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MMP-2 inhibition reduces renal macrophage infiltration with increased fibrosis in UUO

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Abstract

We examined the role of matrix metalloproteinase-2 (MMP-2) in renal fibrosis and its effect on interstitial macrophage infiltration in a mouse model of unilateral ureteral obstruction (UUO). TISAM, a selective inhibitor of MMP-2, was administered during early stage (day −2 to 4; protocol A) and late stage (day 7 to 13; protocol B) after UUO. Treatment with TISAM accelerated fibrosis both at day 5 (A) and at day 14 (B). The degree of macrophage infiltration was decreased by the treatment with TISAM at day 14, but not at day 5. *In vitro* macrophage migration assay showed a greater migration to renal tissue of control UUO kidney (day 14) than to TISAM-treated kidney, which was suppressed by preincubating macrophages with RGDS, a fibronectin degradation peptide. These results suggest that MMP-2 acts to accelerate macrophage infiltration in the late stage of UUO, possibly by degrading extracellular matrix components.

Keywords: Renal fibrosis; Macrophage infiltration; Matrix metalloproteinase-2; Unilateral ureteral obstruction; Obstructive nephropathy; Extracellular matrix

Renal fibrosis is a final common manifestation of every type of chronic kidney disease. The process of renal fibrosis is characterized by an excessive accumulation of extracellular matrix (ECM) components, which leads to glomerulosclerosis and tubulointerstitial fibrosis. Matrix accumulation is a consequence of imbalance between matrix production and its degradation. Thus, several kinds of proteases that may participate in degrading ECM components in the kidney have been considered to be implicated in the evolution of renal fibrosis. Matrix metalloproteinases (MMPs) play a central role in the degradation of ECM macromolecules under various pathological conditions. In fact, the increased expression of MMP-2 as well as the upregulated tissue inhibitor of metalloproteinase-1 (TIMP-1) expression has been shown in an experimental model of obstructive nephropathy [1–3], suggesting the implication of these factors for renal fibrosis. Nevertheless, the precise role of MMPs in renal fibrosis remains to be elucidated.

Another pathological feature that is characteristically noted as an initial event of renal fibrosis is macrophage infiltration to the kidney [4]. Furthermore, because many previous studies showed a correlation between the degree of renal fibrosis and the number of infiltrating macrophages within the injured renal tissue [5], macrophages have classically been considered as active players that promote renal fibrosis. However, recent evidence suggesting that macrophages may also be involved in the resolution of renal fibrosis, as in the case of tissue repair and wound healing in the late stage of inflammatory processes, has superseded this classical view of macrophages [6–8].

Abbreviations: MMP-2, matrix metalloproteinase-2; UUO, unilateral ureteral obstruction.

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Additionally, in settings such as the wound healing process, previous reports further indicated that the degradation products of ECM function as biologically active molecules, i.e., they exhibit chemotactic activity and stimulate migration of macrophages [9,10]. These macrophages finally play a role in tissue repair through phagocytic clearance of these ECM degradation fragments.

Unilateral ureteral obstruction (UUO) is a well-established experimental model of progressive renal interstitial fibrosis. The UUO maneuver leads to many cellular and molecular events related to renal interstitial fibrosis all within 1 week, making it a highly attractive model. These include cellular infiltration to the interstitium composed mostly of macrophages, interstitial myofibroblast accumulation and increased ECM deposition, and upregulated MMP-2 expression in the kidney [3]. In this study, we thus intended to examine the role of MMP-2, which is one of the most abundant MMPs produced by the kidney, in renal fibrosis, focusing on its effect on renal interstitial macrophage infiltration.

Materials and methods

Experimental protocol. Experiments were performed on male C57BL/6 mice (10–12 weeks, 20–24 g). Renal fibrosis was induced in mice by a complete unilateral ureteral obstruction (UUO) as previously described (day 0) [7]. Briefly, under sodium pentobarbital anesthesia, the middle portion of the left ureter was ligated and cut between the two ligated points. To pharmacologically inhibit the MMP-2 activity in the UUO kidney, 5 mg/kg/day of (2*R*)-2-[5-[4-[ethyl-methylamino]phenyl]thiophene-2-sulfonylamino]-3-methylbutyric acid (TISAM) (Shionogi Co., Ltd., Osaka, Japan) in 0.5% (wt/vol) methylcellulose was orally administered once a day daily to mice. This *N*-sulfonylamino acid derivative has been reported to selectively inhibit MMP-2 activity when administered in this manner to mice [11]. In protocol A, two groups of mice were administered TISAM daily or vehicle during day −2 (2 days before the implementation of UUO) and day 4 (4 days after UUO). At day 5 after surgery, the mice were sacrificed, and the obstructed kidneys were harvested and subjected to the studies. In protocol B, mice were administered daily with TISAM or vehicle during day 7 and day 13 after UUO, and the mice were sacrificed and the renal tissues were assessed at day 14. The experimental protocols were performed according to the regulations of the Kyoto Prefectural University of Medicine Animal Care Committee.

Determination of gelatinolytic activity in the kidney. In situ zymography was performed to determine gelatinolytic activity in the kidney. Kidney tissues from TISAM- or vehicle-treated mice were obtained 6 h after the last administration of TISAM or vehicle at day 4 after UUO in protocol A, and at day 13 in protocol B. In situ zymography was performed using MMP in situ Zymo-film or MMP-PT in situ Zymo-film (Wako Pure Chemical Industries Ltd., Osaka, Japan) according to the manufacturer's protocol as previously described [11]. Briefly, frozen sections (6 μm thick) were mounted onto gelatin films that were prepared by coating cross-linked gelatin on polyethylene terephthalate support films. These gelatin films were then incubated at 37 °C for 10 h in protocol A and for 14 h in protocol B. After the incubation, the films were dried and stained with Biebrich-Scarlet stain solution (Wako Pure Chemical Industries Ltd.). Sections were also mounted on gelatin films pretreated with 1,10-phenanthroline, an inhibitor of MMPs, and treated as described above.

Histological study. For histological examinations, kidneys were fixed with 4% buffered paraformaldehyde for 6 h, embedded in paraffin, and sectioned transversely with a thickness of 4 μm. A standard point-counting method was used to quantitate the collagen fractional volume in the renal cortical interstitium on Masson trichrome-stained sections (magnification, 400×), as previously described [7]. The index of interstitial collagen fractional volume was defined as the number of trichrome-positive points in every 1000 points evaluated. To detect infiltrating macrophages, sections were incubated with monoclonal rat anti-mouse F4/80 (Selotec, Oxford, United Kingdom) for 1 h at room temperature, followed by standard ABC immunostaining using ABC-alkaline phosphatase kit (Vector, Burlingame, CA, USA). Macrophage infiltration was determined by enumerating F4/80-positive cells within the cortical interstitium in 10 randomly selected cortical fields under magnification (400×), and the numbers were averaged for each field [7].

Determination of macrophage migrating activity. Peritoneal macrophages were collected from mice 4 days after peritoneal injection of 3% thioglycollate [7]. Chemotaxis was performed in 24-well Matrigel invasion chambers (BD Biosciences, Bedford, MA, USA) according to the manufacturer's protocol as previously described [11]. Briefly, the cells were washed and suspended at a density of 5×10^5 cells/mL in Grey's balanced saline solution containing 2% BSA (GBSS-BSA). The tissue from renal cortex (approximately 1 mg wet weight) was placed in the lower compartment of the chamber in 750 μL of GBSS-BSA. A Matrigel matrix insert (8-μm pores) was used to separate upper and lower compartments. An aliquot (500 μL) of a macrophage suspension (5×10^5 cells/mL) in GBSS-BSA was added to the upper compartment. The chamber was incubated at 37 °C for 22 h in a humidified incubator containing 95% air and 5% CO_2. After incubation, cells remaining on the upper surface of the filter were removed mechanically. Filters were then fixed in methanol and stained with a Diff-Quick stain kit (International Reagent Corp., Kobe, Japan). Migrated cells adhering to the lower surface of the membrane were counted manually under the microscope with an oil-immersion lens (400×). Triplicate wells were used for each experimental condition, and 10 fields were examined for each well.

The effect of intact laminin (Chemicon International, Temecula, CA, USA), intact fibronectin (Chemicon International), and RGDS fibronectin degradation peptide (Calbiochem, San Diego, CA, USA) [9,10], on macrophage migrating activity was examined by addition of these agents (10^{-7} M) into the lower compartment in the macrophage migration assay. The effect of formyl-Met-Leu-Phe (fMLP) (Calbiochem) on macrophage migration activity was also examined by addition of 1 μM fMLP into the lower chamber. The inhibition study of macrophage migration was also performed as previously described [11]. Briefly, peritoneal macrophages (5×10^5 cells/mL) in GBSS-BSA were initially incubated with 10^{-7} M RGDS to block its ligand receptors, or GBSS-BSA alone for 30 min at 37 °C prior to the migration study, and then added to the upper compartment of the Matrigel invasion chamber after being washed with GBSS-BSA. The kidney tissue from control mice or TISAM-treated mice was added to GBSS-BSA in the lower compartment, and a migration assay was performed as described above.

Statistical analysis. Data are presented as means ± SEM. Statistical analysis was performed by ANOVA, and significance was defined as $P < 0.05$.

Results

Effect of TISAM administration on gelatinolytic activity in the UUO kidneys

Strong gelatinolytic activities were demonstrated in the UUO kidneys of control mice both at day 4 and at day 13 after UUO by in situ zymography, seemingly located mostly in the tubulointerstitial region (Fig. 1A, C, D, and F). These gelatinolytic activities were reduced by the treatment with TISAM, a selective inhibitor of MMP-2, both at day 4 and at day 13 after UUO (Fig. 1B and E). The gelatinolytic activity in the UUO kidney of control mice was markedly reduced when sections were incubated

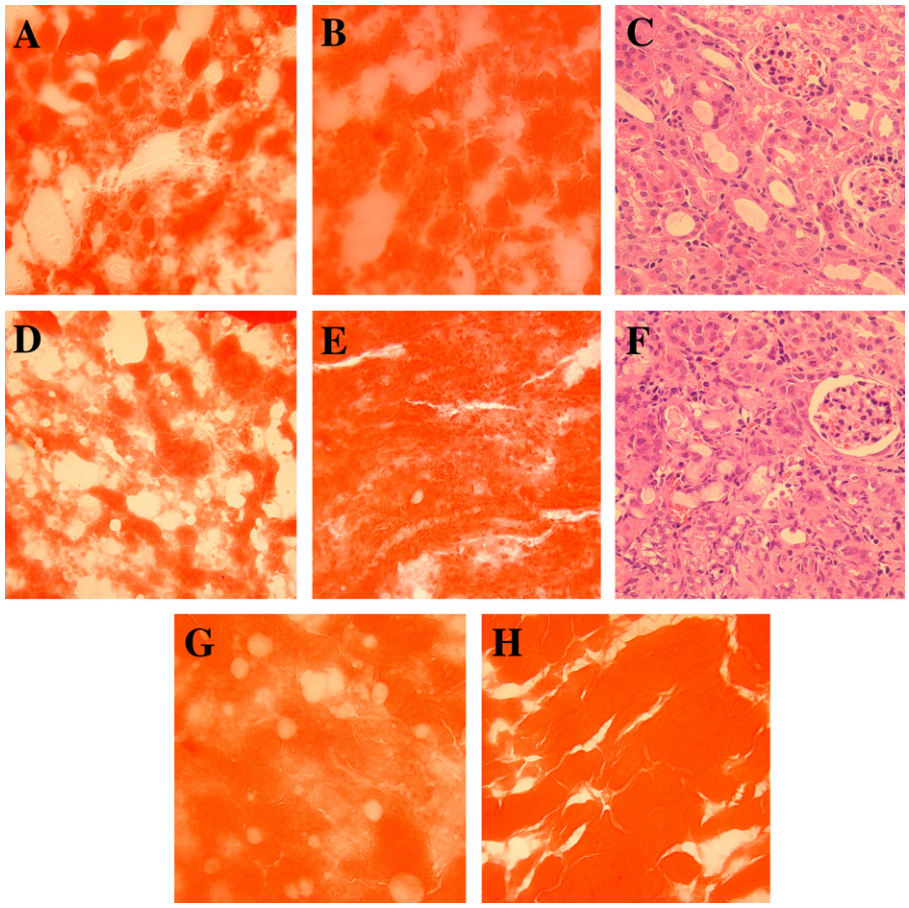

Fig. 1. Determination of gelatinolytic activity in the UUO kidneys. Gelatinolytic activity in the control UUO kidney (A) and TISAM-treated UUO kidney (B) at day 4, in the control UUO kidney (D) and TISAM-treated UUO kidney (E) at day 13, and in the contralateral non-obstructed kidney (H). Representation of counter specimens from the control UUO kidney stained with hematoxylin–eosin at day 4 (C) and at day 13 (F) after UUO. Gelatinolytic activity in the control UUO kidney at day 13 after UUO was also assessed using gelatin films pretreated with 1,10-phenanthroline, an inhibitor of MMPs (G). Original magnification, 400×.

on gelatin films pretreated with 1,10-phenanthroline, an inhibitor of MMPs (Fig. 1G). The gelatinolytic activity was faint in the tissue from contralateral non-obstructed kidney (Fig. 1H).

Effect of TISAM administration on renal fibrosis and interstitial macrophage infiltration in the UUO kidneys

Treatment with TISAM caused significant increases in interstitial collagen indices both at day 5 and at day 14 after UUO compared with those of control (day 5: 44 ± 3 vs. 79 ± 5/1000 points, $P < 0.001$, $N = 7$ in each group; day 14: 95 ± 8 vs. 202 ± 45, $P < 0.05$, $N = 10$ in control and $N = 8$ in TISAM group) (Fig. 2). At day 5 after UUO, no significant change was observed by treatment with TISAM in the number of F4/80-positive macrophages infiltrating to the interstitium (12.7 ± 0.5 vs. 11.7 ± 0.6/400× field, NS). However, at day 14 after UUO, a significant decrease was observed by the treatment with TISAM in the number of F4/80-positive interstitial macrophages (32.0 ± 1.9 vs. 21.6 ± 1.5, $P < 0.001$) (Fig. 3).

Macrophage migrating activity

The number of macrophages that migrated through a Matrigel-coated insert was significantly reduced when tissues from UUO kidneys (day 14) of TISAM-treated mice were placed in the lower compartment instead of those of control mice (40.8 ± 3.3 vs. 28.7 ± 1.5/400× field, $P < 0.001$) (Fig. 4A–C). When tissues from contralateral non-obstructed kidneys were placed in the lower compartment, only a small number of macrophages migrated (7.7 ± 1.2) (Fig. 4C). Migration of macrophages was negligible without addition of any agent to the lower compartment (2.8 ± 0.6), and when intact laminin or intact fibronectin (10^{-7} M) was added to the lower compartment, only a limited number of macrophages migrated (14.9 ± 0.9 in intact laminin and 10.4 ± 2.1 in intact fibronectin) (Fig. 4D). However, when RGDS (10^{-7} M), a fibronectin degradation peptide, or fMLP (1 μM), a chemoattractant for macrophages, was added to the lower compartment, the number of macrophages migrated markedly increased (45.3 ± 6.1 in RGDS peptide, $P < 0.001$ vs. intact laminin or intact fibronectin; 48.0 ± 2.4 in fMLP, $P < 0.001$ vs.

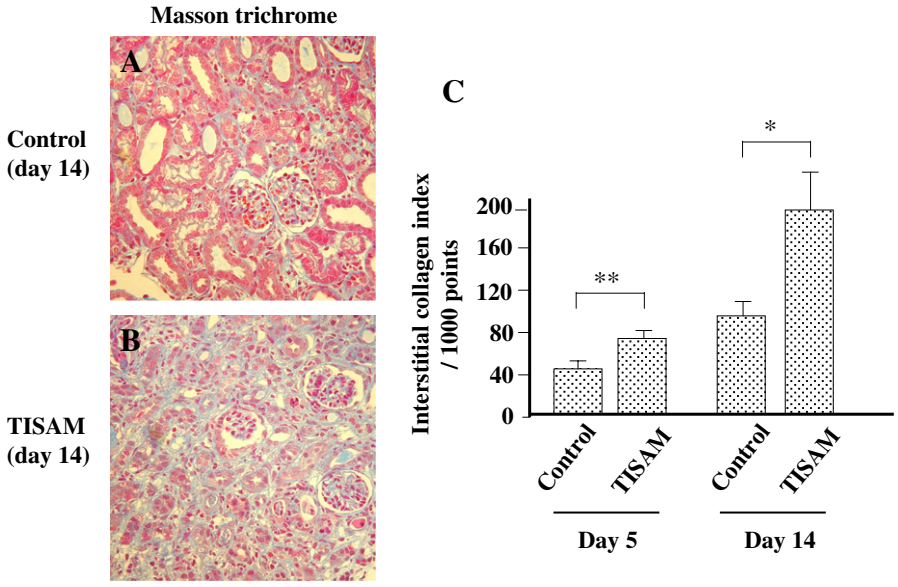

Fig. 2. Effect of TISAM administration on renal fibrosis in the UUO kidneys. Masson trichrome staining of the renal cortex in control mice (A) and in TISAM-treated mice (B) at day 14 after UUO. Original magnification, 400×. (C) Interstitial collagen index assessed by point-counting method on Masson trichrome-stained sections in control mice and in TISAM-treated mice at day 5 (left) and day 14 (right) after UUO. Data are expressed as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.001$.

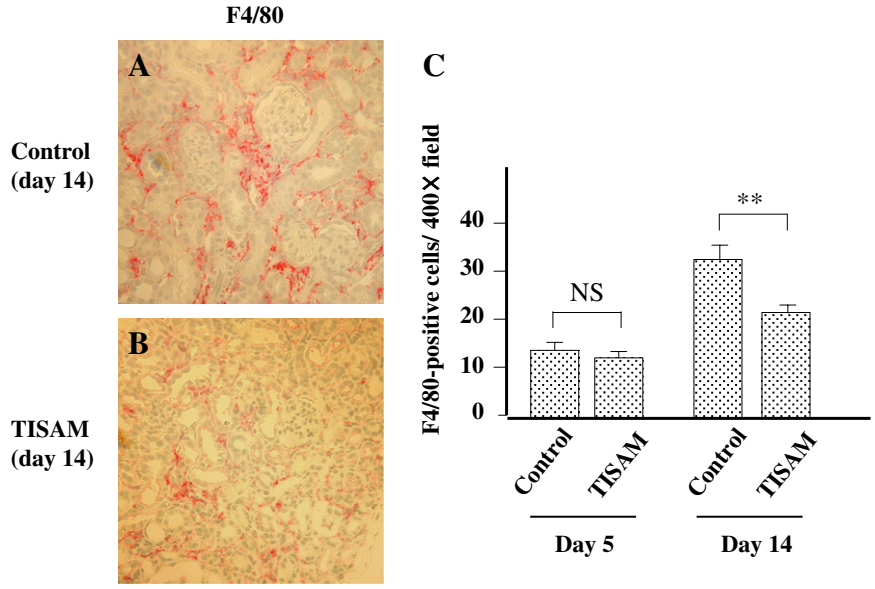

Fig. 3. Effect of TISAM administration on interstitial macrophage infiltration in the UUO kidneys. Immunohistochemical demonstration of macrophages with anti-F4/80 antibody in control mice (A) and in TISAM-treated mice (B) at day 14 after UUO. Original magnification, 400×. (C) Number of macrophages (F4/80-positive cells) infiltrating to the interstitium in control mice and in TISAM-treated mice at day 5 (left) and day 14 (right) after UUO. Data are expressed as means ± SEM. $^{**}P < 0.001$. NS, not significant.

intact laminin or intact fibronectin) (Fig. 4D). Furthermore, when macrophages were preincubated with RGDS peptide (10^{-7} M) to block its ligand receptors prior to the migration assay, the number of macrophages migrated in response to renal tissues of the UUO kidneys (day 14) from control mice was significantly less compared with those when macrophages were preincubated with vehicle alone (41.5 ± 2.7 vs. 24.1 ± 1.3, $P < 0.001$) (Fig. 4E). While in contrast, when tissues from the UUO kidneys (day 14) of TISAM-treated mice were placed in the lower compartment instead of those of control mice, no significant change was observed in the number of macrophages migrated whether macrophages were preincubated with RGDS peptide or with vehicle alone (21.0 ± 1.5 vs. 25.1 ± 2.0, NS) (Fig. 4E).

Discussion

In this study, treatment with TISAM suppressed gelatinolytic activity both at day 5 and at day 14 after

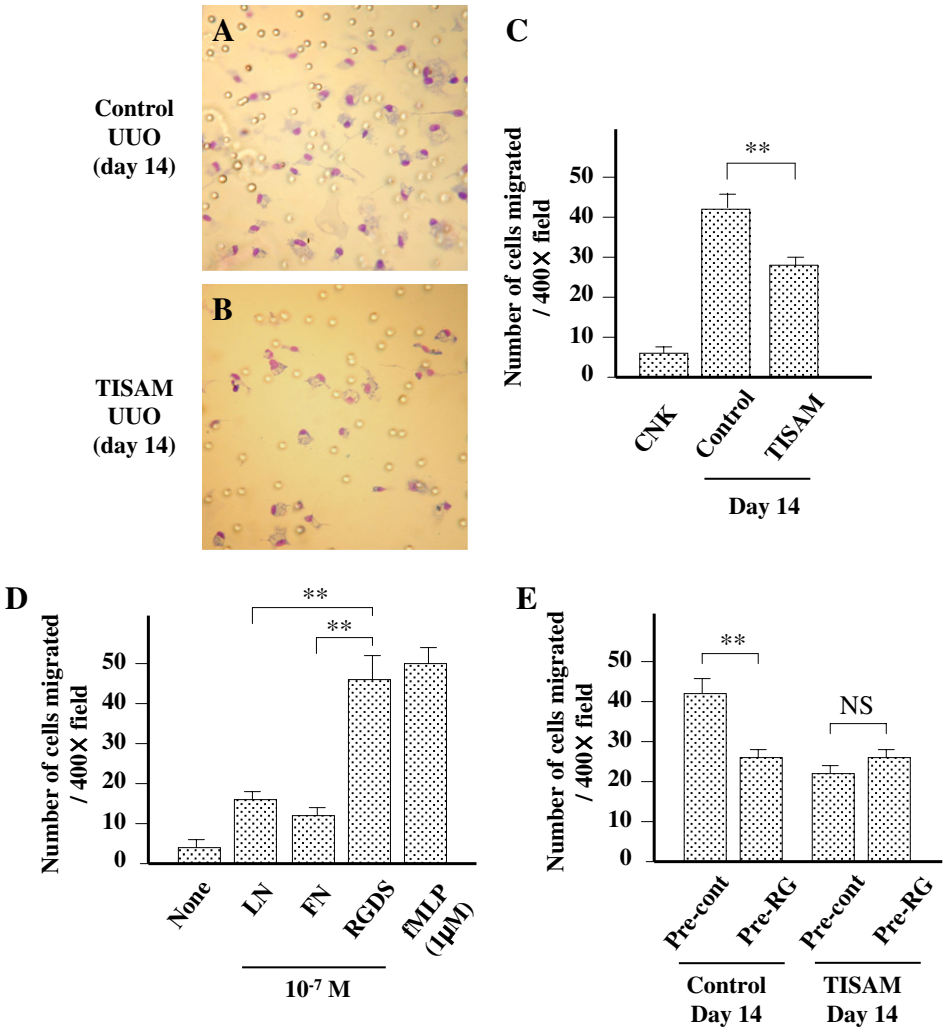

Fig. 4. Determination of macrophage migrating activity. Macrophages migrated through a Matrigel-coated insert in response to tissues of UUO kidneys from control mice (A) and from TISAM-treated mice (B) at day 14 after UUO. Original magnification, 400×. (C) Number of macrophages migrated in response to tissues of contralateral non-obstructed kidneys, of UUO kidneys (day 14) from control mice and from TISAM-treated mice. (D) Number of macrophages migrated in response to 10^{-7} M of laminin, fibronectin, RGDS peptide, and to 1 μM of fMLP. Migration of macrophages was negligible without addition of any agent to the lower compartment. (E) Number of macrophages migrated, following preincubation with 10^{-7} M of RGDS peptide to block its ligand receptors or with vehicle alone prior to the migration assay, in response to tissues of UUO kidneys from control mice (left) and from TISAM-treated mice (right) at day 14 after UUO. Data are expressed as means ± SEM of three independent experiments performed in triplicate. $^{**}P < 0.001$. NS, not significant; CNK, contralateral non-obstructed kidney; LM, laminin; FN, fibronectin; fMLP, formyl-Met-Leu-Phe; Pre-cont, preincubated with control medium; Pre-RG, preincubated with 10^{-7} M of RGDS peptide.

UUO. Because a previous report has shown that this dose of TISAM selectively inhibits MMP-2 activity [11], this suppression of gelatinolytic activity in the UUO kidney is considered due to suppressed MMP-2 activity. This result is consistent with a previous report showing that among two kinds of gelatinases, i.e., MMP-2 and -9, MMP-2 mRNA was upregulated in the UUO kidney, while in contrast, MMP-9 mRNA was significantly decreased [3]. Thus, MMP-2 plays a major role in gelatinolytic activity in the UUO kidney. Another report also showed that, in rat renal allografts, intense MMP-2 mRNA expression was demonstrated in the interstitium and tubular cells, while MMP-9 mRNA expression was located mainly in the glomerulus [12]. This finding is also consistent with our observation that the selective inhibition of MMP-2 activity has led to the suppressed gelatinolytic activity, which was seemingly located mostly in tubulointerstitial region in our model.

In our model, the inhibition of MMP-2 activity during day −2 and day 4 after UUO has led to an accelerated renal fibrosis at day 5, and the inhibition of MMP-2 activity during day 7 and day 13 after UUO has also led to an accelerated renal fibrosis at day 14. Although the action of MMPs for renal fibrosis after UUO is still under debate [13,14], it may be pertinent to consider that, in either case of our experimental protocols, the enhancement of renal fibrosis following inhibition of MMP-2 activity during a certain period after UUO, is due to decreased degradation of ECM. Importantly, our study regarding macrophage infiltration to the interstitium indicated a decreased

number of infiltrating macrophages following treatment with TISAM at the late stage of UUO (day 14), while at the early stage of UUO (day 5), no significant change was observed in the number of infiltrating macrophages following treatment with TISAM. This modification to interstitial macrophage infiltration by MMP-2 inhibition, which was observed only in late stage of UUO, suggested a stage-specific action of MMP-2 for promoting macrophage infiltration.

To explore the mechanisms for this alteration in the degree of macrophage infiltration, we further performed *in vitro* macrophage migration experiments using mouse peritoneal macrophages. Our results showed a markedly increased migration of macrophages in response to renal tissue of the control UUO kidney (day 14) compared with that of the contralateral non-obstructed kidney, and also a significantly decreased migrating response to renal tissue of the TISAM-treated UUO kidney (day 14) compared with that of the control UUO kidney. This result suggested that some potent chemotactic substances for macrophages exist in renal tissue of the UUO kidney, and the inhibition of MMP-2 activity in late stage of UUO suppresses this enhancement in chemotactic activity. Because previous studies indicated that the degradation products of ECM function as a chemotactic factor for macrophages in the settings such as wound healing process [9,10], and experimental myocardial infarction [11], we further studied the migration activity of macrophages in response to ECM components, i.e., laminin and fibronectin, and also that to the degradation products of fibronectin, i.e., RGDS peptide. In this study, RGDS markedly stimulated migrating activity of macrophages, while in contrast, only minor modifications were observed either with laminin or fibronectin, which is consistent with the previous reports indicating that degradation products of fibronectin induce macrophage migration [9,10]. Thus, to study the involvement of RGDS peptide in macrophage migration, we then performed an inhibition assay of macrophage migration to renal tissue of the UUO kidney by preincubating macrophages with RGDS peptide to block its ligand receptors prior to the migration study. As the result, macrophage migration activity in response to renal tissue of the control UUO kidney (day 14) was significantly reduced by preincubating macrophages with RGDS; however, no significant change was observed in the migration activity to the TISAM-treated UUO kidney by the pretreatment with RGDS. This result suggested that RGDS peptide produced through degradation of ECM by MMP-2 contributes, at least in part, to migration of macrophages to renal tissue in the late stage of the UUO kidney.

Several recent reports suggested an alleviative effect of MMP-2 inhibition for renal fibrosis through inhibiting epithelial-mesenchymal transition (EMT) of renal tubular cells by reducing basement membrane injury [14,15]. Lutz et al. also reported that early inhibition of MMP-2 activity in experimental allograft nephropathy resulted in an amelioration of fibrosis, while use of the inhibitor in the setting of established fibrosis resulted in more severe allograft nephropathy [16]. In the present study, the inhibition of MMP-2 activity in early stage after UUO resulted in increased fibrosis, as has been observed in cases when using the inhibitor in the late stage of UUO. The reason for this discrepancy in the result of the early stage of fibrotic change following early inhibition of MMP-2 after the insult is unclear. However, the action of MMP-2 in the evolution of renal fibrosis in each experimental model is believed to be complex, including the effect for promoting EMT and also for promoting proteolytic removal of accumulated ECM material depending on its time course after the insult. Thus, the results of the present study seem to be the subsequent changes of inhibiting these actions of MMP-2 as a whole.

The precise role of macrophage infiltration to renal tissue in the process of renal fibrosis as well as in the UUO kidney still remains to be elucidated. However, recent reports, including ours, suggested the possible role of macrophages to the amelioration of fibrosis and tissue repair following fibrotic change in the kidney [7,8,17]. In this alleviative role of macrophages in renal fibrosis, a mechanism through the phagocytic removal of ECM degradation products by macrophages has also been suggested [7]. The decreased number of infiltrating macrophages by inhibiting MMP-2 activity in the late stage of UUO observed in this study, as well as the increased fibrotic change, may also suggest decreased phagocytic removal of ECM components following its degradation. Further examination for the role of MMP-2 in renal fibrosis is necessary. Nevertheless, our study showed, for the first time, the possible relationship between MMP-2 activity and macrophage infiltration in the setting of renal fibrosis, which may potentially play a critical role in the evolution of renal fibrosis.

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References

[1] A.K. Sharma, S.M. Mauer, Y. Kim, A.F. Michael, Altered expression of matrix metalloproteinase-2, TIMP, and TIMP-2 in obstructive nephropathy, J. Lab. Clin. Med. 125 (1995) 754–761.
[2] C. Duymelinck, S.E. Dauwe, K.E. De Greef, D.K. Ysebaert, G.A. Verpooten, M.E. De Broe, TIMP-1 gene expression and PAI-1 antigen after unilateral ureteral obstruction in the adult male rat, Kidney Int. 58 (2000) 1186–1201.
[3] O. Iimura, H. Takahashi, T. Yashiro, S. Madoiwa, Y. Sakata, Y. Asano, E. Kusano, Effect of ureteral obstruction on matrix metalloproteinase-2 in rat renal cortex, Clin. Exp. Nephrol. 8 (2004) 223–229.
[4] A.A. Eddy, Interstitial macrophages as mediators of renal fibrosis, Exp. Nephrol. 3 (1995) 76–79.
[5] J.R. Diamond, Macrophages and progressive renal disease in experimental hydronephrosis, Am. J. Kidney Dis. 26 (1995) 133–140.

[6] T. Nagaoka, Y. Kaburagi, Y. Hamaguchi, M. Hasegawa, K. Takehara, D.A. Steeber, T.F. Tedder, S. Sato, Delayed wound healing in the absence of intercellular adhesion molecule-1 or L-selectin expression, Am. J. Pathol. 157 (2000) 237–247.
[7] M. Nishida, H. Fujinaka, T. Matsusaka, J. Price, V. Kon, A.B. Fogo, J.M. Davidson, M.F. Linton, S. Fazio, T. Homma, H. Yoshida, I. Ichikawa, Absence of angiotensin II type 1 receptor in bone marrow-derived cells is detrimental in the evolution of renal fibrosis, J. Clin. Invest. 110 (2002) 1859–1868.
[8] M. Nishida, Y. Okumura, S. Fujimoto, I. Shiraishi, T. Itoi, K. Hamaoka, Adoptive transfer of macrophages ameliorates renal fibrosis in mice, Biochem. Biophys. Res. Commun. 332 (2005) 11–16.
[9] D.E. Doherty, P.M. Henson, R.A. Clark, Fibronectin fragments containing the RGDS cell-binding domain mediate monocyte migration into the rabbit lung. A potential mechanism for C5 fragment-induced monocyte lung accumulation, J. Clin. Invest. 86 (1990) 1065–1075.
[10] J. Trial, R.E. Baughn, J.N. Wygant, B.W. McIntyre, H.H. Birdsall, K.A. Youker, A. Evans, M.L. Entman, R.D. Rossen, Fibronectin fragments modulate monocyte VLA-5 expression and monocyte migration, J. Clin. Invest. 104 (1999) 419–430.
[11] S. Matsumura, S. Iwanaga, S. Mochizuki, H. Okamoto, S. Ogawa, Y. Okada, Targeted deletion or pharmacological inhibition of MMP-2 prevents cardiac rupture after myocardial infarction in mice, J. Clin. Invest. 115 (2005) 599–609.
[12] K.A. Inkinen, A.P. Soots, L.A. Krogerus, I.T. Lautenschlager, J.P. Ahonen, Fibrosis and matrix metalloproteinases in rat renal allografts, Transpl. Int. 18 (2005) 506–512.
[13] H. Kim, T. Oda, J. Lopez-Guisa, D. Wing, D.R. Edwards, P.D. Soloway, A.A. Eddy, TIMP-1 deficiency does not attenuate interstitial fibrosis in obstructive nephropathy, J. Am. Soc. Nephrol. 12 (2001) 736–748.
[14] S. Cheng, A.S. Pollock, R. Mahimkar, J.L. Olson, D.H. Lovett, Matrix metalloproteinase 2 and basement membrane integrity: a unifying mechanism for progressive renal injury, FASEB J. 20 (2006) 1898–1900.
[15] S. Cheng, D.H. Lovett, Gelatinase A (MMP-2) is necessary and sufficient for renal tubular cell epithelial-mesenchymal transformation, Am. J. Pathol. 162 (2003) 1937–1949.
[16] J. Lutz, Y. Yao, E. Song, B. Antus, P. Hamar, S. Liu, U. Heemann, Inhibition of matrix metalloproteinases during chronic allograft nephropathy in rats, Transplantation 79 (2005) 655–661.
[17] G. Zhang, H. Kim, X. Cai, J.M. Lopez-Guisa, C.E. Alpers, Y. Liu, P. Carmeliet, A.A. Eddy, Urokinase receptor deficiency accelerates fibrosis in obstructive nephropathy, J. Am. Soc. Nephrol. 14 (2003) 1254–1271.