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Amelioration by beraprost sodium, a prostacyclin analogue, of established renal dysfunction in rat glomerulonephritis model

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Abstract

Effects of beraprost sodium, a chemically stable prostacyclin analogue, on renal dysfunction in an experimental rat model of glomerulonephritis were investigated. Beraprost sodium (30, 100 and 300 μg/kg) was orally given twice daily from the late stage of nephritis in which renal dysfunction was already developed. Beraprost sodium treatment inhibited the increase in urinary protein, serum creatinine and blood urea nitrogen, and the decrease in creatinine clearance. The elevation of serum creatinine was also inhibited by predonisolone (1 mg/kg). However, captopril (25, 50 and 100 mg/kg) and dipyridamole (20 and 60 mg/kg) failed to inhibit the elevation of serum creatinine. In the beraprost sodium-treated nephritic rats, the increase in mRNA levels for monocyte chemoattractant protein-1 (MCP-1) and collagen in the kidney was inhibited. These results suggest that beraprost sodium ameliorates developed renal dysfunction and is possibly an effective agent for the treatment of human glomerulonephritis.

Keywords: Beraprost sodium; Prostacyclin; Renal dysfunction; Nephritis; Anti-glomerular basement membrane; MCP-1 (monocyte chemoattractant protein-1); Collagen

1. Introduction

Prostacyclin, largely produced in vascular endothelial cells, acts on platelets and blood vessels through its specific cell surface receptor whereby inhibiting platelet function, dilating blood vessels and protecting the vascular endothelium (Moncada et al., 1976; Moncada, 1982). Prostacyclin is also known to inhibit leukocyte functions such as migration and reactive oxygen species production (Boxer et al., 1980) and inhibit mesangial cell proliferation (Mene et al., 1990).

Leukocyte infiltration into the glomerulus (Lefkowith et al., 1991), platelet aggregation (Poelstra et al., 1993) and hypertension (Mayer et al., 1993) are important risk factors for the development and progression of renal disorder. Glucocorticoids (Taniguchi et al., 1994), immunosuppressants (Hayashi et al., 1996), anti-platelet drugs (Kincaid-Smith et al., 1970) and angiotensin-converting enzyme inhibitors (Heeg et al., 1991; Ruiz-Ortega et al., 1995) reduce those risk factors and have been clinically used, but the effects are still insufficient. Beneficial effects of prostacyclin and its analogue have been reported on the anti-Thy-1 antibody induced nephritic rat (Poelstra et al., 1993) and the NZB/WF_1 mouse, a model of spontaneous lupus nephritis (Clark et al., 1987; Utsunomiya et al., 1995). However, the pharmacological, clinical studies of prostacyclin have been limited by its in vivo rapid metabolic degradation. Beraprost sodium is a stable prostacyclin analogue (Ohno et al., 1985), acts through prostacyclin receptor and possesses a similar pharmacological profile to prostacyclin (Nishio et al., 1988; Kajikawa et al., 1989). Because beraprost sodium has a long biological half-life and a high oral bioavailability, it has become possible to administer long-term to patients and experimental animals. Beraprost sodium improves walking distance in the patient with peripheral arterial occlusive disease (Lievre et al., 2000) and the survival rates of patients with primary pulmonary hypertension (Nagaya et al., 1999). Recently, the beneficial effect of beraprost sodium on anti-glomerular basement membrane serum-induced nephritic rats was

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reported (Kushiro et al., 1998). They started the administration of beraprost sodium 24 h before the anti-glomerular basement membrane serum injection. They observed the amelioration of urinary protein and crescent formation during the early phase of nephritis up to 14th days after anti-glomerular basement membrane serum injection. It is more important to evaluate the effect of beraprost sodium in the established late phase of nephritis than in a prophylactic setting because of its relevance to clinical applications for human disorders. However, unfortunately, the effects of beraprost sodium in the established late phase have not been explored.

Beraprost sodium has multiple effects directed to improve microcirculation, such as anti-platelet, vasodilatory and anti-inflammatory effects. It is unknown which effects are involved in the improvement of renal dysfunction by beraprost sodium treatment. In order to clarify this issue, we compared beraprost sodium with an anti-platelet drug (dipyridamole), an anti-hypertensive angiotensin-converting enzyme inhibitor (captopril) and an anti-inflammatory glucocorticoid (predonisolone) in the efficacy on the established late phase of experimental glomerulonephritis in the rat. Furthermore, we examined the effects of beraprost sodium on the renal monocyte chemoattractant protein-1 (MCP-1) that is recently proved to play an important role in the aggravation of renal dysfunction in the above model (Wada et al., 1996; Lloyd et al., 1997).

2. Materials and methods

2.1. Animals

Specific pathogen-free Male Wistar–Kyoto (WKY) rats weighting 300–350 g (Charles River Japan, Kanagawa, Japan) were used in the experiments. The rats were treated in accordance with procedures approved by the Animal Ethics Committee in the Toray Pharmaceutical Research Laboratories, Toray Industries, Japan.

2.2. Drugs

Beraprost sodium, (sodium(±)-(1*R**,2*R**,3*aS**,8*bS**)-2,3,3*a*,8*b*-tetrahydro-2-hydroxyl-1-[(*E*)-(3*S**)-3-hydroxy-4-methyl-1-octen-6-ynyl]-1*H*-cyclopenta[*b*]benzofuran-5-butyrate) was synthesized in our chemical laboratory. Predonisolone was purchased from Shionogi Pharmaceutical (Osaka, Japan). Captopril was purchased from Sigma (St Louis, MO, USA). Dipyridamole was purchased from Wako (Osaka, Japan).

2.3. Induction of glomerulonephritis

Glomerular basement membrane of rats was prepared by the method of Krakower et al. (1978). Five albino rabbits were subcutaneously immunized with glomerular basement membrane (1 mg/ml) emulsified with an equal volume of Freund's complete adjuvant (Difco Laboratories, Detroit, MI, USA). The booster was given three times every 2 weeks using the same immunogen. Four days after the final booster, the rabbits were bled from the carotid artery under anesthesia. Anti-glomerular basement membrane sera were heat-decomplemented for 30 min at 56 °C and absorbed with freshly harvested rat erythrocytes. In order to perform preliminary experiments for glomerulonephritis, several anti-glomerular basement membrane serum doses were intravenously injected to rats. The anti-glomerular basement membrane serum diluted 10-fold with saline at a dose of 0.3 ml/100 g body weight was sufficient to induce proteinuria and severe glomerulonephritis (data not shown). At this dose, some rats became proteinuric on the 4th day after the anti-glomerular basement membrane serum injection and all animals showed significant proteinuria on the 7th day and thereafter.

Rats were divided into several groups, each of which consisted of six rats. The rats assigned for the nephritic groups were then injected to the dorsal tail vein with 0.3 ml/100 g body weight of anti-glomerular basement membrane serum diluted 10-fold with saline under ether anesthesia. The day of the anti serum injection was defined as the day 0. On the other hand, the rats assigned for the normal groups were intravenously injected with the same volume of non-immune, normal rabbit serum, for comparison with the nephritic rats. The general condition and body weight of the rats was observed over the course of the experiment.

2.4. Drug administration

Beraprost sodium-treated groups were orally administrated at doses of 30, 100 and 300 μg/kg body weight of beraprost sodium in a volume of 0.1 ml/100 g body weight, twice daily at various timing until sacrifice. Predonisolone (0.1 or 1 mg/kg), captopril (25, 50 or 100 mg/kg) and dipyridamole (20 or 60 mg/kg) were orally administrated to nephritic groups twice daily at the 14th day until sacrifice. The remaining groups were orally given the vehicle distilled water instead of test drugs and served as the nephritic control. Each group consisted of six rats.

2.5. Determination of urinary protein, creatinine and blood urea nitrogen

The 24 h urine samples were obtained at the indicated time points after the anti-glomerular basement membrane serum injection, each rat being kept in an individual metabolic cage with free access to water and food. The urine volume was measured, the urine then centrifuged at $1500 \times g$ for 10 min at 4 °C, and the supernatant used for the determination of protein and creatinine. The amount of

urinary protein was determined by the Pyrogallol Red method using rat serum albumin as a standard, and expressed as mg/day urine. The levels of urine creatinine were determined by creatinine amidohydrolase–*N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-*m*-toluidine (TOOS) method using a commercially available assay kit (Creatinine II-HA Test Wako, Wako). At the end of the urine collection, 0.5 ml of blood was drawn from the dorsal tail vein of each rat lightly anesthetized with ether. The blood was centrifuged at $1500 \times g$ for 10 min at room temperature to obtain serum for the determination of creatinine and blood urea nitrogen. The levels of serum creatinine were determined by the creatinine amidohydrolase–TOOS method and expressed as mg/100 ml serum. The blood urea nitrogen levels in the serum samples were determined by the urease–indophenol method using a commercially available assay kit (Wako Urea N B, Wako) and expressed as mg/100 ml serum. Creatinine clearance was calculated from the measurement of serum and urine creatinine levels.

2.6. Detection and semiquantitation of mRNA levels of MCP-1 and collagen type I by reverse transcription-polymerase chain reaction (RT-PCR)

At the indicated time points after the anti-glomerular basement membrane serum injection, the kidneys were perfused with cold Hanks' balanced salt solution (HBSS), and removed from rats for RNA extraction. Total RNA was prepared from each renal cortex by a guanidinium isothiocyanate method using ISOGEN reagent (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. The total RNA was dissolved in RNase-free water and precisely quantified by UV spectrophotometer at 260 nm and 280 nm. RNA with an $OD_{260/280}$ ratio greater than 1.9 was used for cDNA synthesis.

Total RNA (1 μg) from each sample was reverse-transcribed at 42 °C for 1 h in 20 μl of the buffer (50 mM Tris–HCl, pH 8.3, 75 mM KCl and 3 mM $MgCl_2$) containing 200 U of the reverse transcriptase from moloney-murine leukemia virus (GIBCO BRL, Rockville, MD, USA), 5 μM of random hexamer oligonucleotides (GIBCO BRL), 0.5 mM deoxyribonucleotide triphosphates (dNTP, Amersham Pharmacia Biotech, Buckinghamshire, UK) and 10 mM dithiothreitol. Reverse-transcribed materials were amplified by polymerase chain reaction (PCR), using sense and antisense primer specific for rat MCP-1 (sense primer, 5′-CTC TTC CTC CAC CAC TAT GC-3′; antisense primer, 5′-CTC TGT CAT ACT GGT CAC TTC-3′, which amplify rat MCP-1 fragment), and rat collagen type I (sense primer, 5′-TGA GAA GTG GCA GAG GAG GT-3′; antisense primer, 5′-ATG CCC ACT CCC TAA CAG TG-3′, which amplify rat collagen type I fragment). The rat glyceraldehyde 3-phosphate dehydrogenase (G3PDH) gene (a housekeeping gene) was used as an internal standard gene. Because the internal standard gene was also amplified, relative mRNA levels of MCP-1 and collagen type I were quantified. PCR primers for rat G3PDH were purchased from CLONTECH Laboratories (Palo Alto, CA, USA). PCR was performed 32 cycles for MCP-1, 30 cycle for collagen type I and 30 cycle for G3PDH in 50 μl of the PCR buffer (20 mM Tris–HCl, pH 8.3, 50 mM KCl and 1.5 mM $MgCl_2$) containing 5 μM of the reverse-transcribed RNA solution, 0.25 μM of each primer, 170 μM dNTP and 1.25 U Taq polymerase (Takara Shuzo, Shiga, Japan) using a thermal cycler (GeneAmpTM PCR System 9600, Perkin Elmer Cetus, Norwalk, CT, USA). Each cycle consisting of 30 s denaturation at 95 °C, 30 s annealing at 58 °C, and 1 min extension at 72 °C. After PCR, 10 μl of the reaction mixture was loaded onto a 1.5% agarose minigel, and the PCR products were visualized by ethidium bromide staining after electrophoresis. The levels of mRNA for MCP-1, collagen type I and G3PDH were quantified by densitometric scanning, and the ratio of the MCP-1 and collagen type I mRNA density vs. the G3PDH mRNA density in each point was calculated.

2.7. Measurement of blood pressure

Hypotensive effects of beraprost sodium and captopril on day 14 after anti-glomerular basement membrane serum injection were examined under pentobarbital (50 mg/kg, i.p.) anesthesia. The left carotid artery was inserted with a polyethylene catheter for recording arterial blood pressure. Animals were stabilized for 1 h and the initial values were recorded. Beraprost sodium (300 μg/kg) or captopril (100 mg/kg) was then injected into duodenum through a catheter, and the arterial blood pressure was recorded 5, 10, 15, 30, 45 and 60 min after the injection. Each group consisted of three rats.

2.8. Statistical significance

Results were analyzed for statistical significance by Student's *t*-test for unpaired observations and Dunnett's test for multiple comparison. A P value of <0.05 was considered to be statistically significant.

3. Results

3.1. Effects of beraprost sodium, administered on the day of the anti-glomerular basement membrane serum injection and thereafter on urinary protein excretion in nephritic rats

The extent of proteinuria in the various treatment groups is shown Fig. 1. After the intravenous injection of anti-glomerular basement membrane serum, all untreated nephritic rats (nephritic control rats) showed significant proteinuria exceeding 100 mg/day on the 7th day. In contrast, serum creatinine levels, blood urea nitrogen levels and creatinine clearance appeared to be unchanged by the anti-glomerular basement membrane

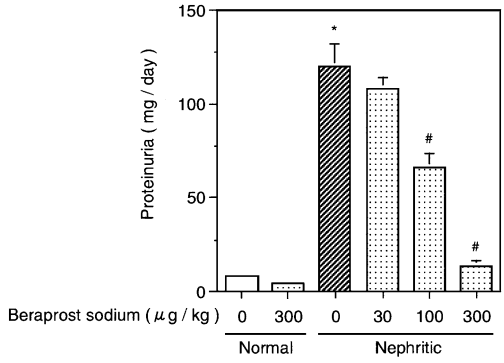

Fig. 1. Effects of beraprost sodium administered on the day of the anti-glomerular basement membrane serum injection and thereafter on urinary protein excretion in nephritic rats. Normal and nephritic rats were orally treated with beraprost sodium (30, 100 or 300 μg/kg) or vehicle, twice daily from the 1st day to the 7th day. The day of anti-glomerular basement membrane serum injection was defined as the day 0. On day 7, urinary protein excretion for 24 h was determined. Open column: normal; hatched column: nephritic control; dotted column: + beraprost sodium (30, 100, 300 μg/kg). Each column and bar denotes the mean with S.E. of six rats. Statistical significance: * $P<0.05$ vs. normal control group; # $P<0.05$ vs. nephritic control group.

serum injection in nephritic rats on the 7th day (data not shown). When the treatment with beraprost sodium was started on the day of the anti-glomerular basement membrane serum injection, beraprost sodium significantly suppressed the urinary protein excretion in a dose-dependent manner. Beraprost sodium at 300 μg/kg prevented proteinuria completely. In normal rats that received beraprost sodium at 300 μg/kg, the urinary protein excretion was not different from that in normal rats that did not receive beraprost sodium.

3.2. Effects of beraprost sodium, administered on the 14th day of the anti-glomerular basement membrane serum injection and thereafter on renal function and body weight in nephritic rats

3.2.1. Urinary protein excretion

As shown in Fig. 2A, the nephritic control rats not receiving beraprost sodium developed a severe proteinuria, showing a value of about 400 mg/day on the 14th day after the injection of anti-glomerular basement membrane serum, reaching a peak on the 35th day. The peak elevated level in urinary protein excretion (35th day) remained elevated, although it was not maintained at the same level throughout the rest of the experiment. When the treatment with beraprost sodium was started on the 14th day after the anti-glomerular basement membrane serum injection, beraprost sodium at 300 μg/kg significantly inhibited the development of urinary protein excretion by 20–40% as compared with that in the nephritic control rats through the 28th to 35th day. Beraprost sodium at 100 μg/kg showed only a tendency of the diminished excretion of the urinary protein. However, beraprost sodium at 30 μg/kg had no effect on the urinary protein excretion.

3.2.2. Serum creatinine levels

On the 14th day, the nephritic control rats had 0.5 to 0.7 mg/100 ml of serum creatinine (Fig. 2B). This serum creatinine level was significantly higher (80–120%) than that of the normal rats. The high level in serum creatinine in nephritic control rats was further increased time-dependently. In rats treated with beraprost sodium, the increase in serum creatinine levels was prevented in a dose-dependent manner. The serum creatinine level was markedly decreased by beraprost sodium 300 μg/kg, although it remained at a level twofold higher than in the normal group. In nephritic rats treated with beraprost sodium at 100 μg/kg, serum creatinine levels were only slightly reduced, compared with the nephritic control rats. Beraprost sodium at 30 μg/kg had no effect on the increase of serum creatinine levels.

3.2.3. Blood urea nitrogen levels

As shown in Fig. 2C, the blood urea nitrogen in the nephritic control rats increased about twofold as compared with that of normal rats on the 14th day after the anti-glomerular basement membrane serum injection, and thereafter, the level was further increased time-dependently. The increase in urea nitrogen levels observed in the nephritic control rats was dose-dependently inhibited by the administration of beraprost sodium. The blood urea nitrogen level was markedly decreased by beraprost sodium 300 μg/kg, although it remained at a level twofold higher than in the normal group. In the group treated with 100 μg/kg beraprost sodium, the urea nitrogen levels were significantly reduced through the 42nd to 49th day. However, 30 μg/kg beraprost sodium had no effect.

3.2.4. Creatinine clearance

On the 14th day, the nephritic control rats showed 0.8 to 1.0 ml/min of creatinine clearance (Fig. 2D). This level was significantly lower (45–55%) than that of the normal rats. The low level in creatinine clearance in nephritic control rats was further decreased time-dependently. In the groups treated with beraprost sodium, the decrease in creatinine clearance was prevented in a dose-dependent manner. Beraprost sodium at 300 μg/kg completely prevented the decrease in creatinine clearance. Beraprost sodium at 100 μg/kg only showed a tendency to diminish the decrease in creatinine clearance over the experimental period, and it was ineffective at 30 μg/kg.

3.2.5. Body weight

Throughout the experimental period, the nephritic control rats gained less weight than the normal rats on the 21st day and thereafter (Fig. 2E). On the other hand, the rats in the beraprost sodium-treated group dose-dependently exhibited a better growth than that of the nephritic control group.

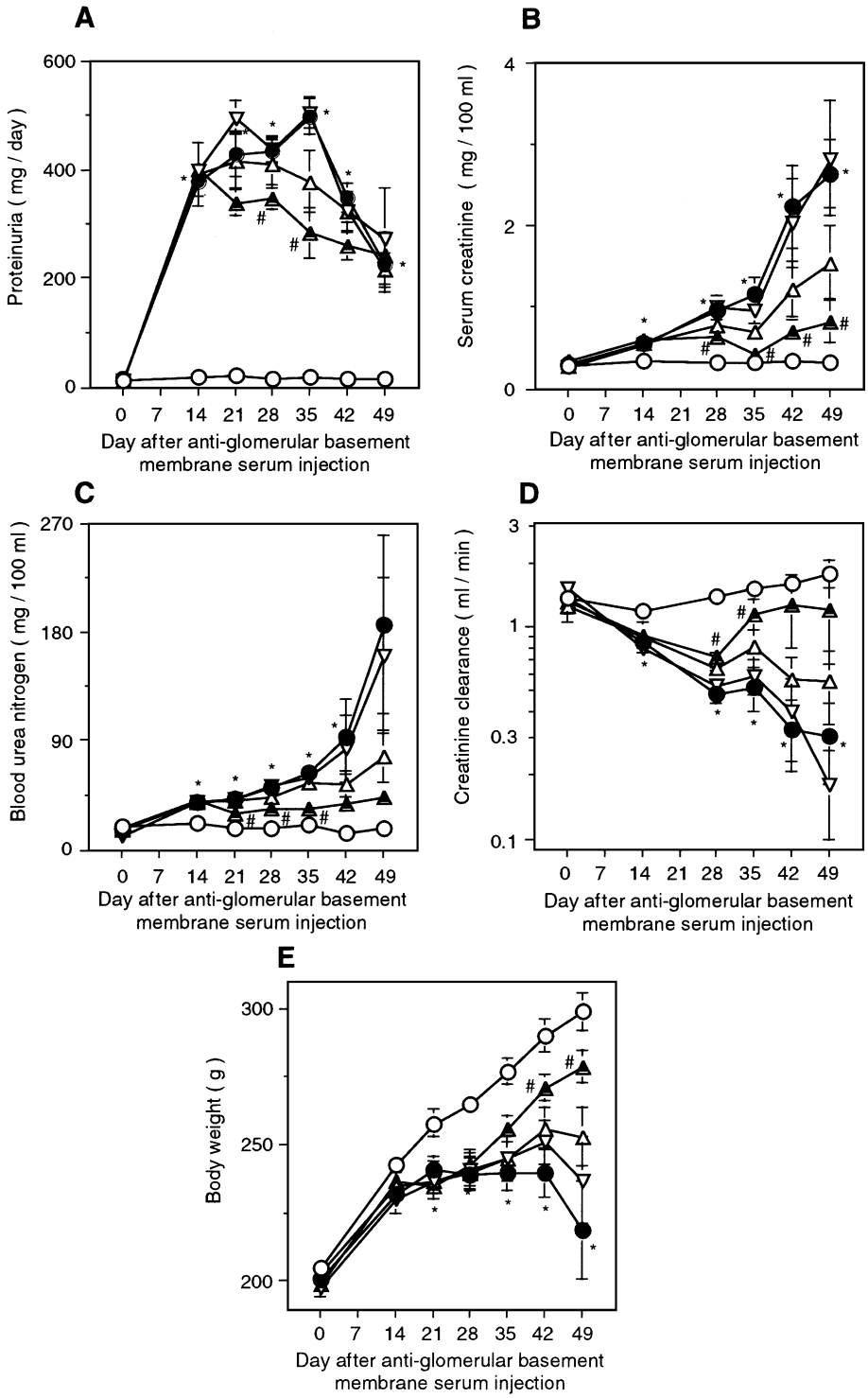

Fig. 2. Effects of beraprost sodium administered on the 14th day of the anti-glomerular basement membrane serum injection and thereafter on renal function and body weight in nephritic rats. Nephritic rats were orally treated with beraprost sodium (30, 100 or 300 μg/kg) or vehicle, twice daily from the 14th day to the 49th day. On days 0, 14, 21, 28, 35, 42 and 49, urinary protein excretion for 24 h (A), serum creatinine levels (B), blood urea nitrogen levels (C), creatinine clearance (D) and body weight (E) were determined. ○: normal; ● nephritic control; ▽: nephritic + beraprost sodium (30 μg/kg); △: nephritic + beraprost sodium (100 μg/kg); ▲: nephritic + beraprost sodium (300 μg/kg). Each symbol and bar denotes the mean with S.E. of six rats. Statistical significance: * $P < 0.05$ vs. normal group; # $P < 0.05$ vs. nephritic control group.

3.3. Mechanisms of the anti-nephritic effects of beraprost sodium

3.3.1. Effects of beraprost sodium and captopril on mean arterial pressure of anti-glomerular basement membrane nephritic rats

Hypotensive effects of intraduodenally administered beraprost sodium (300 μg/kg) and captopril (100 mg/kg) were tested in rats on day 14 after the anti-glomerular basement membrane serum injection under anesthesia. There was no difference between the maximum decreases in the mean arterial pressure by beraprost sodium and captopril (39 ± 7% vs. 39 ± 5%, mean ± S.E. from three rats, $P = 0.34$). One hour after the administration, the decrease in mean arterial pressure of beraprost sodium was not significantly different from that of captopril (30 ± 7% vs. 36 ± 9%, $P = 0.38$).

3.3.2. Effects of predonisolone, captopril and dipyridamole on renal function in anti-glomerular basement membrane nephritic rats

When predonisolone at 0.1 and 1 mg/kg was given on and from the 14th day after the anti-glomerular basement membrane serum injection, predonisolone dose-dependently reduced the level of serum creatinine when compared with the nephritic control rats on the 28th day (Fig. 3A). The inhibitory effect of predonisolone at 1 mg/kg was comparable to that with 300 μg/kg beraprost sodium.

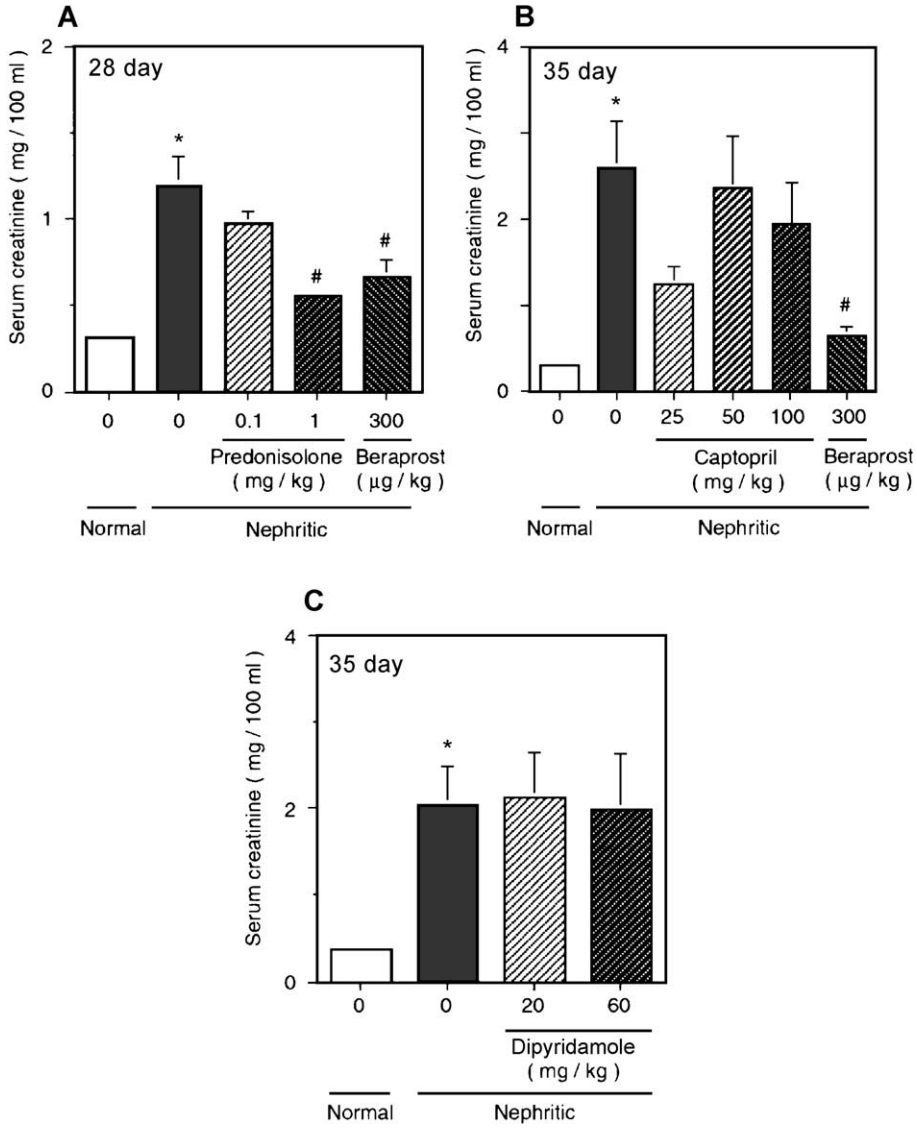

Fig. 3. Effects of predonisolone, captopril, dipyridamole and beraprost sodium on serum creatinine levels in nephritic rats. Nephritic rats were orally treated with predonisolone (0.1 or 1 mg/kg, A), captopril (25, 50 or 100 mg/kg, B), dipyridamole (20 or 60 mg/kg, C), beraprost sodium (300 μg/kg, A and B) or vehicle, twice daily from the 14th day to the 28th or the 35th day. On the indicated days after anti-glomerular basement membrane serum injection, serum creatinine levels were determined. Each column and bar denotes the mean with S.E. of six rats. Statistical significance: * $P < 0.05$ vs. normal group; # $P < 0.05$ vs. nephritic control group.

When captopril at 25, 50 and 100 mg/kg was given on and from the 14th day, captopril reduced the serum creatinine levels by 50–90% of the control level on the 35th day, although the effects were not dose-dependent (Fig. 3B). However, the inhibitory effect with captopril at 25 mg/kg was less than that with 300 μg/kg beraprost sodium.

On the other hand, when dipyridamole at 20 and 60 mg/kg was given on and from the 14th day after the anti-glomerular basement membrane serum injection, dipyridamole at any doses failed to inhibit the elevation of serum creatinine levels observed in the nephritic control rats on the 35th day (Fig. 3C).

3.3.3. Effects of beraprost sodium on mRNA expression of MCP-1 and collagen in nephritic rats

In order to determine whether mRNA levels of MCP-1 and collagen type I are regulated during the development of glomerulonephritis, RT-PCR analysis was conducted with total RNA prepared from renal cortex in the nephritic rats at the various time points after the anti-glomerular basement membrane serum injection. As shown in Fig. 4, the MCP-1 mRNA levels in normal rats, as evaluated by RT-PCR analysis, were very low. In contrast, increased MCP-1 mRNA levels were detected in renal cortex of nephritic control rats compared with the normal rats. The increase in

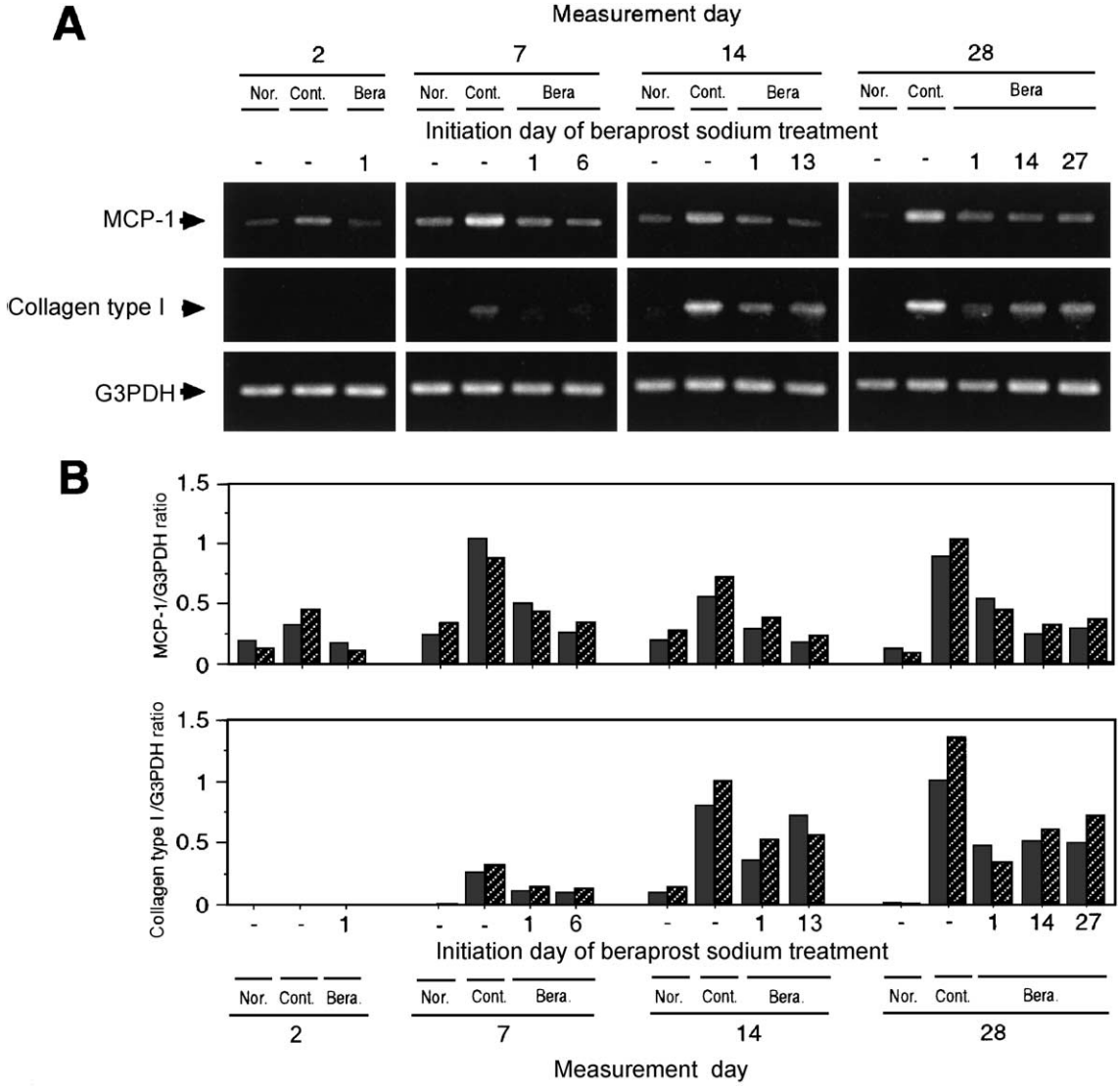

Fig. 4. Effects of beraprost sodium on mRNA levels of MCP-1 and collagen type I in nephritic rats. Nephritic rats were orally treated with beraprost sodium (300 μg/kg) or vehicle, twice daily from the indicated time until sacrifice. The rats were sacrificed on the 2nd, 7th, 14th and 28th days after the anti-glomerular basement membrane serum injection. The total RNA extracted from renal cortex of normal rats (Nor.), nephritic control rats (Cont.) and beraprost sodium-treated nephritic rats (Bera.) was used for RT-PCR analysis to determine mRNA levels of MCP-1, collagen type I and G3PDH. (A) A representative RT-PCR analysis showing mRNA levels of MCP-1, collagen type I and G3PDH. Data shown were reproduced in two independent experiments. (B) Summarized graphs of the densitometry analysis of the RT-PCR-based mRNA levels of MCP-1 and collagen type I relative to that of G3PDH. The double columns represent the two independent experiments.

MCP-1 mRNA levels was detected on the 2nd day after the anti-glomerular basement membrane serum injection, and reached a plateau on the 7th–28th day. When the nephritic rats were treated with 300 μg/kg beraprost sodium daily on the day of the anti-glomerular basement membrane serum injection until sacrifice, beraprost sodium treatment reduced mRNA levels of MCP-1 to less than about 50% of the levels for non-beraprost sodium-treated nephritic control rats at each time point. When beraprost sodium treatment was started 1 day prior to sacrifice, beraprost sodium also reduced the mRNA levels of MCP-1 at every time point after the anti-glomerular basement membrane serum injection.

In non-beraprost sodium-treated nephritic control rats, the signal of collagen type I mRNA was first detected in renal cortex on the 7th day after the anti-glomerular basement membrane serum injection, and increased gradually afterwards. The mRNA levels of collagen type I was reduced when the rats were treated with 300 μg/kg beraprost sodium.

4. Discussion

In this study, we showed the suppressive effects of beraprost sodium on the late phase of a rat anti-glomerular basement membrane nephritis model, which exhibits crescentic-type anti-glomerular basement membrane nephritis and many features similar to that of rapidly progressive glomerulonephritis in human.

In clinical situations, therapies for glomerulonephritis are initiated after the establishment of nephritic symptoms. Therefore, it is important to evaluate the anti-nephritic effect of beraprost sodium in the late phase in which nephritic symptoms has established. In the model used in this study, on the 14th day after anti-glomerular basement membrane serum injection, the rats developed a severe proteinuria, continuous increase in serum creatinine and blood urea nitrogen, and decrease in creatinine clearance. These changes indicated that nephritic symptoms had fully established. We started the treatment with beraprost sodium on the 14th day after anti-glomerular basement membrane serum injection. The treatment with beraprost sodium started on the 14th day markedly and dose-dependently inhibited the aggravation of these parameters. The dose–response relationship in the late phase was similar to that shown in the early phase. These results suggest that beraprost sodium treatment improves renal dysfunction on the progression of nephritis in late phase. In addition, the nephritic rats treated with beraprost sodium at 300 μg/kg exhibited the same growth rate compared with normal rats, while the nephritic control rats gained less weight than the normal ones. These results suggested that beraprost sodium treatment also improve the systemic condition in these rats.

Because beraprost sodium has anti-platelet effects and anti-hypertensive, we compared the effects of beraprost sodium with other drugs that were reported to be effective for renal dysfunction in this model. Dipyridamole (Kincaid-Smith et al., 1970) has been clinically used as an anti-platelet drug. In rats, the administration of 10 mg/kg/day of dipyridamole caused 33.7% inhibition of platelet aggregation (De La Cruz et al., 1994) and significant reduction in thrombus formation (Weichert et al., 1983). In this nephritic rats, dipyridamole up to 60 mg/kg twice a day did not inhibit the increase in serum creatinine levels in late phase of nephritis. It has been reported that dipyridamole improves proteinuria and histological findings only when the administration is started simultaneously with anti-glomerular basement membrane serum injection (Hattori et al., 1989, 1990). Moreover, an angiotensin-converting enzyme inhibitor captopril did not inhibit the increase in serum creatinine levels in late phase of nephritis, although its hypotensive effects was similar to beraprost sodium. It has been reported that captopril improves proteinuria but not blood urea nitrogen, when the administration is started on the day of anti-glomerular basement membrane serum injection (Nagamatsu et al., 1999). Because neither dipyridamole nor captopril was effective in the established renal dysfunction, it is suggested that the anti-platelet and anti-hypertensive effects of beraprost sodium have little contribution to the improvement of renal dysfunction in this late phase model. The treatment of beraprost sodium in rats causes an elevation of plasma renin activity in response to a decrease in blood pressure (Murata et al., 1989), although captopril inhibits renin–angiotensin system. Therefore, this difference might explain the discrepancies in the author report regarding the effects of treatments on serum creatinine levels. The possibility remains that activation of the intrarenal renin–angiotensin system by treatment of beraprost sodium contributes to maintain glomerular filtration rate in this experimental setting. On the other hand, the anti-inflammatory glucocorticoid predonisolone exhibited a dose-dependent effect against the aggravation of renal dysfunction. This result suggested to us that glucocorticoids improved renal dysfunction even when administered after the establishment of renal dysfunction in this model.

MCP-1 is produced by various cells, including fibroblasts, endothelial cells, mesangial cells, smooth muscle cells as well as monocytes/macrophages in kidney. There has been increasing evidence that MCP-1 plays an important role in the aggravation of renal dysfunction in this model (Wada et al., 1996; Lloyd et al., 1997; Segerer et al., 2000). Glucocorticoid is reported to reduce MCP-1 production and to improve renal dysfunction. In order to obtain further insight into the mechanism of the improvement of renal dysfunction by beraprost sodium treatment, we examined the effects of beraprost sodium on MCP-1 mRNA levels in renal cortex using RT-PCR methods. The increase in MCP-1 mRNA levels was inhibited by beraprost sodium, whenever its administration was started after the anti-glomerular basement membrane serum injection. Collagen type I is considered a marker for glomerular sclerosis and interstitial

fibrosis. The levels of collagen type I mRNA was increased as nephritis progressed, and its increase was also inhibited by beraprost sodium treatment. These findings may explain the reason that beraprost sodium exhibited an inhibitory effect even when its administration was started on the 14th day after anti-glomerular basement membrane serum injection. Effects of beraprost sodium are consistent with the view that the inhibition of both intraglomerular coagulation and induction of intercellular adhesion molecules is contributed to the beneficial effect of beraprost sodium in this model (Kushiro et al., 1998). In addition, prostacyclin presumably has a variety of effects such as the improvement renal microcirculation (Natov et al., 1994) and protection of endothelial cells (Moncada, 1982). Therefore, the beneficial effects of beraprost sodium observed in this rat model of glomerulonephritis might be achieved by these multiple mechanisms.

In conclusion, the prostacyclin analogue beraprost sodium inhibits the development and progression of renal dysfunction in the late phase of rat anti-glomerular basement membrane nephritis model, even when its administration was started on evidence of nephritis. Moreover, beraprost sodium administration inhibits the increase in mRNA levels for MCP-1 and collagen in the kidney, suggesting that the inhibition of the progression of renal dysfunction is possibly due to the inhibition of MCP-1 and collagen.

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References

Boxer, L.A., Allen, J.M., Schmidt, M., Yoder, M., Baehner, R.L., 1980. Inhibition of polymorphonuclear leukocyte adherence by prostacyclin. J. Lab. Clin. Med. 95, 672–678.

Clark, W.F., Parbtani, A., McDonald, J.W., Taylor, N., Reid, B.D., Kreeft, J., 1987. The effects of a thromboxane synthase inhibitor, a prostacyclin analog and PGE_1 on the nephritis of the NZB/W F_1 mouse. Clin. Nephrol. 28, 288–294.

De La Cruz, J.P., Ortega, G., Sanchez de la Cuesta, F., 1994. Differential effects of the pyrimido-pyrimidine derivatives, dipyridamole and mopidamol, on platelet and vascular cyclooxygenase activity. Biochem. Pharmacol. 47, 209–215.

Hattori, T., Nagamatsu, T., Ito, M., Suzuki, Y., 1989. Studies on antinephritic effect of TJ-8014, a new Japanese herbal medicine, and its mechanisms (1): effects on original-type anti-GBM nephritis in rats and platelet aggregation. Jpn. J. Pharmacol. 50, 477–485.

Hattori, T., Ito, M., Nagamatsu, T., Suzuki, Y., 1990. Studies on antinephritic effect of TJ-8014, a new Japanese herbal medicine (3): effects on crescentic-type anti-GBM nephritis in rats. Jpn. J. Pharmacol. 52, 131–140.

Hayashi, K., Nagamatsu, T., Ito, M., Suzuki, Y., 1996. Suppression of experimental crescentic-type anti-glomerular basement membrane (GBM) nephritis by FK506 (tacrolimus hydrate) in rats. Jpn. J. Pharmacol. 70, 43–54.

Heeg, J.E., De Jong, P.E., Van der Hem, G.K., De Zeeuw, D., 1991. Angiotensin II does not acutely reverse the reduction of proteinuria by long-term ACE inhibition. Kidney Int. 40, 734–741.

Kajikawa, N., Nogimori, K., Murata, T., Nishio, S., Uchiyama, S., 1989. Specific binding of the new stable epoprostenol analogue beraprost sodium to prostacyclin receptors on human and rat platelets. Arzneim.-Forsch./Drug Res. 39, 495–499.

Kincaid-Smith, P., Laver, M.C., Fairley, K.F., 1970. Dipyridamole and anticoagulants in renal disease due to glomerular and vascular lesions. A new approach to therapy. Med. J. Aust. 1, 145–151.

Krakower, C.A., Nicholes, B.K., Greenspon, S.A., 1978. Proteinuria and the fragility of normal and diseased glomerular basement membrane. Proc. Soc. Exp. Biol. Med. 159, 324–334.

Kushiro, M., Shikata, K., Sugimoto, H., Shikata, Y., Miyatake, N., Wada, J., Miyasaka, M., Makino, H., 1998. Therapeutic effects of prostacyclin analog on crescentic glomerulonephritis of rat. Kidney Int. 53, 1314–1320.

Lefkowith, J.B., Nagamatsu, T., Pippin, J., Schreiner, G.F., 1991. Role of leukocytes in metabolic and functional derangements of experimental glomerulonephritis. Am. J. Physiol. 261, F213–F220.

Lievre, M., Morand, S., Besse, B., Fiessinger, J.N., Boissel, J.P., 2000. Oral Beraprost sodium, a prostaglandin I_2 analogue, for intermittent claudication: a double-blind, randomized, multicenter controlled trial. Beraprost et Claudication Intermittente (BERCI) Research Group. Circulation 102, 426–431.

Lloyd, C.M., Minto, A.W., Dorf, M.E., Proudfoot, A., Wells, T.N., Salant, D.J., Gutierrez-Ramos, J.C., 1997. RANTES and monocyte chemoattractant protein-1 (MCP-1) play an important role in the inflammatory phase of crescentic nephritis, but only MCP-1 is involved in crescent formation and interstitial fibrosis. J. Exp. Med. 185, 1371–1380.

Mayer, G., Lafayette, R.A., Oliver, J., Deen, W.M., Myers, B.D., Meyer, T.W., 1993. Effects of angiotensin II receptor blockade on remnant glomerular permselectivity. Kidney Int. 43, 346–353.

Mene, P., Abboud, H.E., Dunn, M.J., 1990. Regulation of human mesangial cell growth in culture by thromboxane A_2 and prostacyclin. Kidney Int. 38, 232–239.

Moncada, S., 1982. Eighth Gaddum Memorial Lecture. University of London Institute of Education, December 1980. Biological importance of prostacyclin. Br. J. Pharmacol. 76, 3–31.

Moncada, S., Gryglewski, R., Bunting, S., Vane, J.R., 1976. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 263, 663–665.

Murata, T., Murai, T., Kanai, T., Ogaki, Y., Sanai, K., Kanda, H., Sato, S., Kajikawa, N., Umetsu, T., Matsuura, H., Fukatsu, Y., Isogaya, M., Yamada, N., Nishio, S., 1989. General pharmacology of beraprost sodium 2nd communication: effect on the autonomic, cardiovascular and gastrointestinal systems, and other effects. Arzneim.-Forsch./Drug Res. 39, 867–876.

Nagamatsu, T., Hayashi, K., Oka, T., Suzuki, Y., 1999. Angiotensin II type I receptor antagonist suppresses proteinuria and glomerular lesions in experimental nephritis. Eur. J. Pharmacol. 374, 93–101.

Nagaya, N., Uematsu, M., Okano, Y., Satoh, T., Kyotani, S., Sakamaki, F., Nakanishi, N., Miyatake, K., Kunieda, T., 1999. Effect of orally active prostacyclin analogue on survival of outpatients with primary pulmonary hypertension. J. Am. Coll. Cardiol. 34, 1188–1192.

Natov, S., Schmitt, F., Ikeni, A., Lacour, B., Hannedouche, T.P., 1994. Opposite renal effects of a PGE_1 analog and prostacyclin in humans. Kidney Int. 45, 1457–1464.

Nishio, S., Matsuura, H., Kanai, N., Fukatsu, Y., Hirano, T., Nishikawa, N., Kameoka, K., Umetsu, T., 1988. The in vitro and ex vivo antiplatelet effect of TRK-100, a stable prostacyclin analog, in several species. Jpn. J. Pharmacol. 47, 1–10.

Ohno, K., Nagase, H., Matsumoto, K., Nishiyama, H., Nishio, S., 1985. Stereoselective synthesis of 5,6,7-trinor-4,8-inter-*m*-phenylene-PGI_2 derivatives and their inhibitory activities to human platelet aggregation. Adv. Prostaglandin Thromboxane Leukotriene Res. 15, 279–281.

Poelstra, K., Brouwer, E., Baller, J.F., Hardonk, M.J., Bakker, W.W., 1993. Attenuation of anti-Thy1 glomerulonephritis in the rat by anti-inflammatory platelet-inhibiting agents. Am. J. Pathol. 142, 441–450.

Ruiz-Ortega, M., Gonzalez, S., Seron, D., Condom, E., Bustos, C., Largo, R., Gonzalez, E., Ortiz, A., Egido, J., 1995. ACE inhibition reduces proteinuria, glomerular lesions and extracellular matrix production in a normotensive rat model of immune complex nephritis. Kidney Int. 48, 1778–1791.

Segerer, S., Nelson, P.J., Schlondorff, D., 2000. Chemokines, chemokine receptors, and renal disease: from basic science to pathophysiologic and therapeutic studies. J. Am. Soc. Nephrol. 11, 152–176.

Taniguchi, H., Nagamatsu, T., Kojima, R., Ito, M., Suzuki, Y., 1994. Marked antinephritic action and less adverse effects of methylprednisolone suleptanate by intermittent administration in rats. Jpn. J. Pharmacol. 64, 79–88.

Utsunomiya, Y., Ogura, M., Kawamura, T., Mitarai, T., Maruyama, N., Sakai, O., 1995. Attenuation of immune complex nephritis in NZB/WF_1 mice by a prostacyclin analogue. Clin. Exp. Immunol. 99, 454–460.

Wada, T., Yokoyama, H., Furuichi, K., Kobayashi, K.I., Harada, K., Naruto, M., Su, S.B., Akiyama, M., Mukaida, N., Matsushima, K., 1996. Intervention of crescentic glomerulonephritis by antibodies to monocyte chemotactic and activating factor (MCAF/MCP-1). FASEB J. 10, 1418–1425.

Weichert, W., Pauliks, V., Breddin, H.K., 1983. Laser-induced thrombi in rat mesenteric vessels and antithrombotic drugs. Haemostasis 13, 61–71.