ELSEVIER

European Journal of Pharmacology 316 (1996) 73–80

ejp

Effect of DP-1904, a thromboxane A_2 synthase inhibitor, on passive Heymann nephritis in rats

Toshiyuki Nagao, Tadashi Nagamatsu, Yoshio Suzuki *

Department of Pharmacology, Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tenpaku-ku, Nagoya 468, Japan

Received 15 July 1996; revised 12 August 1996; accepted 16 August 1996

Abstract

The antinephritic effect of DP-1904 [6-(1-imidazolylmethyl)-5,6,7,8-tetrahydronaphthalene-2-carboxylic acid hydrochloride], a thromboxane A_2 synthase inhibitor, was evaluated using an experimental model of membranous nephropathy, viz. accelerated passive Heymann nephritis in which the glomerular injury is mediated by immune complexes. DP-1904 markedly inhibited the development of glomerular alteration as well as the elevation of proteinuria and plasma creatinine. When the treatment was started from the 22nd day, at which time proteinuria is fully developed, DP-1904 showed beneficial effects on proteinuria and glomerular histopathological changes. DP-1904 apparently decreased the deposition of both rabbit immunoglobulin G and rat immunoglobulin G on glomerular basement membrane in nephritic rats. A single administration of DP-1904 restored the decreased renal tissue blood flow, inhibited glomerular thromboxane B_2 production and increased glomerular prostaglandin E_2 and 6-keto prostaglandin $F_{1\alpha}$ production in nephritic rats. These results suggest that DP-1904 may be an effective agent for the treatment of idiopathic membranous nephropathy and that the beneficial effect of this drug may be due to the elimination of glomerular immune deposits and to an increase in renal tissue blood flow related to amelioration of the abnormal metabolism of arachidonic acid.

Keywords: DP-1904; Thromboxane A_2 synthase inhibitor; Heymann nephritis; Proteinuria

1. Introduction

In the kidneys, prostaglandins and thromboxane A_2 are synthesized by a variety of circulating inflammatory cells, such as neutrophils (Goldstein et al., 1978), macrophages (Brune et al., 1978) and platelets (Needleman et al., 1976), as well as by glomerular epithelial and mesangial cells (Sraer et al., 1980; Petrulis et al., 1981). Recently, it has been demonstrated in experimental animal models that induction of immunological glomerular injury is associated with an increase in the production of prostaglandins and in thromboxane synthesis in isolated glomeruli (Lianos et al., 1983; Kaizu et al., 1985; Stork and Dunn, 1985) and renal cortex (Kelley et al., 1986). It has been believed that thromboxane A_2 mediates the deterioration of renal function due to its vasoconstrictive, platelet pro-aggregatory and chemotactic actions (Boot, 1976), while prostaglandins, such as prostaglandin E_2 and prostaglandin I_2, improve renal function due to their vasodilator and antiplatelet actions.

On the basis of these findings, we have already investigated the antinephritic effect of DP-1904 [6-(1-imidazolylmethyl)-5,6,7,8-tetrahydronaphthalene-2-carboxylic acid hydrochloride], a thromboxane A_2 synthase inhibitor, on crescentic nephritis in rats, which resembles rapidly progressive glomerulonephritis in humans. The beneficial effect of DP-1904 found in such experiments may be due to the inhibition of platelet aggregation and the restoration of renal tissue blood flow via amelioration of the abnormal metabolism of arachidonic acid (Nagao et al., 1994, 1995).

Of the glomerular diseases other than proliferative glomerulonephritis, idiopathic membranous nephropathy is characterized by diffuse glomerular basement membrane thickening with spike formation without the proliferation of glomerular cells (Couser, 1985). There are few drugs that have a clear effect on human idiopathic membranous nephropathy. Recently, we established an experimental model of accelerated passive Heymann nephritis. Accelerated passive Heymann nephritis is induced by i.v. injections of rabbit antiserum against Fx1A (an antigen from the brush border of the proximal tubles of rat kidney) following immunization of rats with rabbit γ-globulin in

* Corresponding author. Tel.: (81-52) 832-1781; Fax: (81-52) 834-8090.

PII S0014-2999(96)00662-0

Freund's complete adjuvant and used for assessing drug effects in experiments (Ito et al., 1989). This experimental model closely resembles idiopathic membranous nephropathy in humans, which is characterized by diffuse glomerular basement membrane thickening with spike formation. The development of accelerated passive Heymann nephritis in rats has been demonstrated to consist of a heterologous and an autologous phase. Immediately after the injection of rabbit antiserum against Fx1A antigen, the heterologous phase is caused by binding of the injected rabbit anti-Fx1A antibody to the glomerular epithelial antigen, i.e., in situ immune complex formation. Approximately 10 days later, the autologous phase is induced by a reaction between rat antibody against the injected rabbit anti-Fx1A antibody and rabbit anti-Fx1A antibody already bound to the glomeruli.

The aim of the present study was to demonstrate the antinephritic effect of DP-1904, using accelerated passive Heymann nephritis, and to compare it with that of OKY-046 [(E)-[4-(1-imidazolyl) phenyl]-2-propanoic acid hydrochloride monohydrate], another thromboxane A_2 synthase inhibitor on the immunosuppressive action. We also compared DP-1904 with azathioprine [6-[(1-methyl-4-nitroimidazol-5-yl)thio] purine], an immunosuppressive agent, in the autologous study.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley strain specific pathogen-free rats (Nihon, Shizuoka, Japan), weighing approximately 160 g, were used in the experiments. These animals were housed in an air-conditioned room at 22 ± 2°C during the experimental period.

2.2. Drugs

DP-1904 and OKY-046 were kindly provided by Daiichi Pharmaceutical (Tokyo, Japan) and Kittsei Pharmaceutical (Nagano, Japan), respectively. Azathioprine was purchased from Sigma (St. Louis, MO, USA). DP-1904 and OKY-046 were dissolved in distilled water and azathioprine was suspended in 1% gum arabic.

2.3. Experimental protocols

2.3.1. Protocol for evaluation of antinephritic effect of DP-1904

Accelerated passive Heymann nephritis was induced in rats by injecting 1.0 ml of rabbit antiserum directed to Fx1A antigen (anti-Fx1A serum) into the tail vein once a day for 2 days, starting from the day after the injection of 6.5 mg rabbit γ-globulin in 0.25 ml of Freund's complete adjuvant into the hindfoot pads in accordance with the method reported previously (Ito et al., 1989). In this experiment, the effects of test drugs were examined in the heterologous phase and in the autologous phase. In the heterologous study, the animals were then divided into five groups ($n = 9$ or 10), so that the mean body weight of each group was similar. Each of the four groups was given 5, 15 or 45 mg/kg of DP-1904 (APHN + DP-1904) or 200 mg/kg of OKY-046 (APHN + OKY-046) in a volume of 1 ml/100 g of body weight, orally, daily from the day of anti-Fx1A serum injection (the 1st day) to the 45th day. The accelerated passive Heymann nephritic group (APHN) was given distilled water orally. In addition, a control group ($n = 6$) that had not received the anti-Fx1A serum was used for comparison with the nephritic group.

In the autologous study, the animals were then divided into six groups ($n = 7$), so that the mean urinary protein excretion of each group was similar on the 21st day. Each of the five groups was given 5, 15 or 45 mg/kg of DP-1904 (APHN + DP-1904), 200 mg/kg of OKY-046 (APHN + OKY-046) or 20 mg/kg of azathioprine (APHN + azathioprine) from the 22nd day of anti-Fx1A serum injection to the 61st day. The accelerated passive Heymann nephritic group (APHN) was given distilled water orally. In addition, a control group ($n = 7$) that had not received the anti-Fx1A serum was used for comparison with the nephritic group.

2.3.2. Protocol for exploration of antinephritic mechanisms of DP-1904

To investigate the effect of a single p.o. administration of test drugs on renal tissue blood flow, we used five groups of five rats each: control group, APHN group, APHN + DP-1904 group (5 and 45 mg/kg) and APHN + OKY-046 group (200 mg/kg).

We next studied the effect of a single administration of test drugs on glomerular thromboxane B_2, prostaglandin E_2 and 6-keto prostaglandin $F_{1\alpha}$ production in nephritic rats. We used seven groups of four rats each: control group (control), accelerated passive Heymann nephritic group (APHN), APHN + DP-1904 group (5, 15 and 45 mg/kg), APHN + OKY-046 group (200 mg/kg) and APHN + azathioprine group (20 mg/kg).

2.4. Urine and blood collection

The 24-h urine samples were obtained by keeping each animal in an individual metabolic cage for 24 h without food and water at various intervals after the induction of nephritis. The urine was then centrifuged and the supernatant was used for the determination of protein. Immediately after the last collection of urine, blood samples were taken from the renal vein under pentobarbital anesthesia with a heparinized microsyringe. The blood was centrifuged at 2250 × g for 20 min at 4°C to obtain plasma for the determination of creatinine and antibody titer against rabbit γ-globulin.

2.5. Measurement of urinary protein and plasma creatinine and antibody titer

The urinary protein content was determined by the method of Kingsbury et al. (1926) and expressed as mg/24-h urine. The plasma creatinine content was determined with a commercial assay kit (Kainos creatinine, Kainos Tokyo, Japan). The plasma antibody titer against rabbit γ-globulin was determined by the method of McLeish et al. (1982).

2.6. Assessment of histopathological parameters in the glomeruli

For light microscopic study, the kidneys were fixed in alcohol and the tissues embedded in paraffin were then cut into 2–3 μm thick sections. The sections were stained with periodic acid-methenamine silver. Twenty-five glomeruli/section were examined under a light microscope to evaluate the thickening of glomerular basement membrane (thickening) and the spike formation in the glomerular basement membrane. These were evaluated by a different person in a blinded fashion. For assessment of the histopathological parameters, the degrees of thickening and spike formation were scored as 1 (mild), 2 (moderate) and 3 (severe) (Hattori et al., 1990). The number of glomeruli corresponding to each score is shown as n_1, n_2 and n_3. A thickening index and a spike formation index were calculated from the following formula.thickening index and spike formation index $= 1 \times n_1 + 2 \times n_2 + 3 \times n_3$.

2.7. Immunoperoxidase studies

An indirect immunoperoxidase technique using avidin-biotin peroxidase kits (Vectastain, Vector Laboratories, Burlingame, CA, USA) was used (Hayashi et al., 1994). In brief, the paraffin sections were consecutively incubated with normal rabbit serum, individual mouse monoclonal antibody for rabbit immunoglobulin G or rat immunoglobulin G (Cappel Laboratories, PA, USA), affinity-purified rabbit anti-mouse peroxidase conjugated immunoglobulin and finally with diamino-benzidine tetrahydrochloride (0.5 mg/ml in PBS + 0.01% H_2O_2). We selected the glomeruli that were equatorially sectioned and had the vascular pole under a light microscope.

Intraglomerular rabbit immunoglobulin G or rat immunoglobulin G deposition was measured by a image-analyzer (Toyobo Imageanalyzer VI, Toyobo, Tokyo, Japan).

2.8. Effect of a single administration of DP-1904 on renal tissue blood flow in nephritic rats

The effect of a single p.o. administration of test drugs on renal tissue blood flow was examined at the 22nd and 61st days after the anti-Fx1A serum injection. Renal tissue blood flow was measured by the hydrogen clearance method, with hydrogen gas generated by the electrolysis of body fluid (RBF-2, Biomedical Science, Ishikawa, Japan). A clearance curve indicating the decline of hydrogen concentration was then obtained and analyzed by the method of Aukland et al. (1964). Renal tissue blood flow was measured at 30 min after the treatment with the test drug. Renal tissue blood flow measured by this method is expressed as ml/min per 100 g kidney weight. The result was obtained as a percentage of the control group.

2.9. Effect of a single administration of DP-1904 on glomerular thromboxane B_2, prostaglandin E_2 and 6-keto prostaglandin $F_{1\alpha}$ production in nephritic rats

The effect of a single p.o. administration of test drugs on glomerular thromboxane B_2, prostaglandin E_2 and 6-keto prostaglandin $F_{1\alpha}$ production was examined at the 22nd and 61st days after anti-Fx1A serum injection.

At 4 h after the administration of test drug, the glomeruli were isolated by the differential sieving technique of Zoja et al. (1987) from kidneys obtained from nephritic rats and perfused with Krebs-Ringer phosphate-buffered saline (pH 7.2). The isolated glomeruli (the purity was > 80% when observed under light microscopy) were then incubated in Krebs-Ringer phosphate-buffered saline at 37°C for 30 min. The incubation mixture was then centrifuged and the supernatant was frozen at −70°C for the determination of thromboxane B_2, prostaglandin E_2 and 6-keto prostaglandin $F_{1\alpha}$.

The amounts of thromboxane B_2, prostaglandin E_2 and 6-keto prostaglandin $F_{1\alpha}$ in the glomeruli were determined by radioimmunoassay (New England Nuclear, Beverly, MA, USA). The protein content in the glomeruli was assayed by a colorimetric method (Bio-Rad, Richmond, CA, USA).

2.10. Statistical analysis

The results obtained are expressed as the means ± S.D. The data were analyzed by one-way analysis of variance (ANOVA) or the Kruskal-Wallis test. To determine the significance of differences among the groups Dunnett's test or Tukey's test was used.

3. Results

3.1. Antinephritic effect of DP-1904 in the heterologous study

3.1.1. Urinary protein excretion and plasma creatinine (Fig. 1A, Table 1)

When test drugs were given from the day after the anti-Fx1A serum (the 1st day), DP-1904 at 5, 15 and 45 mg/kg significantly suppressed urinary protein excretion

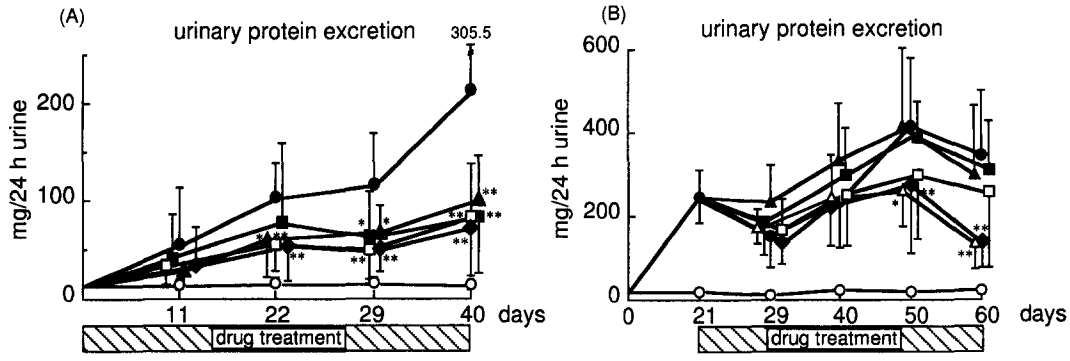

Fig. 1. Effects of DP-1904 administered in the heterologous (A) and autologous (B) phases on the urinary protein excretion of accelerated passive Heymann nephritic rats. ○–○: control; ●–●: APHN; ■–■: APHN + DP-1904 (5 mg/kg); □–□: APHN + DP-1904 (15 mg/kg); ◆–◆: APHN + DP-1904 (45 mg/kg); ▲–▲: APHN + OKY-046 (200 mg/kg); △–△: APHN + azathioprine (20 mg/kg). Each plot or each column denotes the mean with S.D. obtained from 6–10 rats. * $P < 0.05$, ** $P < 0.01$ vs. APHN.

by 54–70% throughout the 40-day experimental period. In addition, on the 45th day, this agent at the three doses restored plasma creatinine to control levels. OKY-046 also significantly inhibited the elevation of proteinuria and plasma creatinine.

3.1.2. Histopathological parameters in the glomeruli (Table 1)

Regarding the histopathological alterations of the glomeruli, DP-1904 at 5, 15 and 45 mg/kg reduced the thickening index and the spike formation index by 38–50 and 28–60%, respectively. OKY-046 (200 mg/kg) reduced them to a similar extent as DP-1904 at 5 mg/kg did.

3.1.3. Effect on plasma antibody titer against rabbit γ-globulin

The effects of the test drugs on the plasma antibody titer were evaluated on the 45th day after anti-Fx1A serum injection.

Table 1
Effect of DP-1904 administered in the heterologous phase on plasma creatinine and glomerular histopathological parameters in accelerated passive Heymann nephritis in rats

Treatment	(mg/kg)	n	Creatinine (μmol/l per 100 g bw)	Thickening	Spike formation
Control	–	6	12.2±0.9		
APHN	–	10	13.9±1.7 [a]	20.0±5.0	24.0±6.8
+DP-1904	5	9	12.2±0.9 [b]	14.4±4.3 [c]	15.0±6.3 [b]
	15	10	11.3±1.7 [c]	8.3±3.3 [c]	12.8±6.0 [c]
	45	10	11.3±0.9 [c]	8.5±4.8 [c]	12.3±3.4 [c]
+OKY-046	200	9	11.3±0.9 [c]	12.5±4.0 [c]	14.0±6.0 [c]

Test drugs were given p.o. daily to rat from the day after injection of anti-Fx1A serum to the 45th day. Plasma and kidney were taken on the 45th day for biochemical and light microscopic studies, repectively.
Value indicates the mean±S.D. and n indicates the number of rats used; bw indicates body weight.
[a] Significant difference from the control at $P < 0.01$.
[b,c] Significant differences from the rats given APHN at $P < 0.05$ and 0.01, respectively.

DP-1904 at 5, 15 and 45 mg/kg showed a tendency to reduce the elevation of antibody titer by 18–19%.

3.2. Antinephritic effects of DP-1904 in the autologous study

3.2.1. Urinary protein excretion and plasma creatinine (Fig. 1B, Table 2)

When test drugs were given from the 22nd day after the anti-Fx1A serum, DP-1904 (45 mg/kg per day) and azathioprine (20 mg/kg) significantly suppressed urinary protein excretion by 35–64 and 39–64%, respectively, through the 50–60th days. In addition, on the 60th day, DP-1904 at 15 and 45 mg/kg inhibited the increase in plasma creatinine to a near-control level. OKY-046 (200 mg/kg) showed only a tendency to diminish the urinary protein excretion.

3.2.2. Histopathological parameters in the glomeruli (Table 2, Fig. 2)

Regarding the histopathological alterations of the glomeruli, DP-1904 at 15 and 45 mg/kg reduced the thickening index by 25–40%. In addition, DP-1904 at 15 and 45 mg/kg reduced the spike formation index by 19–31%. OKY-046 (200 mg/kg) significantly reduced only the spike formation index by 21% and azathioprine (20 mg/kg) reduced both the thickening index and the spike formation index. Representative micrographs of the glomeruli from drug-treated and untreated accelerated passive Heymann nephritic rats are given in Fig. 2.

3.2.3. Effect on plasma antibody titer against rabbit γ-globulin

The effects of the test drugs on the plasma antibody titer were evaluated on the 61st day after anti-Fx1A serum injection.

DP-1904 (15 and 45 mg/kg) significantly inhibited the antibody titer ($P < 0.05$ vs. APHN). However, OKY-046

Table 2
Effect of DP-1904 administered in the autologous phase on plasma creatinine, glomerular histopathological parameters and glomerular rabbit and rat immunoglobulin G deposition in accelerated passive Heymann nephritis in rats

Treatment	(mg/kg)	n	Creatinine (μmol/l per 100 g bw)	Thickening	Spike formation	Rabbit IgG ($\times 10^{-3}$ mm^2/GCS)	Rat IgG ($\times 10^{-3}$ mm^2/GCS)
Control	–	7	11.3 ± 1.7				
APHN	–	7	15.7 ± 2.6 [a]	30.0 ± 4.3	28.9 ± 7.4	1.42 ± 0.17	2.30 ± 0.31
+ DP-1904	5	7	13.9 ± 1.7	25.9 ± 4.5	27.1 ± 5.9	1.43 ± 0.20	2.10 ± 0.49
	15	7	11.3 ± 0.9 [b]	22.4 ± 6.4 [c]	23.3 ± 4.2 [b]	1.29 ± 0.25	1.81 ± 0.18 [b]
	45	7	10.4 ± 0.9 [c]	18.0 ± 3.8 [c]	19.9 ± 3.1 [c]	1.10 ± 0.13 [c]	1.72 ± 0.16 [c]
+ OKY-046	200	7	14.8 ± 2.6	26.0 ± 5.5	22.9 ± 3.2 [b]	1.37 ± 0.21	2.00 ± 0.23
+ Azathioprine	20	7	17.4 ± 3.5	20.6 ± 3.7 [c]	18.6 ± 3.4 [c]	1.42 ± 0.21	1.58 ± 0.12 [c]

Test drugs were given p.o. daily to rat from the 22nd day after injection of anti-Fx1A serum to the 61st day. Plasma and kidney were taken on the 61st day for biochemical and light microscoopic studies, respectively.
Values indicate the mean ± S.D. and n indicates the number of rats used; bw and GCS indicate body weight and glomerular cross-section, respectively.
[a] Significant difference from the control at $P < 0.05$.
[b,c] Significant differences from the rat given APHN at $P < 0.05$ and 0.01, respectively.

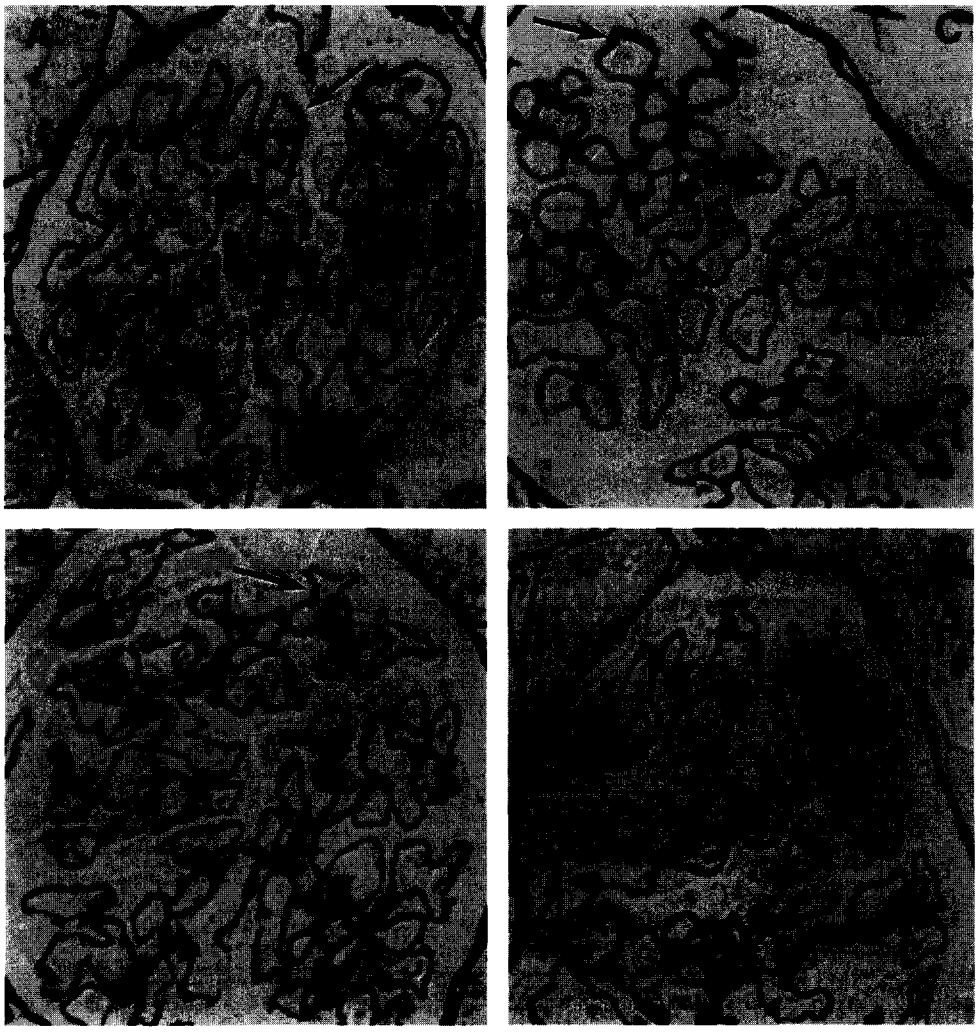

Fig. 2. Light micrographs of glomeruli from rats treated with APHN (A), APHN + DP-1904 (45 mg/kg) (B), APHN + OKY-046 (200 mg/kg) (C) and APHN + azathioprine (20 mg/kg) (D). Test drugs were given p.o. daily to rats from the 22nd day after i.v. injection of anti-Fx1A serum to the 61st day. The thickening and diffuse spike formation were observed in the glomerular basement membrane. Arrows indicate the area that has thickening or spike formation.

(200 mg/kg) was found to have little effect. The elevation of the antibody titer due to nephritis was markedly inhibited by azathioprine (20 mg/kg) ($P < 0.01$ vs. APHN). The antibody titers on the 61st day were as follows: APHN, 6.4 ± 1.3; APHN + DP-1904 5 mg/kg, 6.0 ± 0.8; 15 mg/kg, 5.4 ± 0.6; 45 mg/kg, 5.3 ± 0.8; APHN + OKY-046, 6.1 ± 1.2; APHN + azathioprine, 4.6 ± 0.8.

3.2.4. Effects on the deposition of rabbit immunoglobulin G and rat immunoglobulin G in the glomeruli (Table 2)

In the sections from accelerated passive Heymann nephritic animals on the 61st day, granular depositions of rabbit immunoglobulin G and rat immunoglobulin G were observed along the glomerular basement membrane. DP-1904 (45 mg/kg) significantly reduced the deposition of both rabbit immunoglobulin G and rat immunoglobulin G. However, OKY-046 (200 mg/kg) showed only a tendency to reduce the deposition of both rabbit immunoglobulin G and rat immunoglobulin G. Azathioprine apparently reduced only the deposition of rat immunoglobulin G.

3.3. Mechanisms of the antinephritic action of DP-1904

3.3.1. Effects on renal tissue blood flow (Fig. 3)

The effects of DP-1904 and OKY-046 on renal tissue blood flow were examined on the 22nd and 61st days after anti-Fx1A serum injection. The decrease in renal tissue blood flow of the accelerated passive Heymann nephritic rats on the 22nd and 61st days was markedly reversed by a single p.o. administration of DP-1904 (5 and 45 mg/kg). OKY-046 also increased renal tissue blood flow to a level similar to that of DP-1904 at 5 mg/kg.

3.3.2. Effects on glomerular thromboxane B_2, prostaglandin E_2 and 6-keto prostaglandin $F_{1\alpha}$ production (Table 3)

The effects of DP-1904 and OKY-046 on glomerular thromboxane B_2, prostaglandin E_2 and 6-keto prostaglandin $F_{1\alpha}$ production were examined at 4 h after the single p.o. administration of test drugs to nephritic rats on the 22nd and 61st days after the anti-Fx1A serum injection. Glomerular thromboxane B_2, prostaglandin E_2 and 6-keto prostaglandin $F_{1\alpha}$ production in the APHN group was approximately 2-fold higher than that of the control group on the 22nd and 61st days. At 4 h after the drug treatment, DP-1904 at 5, 15 and 45 mg/kg dose-dependently inhibited the increase in glomerular thromboxane B_2 production by 49–88% at on 22nd and 61st days. In addition, DP-1904 at 5, 15 and 45 mg/kg caused increases of 13–27%, in prostaglandin E_2 and increases of 10–27%, in 6-keto prostaglandin $F_{1\alpha}$. OKY-046 (200 mg/kg) also decreased thromboxane B_2 production by 45% on the 61st day, while it increased prostaglandin E_2 by 19% on the 22nd day.

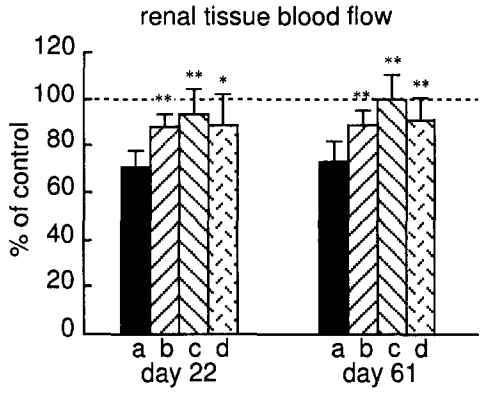

Fig. 3. Effects of a single administration of DP-1904 on renal tissue blood flow of accelerated passive Heymann nephritic rats. Columns (a): APHN; columns (b): APHN + DP-1904 (5 mg/kg); columns (c): APHN + DP-1904 (45 mg/kg); columns (d): APHN + OKY-046 (200 mg/kg). Broken line indicates control levels (95.6 ± 6.2 at the 22 day and 91.2 ± 7.0 ml/min per 100 g kidney weight at the 61st day). Each column denotes the mean with S.D. for five rats. * $P < 0.05$, ** $P < 0.01$ vs. APHN.

Table 3
Effects of a single administration of DP-1904 on glomerular thromboxane B_2, prostaglandin E_2 and 6-keto prostaglandin F_{1a} production of accelerated passive Heymann nephritic rats

Treatment	(mg/kg)	*n*	22nd day			61st day		
			TxB_2	PGE_2 (pg/mg protein per 30-min incubation)	6-keto $PGF_{1\alpha}$	TxB_2	PGE_2 (pg/mg protein per 30 min incubation)	6-keto $PGF_{1\alpha}$
Control	-	4	61.0 ± 7.1	560.4 ± 26.5	160.2 ± 19.7	59.7 ± 7.1	582.3 ± 22.0	164.6 ± 16.6
APHN	-	4	106.4 ± 17.5 [a]	1008.4 ± 95.6 [a]	270.4 ± 21.0 [a]	104.6 ± 15.3 [a]	1021.0 ± 86.3 [a]	286.6 ± 14.8 [a]
+ DP-1904	5	4	80.6 ± 7.2	1142.3 ± 83.5 [b]	296.4 ± 17.7 [b]	82.6 ± 9.8 [c]	1110.1 ± 97.2	290.0 ± 8.6
	15	4	71.4 ± 3.4 [b]	1206.8 ± 38.9 [c]	312.2 ± 21.5 [c]	70.2 ± 11.9 [c]	1236.2 ± 100.5 [b]	314.6 ± 12.2 [c]
	45	4	66.6 ± 5.3 [c]	1280.7 ± 62.4 [c]	343.2 ± 19.3 [c]	65.4 ± 4.9 [c]	1301.4 ± 95.6 [c]	340.4 ± 17.3 [c]
+ OKY-046	200	4	82.4 ± 3.8	1200.4 ± 66.6 [c]	300.6 ± 7.4 [b]	84.4 ± 4.6 [c]	1208.3 ± 80.8 [b]	298.6 ± 6.8
+ Azathioprine	20	4	100.2 ± 30.0	1012.8 ± 133.4	268.0 ± 21.3	103.3 ± 6.7	1012.1 ± 275.9	270.4 ± 14.5

Test drugs were administered singly to nephritic rats on the 22nd or 61st day after injection of anti-Fx1A serum. At 4 h after the administration of test drug, the glomeruli were isolated from nephritic rats.

Values indicate the mean ± S.D. and *n* indicates the number of rats used. TxB_2, PGE_2 AND 6-keto $PGF_{1\alpha}$ indicate thromboxane B_2, prostaglandin E_2 and 6-keto prostaglandin $F_{1\alpha}$, respectively.

[a] Significant differences from the control at $P < 0.01$.

[b,c] Significant differences from the rats given APHN at $P < 0.01$ and 0.01, respectively.

4. Discussion

In the present study, DP-1904, a thromboxane A_2 synthase inhibitor, apparently inhibited the development of proteinuria and glomerular histopathological changes, such as spike formation and thickening, when we started the administration in the heterologous or autologous phase of accelerated passive Heymann nephritis. The effects of DP-1904 on accelerated passive Heymann nephritis were more potent than those of OKY-046 or azathioprine.

Regarding the mechanisms of the antinephritic action of DP-1904, we demonstrated that a single p.o. administration of DP-1904 or OKY-046 markedly restored renal tissue blood flow in accelerated passive Heymann nephritic rats. When the effects of DP-1904 or OKY-046 on the synthesis of glomerular prostanoids were evaluated, both drugs were found to have markedly suppressed glomerular thromboxane B_2 production and significantly increased glomerular prostaglandin E_2 and 6-keto prostaglandin $F_{1\alpha}$ production in accelerated passive Heymann nephritic rats. Accordingly, it is suggested that the increase in renal tissue blood flow produced by DP-1904 or OKY-046 may be due to the suppression of excessive glomerular thromboxane A_2 synthesis or the increase in the ratio of prostaglandin E_2 and prostaglandin I_2 to thromboxane A_2 produced by both drugs. Although the precise mechanism by which DP-1904 and OKY-046 increase prostaglandin E_2 and prostaglandin I_2 production remains unclear, it seems reasonable to consider that the inhibition of thromboxane A_2 synthesis by thromboxane A_2 synthase inhibitors promotes the transformation of prostaglandin endoperoxides (prostaglandin G_2 and prostaglandin H_2) to prostaglandin E_2 and prostaglandin I_2.

Our previous study indicated that the inhibitory effect of DP-1904 on glomerular thromboxane B_2 synthesis was more potent and longer lasting than that of OKY-046 in normal and nephritic rats (Nagao et al., 1994). In accelerated passive Heymann nephritic rats, the potency of OKY-046 at 200 mg/kg regarding the inhibition of thromboxane B_2 production was similar to that of DP-1904 at 5 mg/kg. It is possible that the difference between DP-1904 and OKY-046 in antinephritic effect may result from the difference in the ability to inhibit thromboxane A_2 synthase of these drugs.

In the autologous study, azathioprine markedly reduced the antibody titer and reduced the deposition of only rat immunoglobulin G in the glomeruli of accelerated passive Heymann nephritic rats. On the other hand, long-term administration of thromboxane A_2 synthase inhibitors slightly reduced the elevation of antibody titer and the deposition of both rabbit immunoglobulin G and rat immunoglobulin G. Regarding the effect on glomerular rabbit (hetero) immunoglobulin G deposition of thromboxane A_2, Nagamatsu et al. (1994) examined the effect of thromboxane A_2 receptor antagonists on the clearance of glomerular aggregated bovine serum albumin and suggested that thromboxane A_2 delays the disposal of macromolecules deposited in the glomeruli in vivo. Mattana and Singhal (1992) also reported that thromboxane A_2 analogue enhanced immune complex uptake. Moreover, prostaglandin E_2 has been shown to down-regulate T lymphocyte function (Goodwin and Ceuppens, 1983), likely through its ability to decrease interleukin 1 and interleukin 2 production (Minakuchi et al., 1990), its capacity to decrease major histocompatibility complex class II expression on antigen-presenting cells, such as the macrophage (Snyder et al., 1982), and the capacity to enhance certain aspects of the immune response, such as immunoglobulin E production (Phipps et al., 1991). Therfore, long-term administration of thromboxane A_2 synthase inhibitor may cause an immunosuppressive action, such as the reduction of glomerular immunoglobulin G deposition through suppression of thromboxane A_2 synthesis and an increase in prostaglandin E_2 synthesis.

Several reports have implied a beneficial effect of thromboxane A_2 synthase inhibitors on renal function or an antinephritic effect (Lianos et al., 1983; Suzuki et al., 1987; Remuzzi et al., 1985), although this has been disputed (Shinkai and Cameron, 1987; Stahl et al., 1987; Thaiss et al., 1989). However, most studies that failed to find an antinephritic effect of thromboxane A_2 synthase inhibitors were carried out with short-term administration only. The present antinephritic effect seen after long-term administration of a thromboxane A_2 synthase inhibitor may have resulted from an immunosuppressive effect in addition to an inhibition of thromboxane A_2 synthesis.

The present data suggest that the antinephritic action of DP-1904 may be due to a decrease in the deposition of rabbit and rat immunoglobulin G in the glomeruli, an inhibition of antibody formation and an increase in renal tissue blood flow. We have demonstrated that DP-1904 has a more potent antinephritic action than OKY-046 and azathioprine in rat accelerated passive Heymann nephritis. Azathioprine has been often used for the treatment of various types of human glomerulonephritis and OKY-046 has been reported to be clinically effective for the treatment of chronic glomerulonephritis accompanied by a nephrotic syndrome (Niwa et al., 1987). Therefore, DP-1904 is expected to have a clinical effect on human chronic glomerulonephritis, especially membranous nephropathy.

Acknowledgements

We wish to thank Dr. M. Ito for his helpful discussion and Ms. H. Mizusaki, Ms. C. Imamura and Ms. K. Mizutani for their technical assistance.

References

Aukland, K., B.F. Bower and R.W. Berliner, 1964, Measurement of local blood flow with hydrogen gas, Circ. Res. 14, 164.

Boot, J.R., 1976, The chemotactic activity of thromboxane B_2: a possible role in inflammation, J. Physiol. 257, 47.

Brune, K., M. Galah and H. Kahn, 1978, Pharmacological control of prostaglandin and thromboxane release from macrophages, Nature 274, 261.

Couser, W.G., 1985, Glomerular disorder, in: Cecil Textbook of Medicine (Chapter 80), 17th Ed., ed. J.B. Wyngaaren (W.B. Saunders, Philadelphia, PA) p. 568.

Goldstein, J.M., C.L. Malmsten, H. Kindahl, H.P. Kaplan, D. Radmarh, B. Samuelsson and G. Weissman, 1978, Thromboxane generation by human peripheral blood polymorphonuclear leukocytes, J. Exp. Med. 148, 787.

Goodwin, J. and J. Ceuppens 1983, Regulation of the immune response by prostaglandins, J. Clin. Immunol. 3, 295.

Hattori, T., M. Ito and Y. Suzuki, 1990, Studies on antinephritic effect of TJ-8014, a new Japanese herbal medicine (4): effects on accelerated passive Heymann nephritis in rats, Jpn. J. Pharmacol. 54, 265.

Hayashi, K., T. Nagamatsu, M. Ito, T. Hattori and Y. Suzuki, 1994, Acteoside, a component of *Stachys sieboldii* MIQ, may be a promising antinephritic agent: effect of acteoside on crescentic-type anti GBM nephritis in rats, Jpn. J. Pharmacol. 65, 143.

Ito, M., Y. Aono, A. Suzuki, T. Nagamatsu and Y. Suzuki, 1989, Accelerated passive Heymann nephritis in rats as experimental model for membranous glomerulonephritis and effects of azathioprine and prednisolone on the nephritis, Jpn. J. Pharmacol. 49, 101.

Kaizu, K., D. Marsh, R. Zipser and R.J. Glassock, 1985, Role of prostaglandins and angiotensin II in experimental glomerulo-nephritis, Kidney Int. 28, 629.

Kelley, V.E., S. Sneve and S. Musinski, 1986, Increased renal production in murine lupus nephritis, J. Clin. Invest. 77, 252.

Kingsbury, F.B., C.P. Clark, G. Williams and A.L. Post, 1926, The rapid determination of albumin in urine, J. Lab. Clin. Med. 11, 981.

Lianos, E.A., G.A. Andres and M.J. Dunn, 1983, Glomerular prostaglandin and thromboxane synthesis in rat nephrotoxic serum nephritis. Effects on renal hemodynamics, J. Clin. Invest. 72, 1439.

Mattana, J. and P.C. Singhal, 1992, Effects of prostaglandin E_2 and cyclic AMP on uptake of immunoglobulin G complexs by cultured macrophages, Eicosanoids 5, 67.

McLeish, K.R., A.F. Gohara and W.T. Gunning, 1982, Suppression of antibody synthesis by prostaglandin E as a mechanism for preventing murine immune complex glomerulonephritis, Lab. Invest. 47, 147.

Minakuchi, R., M.C. Wacholtz, L.S. Davis and D.E. Lipsky, 1990, Delineation of the mechanism of inhibition of human T cell activation by PGE_2, J. Immunol. 145, 2616.

Nagamatsu, T., Y. Nomura and Y. Suzuki, 1994, Thromboxane A_2 delays disposal of aggregated protein in glomeruli, Can. J. Physiol. Pharmacol. 72 (Suppl. 1), 276.

Nagao, T., M. Ito, T. Nagamatsu and Y. Suzuki, 1994, Effect of DP-1904, a thromboxane synthetase inhibitor, on crescentic nephritis in rats, Eur. J. Pharmacol. 259, 233.

Nagao, T., T. Nagamatsu and Y. Suzuki, 1995, Effect of DP-1904, a thromboxane synthase inhibitor, administered from the autologous phase on crescentic-type anti-GBM nephritis in rats, Jpn. J. Pharmacol. 68, 137.

Needleman, P.S., S. Moncada, S. Bunting, J.R. Vane, M. Hamberg and B. Samuelsson, 1976, Identification of an enzyme in platelet microsomes which generates thromboxane A_2 from prostaglandin endoperoxides, Nature 261, 558.

Niwa, T., K. Maeda and M. Shibata, 1987, Urinary prostaglandins and thromboxane in patients with chronic glomerulonephritis, Nephron 46, 281.

Petrulis, A.S., G. Aikawa and M.J. Dunn, 1981, Prostaglandin and thromboxane synthesis by rat glomerular epithelial cells, Kidney Int. 20, 469.

Phipps, R.P., S.H. Stein and R.L. Roper, 1991, A new view of prostaglandin E regulation of the immune response, Immunol. Today 12, 349.

Remuzzi, G., L. Imbert, M. Rossini, C. Morelli, C. Carminati, G.M. Cattaneo and T. Bertani, 1985, Increased glomerular thromboxane synthesis as a possible cause of proteinuria in experimental nephrosis, J. Clin. Invest. 75, 94.

Shinkai, Y. and J.S. Cameron, 1987, Rabbit nephrotoxic nephritis: effect of a thromboxane synthetase inhibitor on evolution and prostaglandin excretion, Nephron 47, 211.

Snyder, D.S., D.I. Beller and E.R. Unaue, 1982, Prostaglandins modulate macrophage Ia expression, Nature 299, 163.

Sraer, J., J. Foidart, D. Chansel, P. Mahieu and R. Ardaillou, 1980, Prostaglandin synthesis by rat isolated glomeruli and glomerular cultured cells, Int. J. Biochem. 12, 203.

Stahl, R.A.K., S. Adler, P.J. Baker, Y.P. Chen, P.M. Pritzl and W.G. Couser, 1987, Enhanced glomerular prostaglandin formation in experimental membranous nephropathy, Kidney Int. 31, 1126.

Stork, J.E. and M.J. Dunn, 1985, Hemodynamic roles of thromboxane A and prostaglandin E in glomerulonephritis, J. Pharmacol. Exp. Ther. 233, 672.

Suzuki, Y., Y. Tsukushi, M. Ito and T. Nagamatsu, 1987, Antinephritic effect of Y-19018, a thromboxane A synthetase inhibitor, on crescentic-type anti-GBM nephritis in rats, Jpn. J. Pharmacol. 45, 177.

Thaiss, F., P.J. Germann, S. Kahf, W. Schoeppe, U. Helmchen and R.A.K. Stahl, 1989, Effect of thromboxane synthesis inhibition in a model of membranous nephropathy, Kidney Int. 35, 76.

Zoja, C., A. Benigni, P. Verroust, P. Ronco, T. Bertani and G. Remuzzi, 1987, Indomethacin reduced proteinuria in passive Heymann nephritis in rats, Kidney Int. 31, 1335.