Role of PDGF B-Chain and PDGF Receptors in Rat Tubular Regeneration after Acute Injury

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Various polypeptide growth factors are generally considered to be involved in the regulation of the nephrogenic process both after acute renal injury and during renal development. Because platelet-derived growth factor B-chain (PDGF-B) has been reported to be expressed in immature tubulus of the developing kidney, PDGF-B could play a role in the process of tubulogenesis. We examined the expression of PDGF-B and PDGF receptors α and β and their localization in kidneys after ischemia/reperfusion injury. The mRNA expressions of PDGF-B, PDGFR- α , and PDGFR- β were enhanced after injury. In the immunohistochemical analysis and/or *in situ* hybridization, PDGF-B and PDGFR- α , β were expressed after reperfusion in the S3 segment of the proximal tubuli, where they were not expressed normally. The expressions of proliferating cell nuclear antigen and vimentin were concomitantly observed with PDGF-B and PDGFRs in the tubular cells of injured S3 segment at 48 hours after injury. Next, the inhibition of the PDGF-B/PDGFRs axis with either Trapidil or Ki6896, which was found to inhibit the phosphorylation of PDGFR- β selectively, resulted in a rise of serum creatinine, higher mortality rate, abnormal regenerating process, and suppressed proliferation of tubular epithelial cells. These findings suggest that the PDGF-B/PDGFRs axis is involved in the proliferation of injured tubular cells and plays an important role in the regeneration of tubular cells from acute ischemic injury. (Am J Pathol 1999, 155:1689–1699)

Ischemia- and nephrotoxin-induced renal damages are the main causes of acute renal failure and are characterized by structural and functional damages to renal tubular epithelial cells, predominantly to the proximal tubuli.¹ Damage to the proximal tubular epithelium is repaired by a complex regeneration process. After cell desquamation, dedifferentiated proximal tubular cells proliferate and migrate into the denuded area of the basement

membrane to establish a new epithelium.² Through this nephrogenic repair process, a functional tubular epithelium is regenerated in as little as 2 weeks. Moreover, in many respects, this nephrogenic repair process resembles the late stage of the development of nephrons, when the embryonic mesenchyme converts to a tubular epithelium.^{2,3}

Various polypeptide growth factors are generally considered to regulate the proliferation, motilization, and differentiation of renal epithelial cells during both the repair from acute tubular injury and the development of a tubular epithelium.^{3,4} Indeed, the expression of insulin-like growth factor I (IGF-I), heparin-binding epidermal growth factor-like growth factor (HB-EGF), transforming growth factor- β 1 (TGF- β 1), or hepatocyte growth factor (HGF) was found to be enhanced in regenerating rat kidneys after the damage by toxicants or ischemia.^{5–11} HB-EGF and HGF were shown to be induced as early as 1 hour and 6 hours, respectively,^{5,9} whereas the expression of IGF-I was found to be delayed, with a peak expression 3 to 7 days after ischemic injury.^{6,11} Therefore, the timed and sequential expression of these growth factors is considered to be important in the repair process of damaged renal tubular cells as well as in the development process of renal tubular epithelium.⁴

Recently, the immunoreactivity of platelet-derived growth factor B-chain (PDGF-B) was demonstrated in the immature tubuli of the developing human kidney,¹² suggesting that PDGF-B would be involved in the tubulogenesis. This finding raises the possibility that PDGF-B and PDGF receptors (PDGFRs) may be involved in the regeneration of tubuli after acute tubular injury. Although the role of PDGF-B in the development and proliferation of glomerular mesangial cells and fibroblasts in the kidney has been extensively studied,^{13–18} little is known about the roles of PDGF-B and PDGFRs in the tubuli of both normal and injured kidneys. Thus, to clarify the role of PDGF-B in the acute tubular injury, we examined both the expressions of the PDGF-B/PDGFRs axis and the effect of the inhibition of PDGF-B action in kidneys with acute tubular injury.

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Materials and Methods

Animal Studies

Male Sprague-Dawley rats weighing 250 to 300 g were used. Ischemic tubular injury was induced by clamping bilateral renal arteries for exactly 50 minutes. Core body temperature was maintained at $37 \pm 1^\circ\text{C}$ by placing the animal on a homeothermic table and monitoring with a temperature-sensing rectal probe. After the clamp was released, the kidneys were reperfused for various time intervals before the following examination.

Preparation of Kidneys

A 22 gauge needle was inserted into aorta caudal to the renal arteries. The kidneys were perfused with 250 ml of Ringer's solution to remove all blood from the organ. The kidney was excised, quickly frozen in liquid nitrogen, and stored at -80°C for RNA extraction. For morphological study, kidneys were fixed in 4% paraformaldehyde (PFA) solution or methyl-Carnoy's solution for immunohistochemistry, or in the 10% formalin for *in situ* hybridization before kidneys were embedded in paraffin. Sections 4 μm thick were cut for immunohistochemical and *in situ* hybridization studies.

Northern Blot Analysis

After total RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform method, poly A⁺ RNA was extracted by use of a Micro Poly(A) Pure (Ambion, Austin, TX). Northern Blot analysis was performed as we previously described.^{19,20} Briefly, aliquots of poly A⁺ RNA (4 μg) were subjected to electrophoresis and transferred to Nytran nylon membrane. The filters were hybridized with ³²P-labeled cDNA probes for rat PDGF-B,²¹ human PDGF receptor- β ,²² human PDGF receptor- α (a generous gift of C.-H. Heldin), or 36B4, respectively. Hybridization for 36B4 was used as reference for relative mRNA per lane.²³

Immunohistochemistry

Methyl-Carnoy's-fixed tissue was used for PDGF-B immunohistochemistry with a monoclonal antibody, PGF-007 (Mochida, Tokyo).²¹ Tissue fixed in 4% PFA was used for PDGFRs immunohistochemistry. Two rabbit polyclonal antibodies, Ab-1 (Oncogene, NY) or P-20-R (Santa Cruz Biotechnology, Santa Cruz, CA), were used for PDGFR- β staining. These antibodies are raised against the synthetic peptides that correspond to amino acid 425–446 of murine or 1082–1101 of human PDGFR- β . For PDGFR- α staining, rabbit anti-human polyclonal antibody, PDGF(R)-A 951 (Santa Cruz Biotechnology), which corresponds to amino acid 425–446 of human PDGFR- α , was used. The specificities of antibodies for both PDGFR- α and PDGFR- β have been already demonstrated with rat tissue by Western blot analysis.^{22,24} Both Methyl-Carnoy's-fixed tissue and 4% PFA-fixed tissue

were also stained using mouse monoclonal antibody against rat proliferating cell nuclear antigen (PCNA; Organon Teknika, USA) or mouse monoclonal antibody against porcine vimentin as a primary antibody.

Immunohistochemical staining was performed by the streptavidin-biotin immunoperoxidase method. Immuno-reactive products were visualized using diaminobenzidine as a chromogen. Control staining was performed using nonimmune serum and the appropriate secondary antibody. To check the specificity of PDGFR- β , an absorption test was performed; sections were pre-incubated in the serum containing PDGFR- β antibody and a 10-fold excess amount of immunizing PDGFR- β peptide and then processed for further immunohistochemical staining as described above.

In Situ Hybridization

In situ hybridization was performed as we previously described.²⁰ The probe preparation was described previously.²¹ Briefly, sections of the 10% formalin-fixed kidney on polyL-lysine-coated glass slides were treated with proteinase K at 37°C , and then with 0.1 mol/L triethanolamine in 0.25% acetic anhydride. After these sections were incubated with prehybridization solution, they were hybridized overnight at 48°C with digoxigenin-labeled cRNA probe. After hybridization, sections were washed in $2\times$ SSC for 25 minutes, in $1\times$ SSC for 25 minutes at 55°C , in $0.5\times$ SSC for 25 minutes at 55°C , and finally in $0.5\times$ SSC for 25 minutes at room temperature. Slides were then incubated with alkaline phosphatase-conjugated anti-digoxigenin antibody for 1 hour. Nitro blue tetrazolium development solution was applied for 20 minutes. Slides were air-dried and coverslipped over Entellan.

To evaluate the specificity of the technique, a study with a sense probe that was complementary to the anti-sense probe and a competitive study using large amounts of unlabeled probes were performed.

Identification of Nephron Segments

As we previously described,¹⁹ kidney tissues stained with hematoxylin-eosin (HE) were examined morphologically. To further identify the individual nephron segments, Tamm-Horsfall protein (THP) and peanut agglutinin (PNA) stainings were performed to identify the individual nephron segments.²⁵

Inhibition of PDGF-B/PDGFRs Axis

To clarify the role of PDGF-B/PDGFRs axis in the acute tubular injury further, the effect of the inhibition of PDGF-B action was examined. Two kinds of inhibitors were used: Trapidil, which was shown to inhibit the binding of PDGF-B to PDGFR- β competitively,^{26–29} and Ki 6896, which was found to inhibit the phosphorylation of PDGFR- β selectively.^{30,31} Trapidil (30, 60, or 90 mg/kg) was given daily by an intraperitoneal injection from 2 days before the ischemic injury. Ki 6896 (100 mg/kg/day) was administered per os from the day when the ischemic

Figure 1. Northern analysis for PDGF-B and PDGFRs in rat kidney after ischemia/reperfusion. Representative result of independent experiments are shown in **A**. The means and SE bars of the signal intensities from independent experiments are summarized for PDGF-B (**B**), PDGFR-β (**C**), and PDGFR-α (**D**). Data are expressed as mean ± SE ($n = 3-7$). Significant difference between control and injured rat groups is indicated by asterisks (*, $P < 0.05$; **, $P < 0.01$).

injury was induced. Blood samples were obtained daily from the tail vein for 7 days. The concentrations of serum creatinine were measured by an enzyme method (Boehringer Mannheim, Mannheim, Germany). To investigate the morphological change, HE staining and immunohistochemistry for PCNA were performed in kidney of rats treated with Ki 6896 and vehicle at day 6.

Statistical Analyses

Analysis of variance followed by Scheffé's test was used to determine significant difference in multiple comparisons. Comparisons between two groups were analyzed by Student's unpaired t -test. Difference in the mortality rate was analyzed by χ^2 test.

Results

Induction of PDGF-B and PDGFRs mRNA in Rat Kidney after Ischemia/Reperfusion

We first examined the expression of PDGF-B mRNA in kidneys subjected to ischemia/reperfusion injury. In post-ischemic-injured kidney, the expression of PDGF-B mRNA was significantly enhanced with a peak expression at 6 hours ($P < 0.01$, Figure 1). Similar to PDGF-B, the expression of both PDGFR-α and -β was also significantly enhanced with a peak at day 1 ($P < 0.05$, Figure 1). The expression of PDGF-B, PDGFR-α, and -β mRNA remained unchanged after sham operation (data not shown).

Figure 2. Immunohistochemistry for PDGF-B in rat kidney. In the control kidney (**A**; $\times 50$), immunoreactive products were recognized in the convoluted proximal tubuli and distal tubuli. However, the S3 segment of the proximal tubuli was completely negative for PDGF-B. In kidneys at 6 hours after ischemia/reperfusion (**B**; $\times 100$), some cells of the S3 segment were positive for PDGF-B. The TAL adjacent to the injured tubuli showed strong immunoreactivities. In kidneys at 48 hours (**C**; $\times 100$), injured proximal tubuli were positive. MR, Medullary ray; S3, the S3 segment of the proximal tubuli; TAL, the thick ascending limb.

Localization of PDGF-B Protein and mRNA

In the normal kidney, PDGF-B immunoreactivity was observed in the parietal epithelial cells, the convoluted proximal tubuli, the thick ascending limb (TAL), distal tubuli, and to a lesser extent in the collecting ducts. In the collecting ducts, immunoreactive products were localized in the basolateral membrane, whereas they were distributed in the cytoplasm of the epithelial cells in the proximal tubuli, TAL, and distal tubuli. No immunoreac-

Figure 3. *In situ* hybridization for expression of PDGF-B mRNA in rat kidney after ischemia/reperfusion. Strong mRNA signals were detected in the injured tubuli of the outer stripe of the outer medulla, and moderate signals were observed in the tubuli adjacent to the injured tubuli (**A**; $\times 50$). Signals of PDGF-B were not observed in the preparation hybridized with sense probe (**B**; $\times 50$).

tivity was observed in the S3 segment of the proximal tubuli in both medullary rays and the outer stripe of the outer medulla (OSOM; Figure 2A). In high power view, glomerular tuft was also positive. These findings are concordant with those in previous studies.³²⁻³⁴

The immunoreactivity for PDGF-B was strong in the TAL both adjacent to the injured S3 segment (Figure 2B) and in the inner stripe of the outer medulla 6 hours after ischemia/reperfusion, but signals became moderate in this portion at 48 hours. On the other hand, PDGF-B immunoreactivity appeared at 6 hours in some cells of the S3 segment of the proximal tubuli. At 48 hours, the injured tubuli at the S3 segment markedly expressed PDGF-B protein (Figure 2C). The immunoreactivity for PDGF-B observed in other tubular systems in the normal kidney did not alter significantly after ischemia/reperfusion injury (data not shown).

The localization of PDGF-B mRNA was demonstrated by *in situ* hybridization. The pattern of signals for PDGF-B mRNA correlated closely with the pattern of staining of PDGF-B protein seen in immunohistochemistry. In the normal kidney, no hybridization signal was observed in the S3 segment of the proximal tubuli (data not shown). Strong signals were detected in the injured tubuli of the S3 proximal tubule (Figure 3A). Moderate signals were also observed in the tubuli adjacent to the injured tubuli

Figure 4. Immunohistochemistry for PDGFR- β in the normal kidney. Immunoreactivities for PDGFR- β were observed in the glomerulus, the distal tubuli, the TAL, and the collecting ducts. The convoluted proximal tubuli in the cortex were completely negative (**A**; $\times 100$). The proximal tubuli of the S3 segment in the outer stripe of the outer medulla were also negative (**B**; $\times 50$). The TAL was positive in the inner stripe of the outer medulla (**C**; $\times 100$). The strong immunoreactivity was observed in the collecting ducts of the inner medulla (**D**; $\times 100$). Cx, cortex; OS, outer stripe of the outer medulla; IS, inner stripe of the outer medulla; IM, inner medulla; G, glomerulus; DT, distal tubule; CD, collecting duct.

(Figure 3A). The PDGF-B sense probe yielded no positive signal (Figure 3B).

Localization of PDGFR- β Protein

Because of the lack of the systematic study on the tubular localization of PDGFR- β in the kidney, we first examined the localization of PDGFR- β in the normal kidney. We used two different antibodies against PDGFR- β to ensure the localization of PDGFR- β , and the pattern of the staining with both antibodies was identical. In the normal rat kidney, immunoreactivity for PDGFR- β was observed in the glomerulus, TAL, distal tubule, and collecting duct, and most strongly in the apical membrane of the distal tubuli and collecting ducts (Figure 4, A-D). The specificity of these results was confirmed by complete disappearance of the staining after the pre-incubation of primary antibodies with a 10-fold excess of corresponding immunizing peptides (data not shown). Significant staining was not recognized in the proximal tubuli (Figure 4, A and B).

After ischemic injury, immunoreactivity for PDGFR- β could be detected in some of the damaged proximal tubuli at 6 hours (Figure 5, A and B). After 48 hours postischemia, the S3 segments showed strong immunoreactivity for PDGFR- β with a marked positive staining in

most of proximal tubular cells of the S3 segments (Figure 5, C and D).

Relationship between PDGF-B/PDGFRs Expression and Proliferation in the Injured Tubuli

To investigate the role of PDGF-B/PDGFRs axis in the proliferation and phenotypic changes of tubular epithelial cells, we examined the expression of PCNA and vimentin with serial sections by immunohistochemistry. As previously reported,^{28,29} the proliferation, as detected by PCNA expression, was more abundant in the proximal tubuli of S3 segment, peaking at 48 hours after reperfusion, than in the S1 or S2 segments, TAL, and collecting ducts. The expression of PCNA was concomitantly observed with the expression of both PDGF-B and PDGFR- β in the tubular cells of the OSOM at 48 hours (Figure 6, A-D). Moreover, immunoreactivity for vimentin was observed in the tubular cells with intense PDGFR- β immunoreactivity, whereas it was negative in cells with subtle immunoreactivity for PDGFR- β (Figure 6E). PDGFR- α was also expressed in the proliferating proximal tubular cells of the S3 segment in the OSOM (Figure 6, F and G).

Figure 5. Immunohistochemistry for PDGFR- β in the injured tubuli after ischemia/reperfusion. In low power view ($\times 15$) of kidneys 6 hours after ischemia/reperfusion (**A**), immunoreactive products for PDGFR- β were observed in some of the proximal tubuli of the S3 segments both in the medullary ray and in the outer stripe of the outer medulla. In high power view (**B**; $\times 100$), immunoreactivities for PDGFR- β appeared in some cells of the S3 segment of the proximal tubuli. In low power view ($\times 15$) of kidneys 48 hours after ischemia/reperfusion (**C**), intense signals were observed in most of the proximal tubuli of the S3 segments both in the medullary ray and in the outer stripe of the outer medulla. In high power view (**D**; $\times 100$), strong immunoreactivities were recognized in the whole cytoplasm of proximal tubular cells in the S3 segments. MR, medullary ray; OS, outer stripe of the outer medulla.

Inhibition of PDGF-B/PDGFRs Axis in Acute Tubular Injury

To characterize the role of PDGF-B in acute tubular injury, the effect of the inhibition of PDGF/PDGFRs axis was examined in rats with ischemia/reperfusion injury by measuring the concentrations of serum creatinine. The levels of serum creatinine at day 1 of ischemia/reperfusion injury increased in a dose-dependent manner with Trapidil, a competitive inhibitor of PDGF-B binding to PDGFR- β ,^{26–29} with a significant increase in rats treated with 90 mg/kg Trapidil (Figure 7A). A significant elevation of the levels of serum creatinine was also observed in rats treated with Ki 6896, a selective inhibitor of the autophosphorylation of PDGFR- β (Figure 7B). The values of serum creatinine remained unchanged when control rats were treated with Trapidil (Figure 7A) or Ki 6896 (data not shown). We also evaluated the mortality rate of rats with ischemia/reperfusion injury. The mortality rate was significantly higher in rats treated with Trapidil in a dose-dependent manner than in rats treated with vehicle (Table 1). The mortality rate of rats with ischemia/reperfusion injury treated with Ki 6896 was also slightly elevated. Morphologically, it was frequently observed that hypertrophic tubular epithelial cells are clustered and obliterate tubular lumina in rats treated with Ki 6896 (Figure 8, A and B), although it was rarely seen and tubular lamina

were preserved in vehicle group. Papillary regenerating epithelial cells and cellular debris were included in the tubular limina in vehicle group (Figure 8, C and D). PCNA-positive cells were scattered within single-layered tubule-lining epithelial cells at low frequency, mainly in the S3 segment of Ki 6896 group (Figure 8E). Tubular epithelial cells, which are clustered and obliterate tubular lumina, did not proliferate (Figure 8E). In contrast, numerous PCNA-positive cells were distributed within the surface-lining epithelial cells frequently, not only in S3 but also in the cortical area in vehicle group. Further, PCNA-positive cells were often clustered in multilayered concentric form in the regenerating tubules of vehicle group (Figure 8F).

Discussion

We investigated the role of PDGF-B and PDGFRs in both normal kidney and kidneys damaged by ischemia/reperfusion. We showed that both PDGF-B and PDGFRs were distributed predominantly in tubuli and, to a lesser extent, the glomerulus in the normal kidney. The present study clearly demonstrated that the expression of PDGF-B, PDGFR- α , and PDGFR- β mRNA was enhanced in kidneys with ischemic tubular injury. *In situ* hybridization and/or immunohistochemistry revealed that both PDGF-B

Figure 6. Immunohistochemistry for PDGF-B/PDGFRs, PCNA, and vimentin in rat kidney after ischemia/reperfusion. In kidneys 48 hours after ischemia/reperfusion, expression of PDGF-B (A; $\times 100$) and PCNA (B; $\times 100$) was concomitantly observed in the injured tubular cells of the S3 segment. In the S3 segment, PDGFR- β (C; $\times 150$) was observed in accordance with PCNA expression (D; $\times 150$). The immunoreactivity for vimentin (E; $\times 150$) was observed in the tubular cells where PDGFR- β immunoreactivity was strong, although tubular cells with weak immunoreactivity for PDGFR- β were negative for vimentin. At 48 hours after ischemia/reperfusion, PDGFR- α (F; $\times 100$) was observed in accordance with PCNA expression (G; $\times 100$) in the S3 segment.

and PDGFRs were induced in the tubular epithelial cells of the S3 segment of the proximal tubule after acute renal injury, whereas this segment was negative for them in the normal kidney. In contrast, changes in glomeruli were relatively subtle compared with those observed in tubuli. Moreover, the cellular proliferation, as demonstrated by

the staining for PCNA, and the phenotypic change, as demonstrated by vimentin, were well correlated with the expression of PDGF-B and PDGFRs in the injured tubular cells after acute injury. Finally, the inhibition of PDGF-B/PDGFRs axis in rats with acute tubular injury resulted in a rise of serum creatinine, higher mortality

Figure 7. The effect of the inhibition of the PDGF/PDGFRs axis on the concentrations of serum creatinine in rats with ischemia/reperfusion injury. **A:** Serum creatinine values in injured rats treated with Trapidil on day 1. **B:** Time course of serum creatinine values in injured rats treated with Ki 6896. (*, $P < 0.05$; **, $P < 0.01$). ARF, acute renal failure due to ischemia/reperfusion.

rate, abnormal regenerating process, and suppressed proliferation of tubular epithelial cells.

The importance of PDGF-B/PDGFRs axis in the repair of renal tubular epithelial cells from acute injury was suggested by an increase in mRNA levels in the injured kidneys. Although the expression of mRNA of various growth factors was found to be induced by ischemic injured kidneys, the time course of the induction was different among them. HB-EGF was found to be induced by 1 hour,⁹ HGF as early as 6 hours,⁵ and TGF- β 1 at 12 hours,¹⁰ whereas the induction of IGF-I was shown to be delayed, with maximal induction from 3 to 7 days after injury.^{6,11} These data suggest that the timed and sequential expression of various growth factors including PDGF-B shown in the present study would be important for the repair process from acute tubular injury.

The localization of PDGF-B immunoreactivity in the normal kidney in the present study was similar to that in previous studies.^{32-34,36} Although the distribution of PDGFR- β in this study was different from that in most previous studies, which could not detect the immunoreactivity for PDGFR- β in

the tubuli,^{16,37,38} our results are partly in agreement with the recent report of Kliem et al, who showed that PDGFR- β was expressed in the distal tubuli and collecting ducts in normal rat kidney.³² The difference might be due to the difference in the antibodies. Thus, we used two different antibodies and obtained identical findings. We also confirmed our results by the neutralization of immunoreactivity with immunizing peptides. Moreover, we previously demonstrated the specific binding of Ab-1 antibody to rat tissue by Western blotting.²²

Colocalization of PDGF-B and PDGFRs was observed in the distal tubuli, TAL, and collecting ducts, whereas only PDGF-B was recognized in the proximal tubuli of the S1 and S2 segments in the normal kidney. Considering those localizations, PDGF-B/PDGFRs axis would be expected to play some roles to maintain the normal tubular function in an autocrine and paracrine manner. The physiological role of PDGF-B in the tubuli has not been clarified yet. PDGF-B/PDGFRs axis may be involved either in the renewal of normal tubular cells as a mitogen or in the ion transporter as a nonmitogen under the normal condition. The latter hypothesis might be supported by the findings that another growth factor, such as EGF, could be involved in the nonmitogenic tubular function.^{39,40}

In the acutely injured kidney, strong immunoreactivity for PDGF-B was observed in injured epithelial cells of S3 segment, where PDGF-B was not usually expressed, and in TAL segment both adjacent to the injured S3 segment and in the inner stripe after ischemia/reperfusion injury. The expression of PDGF-B mRNA in the same segments was confirmed by *in situ* hybridization. Like PDGF-B,

Table 1. Mortality in Rats with Ischemic Injured Kidney at Day 7

Treatment	Rats	Deaths	% mortality	P
Vehicle	20	4	20.0	
Trapidil, 30 mg/kg	5	2	40.0	N.S.
Trapidil, 60 mg/kg	4	3	75.0	<0.05
Trapidil, 90 mg/kg	5	5	100.0	<0.01
Ki 6896	11	5	45.5	N.S.

N.S., not significant.

Figure 8. The effect of the inhibition of PDGF/PDGFRs axis on morphology in rats with ischemia/reperfusion injury at day 6. In HE staining, hypertrophic tubular epithelial cells are clustered and obliterate tubular lumina in rats treated with Ki 6896 (**A**; $\times 25$, **B**; $\times 100$). Tubular lamina were well preserved in vehicle groups, although they included papillary regenerating epithelial cells and cellular debris (**C**; $\times 25$, **D**; $\times 50$). PCNA-positive cells were scattered within single-layered tubule-lining epithelial cells at low frequency, mainly in S3 segment of Ki 6896 group (**E**; $\times 50$). In contrast, numerous PCNA-positive cells were distributed within the surface-lining epithelial cells frequently, not only in S3 but also cortical area in vehicle group. Furthermore, PCNA-positive cells was often clustered in multilayered concentric form in the regenerating tubules of vehicle group (**F**; $\times 50$).

PDGFR- β was dramatically induced in the S3 segment of the proximal tubuli, where PDGFR- β was not usually expressed. Moreover, the expression of PDGFR- α was induced in the tubular epithelial cells of the same segment. These observations indicate that PDGF-B/PDGFRs axis may play an important role in the repair of the injured tubular cells in an autocrine and paracrine manner.

To clarify this hypothesis, we first examined the spatial relationship between PDGF-B/PDGFRs and regeneration. In ischemia/reperfusion model, both PDGF-B and PDGFRs were expressed in the proximal tubular cells undergoing proliferation, which was demonstrated by the PCNA staining. Furthermore, vimentin and PDGFR- β were concomitantly expressed in the same injured tubu-

lar cells. Because vimentin is usually expressed in mesenchymal cells and is thought to be a marker of dedifferentiation of the tubular epithelium,^{35,41} these data suggest that the activation of PDGFRs might be involved in the phenotypic changes of injured tubular cells. Therefore, PDGF-B and PDGFRs expressed in the injured tubular cells might be considered to promote the repair process by inducing the proliferation and phenotypic changes of the injured cells.

To characterize further the potential role of PDGF-B/PDGFRs axis in the recovery from acute tubular injury, we attempted to inhibit PDGF-B/PDGFRs axis using two kinds of inhibitors, Trapidil and Ki 6896. Trapidil was shown to inhibit the action of PDGF-B,²⁶⁻²⁹ including

PDGF-induced mitogenesis,²⁶ by inhibiting the binding of PDGF to PDGFR- β competitively.²⁷ In the present study, the inhibition of PDGF-B/PDGFRs axis with Trapidil in rats with injured kidney resulted in more severe renal damage on day 1 and a high mortality rate. To confirm these results, we also used a different inhibitor, Ki 6896, which was found to selectively inhibit the autophosphorylation of PDGFR- β without inhibiting the phosphorylation of receptors for other growth factors such as EGF, insulin, and FGF.^{30,31} Blockade of PDGF-B/PDGFR axis with Ki 6896 in rats with acute tubular injury resulted in significantly higher levels of serum creatinine than vehicle. Morphologically, tubular obliteration by clustered hypertrophic epithelial cells was observed in the Ki 6896 group at day 6. This finding was considered to be evidence of an abnormal process of regeneration. Further, cell proliferation was severely suppressed in this group. Considering these facts, PDGF-B/PDGFR axis appears to be involved in the functional recovery of the kidney after acute tubular injury. And it was suggested that PDGF stimulates the regenerating process of tubular epithelial cells including cellular proliferation. Because PDGF also exerts hemodynamic effects,⁴² vasodilatation induced by inhibition of PDGF might partly contribute to the deterioration of renal function.

The bulk of the studies about PDGF-B and/or PDGFR- β have tended to focus on the biology of mesangial cells in the kidney. Their role in the mesangial cells is well understood by use of knockout mice,^{17,18} the model animals for studying glomerulonephritis^{33,43}, and cultured mesangial cells.¹³ However, little is known about the importance of PDGF-B and PDGFRs in tubular cells. The current study determined the abundant localization of both PDGF-B and PDGFRs in normal rat tubuli and further demonstrated marked induction of them after acute tubular injury, suggesting the importance of PDGF-B/PDGFRs axis in renal tubuli as well as mesangial cells.

In conclusion, the current study provides evidence of the importance of PDGF-B/PDGFRs axis in kidneys in both the normal condition and acute tubular injury. In the repair process from acute renal injury, both PDGF-B and PDGFRs were expressed in the injured tubular cells which were undergoing proliferation, suggesting that the PDGF-B/PDGFRs axis could be involved in the regeneration from acute tubular injury. Moreover, inhibition of PDGF-B/PDGFRs axis resulted in more severe renal damage, suggesting that PDGF-B/PDGFRs axis could contribute to the repair process from acute tubular injury.

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