. 1A), whereas TGF-β1 treatment (10 ng/ml) for 3 days induced a complete conversion to spindle-like morphology (Fig. 1B). Strikingly, the TGF-β1-induced morphological changes were completely prevented by treatment with 2 μM PGD2 (Fig. 1C). In contrast, CTA2 (Fig. 1D), PGF2α (Fig. 1E), PGI2 (Fig. 1F), and the stable PGI2 analog beraprost (Fig. 1G) had no obvious effect on EMT.

E-cadherin, a classic epithelial cell marker, is a membrane-bound protein involved in cell-cell interactions in intact renal tubular epithelial cells. We then performed immunocytochemical analyses to monitor changes in E-cadherin protein expression. As expected, E-cadherin was expressed exclusively in the basolateral membrane of MDCK cells in basal state (Fig. 2A).
In contrast, incubation with 10 ng/ml TGF-β1 for 3 days dramatically reduced E-cadherin expression (Fig. 2B). The reduction of E-cadherin was completely prevented in the presence of 2 μM PGD2 (Fig. 2C), but not in CTA2 (Fig. 2D), PGF2α (Fig. 2E), or PGI2 (Fig. 2F), or beraprost (Fig. 2G).

α-SMA is an actin isoform specific to myofibroblasts and its expression undergoes characteristic changes during EMT. The effect of PGD2 on EMT was further analyzed by immunoblotting analysis of α-SMA expression; n = 3 for each group. *P < 0.01 vs. TGF-β1-stimulated cells.

The EMT induced by TGF-β1 appeared to be associated with reduced cell proliferation rate. Therefore, we tested whether TGF-β1 (10 ng/ml) in the presence or absence of PGD2 (2 μM) induced apoptosis in MDCK cells. Caspase 3/7/10 activities, detected by using the Cas-PASE-3/7/10 assay kit, were not significantly affected by TGF-β1 regardless of the presence or absence of PGD2 (control: 1.00 ± 0.24; TGF-β1 alone: 1.13 ± 0.15; TGF-β1 + PGD2: 1.01 ± 0.2, n = 5 in each group, P > 0.05). The values represent fold changes over controls.

Examination of DP receptors involved. The biological effects of PGD2 are transduced by D prostanoid receptor (DP) 1 and DP2, two G protein-coupled receptors. To determine the mechanisms involved in the PGD2 inhibition of EMT, we first examined expression of the two PGD2 receptors using immunoblotting. As shown in Fig. 4A, DP1 receptor was detected as a single 43-kDa protein. DP2 receptor protein was detected as a double band; the smaller molecular weight band of 39 kDa.
was of predicted size while the identity of the slightly larger molecular weight band (~50 kDa) is unclear. Cayman’s DP2 receptor polyclonal antibody detects both unglycosylated and glycosylated protein from human ranging from 35–40 to 55–70 kDa, as reported by Nagata et al. (27). This raises a possibility that the ~50-kDa band detected in MDCK cells might be the glycosylation product. To address the functional aspect of the DP receptors, we determined effects of PGD2 on intracellular cAMP, along with the use of DP receptor agonists and antagonists to discriminate the types of DP receptors. PGD2 at 2 μM significantly elevated intracellular cAMP levels that were blocked by DP1 antagonist BW-A868C (10 μM), but not DP2 antagonist BAY-u3405 (10 μM), suggesting involvement of the DP1 receptor. Indeed, like PGD2, the selective DP1 agonist BW-245C (10 μM) was able to elevate intracellular cAMP (Fig. 4B), thereby confirming the presence of a functional DP1 receptor in MDCK cells.

To investigate the involvement of DP receptors in the PGD2 inhibition of EMT, we used respective DP receptor antagonists and agonists to assess the roles of individual DP receptors. Unexpectedly, neither the DP1 antagonist BW-A868C (10 μM) nor DP2 antagonist BAY-u3405 (10 μM) was able to block the PGD2 effect as assessed by morphological changes (Fig. 5, D and E), E-cadherin expression (Fig. 5, J and K), and α-SMA expression (Fig. 6, D, E, and G). Unlike PGD2, DP1 agonist BW-245C (10 μM) did not affect TGF-β1-induced EMT as shown in Fig. 5F for morphology, Fig. 5L for E-cadherin immunostaining, and Fig. 6F for α-SMA immunostaining and Fig. 6G for α-SMA immunoblotting.

Role of cAMP. CAMP pathway has been shown to antagonize the TGF-β1-elicted signaling (21). Thus we examined whether the cAMP pathway affected TGF-β1-induced EMT and whether this mechanism was applicable to the PGD2 action. We examined the effect of cAMP on EMT. MDCK cells were preincubated with cell membrane-permeable cAMP analog 8-Br-cAMP (50 μM), or an adenylyl cyclase activator, forskolin (50 μM), for 30 min, and then treated with TGF-β1 for 3 days. As seen in Fig. 7, both 8-Br-cAMP and forskolin

Fig. 7. Effect of cAMP-elevating agents on TGF-β1-induced EMT. A–D: morphologic changes. The cells were grown in 6-well plates until 80% confluence and then treated with vehicle (A), TGF-β1 alone (10 ng/ml; B), TGF-β1 plus 50 μM 8-Br-cAMP (50 μM; C), or TGF-β1 plus forskolin (50 μM; D) for 3 days. Photographs were taken using a Nikon microscope (phase contrast). E: cells were treated as aforementioned and α-SMA expression was detected by Western blotting. Top: representative immunoblots. Bottom: densitometric analysis of α-SMA expression; n = 3 for each group. *P < 0.01 vs. TGF-β1-stimulated cells.
completely blocked TGF-β₁-induced EMT as assessed by morphological changes (Fig. 7, C and D) and α-SMA expression (Fig. 7E). Both Rp-cAMP and PKA inhibitor H89 blocked the effect of forskolin on the inhibition of EMT induced by TGF-β₁, indicating that cAMP was involved in the inhibition of forskolin on EMT (Fig. 8). We next used Rp-cAMP and H89 to block the cAMP activity and determined their impact on the PGD₂ effect. As shown in Fig. 9, neither Rp-cAMP (200 μM) nor H89 (10 μM) was able to block the PGD₂ effect as assessed by morphological changes and α-SMA expression.

**Measurement of production of 15d-PGJ₂ from PGD₂.** To address the possibility that PGD₂ may act via its end metabolite 15d-PGJ₂, we determined the levels of 15d-PGJ₂ in the medium from PGD₂-treated MDCK cells. MDCK cells were treated with 3 μM PGD₂ for 48 and 72 h, and the medium was subjected to 15d-PGJ₂ EIA. Our data showed that the concentrations of 15d-PGJ₂ in the medium at both 48 and 72 h following the addition of 3 μM PGD₂ were 0.12 ± 0.02 μM (n = 3 in each group). The actual concentration of 15d-PGJ₂ was well below the values predicted from the cross activity of 15d-PGJ₂ antibody to PGD₂ (5% cross-reactivity equivalent to 0.16 μM). Moreover, 15d-PGJ₂ at the dose of 0.1 μM had no effect on EMT in cultured MDCK cells (data not shown).

**Effect of PGD₂ on ROS production.** In light of the recent reports on an important role of ROS in mediating EMT (33, 38), we examined the possibility that PGD₂ may act via inhibition of ROS production. We found that cellular ROS levels at 30 min after TGF-β₁ treatment were threefold higher than the basal value (Fig. 10). This increase was completely blocked by treatment with 2 μM PGD₂ (Fig. 10).

**DISCUSSION**

EMT has emerged as a critical event in the pathogenesis of TIF. Knowledge concerning the regulatory mechanism of EMT may lead to the development of effective therapies to halt the progression of ESRD. The identification of a limited number of negative regulators of EMT, namely, HGF (23, 24) and bone morphogenic protein 7 (51), has already shed light on the therapeutic interventions of the disease process. In an earlier study, we identified PGE₂ as a potent inhibitor of EMT, suggesting that eicosanoids may represent a novel class of EMT regulators (52). Inspired by this finding, we moved forward to examining the effects of other prostanoids on EMT in the same cell culture model. Our results showed that PGD₂ remarkably blocked EMT but PGF₂α, PGI₂, and TXA₂ had no
effect. The PGD₂ effect appeared independent of DP₁ and DP₂ receptors. PGD₂ exhibited an antioxidant property that was likely relevant to the antifibrotic action of this PG.

As expected, exposure of MDCK cells to TGF-β₁ for 3 days induced a complete conversion of the epithelial cells to myofibroblasts as evidenced by acquisition of spindle-like morphology, loss of E-cadherin, and activation of α-SMA. Additionally, the TGF-β₁-induced EMT in MDCK cells appeared to be associated with reduced cell number. In a general agreement with this observation, reduced cell number has been noticed during EMT in primary proximal tubular cells and HK-2 cells (40) and in mouse mammary epithelial cells (33). We performed the caspase-3/7/10 assay but did not find evidence for apoptosis. Whether there is a cause-effect relationship between reduced cell number and EMT is unclear but certainly warrants further investigation.

Among various prostanoids tested, PGD₂ was highly efficient in inhibiting TGF-β₁-induced EMT. In contrast, PGD₂ treatment induced a full restoration of epithelial morphology and E-cadherin expression and a complete abolishment of α-SMA stimulation. This effect was observed with PGD₂ at the range of 0.25 to 2 μM, which is likely to be within the range of physiological concentrations of PGD₂ in the renal tissues. Tissue concentration of PGD₂ in the rat kidney was detected at

Fig. 9. Effects of cAMP blockade on the PGD₂ inhibition of EMT. A-E: morphologic changes. The cells were grown in 6-well plates until 80% confluence and then treated with vehicle (A), TGF-β₁ alone (B), TGF-β₁ plus 2 μM PGD₂ (C), TGF-β₁ plus 2 μM PGD₂ and 200 μM Rp-cAMP (D), or TGF-β₁ plus 2 μM PGD₂ and 10 μM H89 (E) for 3 days. Photographs were taken using a Nikon microscope (phase contrast). F: α-SMA expression. MDCK cells were treated as aforementioned, and α-SMA expression was determined by immunoblotting. Top: representative immunoblots. Bottom: densitometric analysis of α-SMA expression; n = 3 for each group. *P < 0.01 vs. TGF-β₁-stimulated cells.
~200 ng/g tissue weight which was even higher than that of PGE2 (46). This raises a possibility that PGD2 may function as an endogenous inhibitor of EMT in the kidney.

PGD2 elicits its effects through two G protein-coupled receptors (GPCRs), the DP1 and the recently discovered chemotactant receptor homologous molecule expressed on Th2 cells (CRTH2, also named DP2 receptor). The DP1 and DP2 receptors are coupled to Gs and Gi, respectively, mediating divergent effects; DP2 is primarily responsible for the proinflammatory action of PGD2 in allergic inflammation (43), whereas DP1 activation decreases inflammation (1, 13, 16). To determine the DP receptors involved, we examined effects of the DP1 antagonist BW-A868C and the DP2 antagonist BAY-u3405 on PGD2-induced inhibitory effects on EMT. Unexpectedly, neither BW-A868C nor BAY-u3405 was able to block the effect of PGD2. In line with this finding, the DP1 agonist BW-245C did not affect TGF-β1-induced EMT. We also provided evidence that the lack of responses to the DP receptor agonist and antagonist was not due to the absence of the DP receptors in MDCK cells. Taken together, these findings may suggest the existence of additional DP receptors responsible for PGD2-dependent regulation of EMT.

Recently, Lin and colleagues (21) demonstrated that PKA activators, db-cAMP and forskolin, all efficiently inhibited TGF-β1-induced, Smad3/4-dependent connective tissue growth factor (CTGF) expression. Smad3/4 activation is the common mediator of TGF-β1-induced EMT. Thus it is possible that cAMP may exhibit an inhibitory effect on EMT. Our data showed that 8-Br-cAMP and forskolin completely blocked TGF-β1-induced EMT as assessed by morphologic change and α-SMA expression. Moreover, both Rp-cAMP and PKA inhibitor H89 blocked the inhibitory effect of forskolin on EMT, indicating a counterregulatory role of cAMP pathway during EMT. Although PGD2 stimulated production of intracellular cAMP, blockade of endogenous cAMP activity had no effect of PGD2-induced inhibition of EMT, nearly ruling out the involvement of cAMP. Despite this negative aspect of the finding, to our knowledge, the counterregulatory role of cAMP in EMT has not been reported. It is possible that the cAMP pathway may be utilized by other antifibrotic factors with more effective cAMP-elevating capability. More importantly, this finding lends support to the use of cAMP-elevating agents for treatment of chronic renal disease.

PGD2 can be degraded nonenzymatically to biologically active J-series cyclopentene PGs, especially the end product of 15d-PGJ2 which is considered as a naturally occurring endogenous ligand of peroxisome proliferator-activated receptor γ (PPARγ) (14, 20). 15d-PGJ2 and other synthetic PPARγ ligands are reported to exert anti-inflammatory and antifibrotic effects in a wide variety of tissues including the kidney (7, 15, 36). There is a possibility that PGD2 may act through its metabolite 15d-PGJ2 in renal cells. To rule out this possibility, we determined the levels of 15d-PGJ2 in the medium of MDCK cells 48 and 72 h after adding 3 μM PGD2. The enzyme immunoassay detected a substantially low medium concentration of 15d-PGJ2 that can be fully attributed to the cross activity of 15d-PGJ2 antibody to PGD2. This finding substantiates the notion that PGD2 may act via its receptors rather than its metabolite 15d-PGJ2.

Whatever DP receptors are involved, we provide evidence for antioxidant activity of PGD2 that likely accounts for the antifibrotic effect of this PG. ROS have an established role in the pathogenesis of chronic renal disease. Two recent studies have made a landmark observation that ROS mediate EMT in...
both cancer cells and renal proximal tubule cells (33, 38). We found that TGF-β1 at 10 ng/ml induced a threefold increase in DCF-sensitive cellular ROS that was completely blocked by treatment with 2 μM PGD2. This finding suggests that ROS serve as a target of PGD2 during EMT. To our knowledge, this is the first report on the antioxidant property of PGD2. A previous report on antioxidant capability of PGD2 in cultured aortic smooth muscle cells provides an important insight into the atheroprotection of PGD2 (11).

PGD2 is formed from the product of COX activity, PGH2, by two distinct PGD synthases, the lipocalin-type synthase (L-PGDS) and the glutathione-dependent hematopoietic PGD synthase (H-PGDS). PGD2 is the major arachidonic acid product in mast cells, playing a critical role in allergic inflammation. Other pharmacological activities of PGD2 include vasodilation and bronchoconstriction, inhibition of platelet aggregation, sleep induction, hypothermia, and reduction of intraocular pressure. In contrast, there is scant information concerning PGD2 actions in the kidney. The demonstration of the antifibrotic and antioxidant properties of PGD2 in cultured renal epithelial cells indicates potential beneficial effects of this PG on renal injury. In line with this notion, systemic administration of PGD2 improves renal blood flow and function in dogs (35). In Dahl salt-sensitive rats susceptible to hypertension-induced kidney injuries, tissue concentration of PGD2 in the outer medulla was significantly lower than those of salt-sensitive rats (46). A large number of studies report a creatinine-like increase in plasma L-PGDS in chronic renal disease (18, 25, 32). More importantly, urinary L-PGDS excretion markedly increases in the early stage of kidney injury such as diabetic nephropathy, and urinary L-PGDS is considered as a useful predictor of the forthcoming renal injury (47, 48). There is debate, however, as to whether the increase in L-PGDS levels is a consequence of reduced glomerular filtration or due to de novo synthesis as a compensatory mechanism. The latter is supported by recent genetic evidence that L-PGDS knockout mice develop glomerular hypertrophy, tubular damage, and renal fibrosis (34). It seems likely that in response to kidney injury the PGDS-PGD2 pathway becomes activated, functioning as a renoprotective mechanism. In line with this notion, NSAIDs are well known to be associated with various types of nephrotoxicities, including interstitial nephritis (2, 49) and COX-2-deficient mice develop severe renal pathologies and progressive renal failure (8, 26). Whether COX activity in these situations is related to regulation of EMT is unknown but certainly represents an interesting area for future studies. Contrary to this hypothesis, however, it has been shown that selective COX-2 inhibitors exhibit renoprotective effects in animal models of 5/6 nephropathy (39) and diabetic and hypertensive nephropathy (5). The reason for the discrepancy is unclear but appears, in part, to be related to a distinct heterogeneity of coxibs. In this regard, Hermann et al. (17) recently compared the effects of two selective COX-2 inhibitors, celecoxib and rofecoxib, and a nonselective COX inhibitor, diclofenac, on renal morphology and function in salt-sensitive hypertension and demonstrated distinct renal effects of the two coxibs, celecoxib and rofecoxib, with the former showing protective effects while the latter showing detrimental effects. This finding raises a concern that some of renal effects of the coxibs may not be entirely related to the blockade of COX-2 activity.

It is somewhat surprising that PGI2 did not exhibit any effect on TGF-β1-induced EMT despite increasing reports on the role of PGI2 in renal fibrotic response (29). In cultured mesangial cells, the PGI2 analog cicaprost reduces fibronectin levels by 40% and induces a threefold increase in the level of matrix metalloproteinase-2, a key enzyme in matrix degradation (30, 31). In a general agreement with this observation, prostacyclin synthase knockout mice develop severe renal fibrosis (50). We speculate that different mechanisms may underlie the beneficial effects of various types of prostanoids.

In summary, the present study complements an earlier observation on PGE2 inhibition of EMT in cultured MDCK cells (52) by examining the roles of other prostanoids: PGD2, PGF2α, PGI2, and TXA2. Like PGE2, PGD2 completely reversed TGF-β1-induced EMT while PGF2α, PGI2, and CTX were without an effect. The mechanism of the PGD2 effect is unclear but appears to be independent of DP1 or DP2 receptors and may be related to its antioxidant property. The new information will help in an understanding of the role and mechanism of prostanoid-dependent pathway in chronic renal disease and will also shed light on the use of prostanoids for therapeutic interventions of the devastating disease.

GRANTS

This work was supported by National Institutes of Health Grants RO-1-HL-079453, RO-1-DK-066592, R21-DK-069460, and KO-1-DK-064981 (to T. Yang).

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