# Inhibition of Matrix Metalloproteinases During Chronic Allograft Nephropathy in Rats

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**Background.** Chronic allograft nephropathy (CAN) belongs to the major causes of long-term kidney allograft failure. One of the histologic hallmarks of CAN is interstitial fibrosis, influenced by matrix metalloproteinases (MMPs) that are controlling extracellular matrix (ECM) degradation. Whether MMPs affect the development and progression of CAN is not clear so far. To analyze the role of MMPs in CAN, we investigated the effects of an early and a late application of BAY 12–9566, an inhibitor of MMP-2, -3, and -9 on the development and progression of CAN in a rat kidney-transplantation model.

**Methods.** Fisher kidneys were orthotopically transplanted into Lewis recipients that were treated with BAY 12–9566 (15 mg/kg per day) or vehicle either for the first 10 days after transplantation (early treatment) or from week 12 to week 20 after transplantation (late treatment). Proteinuria was analyzed every 4 weeks up to week 20 after transplantation when kidney grafts were removed for further analysis.

**Results.** Early MMP-inhibition resulted in a significantly reduced 24-hour protein excretion that was paralleled by a lower grade of CAN after 20 weeks. However, late MMP inhibition starting at week 12 after transplantation resulted in significantly higher proteinuria and a higher grade of CAN as compared with controls. Furthermore, transforming growth factor-$\beta$ and platelet-derived growth factor-B chain mRNA levels were significantly increased in these animals.

**Conclusions.** Inhibition of MMPs early after transplantation reduced the development and progression of CAN but promoted CAN if initiated at later stages. Thus, MMPs are involved in the development and progression of CAN.

**Keywords:** Chronic allograft nephropathy, Extracellular matrix, Metalloproteinase inhibition.

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The accumulation of extracellular matrix (ECM), a histologic hallmark of chronic allograft nephropathy (CAN), is responsible for progressive tubulointerstitial fibrosis (*1*). ECM accumulation can result from an increased synthesis or a decreased degradation of ECM as well as a combination of both (*2*).

The major regulators of ECM turnover are matrix metalloproteinases (MMPs) (*3*) and growth factors. MMPs form a large family of zinc-dependent matrix-degrading enzymes, acting on the ECM of connective tissue and basement membranes (*4*), and are inhibited by so-called tissue inhibitors of MMPs. MMPs are important in tissue remodeling and repair in various renal diseases such as glomerulonephritis and progressive renal fibrosis, respectively (*5–7*). They are divided into subclasses according to their substrate specificity: collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9), and stromelysins (MMP-3, -7, -10, -11). Furthermore, membrane-type (MT)-MMPs (MT-1–4-MMP) exist. Especially the gelatinases MMP-2 and -9 have been studied in the kidney (*7*) as well as in renal-transplantation models for acute allograft rejection (*8*) and CAN (*9*).

MMP-2 (gelatinase A) predominantly degrades fibronectin and laminin, whereas MMP-9 (gelatinase B) degrades collagen type IV and V (*10*). MMP-3 (stromelysin-1) favors basement membrane proteins, particularly fibronectin (*10*). In the kidney, collagen type IV is present in basal membranes, whereas fibronectin, laminin, and collagen type V occur additionally in the tubulointerstitial matrix (*10*). So far, the role of MMPs in the development and progression of CAN is not clear. We hypothesized that MMPs, particularly MMP-2 and -9 through their effects on the tubulointerstitial ECM and the basement membranes, could enhance tissue damage during the acute phase after transplantation through a faster breakdown of normal ECM/glomerular basement membranes, whereas MMP activity might have protective effects regarding the progression of CAN during later stages after transplantation caused by a reduction of ECM accumulation and fibrosis.

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What is known about the intragraft kinetics of the expression of MMP-2 and -9 during the early phase after transplantation? Recently, increased levels of MMP-2 and -9 have been observed during the early phase in a model of lung transplantation (*11*) as well as after renal-ischemia reperfusion (*12*, *13*). A differential expression of MMPs was present in a model of acute renal-allograft rejection with increased MMP-2 and decreased MMP-9 during the first 7 days after transplantation (*8*). Moreover, MMP-2 and MMP-9 were enhanced in postischemic kidney tissue and were localized to tubular epithelial cells, interstitial cells, and the tubulointerstitial space (*14*). Furthermore, MMP-2 and -9 were up-regulated during the early phase after ureteral obstruction (*15*, *16*).

MMPs, particularly MMP-3 and elastase, are also capable of increasing the albumin permeability of isolated rat glomeruli in vitro, possibly by degradation of the glomerular basement membrane (*17*). Another group identified stromelysin and gelatinase as potent destructors of the glomerular basement membrane (*18*). This is supported by the localization of mesangial MMPs at areas of mesangiolysis and sites of glomerular basement membrane disruption in correlation with elevated MMP levels in anti-Thy 1.1 nephritis (*19*). Although this form of nephritis is not directly comparable with the acute situation after transplantation, it also represents a direct glomerular damage similar to the ischemia-reperfusion injury that occurs after transplantation. Moreover, the MMP inhibitor BB-94 (batimastat) effectively inhibited the proliferation and migration of smooth-muscle cells as well as the formation of neointima in a balloon-injury model in arteries (*20*). Such data stress the importance of MMPs in mediating effects of acute tissue injury by way of different cell types in different organs.

On the other hand, the question arises whether the reduced MMP activity observed during later stages of progressive renal diseases is cause or consequence of this process (*21*, *22*). The deposition of ECM after decreased MMP expression, respectively, in progressive fibrosis may disrupt the interaction between tubular epithelial cells and interstitial fibroblasts. This interaction is important for the maintenance of a normal ECM homeostasis. ECM deposition may become self-perpetuating because collagen IV, which is also a major component of the ECM, interacts with growth factors such as platelet-derived growth factor (PDGF), thus stimulating the proliferation of interstitial fibroblasts and leading to progressive tissue injury and organ dysfunction (*23*).

Growth factors such as transforming growth factor (TGF)-$\beta$ and PDGF-BB have been associated with progressive fibrosis during the development of CAN and after acute renal ischemia-reperfusion injury (*13*, *24*, *25*). Particularly, TGF-$\beta$ was increased in kidney tissues of grafts with CAN and after ischemia-reperfusion during the development of fibrosis (*13*, *25*). Interestingly, the activity and expression of MMPs was decreased in the latter.

BAY 12–9566 is a butanoic acid analogue that, as a nonpeptidic compound, inhibits the following MMPs with decreasing efficacy: MMP-2>MMP-3>MMP-9 (*26*). MMP inhibitors have been discovered in the field of oncology as substances capable of impairing tumor invasion, angiogenesis, and metastasis, thus being promising tools in the treatment of malignancies (*26*, *27*). However, they could also influence tissue fibrosis, particularly in the context of the development and progression of CAN.

We hypothesize that inhibition of MMP-2, -3, and -9 during the early posttransplant phase reduces tissue damage and delays the progression of CAN, whereas late inhibition of MMP-2, -3, and -9 increases tissue damage with progressive fibrosis and accelerates the progression of CAN. Thus, we administered the MMP inhibitor BAY 12–9566 at different phases in an experimental model of CAN.

## MATERIALS AND METHODS

### Animals and Surgery

Kidneys of Fisher rats (F344, RT1$^{lvl}$) (170–210 g) (Charles River, Sulzfeld, Germany) were orthotopically transplanted into Lewis rats (LEW, RT1 [1]) (170–210 g) (Charles River, Sulzfeld, Germany) as previously described (*28*). All animals received 1.5 mg/kg per day of cyclosporine A (Sigma Chemical Co., Steinheim, Germany) subcutaneously administered from day 0 to day 10 after transplantation, at what time the right recipient kidney was removed (*29*). Total ischemia of the grafts was adjusted to 25 minutes. Rats received Cephtriaxone (Rocephine, 20 mg/kg per day, intramuscularly, Hoffmann-la Roche Ag, Grenzach-Wyhlen, Germany) on the first postoperative day. Animals were housed under standard conditions and fed rat chow and water as desired. Animals were weight matched between the experimental groups throughout the experiment. All experiments were approved by a governmental committee on animal welfare.

Animals were assigned to four experimental groups treated either with BAY 12–9566 (BAY) (*26*, *27*) 15 mg/kg per day or vehicle: (1) treatment with BAY from day 0 to day 10 after transplantation (BAY 0–10) (n=7); (2) treatment with BAY from week 12 to week 20 after transplantation (BAY 12–20) (n=10). Control groups included animals treated with vehicle from day 0 to day 10 (VEH 0–10) (n=7) and from week 12 to week 20 (VEH 12–20) (n=10) after transplantation. BAY was suspended in carboxymethyl cellulose sodium–Tween 80 according to the manufacturer's instructions and administered daily by oral gavage (1 mL solute/animal).

After 20 weeks, rats were anesthetized with diethylether, and intraaortic blood pressure was measured using a DPT 3003-S/3cc arterial transducer (Peter von Berg Medizintechnik GmbH, Germany). Rats were bled thereafter, and the transplanted kidneys were removed, according to a standard protocol. Samples were snap frozen in liquid nitrogen for immunohistologic staining and for RNA preparation or fixed in buffered formalin (4%) for light microscopy.

### Functional Measurements

The 24-hour urine protein excretion, serum, and urine creatinine concentrations were determined every 4 weeks, as previously described (*28*).

### Histology

Morphologic studies were performed on paraformaldehyde (4%)-fixed, paraffin-embedded tissue sections stained with hematoxylin-eosin to evaluate tubulointerstitial fibrosis, vasculopathy, and tubular atrophy. Periodic acid Schiff reaction was performed to evaluate glomerulosclerosis. All glomeruli in each section were counted, and the propor-

tion of sclerotic glomeruli to the total number of glomeruli was expressed as a percentage.

CAN was graded according to parameters adapted from the BANFF 97 classification (*30*) as follows: grade 1, mild CAN with mild fibrosis, tubular atrophy, and vasculopathy; grade 2, moderate CAN with moderate fibrosis, tubular atrophy, and vasculopathy; grade 3, severe CAN with severe fibrosis, tubular atrophy, and vasculopathy. Two independent observers examined the slides by light microscopy in a blinded fashion.

### Immunohistochemical Studies

Immunocytochemical studies were performed on frozen sections fixed in acetone. Sections were incubated with mouse primary monoclonal antibodies against macrophages (ED1) and CD5+ T cells (OX19) (Serotec Ltd., Oxford, UK) followed by incubation with a secondary rabbit anti-mouse antibody (Dako A/S, Glostrup, Denmark) and development of the color signal by an alkaline phosphatase anti-alkaline phosphatase complex (Dako A/S, Glostrup, Denmark). ED-1 and OX-19 immunoreactive cells were counted (cells/ field of view; mean±SEM) (>20 fields of view/section at ×400).

### RNAse Protection Assay

Total RNA, prepared as described before (*28*), was used for the analysis of mRNA levels of TGF-$\beta_1$, PDGF-B chain, and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH). Antisense riboprobes (Pharmingen, San Diego, CA) were prepared by in vitro transcription with the incorporation of $^{32}$P-UTP according to the manufacturers protocol using the RiboQuant Kit (RiboQuant Multi-Probe Ribonuclease Protection Assay System, Pharmingen, San Diego, CA) (*31*). Total RNA samples were hybridized according to the manufacturers protocol with their respective rat antisense riboprobes, followed by RNA- and RNAse digestion and separation on a 6% polyacrylamide gel. Dried radioactive blots were scanned on a Fuji-BAS phosphor imager (Fuji, Düsseldorf, Germany). The blot was computer digitized, and the density of bands was measured. Data represent a ratio between specific mRNA density to housekeeping gene mRNA (GAPDH).

### Statistical Analysis

Data are expressed as mean±standard error of mean (SEM). Parametric data were compared with one-way analysis of variance. Nonparametric data were tested using the Kruskal-Wallis one-way analysis of ranks or Mann-Whitney test. A *P* value of less than 0.05 was considered significant.

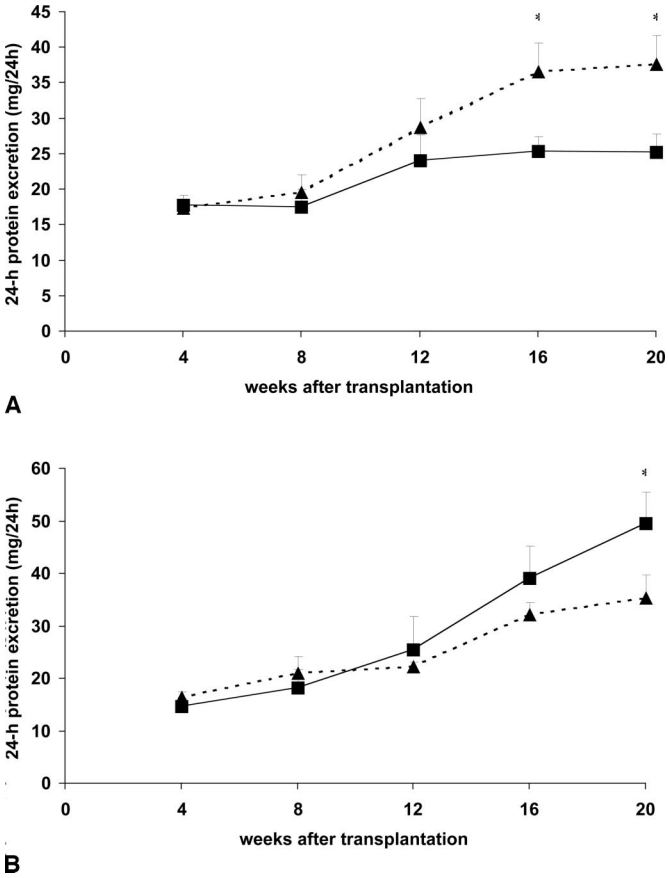


**FIGURE 1.** (A) Protein excretion from 4 to 20 weeks after transplantation in animals treated with BAY 12–9566 from day 0 to day 10 (BAY 0–10, ▲) after transplantation or vehicle (VEH 0–10, ■). Matrix metalloproteinase (MMP) inhibition during the first 10 days after transplantation resulted in a significantly lower proteinuria as compared with controls (*$P$<0.05). (B) Protein excretion from 4 to 20 weeks after transplantation in animals treated with BAY 12–9566 from week 12 to 20 (BAY 12–20, ▲) after transplantation or vehicle (VEH 12–20, ■). Animals treated from week 12 to week 20 after transplantation had a significantly higher proteinuria as compared with controls after 20 weeks (*$P$<0.05).

## RESULTS

### 24-Hour Protein Excretion was Decreased by Early MMP Inhibition

MMP inhibition with BAY during the first 10 days after transplantation (BAY 0–10) resulted in significantly lower proteinuria as compared with controls (VEH 0–10) at the end of the follow-up period ($P$<0.05) (Fig. 1A). In contrast, animals treated from week 12 to week 20 after transplantation (BAY 12–20) developed a significantly higher proteinuria as compared with controls (VEH 12–20) ($P$<0.05) (Fig. 1B).

Creatinine clearance was higher in animals treated early with BAY (BAY 0–10) as compared with controls, whereas it was markedly decreased in animals treated at a later time ($P$<0.05) (Table 1). Differences in body weight and arterial blood pressure between the groups did not reach statistical significance throughout the follow-up time (Table 1).

### Early MMP Inhibition Reduced Grade of CAN and Glomerulosclerosis

The grade of CAN was lower in early treated animals (BAY 0–10) ($P$<0.05), whereas animals treated at a later time point (BAY 12–20) developed higher grades as compared with controls ($P$<0.05) (Fig. 2, A and B). This was paralleled by a significantly lower glomerulosclerosis in animals with early treatment (BAY 0–10) than in controls (VEH 0–10) ($P$<0.05). On the other hand, animals with late treatment (BAY 12–20) developed significantly more sclerotic glomeruli as compared with controls (VEH 12–20) ($P$<0.01) (Fig. 2, C and D).

**TABLE 1.** Creatinine clearance, mean arterial pressure, and body weight 20 weeks after transplantation in animals treated either with BAY 12-9566 or vehicle from day 0 to 10 (BAY 0–10; VEH 0–10) or week 12 to 20 (BAY 12–20; VEH 12–20) (mean±SEM)

| | BAY 0–10 | VEH 0–10 | BAY 12–20 | VEH 12–20 |
|---|---|---|---|---|
| Creatinine clearance (mL/min) | 1.7±0.2 | 1.5±0.2 | 1.2±0.1 | 1.6±0.2 |
| Mean arterial pressure (mm Hg) | 103.4±12.1 | 102.2±6.8 | 93.2±5.9 | 108.8±4.8 |
| Body weight (g) | 444.4±17.2 | 435.8±15.1 | 418.6±8.9 | 438.8±5.5 |

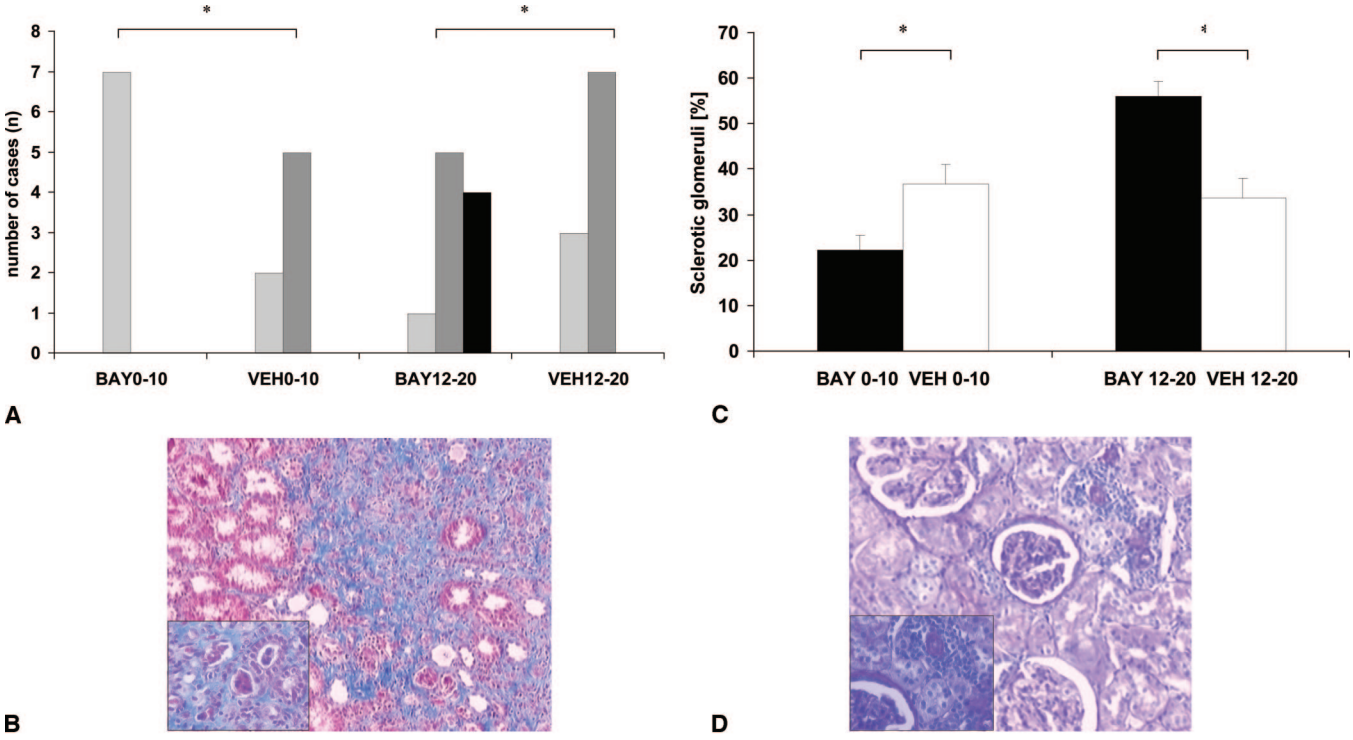


**FIGURE 2.** (A) Grade of chronic allograft nephropathy (CAN) 20 weeks after transplantation groups BAY 0–10, BAY 12–20, VEH 0–10, and VEH 12–20 (□, mild CAN; □, moderate CAN; ■, severe CAN). Animals with BAY treatment from day 0 to day 10 after transplantation (BAY 0–10) had a significantly lower grade of CAN as compared with controls (VEH 0–10) ($P<0.05$). Animals with late BAY treatment from week 12 to week 20 after transplantation (BAY 12–20), on the other hand, developed more severe CAN than controls (VEH 12–20) (*=$P<0.05$). (B) Tubulointerstitial fibrosis wit tubular atrophy (×100; insert ×400; Masson trichrome). (C) Glomerulosclerosis 20 weeks after transplantation in animals treated either with BAY 12–9566 (■) or vehicle (□) from day 0 to 10 (BAY 0–10, VEH 0–10) or week 12 to 20 (BAY 12–20, VEH 12–20). Animals with BAY treatment from day 0 to day 10 after transplantation (BAY 0–10) had a significantly lower glomerulosclerosis as compared with controls (VEH 0–10) ($P<0.05$). Animals treated with BAY from week 12 to week 20 after transplantation (BAY 12–20) developed more sclerotic glomeruli as compared with controls (VEH 12–20) (*$P<0.05$). (D) Sclerotic glomerulus, small arteries with vasculopathy (insert), and perivascular lymphocyte infiltrations (×200, periodic acid Schiff reaction).

### Early BAY Treatment Reduced Macrophage Infiltration of Graft Tissue

Animals with early treatment (BAY 0–10) had significantly lower numbers of ED1-positive macrophages in their graft tissue (VEH 0–10) ($P<0.05$), whereas late treatment (BAY 12–20) resulted in a higher number of graft infiltrating macrophages as compared with controls (VEH 12–20) ($P<0.05$) (Fig. 3). The number of graft-infiltrating OX–19-positive lymphocytes did not differ significantly between treated animals and controls (data not shown).

### Growth Factor mRNA Levels Elevated in Animals Receiving Late BAY Treatment

Animals with early treatment (BAY 0–10) developed a trend toward lower TGF-$\beta_1$ mRNA levels as compared with controls (VEH 0–10) ($P>0.05$) (Fig. 4A). PDGF-B mRNA levels were similar in the early treatment group (BAY 0–10) and controls (VEH 0–10) (Fig. 4B). Animals that received late treatment (BAY 12–20) had significantly higher TGF-$\beta_1$ and PDGF-B mRNA levels than controls (VEH 12–20) ($P<0.05$). (Fig. 4).

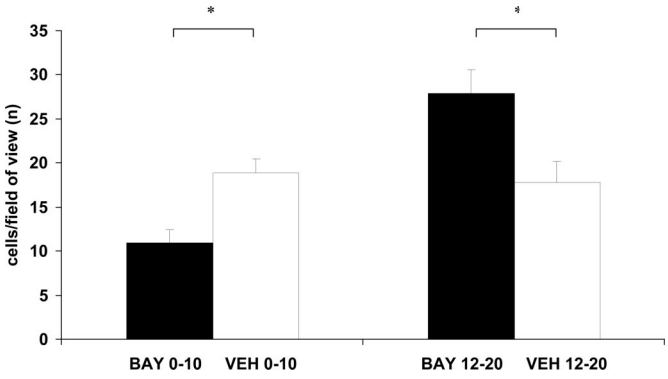


**FIGURE 3.** Infiltration of macrophages in the graft tissue 20 weeks after transplantation in animals treated either with BAY 12–9566 (■) or vehicle (□) from day 0 to 10 (BAY 0–10, VEH 0–10) or week 12 to 20 (BAY 12–20, VEH 12–20). Animals with early BAY treatment (BAY 0–10) had a significantly lower number of ED1-positive macrophages in their graft tissue as compared with controls (VEH 0–10) (*$P<0.05$), whereas late treatment (BAY 12–20) resulted in a significantly higher number of graft infiltrating macrophages as compared with controls (VEH 12–20) (*$P<0.05$).

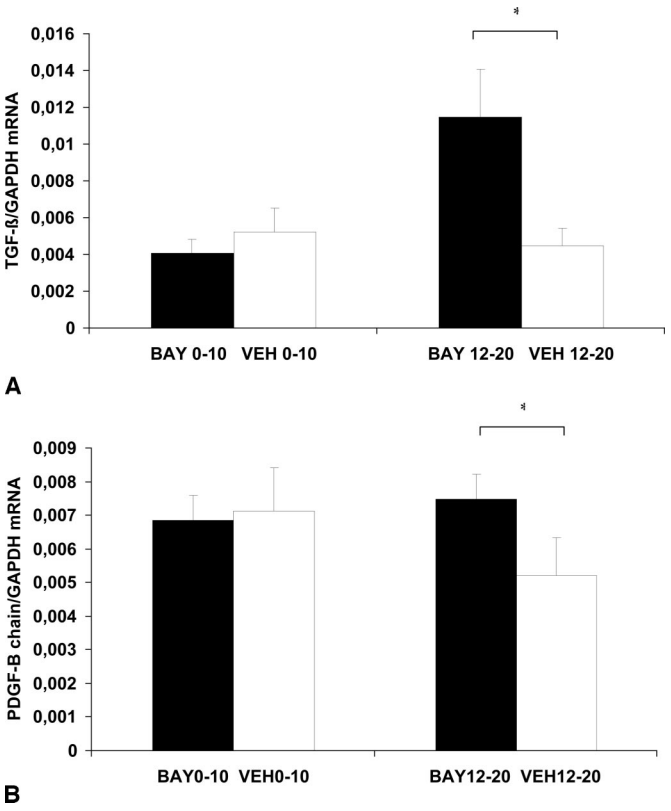


**FIGURE 4.** (A) Transforming growth factor (TGF)-$\beta$ mRNA levels 20 weeks after transplantation in animals treated either with BAY 12–9566 (■) or vehicle (□) from day 0 to 10 (BAY 0–10, VEH 0–10) or week 12 to 20 (BAY 12–20, VEH 12–20). Animals with early BAY treatment (BAY 0–10) developed a trend toward lower TGF-$\beta_1$ mRNA levels as compared with controls (VEH 0–10), whereas animals that received late treatment (BAY 12–20) had significantly higher TGF-$\beta_1$ mRNA levels as compared with controls (VEH 12–20) (*$P<0.05$). (B) Platelet-derived growth factor (PDGF)-B mRNA levels 20 weeks after transplantation in animals treated either with BAY 12–9566 (■) or vehicle (□) from day 0 to 10 (BAY 0–10, VEH 0–10) or week 12 to 20 (BAY 12–20, VEH 12–20). PDGF-B mRNA levels were similar in the early treatment group (BAY 0–10) and controls (VEH 0–10), whereas animals that received late BAY treatment from week 12 to 20 after transplantation (BAY 12–20) had significantly higher PDGF-B mRNA levels as compared with controls (VEH 12–20) (*$P<0.05$).

## DISCUSSION

In the present study, we applied BAY 12–9566, an inhibitor of MMP-2, -3, and -9, in a rat model of CAN. Inhibition of MMPs had different effects depending on the time after kidney transplantation. Whereas early inhibition of MMPs significantly improved graft function and morphology, late MMP inhibition significantly deteriorated graft function as compared with controls.

Interestingly, the beneficial long-term effects were achieved by a short treatment during the early posttransplantation phase when graft injury caused by alloantigen-dependent factors is most pronounced. Our results are in accordance with findings in a lung-transplantation model, in which early MMP inhibition improved graft outcome, particularly with respect to ischemia-reperfusion injury (*11*). The importance of MMPs in mediating acute tissue injury is also highlighted by the protective effects of a targeted deletion of MMP-9 regarding the tissue injury in a model of myocardial ischemia-reperfusion (*32*). Furthermore, proteinuria was lowered in animals with MMP inhibition 7 days after transplantation in a model of acute renal-allograft rejection (*8*).

In our experimental setting, the early treatment with BAY 12–9566 from day 0 to day 10 after transplantation coincided with the cyclosporine A treatment that is part of this experimental transplantation model of CAN. However, it is not likely that cyclosporine A treatment had an independent effect because control animals also received the same dose of cyclosporine without a beneficial effect regarding the development of CAN. On the other hand, BAY 12–9566 itself could influence the plasma levels of cyclosporine that could in turn effect inflammatory processes in the graft as well as calcineurin–inhibitor-related nephrotoxicity. However, both BAY 12–9566 as well as vehicle-treated animals had comparable blood pressure values and T-cell infiltrates. Thus, it seems to be unlikely that BAY 12–9566 leads to an increase of cyclosporine levels.

Two mechanisms could be involved in the fibrosis-promoting effects of ECM degradation during the early phase after an acute injury to the graft. First, disruption of the normal ECM network upon acute injury to the kidney (i.e., after ischemia-reperfusion or transplantation) (*8*), can activate mesangial cells (*33*), which are usually maintained in a quiescent, nonfibrogenic phenotype. This is because of their contact with ECM comprised of type IV collagen, laminin, and proteoglycans (*34*). The activation of mesangial cells upon contact loss with normal ECM is supported by in vitro observations, where removal of matrix transformed resting mesangial cells into a profibrogenic myofibroblastic phenotype, contributing to glomerulosclerosis and progressive fibrosis (*35*, *36*). Thus, early MMP inhibition could help to maintain

a physiologic ECM in the allografts. This could result in a lower state of activation of profibrogenic cells such as mesangial cells or interstitial fibroblasts, leading to a lower degree of CAN.

Second, fragments of ECM produced by MMP cleavage could function as chemotactic factors and costimulators for leukocytes and, thus, may induce their recruitment, extravasation, and activation. Moreover, chemokines can be cleaved by MMPs, thus potentiating their proinflammatory effects (*37*). MMP-2 and-9, also secreted by activated T-cells, can facilitate extravasation and migration of inflammatory cells toward the inflammatory site (*38*). Particularly, fibronectin can promote binding of inflammatory cells such as monocytes and enhance cytokine and MMP secretion, thus promoting inflammation and fibrosis (*39*). Furthermore, a disruption of basement membranes by MMPs activates endothelial cells (*40*). This could again contribute to leukocyte/monocyte infiltration, thus promoting interstitial fibrosis. In fact, early inhibition of MMPs reduced macrophage infiltration in our experiments. Thus, the beneficial effects of early MMP inhibition could also result from a reduced leukocyte/monocyte infiltration, possibly mediated by a reduced production of chemoattractants or activation of endothelial cells.

In contrast with our results, inhibition of MMPs in a model of renal ischemia-reperfusion did not improve organ function or morphologic outcome (*41*). However, the authors applied a different MMP inhibitor, and an observation time of 24 hours after onset of the injury might be too short to detect significant differences between the experimental groups.

In contrast with the beneficial effects of early MMP inhibition, delayed treatment with BAY 12–9566 decreased graft function and aggravated CAN as paralleled by increased glomerulosclerosis of the grafts. This is supported by the data, indicating that a reduced activity of MMPs was associated with progression of fibrosis in chronic renal disease models such as diabetes mellitus (*42*, *43*), aging (*44*), or nephritic syndrome (*45*).

These data suggest that accumulation of intracellular proteins and ECM components in conditions of reduced MMP activity or expression contribute to the development of glomerulosclerosis and tubulointerstitial fibrosis. A reduced ECM degradation results in a net deposition of fibrillar matrix that is predominantly comprised of collagen type I and III, predominantly present in the interstitium, which further induces glomerular hypertrophy, glomerular basement membrane thickening, and mesangial expansion (*46*).

In our present study, along with pathologic changes and impaired renal function, late inhibition of MMPs was associated with increased levels of TGF-$\beta$ and PDGF-B chain mRNA. This may derive from an enhanced infiltration of mononuclear cells, such as lymphocytes and macrophages, that can also stimulate residential cells to release such growth factors.

TGF-$\beta$ and PDGF have been localized to infiltrating cells and fibroblasts as well as glomeruli and vessel walls in transplanted kidneys (*25*). Indeed, we also observed an increased infiltration of macrophages after late MMP inhibition. Furthermore, TGF-$\beta$ not only enhances the synthesis of numerous matrix components such as type I, III, IV, and V collagen, fibronectin, laminin, and proteoglycans (*47*) but also reduces MMP activities (*48*). As a result, the involvement of the growth factors may result in a vicious cycle of MMP inhibition, which ultimately promotes allograft fibrosis and the progression of CAN. However, growth-factor mRNA levels were not significantly different in animals receiving early MMP inhibition in our experiments. This could have been casued by a situation of slowly increasing growth factor levels late after transplantation.

In conclusion, MMP inhibition during the immediate posttransplant period may ameliorate CAN possibly through a reduced breakdown of physiologic ECM with a reduced production of chemoattractants and profibrogenic factors, whereas it promotes the progression of CAN if administered at later stages after transplantation, possibly through a reduced degradation of excessively formed interstitial ECM. This suggests a pathogenetic time–course-dependent differential role of MMPs in the development and progression of CAN.

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## REFERENCES

1. Inkinen K, Soots A, Krogerus L, et al. Collagens in chronic rejection of rat renal allografts. *Transplant Proc* 1997; 7: 2495.
2. Norman JT, Lewis MP. Matrix metalloproteinases (MMPs) in renal fibrosis. *Kidney Int* 1996; 49: 61.
3. Baricos WH. Chronic renal disease: do metalloproteinase inhibitors have a demonstrable role in extracellular matrix accumulation? *Curr Opin Neph Hyperten* 1995; 4: 365.
4. Chapman KT, Kopka IE, Durette PL, et al. Inhibition of Matrix Metalloproteinases by N-carboxyalkyl peptides. *J Med Chem* 1993; 36: 4293.
5. Rodrigo E, Lopez-Hoyos M, Escallada R, et al. Circulating levels of matrix metalloproteinases MMP-3, and MMP-2 in renal transplant recipients with chronic transplant nephropathy. *Nephrol Dial Transplant* 2000; 15: 2041.
6. Akiyama K, Shikata K, Sugimoto H, et al. Changes in serum concentrations of matrix metalloproteinases. Tissue inhibitors of metalloproteinases and type IV collagen in patients with various types of glomerulonephritis. *Res Commun Mol Pathol Pharmacol* 1997; 95: 115.
7. Lenz O, Elliot SJ, Stevenson S. Matrix metalloproteinases in renal development. *J Am Soc Nephrol* 2000; 11: 574.
8. Ermolli M, Schumacher M, Lods N, et al. Differential expression of MMP-2/MMP-9 and potential benefit of an MMP inhibitor in experimental acute kidney allograft rejection. *Transplant Immunol* 2003; 11: 137.
9. Inkinen K, Soots A, Krogerus L, et al. Collagens in chronic rejection of rat renal allografts. *Transplant Proc* 1997; 29: 1543.
10. Johnson TS, Haylor JL, Thomas GL, et al. Matrix metalloproteinases and their inhibitions in experimental renal scarring. *Exp Nephrol* 2002; 10: 182.
11. Soccal PM, Gasche Y, Miniati DN, et al. Matrix metalloproteinase inhibition decreases ischemia-reperfusion injury after lung transplantation. *Am J Transplant* 2004; 4: 41.
12. Forbes JM, Hewitson TD, Becker GJ, et al. Ischemic acute renal failure: long-term histology of cell and matrix changes in the rat. *Kidney Int* 2000; 57: 2375.
13. Jain S, Bicknell GR, Nicholson NL. Molecular changes in extracellular matrix turnover after renal ischaemia-reperfusion injury. *Br J Surg* 2000; 87: 1188.
14. Basile DP, Fredrich K, Weihrauch D, et al. Angiostatin and matrix metalloproteinase expression following ischemic acute renal failure. *Am J Physiol Renal Physiol* 2004; 286: F893.
15. Sharma AK, Mauer SM, Kim Y, et al. Altered expression of matrix

metalloproteinase-2, TIMP, and TIMP-2 in obstructive nephropathy. *J Lab Clin Med* 1995; 125: 754.
16. Gonzalez AG, Iturria C, Vadillo OF, et al. Changes in matrix metalloproteinases during the evolution of interstitial renal fibrosis in a rat experimental model. *Pathobiology* 1998; 66: 196.
17. Sharma R, Suzuki K, Nagase H, et al. Matrix metalloproteinase (stromelysin-1) increase the albumin permeability of isolated rat glomeruli. *J Lab Clin Med* 1996; 128: 297.
18. Baricos WH, Murphy G, Zhou Y, et al. Degradation of glomerular basement membrane by purified mammalian metalloproteinases. *Biocehm J* 1998; 254: 609.
19. Le Q, Cortez S, Nguyen H, et al. Glomerular neutral metalloproteinase: characterization and activity in animal models of human glomerular disease. *Matrix (Suppl)* 1992; 1: 415.
20. Zempo N, Koyama N, Kenagy RD, et al. Regulation of vascular smooth muscle cell migration and proliferation in vitro and in injured rat arteries by a synthetic matrix metalloproteinase inhibitor. *Arterioscler Thromb Vasc Biol* 1996; 16: 28.
21. Carome M, Striker L, Peten E, et al. Human glomeruli express TIMP-1 mRNA, and TIMP-2 protein and mRNA. *Am J Physiol* 1993; 264: F923.
22. Carome M, Striker L, Peten E, et al. Assessment of 72-kilodalton gelatinase and TIMP-1 gene expression in normal and sclerotic murine glomeruli. *J Am Soc Nephrol* 1994; 4: 1391.
23. Somasundaram R, Schuppan D. Type I, II, III, IV, V, and VI collagens serve as extracellular ligands for the isoforms of platelet-derived growth factor (AA, BB, and AB). *J Biol Chem* 1996; 271: 26884.
24. Song E, Zou H, Yao Y, et al. Early application of Met-RANTES ameliorates chronic allograft nephropathy. *Kidney Int* 2002; 61: 676.
25. Paul LC, Saito K, Davidoff A, et al. Growth factor transcripts in rat renal transplants. *Am J Kidney Dis* 1996; 28: 441.
26. Rothenberg ML, Nelson AR, Hande KR. New drugs on the horizon: matrix metalloproteinase inhibitors. *Stem Cells* 1999; 17: 237.
27. Rowinsky EK, Humphrey R, Hammond LA, et al. Phase I and pharmacologic study of the specific matrix metalloproteinase inhibitor BAY 12–9566 on a protracted oral daily dosing schedule in patients with solid malignancies. *J Clin Oncol* 2000; 18: 178.
28. Szabo A, Lutz J, Schleimer K, et al. Effect of angiotensin-converting enzyme inhibition on growth factor mRNA in chronic renal allograft rejection in the rat. *Kidney Int* 2000; 57: 982.
29. Heemann U, Tullius SG, Tamatami T, et al. Infiltration patterns of macrophages and lymphocytes in chronically rejecting rat kidney allografts. *Transpl Int* 1994; 7: 349.
30. Racusen LC, Solez K, Colvhin RB, et al. The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999; 55: 713.
31. Antus B, Yao Y, Liu S, et al. Antiandrogens ameliorate chronic allograft nephropathy. *Kidney Int* 2001; 60: 1955.
32. Romanic AM, Harrison SM, Bao W, et al. Myocardial protection from ischemia/reperfusion injury by targeted deletion of matrix metalloproteinase-9. *Cardiovasc Res* 2002; 54: 549.
33. Yamada H, Saito F, Fukuta-Ohi H, et al. Processing of beta-dystroglycan by matrix metalloproteinase disrupts the link between the extracellular matrix and cell membrane via dystroglycan complex. *Hum Mol Genet* 2001; 10: 1563.
34. McLennan SV, Fisher E, Martell SY, et al. Effects of glucose on matrix metalloproteinase and plasmin activities in mesangial cells: possible role in diabetic nephropathy. *Kidney Int* 2000; 58: S81.
35. Pawluczyk IZ, Harris KP. Cytokine interactions promote synergistic fibronectin accumulation by mesangial cells. *Kidney Int* 1998; 54: 62.
36. Pawluczyk IZ, Harris KP. Macrophages promote prosclerotic responses in cultured rat mesangial cells: mechanism for the initiation of glomerulosclerosis. *J Am Soc Nephrol* 1997; 8: 152.
37. Van Den Steen PE, Wuyts A, Husson SJ, et al. Gelatinase B/MMP-9 and neutrophil collagenase/MMP-8 process the chemokines human GCP-2/CXCL6, ENA-78/CXCL5 and mouse GCP-2/LIX and modulate their physiological activities. *Eur J Biochem* 2003; 270: 3739.
38. Yakubenko VP, Lobb RR, Plow EF, et al. Differential induction of gelatinase B (MMP-9) and gelatinase A (MMP-2) in T lymphocytes upon alpha(4)beta(1)-mediated adhesion to VCAM-1 and the CS-1 peptide of fibronectin. *Exp Cell Res* 2000; 10: 73.
39. Chana RS, Martin J, Rahman EU, et al. Monocyte adhesion to mesangial matrix modulates cytokine and metalloproteinase production. *Kidney Int* 2003; 63: 889.
40. Hudlicka O, Brown MD. Postnatal growth of the heart and its blood vessels. *J Vasc Res* 1996; 33: 266.
41. Ziswiler R, Daniel C, Franz E, et al. Renal matrix metalloproteinase activity is unaffected by experimental ischemia-reperfusion injury and matrix metalloproteinase inhibition does not alter outcome of renal function. *Exp Nephrol* 2001; 9: 118.
42. Reckelhoff JF, Tygart VL, Mitias MM, et al. STZ-induced diabetes results in decreased activity of glomerular cathepsin and metalloprotease in rats. *Diabetes* 1993; 42: 1425.
43. Schaefer L, Han X, August C, et al. Differential regulation of glomerular gelatinase B (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in obese Zucker rats. *Diabetologica* 1997; 40: 1035.
44. Gaglliano N, Arusio B, Santambrogio D, et al. Age-dependent expression of fibrosis-related genes and collagen deposition in rat kidney cortex. *J Gerontol A Biol Sci Med Sci* 2000; 55: B365.
45. Uchio K, Manabe N, Tamura K, et al. Decreased matrix metalloproteinase activity in the kidneys of hereditary nephrotic mice (ICGN strain). *Nephron* 2000; 86: 145.
46. Heidland A, Sebekova K, Paczek L, et al. Renal fibrosis: role of impaired proteolysis and potential therapeutic strategies. *Kidney Int* 1997; 62(Suppl): S32.
47. Cuhaci B, Kumar MS, Bloom RD, et al. Transforming growth factor-beta levels in human allograft chronic fibrosis correlate with rate of decline in renal function. *Transplantation* 1999; 68: 785.
48. Mozes MM, Bottinger EP, Jacot TA, et al. Renal expression of fibrotic matrix proteins and of transforming growth factor-beta (TGF-$\beta$) isoforms in TGF-$\beta$ transgenic mice. *J Am Soc Nephrol* 1999; 10: 271.