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Effect of lipo-prostaglandin E_1 on crescentic-type anti-glomerular basement membrane nephritis in rats

Toshiyuki Nagao, Tadashi Nagamatsu, Yoshio Suzuki *

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

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Abstract

The antinephritic effect of lipo-prostaglandin E_1, prostaglandin E_1 ((1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3*S*)-3-hydroxy-1-octenyl]-5-oxocyclopentane heptanoic acid) incorporated in lipid microspheres was investigated using an experimental model of nephritis, crescentic-type anti-glomerular basement membrane nephritis. Lipo-prostaglandin E_1 was given i.v. twice a day at 20, 40 and 80 μg/kg and azathioprine, an immunosuppressive agent, at 20 mg/kg was given p.o. once daily from the autologous phase, in which glomerulonephritis was fully developed (the 21st day after injection of the anti-glomerular basement membrane serum), to the 50th day. Lipo-prostaglandin E_1 (40 and 80 μg/kg $\times$ 2 per day) significantly inhibited the development of glomerular alterations as well as the elevation of proteinuria and plasma creatinine. Lipo-prostaglandin E_1 (20 μg/kg $\times$ 2 per day) and azathioprine (20 mg/kg per day) significantly inhibited only the glomerular histopathological changes. Lipo-prostaglandin E_1 at three doses significantly decreased the deposition of both rabbit immunoglobulin G and rat immunoglobulin G on the glomerular basement membrane in nephritic rats, but azathioprine apparently inhibited only the deposition of rat immunoglobulin G. A single administration of lipo-prostaglandin E_1 inhibited the elevation of platelet aggregation and restored the decrease in renal tissue blood flow in nephritic rats. In addition, a single administration of lipo-prostaglandin E_1 inhibited the elevation of glomerular thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$ production in nephritic rats. These results suggest that lipo-prostaglandin E_1 may be an effective agent for the treatment of glomerulonephritis. Its antinephritic effect may be due to the inhibition of platelet aggregation, an increase in renal tissue blood flow, a decrease in rabbit and rat immunoglobulin G deposition, and amelioration of the abnormal metabolism of arachidonic acid. © 1998 Elsevier Science B.V.

Keywords: Lipo-prostaglandin E_1; Lipid microsphere; Anti-glomerular basement membrane nephritis; Crescentic-type; Proteinuria

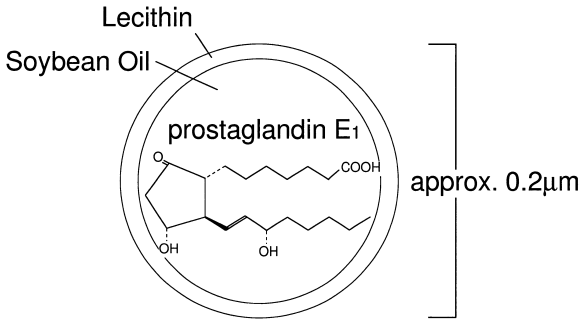
1. Introduction

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 thromboxane in isolated glomeruli (Lianos et al., 1983; Kaizu et al., 1985; Stork and Dunn, 1985) and renal cortex (Kelley et al., 1986). It is believed that thromboxane A_2 mediates deterioration of renal function by 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. First demonstrated by Zurier et al. (1977), other investigators (Kelley et al., 1979; Kunkel et al., 1982; McLeish et al., 1982) have since demonstrated the beneficial effect of prostaglandin E_1, which is a potent vasodilator as well as an inhibitor of platelet aggregation, on experimental nephritis. Nagamatsu et al. (1989a) also previously reported that twice daily s.c. administration of prostaglandin E_1 had a curative effect on anti-glomerular basement membrane nephritis in rats.

Since prostaglandin E_1 is rapidly inactivated in the lung, prostaglandin E_1 must be administered into the obstructed artery or a large amount has to be given intravenously. Prostaglandin E_1 also causes irritation in blood vessels near the site of injection. To avoid these clinical problems of prostaglandin E_1, Mizushima et al. (1982, 1983) incorporated prostaglandin E_1 in lipid microspheres (small lipid particles) with an average particle size of 0.2 μm, suspensions of which are in widespread use for parenteral nutrition in humans (Fig. 1).

The aim of the present study was to determine whether lipo-prostaglandin E_1 has a beneficial effect in the treatment of glomerulonephritis. To this end rats with crescen-

* Corresponding author. Tel.: +81-52-832-1781; fax: +81-52-834-8090.



(1R, 2R, 3R)-3-hydroxy-2-[(E)-(3S)-3-hydroxy-1-octenyl]-5-oxocyclopentane heptanoic acid.

Fig. 1. Structure of lipo-prostaglandin E_1. Lipo-prostaglandin E_1 was prepared by emulsifying prostaglandin E_1 dissolved in soybean oil, using lecithin as surfactant.

tic-type anti-glomerular basement membrane nephritis were used. In this model, the glomerular injury has been demonstrated to consist of a heterologous and an autologous phase. Immediately after the injection of anti-glomerular basement membrane serum (rabbit anti-glomerular basement membrane antibody; hetero-antibody), the heterologous phase is caused by the binding of the injected rabbit anti-glomerular basement membrane antibody to the glomeruli, followed by the activation of complement. Following this, 7 to 10 days later, the autologous phase is induced by a reaction between rat antibody against the injected rabbit anti-glomerular basement membrane antibody and rabbit anti-glomerular basement membrane antibody already fixed on the glomeruli. Crescentic-type anti-glomerular basement membrane nephritis is induced by enhancement of the immune response in the autologous phase via the persistent formation of auto-antibodies against rabbit gamma-globulin following the i.v. injection of anti-glomerular basement membrane serum. This experimental model closely resembles rapidly progressive glomerulonephritis in humans, which is characterized by severe glomerular lesions with extensive formation of crescents in the glomeruli.

Therefore, we investigated the antinephritic effect of lipo-prostaglandin E_1 administered from the autologous phase, in which glomerulonephritis was fully developed, onwards in order to estimate the benefit of this drug in clinical situations.

2. Materials and methods

2.1. Animals

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

2.2. Drugs

Lipo-prostaglandin E_1 was kindly provided by Taisho Pharmaceutical (Tokyo, Japan). Azathioprine was purchased from Sigma Chemical (St. Louis, MO, USA). The method of preparing a suspension of lipid microspheres containing prostaglandin E_1 was that of Mizushima et al. (1982, 1983). Briefly, prostaglandin E_1 was dissolved in soybean oil containing yolk phospholipid (10:1.2). The mixture was emulsified in water (1:9) with a Manton–Gaulin homogenizer. The resultant emulsion was put in 1-ml glass ampoules and sterilized at 121°C for 10 min. The final concentration of prostaglandin E_1 in the lipo-prostaglandin E_1 preparation was 20 μg/ml. Azathioprine was suspended in 1% gum arabic.

2.3. Experimental protocols

2.3.1. Protocol for evaluation of antinephritic effect of lipo-prostaglandin E_1

Crescentic-type anti-glomerular basement membrane nephritis was induced in rats by injecting 6.5 mg rabbit gamma-globulin in 0.25 ml of Freund's complete adjuvant into the hind foot pads, following the injection of 0.6 ml of rabbit anti-rat glomerular basement membrane serum into the tail vein, in accordance with the method reported previously (Ito et al., 1983). On the 20th day after injection of the anti-glomerular basement membrane serum, urine samples were collected for 24 h. The animals were then divided into 6 groups ($n = 8$) such that the average protein content in the 24-h urine samples of each group was similar. Each of the four groups was given 20, 40 or 80 μg/kg of lipo-prostaglandin E_1 (anti-GBM + lipo-PGE_1) in a volume of 0.4 ml per 100 g of body weight, i.v. twice a day, and 20 mg/kg of azathioprine (anti-GBM + azathioprine) in a volume of 1 ml per 100 g of body weight, orally, daily from the 21st day after injection of the anti-glomerular basement membrane serum to the 50th day. The remaining two groups were given i.v. physiological saline (anti-GBM) or lipid microspheres without prostaglandin E_1 (anti-GBM + vehicle) instead of test drugs and served as the anti-GBM nephritic group and anti-GBM + vehicle group. In addition, a control group ($n = 8$) that did not receive the anti-glomerular basement membrane serum was used for comparison with the nephritic group.

2.3.2. Protocol for evaluation of antinephritic mechanisms of lipo-prostaglandin E_1

Crescentic-type anti-glomerular basement membrane nephritis was induced by the same method as indicated in Section 2.3.1.

To investigate the effect of a single i.v. administration of test drugs on platelet aggregation and renal tissue blood flow, we used 5 groups of five rats each: control group, anti-GBM nephritic group, anti-GBM + vehicle group and

anti-GBM + lipo-prostaglandin E_1 groups (20 and 80 μg/kg). Renal tissue blood flow was measured before and 30 min after treatment with the test drugs. After the measurement of renal tissue blood flow, the animals were killed 2 h following the administration of each test drug for measurement of platelet aggregation.

We next investigated the effect of a single i.v. administration of test drugs on glomerular thromboxane B_2 (the stable metabolite of thromboxane A_2) and 6-keto prostaglandin $F_{1\alpha}$ (the stable metabolite of prostaglandin I_2) production on the 20th and the 50th days, using 7 groups of 4 rats each: control group, anti-GBM nephritic group, anti-GBM + vehicle group, anti-GBM + lipo-PGE_1 group (20, 40 and 80 μg/kg) and anti-GBM + azathioprine group (20 mg/kg). Control rats were given i.v. physiological saline. Animals were killed at 4 h following the administration of each test drug or saline. Glomeruli were then isolated from their kidneys.

2.4. Urine and blood collections

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. At the beginning of the urine collection, each animal received 8 ml of distilled water orally. The urine was then centrifuged at $810 \times g$ for 15 min at 4°C and the supernatant was used for the determination of protein. Immediately after the last collection of urine samples, blood was taken from the renal vein under pentobarbital anesthesia. The blood was centrifuged at $2250 \times g$ for 20 min at 4°C to obtain plasma for the determination of creatinine and antibody titer against rabbit gamma-globulin.

2.5. Measurement of urinary protein, 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 (CRE-EN, Kainos, Tokyo, Japan) and expressed as mg/dl per 100 g body weight. The plasma antibody titer against rabbit gamma-globulin was determined by indirect hemagglutination, using sensitized sheep red blood cells (McLeish et al., 1982), and expressed as the $\log_2$ of the hemagglutination titer.

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- to 3-μm thick sections. The sections were stained with hematoxylin and eosin or Masson's trichrome. Twenty-five glomeruli per section were observed under a light microscope to evaluate crescent formation, adhesion of Bowman's capsule to capillary walls (adhesion) and fibrinoid necrosis, respectively, by a different person who did not know the identity of sections. To assess each of the histopathological parameters, the extent of crescent formation, adhesion and fibrinoid necrosis was scored as 1 (mild), 2 (moderate) and 3 (severe) (Hattori et al., 1990). The number of the glomeruli corresponding to each score is given as n_1, n_2 and n_3.

A crescent formation index (CI), an adhesion index (AI) and a fibrinoid necrosis index (FI) were calculated from the following formula.

$$\text{CI, AI and FI} = 1 \times n_1 + 2 \times n_2 + 3 \times n_3.$$

The index of glomerular lesions (IGL) was calculated to evaluate the extent of glomerular lesions as follows:

$$\text{IGL} = \frac{(3 \times \text{CI}) + (2 \times \text{AI}) + (1 \times \text{FI})}{(3 + 2 + 1) \times 25}$$

2.7. Immunoperoxidase studies

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

The deposition of intraglomerular rabbit immunoglobulin G or rat immunoglobulin G was measured with an image-analyzer (TOYOBO Imageanalyzer VI, TOYOBO, Tokyo, Japan) and expressed as $\times 10^{-3}$ mm^2/glomerular cross section (GCS).

2.8. Effects of a single administration of lipo-prostaglandin E_1 on glomerular thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$ production in nephritic rats

The glomeruli were isolated by the differential sieving technique of Zoja et al. (1987) from kidneys perfused with Krebs–Ringer phosphate-buffered saline (pH 7.2) and obtained from normal and nephritic rats treated as mentioned in Section 2.3.2. The isolated glomeruli (the purity was more than 80% when observed under a light microscope) 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 and 6-keto prostaglandin $F_{1\alpha}$. The amount of thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$ in the glomeruli was determined by

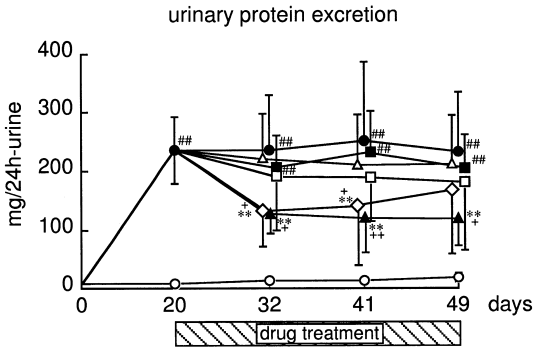


Fig. 2. Effects of lipo-prostaglandin E_1 on urinary protein excretion of rats with crescentic-type anti-glomerular basement membrane nephritis. ○-○: control; ●-●: anti-GBM; ■-■: anti-GBM + vehicle; □-□: anti-GBM + lipo-PGE_1 (20 μg/kg × 2 per day); ◇-◇: anti-GBM + lipo-PGE_1 (40 μg/kg × 2 per day); ▲-▲: anti-GBM + lipo-PGE_1 (80 μg/kg × 2 per day); △-△: anti-GBM + azathioprine (20 mg/kg per day). Each plot denotes the mean with S.D. for eight rats. ##$P < 0.01$ vs. control. ∗ $P < 0.05$, ∗∗ $P < 0.01$ vs. anti-GBM. + $P < 0.05$, ++ $P < 0.01$ vs. anti-GBM + vehicle.

radio-immunoassay (New England Nuclear, MA, USA). The protein content in the glomeruli was assayed by a colorimetric method (Bio-Rad, Richmond, CA, USA).

2.9. Effects of lipo-prostaglandin E_1 on platelet aggregation and renal tissue blood flow in nephritic rats

The effects of a single i.v. administration of lipo-prostaglandin E_1 on platelet aggregation and renal tissue blood flow were examined 20 and 50 days after injection of anti-glomerular basement membrane serum. 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) (Nagao et al., 1994). Briefly, a rat was anesthetized with sodium pentobarbital (32.4 mg/kg, i.p.) and its left kidney was exposed through a flank incision. An electrode was superficially implanted into the renal cortex and a reference electrode was inserted into the abdomen far from the electrode. The electrode consisted of two wires. One of the wires was prepared for electrolysis of body fluid, and the other was prepared for detecting hydrogen. When the electrode is inserted into the tissue, electrolysis occurs around the wire and hydrogen gas is produced. The micro-electric current resulting from ionization of the hydrogen is measured by the other wire. A clearance curve indicating the decline of hydrogen concentration is then obtained and analyzed by the method of Aukland et al. (1964). Renal tissue blood flow was measured before and 30 min after treatment with the test drug. Renal tissue blood flow measured by this method is obtained as milliliters per minute per 100 gram kidney weight and is expressed as a percentage of that of the control group.

After the measurement of renal tissue blood flow, platelet aggregation was measured with a whole blood aggregometer, model C-506 (Crono-log, Tokyo, Japan). Two hours after treatment with the test drug, blood was taken from the renal vein. 500 μl of blood was added to 495 μl of 0.9% saline in a small aggregometer cuvette. This cuvette was maintained at 37°C in a heated block and the content was stirred with a flea magnet. Five microliters of collagen (Collagen reagent Horm, Hormon-Chemie, Bavaria, Germany) was then added to the sample in the cuvette. The results are presented as the impedance (ohm) between the two electrodes 5 min after the addition of the collagen.

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

Table 1
Effect of lipo-prostaglandin E_1 on plasma creatinine content and antibody titer against rabbit gamma-globulin in rats with crescentic-type anti-glomerular basement membrane nephritis

Treatment	Dose	*n*	Creatinine content (mg/dl per 100 g bw)	Antibody titer ($\log_2$ of the HT)
Control	−	8	0.10 ± 0.01	
anti-GBM	−	8	0.17 ± 0.05[a]	7.1 ± 0.6
+ vehicle	−	8	0.15 ± 0.03[a]	6.9 ± 1.7
+ Lipo-PGE_1	20 μg/kg × 2 per day, i.v.	8	0.11 ± 0.02[bc]	7.1 ± 0.8
	40 μg/kg × 2 per day, i.v.	8	0.11 ± 0.02[bc]	6.6 ± 1.6
	80 μg/kg × 2 per day, i.v.	8	0.09 ± 0.03[bc]	6.1 ± 0.8[b]
+ Azathioprine	20 mg/kg per day, p.o.	8	0.12 ± 0.02	5.3 ± 0.9[b]

Lipo-prostaglandin E_1 and azathioprine were given i.v. twice a day and p.o. daily to rats, respectively, from the 21st day after injection of anti-glomerular basement membrane serum to the 50th day. Plasma taken on the 50th day for the determination of creatinine and antibody titer against rabbit gamma-globulin. Values indicate the means ± S.D. and *n* indicates the number of rats used.
bw = Body weight; HT = hemagglutination titer.
[a]Significant difference from the control at $P < 0.05$.
[b]Significant difference from the anti-GBM at $P < 0.05$.
[c]Significant difference from the anti-GBM + vehicle at $P < 0.05$.

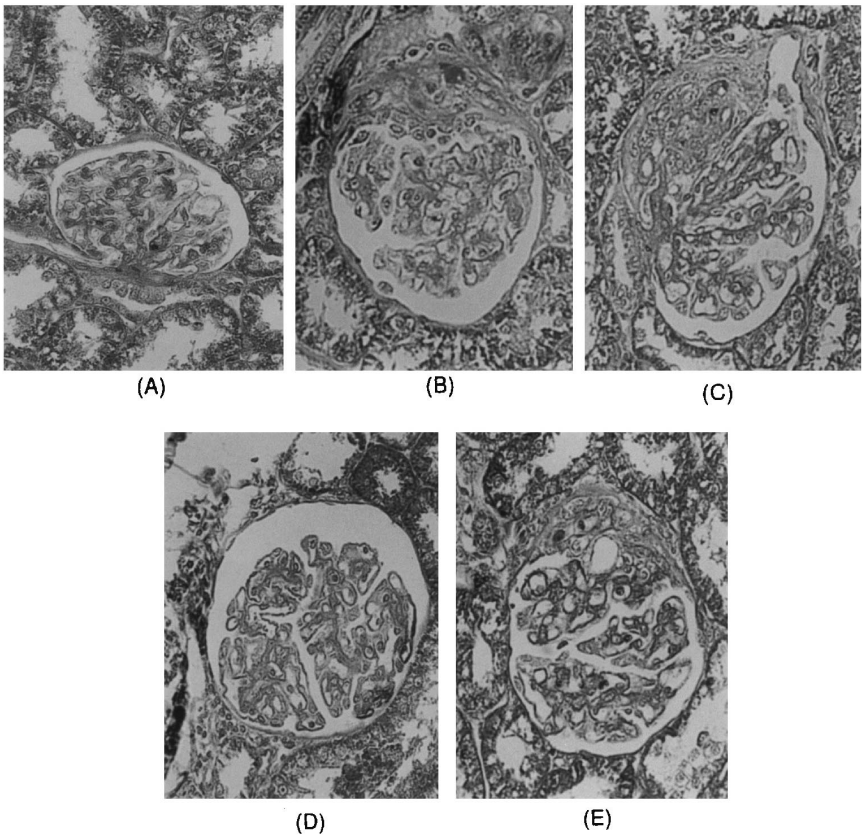


Fig. 3. Light micrographs of glomeruli from control (A) and anti-GBM (B), anti-GBM + vehicle (C), anti-GBM + lipo-PGE_1 (80 $\mu g/kg \times 2$ per day) (D) and anti-GBM + azathioprine (20 mg/kg per day)-treated rats (E). Lipo-prostaglandin E_1 and azathioprine were given i.v. twice a day and p.o. daily to rats, respectively, from the 21st day after injection of anti-glomerular basement membrane serum to the 50th day.

significance of differences among the groups, Dunnett's test or Tukey's test was used.

3. Results

3.1. Antinephritic effects of lipo-prostaglandin E_1 on crescentic-type anti-glomerular basement membrane nephritis

3.1.1. Urinary protein excretion and plasma creatinine content (Fig. 2 and Table 1)

When test drugs were given from the 21st day after injection of the anti-glomerular basement membrane serum, lipo-prostaglandin E_1 at 40 and 80 $\mu g/kg \times 2$ significantly suppressed urinary protein excretion by 47 and 54%, respectively, through the 32nd to the 50th day (Fig. 2, Table 1). In addition, on the 50th day this agent at the

Table 2
Effect of lipo-prostaglandin E_1 on glomerular histological parameters and glomerular rabbit and rat immunoglobulin G deposition in rats with crescentic-type anti-glomerular basement membrane nephritis

Treatment	Dose	*n*	Index of glomerular lesions	Rabbit IgG ($\times 10^{-3}$ mm^2/GCS)	Rat IgG ($\times 10^{-3}$ mm^2/GCS)
anti-GBM	−	8	2.44 ± 0.27	1.52 ± 0.16	2.40 ± 0.30
+ vehicle	−	8	2.44 ± 0.32	1.54 ± 0.17	2.40 ± 0.28
+ Lipo-PGE_1	20 $\mu g/kg \times 2$/day, i.v.	8	1.71 ± 0.23^{bc}	1.22 ± 0.19^{bc}	1.91 ± 0.17^{bc}
	40 $\mu g/kg \times 2$/day, i.v.	8	1.48 ± 0.25^{bc}	1.07 ± 0.16^{bc}	1.72 ± 0.14^{bc}
	80 $\mu g/kg \times 2$/day, i.v.	8	1.36 ± 0.24^{bc}	1.00 ± 0.19^{bc}	1.60 ± 0.06^{bc}
+ Azathioprine	20 mg/kg/day, p.o.	8	1.95 ± 0.42^{b}	1.54 ± 0.14	1.56 ± 0.11^{b}

Lipo-prostaglandin E_1 and azathioprine were given i.v. twice a day and p.o. daily to rats, respectively, from the 21st day after injection of anti-glomerular basement membrane serum to the 50th day. Kidney taken on the 50th day for light microscopic study. Values indicate the means ± S.D. and *n* indicates the number of rats used. GCS indicates glomerular cross-section.
[b]Significant difference from the anti-GBM at $P < 0.05$.
[c]Significant difference from the anti-GBM + vehicle at $P < 0.05$.

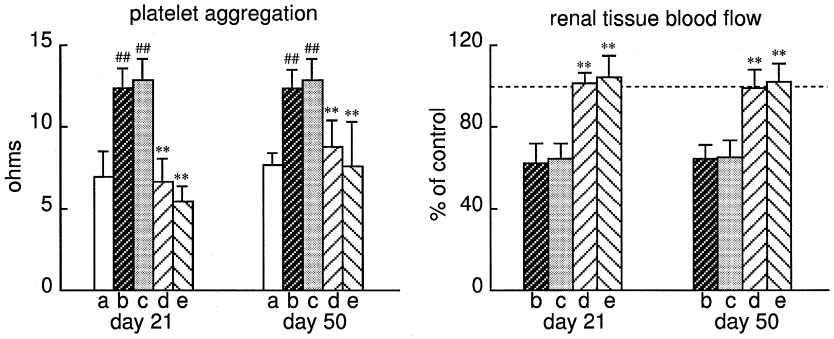


Fig. 4. Effect of a single administration of lipo-prostaglandin E_1 on platelet aggregation and renal tissue blood flow of rats with crescentic-type anti-glomerular basement membrane nephritis. Columns (a) control; columns (b) anti-GBM; columns (c) anti-GBM + vehicle; columns (d) anti-GBM + lipo-PGE_1 (20 μg/kg × 2 per day); columns (e) anti-GBM + lipo-PGE_1 (80 μg/kg × 2 per day). Broken line indicates the renal tissue blood flow of control rats (87.1 ± 12.4 at 20 days and 101.8 ± 13.9 ml/min per 100 g kidney weight at 50 days). Each column denotes the mean with S.D. for 5 rats. ##$P < 0.01$ vs. control. ∗∗ $P < 0.01$ vs. anti-GBM. ++ $P < 0.01$ vs. anti-GBM + vehicle.

three doses used inhibited the increase in plasma creatinine content almost to the control level. Vehicle and azathioprine at 20 mg/kg had no effect on the proteinuria and the plasma creatinine content.

3.1.2. Effects on plasma antibody titer against rabbit τ-globulin (Table 1)

On the 50th day, the elevation of the antibody titer due to nephritis was significantly inhibited by lipo-prostaglandin E_1 (80 μg/kg × 2) ($P < 0.05$ vs. anti-GBM). Azathioprine (20 mg/kg) markedly suppressed the antibody titer ($P < 0.01$ vs. anti-GBM). Vehicle had no effect on the antibody titer.

3.1.3. Histopathological parameters in the glomeruli (Fig. 3 and Table 2)

Regarding histopathological alterations of the glomeruli, lipo-prostaglandin E_1 at 20, 40 and 80 μg/kg × 2 dose dependently reduced the IGL by 30 to 44% (Fig. 3, Table 2). Azathioprine (20 mg/kg) also reduced the IGL by 20%. Vehicle had no effect on the histopathological parameters. Representative micrographs of glomeruli from drug-treated and untreated anti-glomerular basement membrane nephritic rats are given in Fig. 3.

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

In the sections of glomeruli from anti-glomerular basement membrane nephritic rats, linear deposition of rabbit immunoglobulin G (hetero-antibody) and rat immunoglobulin G (auto-antibody) was observed along the glomerular basement membrane. Lipo-prostaglandin E_1 dose dependently reduced the deposition of both rabbit immunoglobulin G and rat immunoglobulin G. Azathioprine (20 mg/kg) markedly reduced only the deposition of rat immunoglobulin G. Vehicle had no effects on these parameters.

3.2. Mechanisms of the antinephritic action of lipo-prostaglandin E_1

3.2.1. Effects of a single administration of lipo-prostaglandin E_1 on platelet aggregation and renal tissue blood flow (Fig. 4)

The effect of lipo-prostaglandin E_1 on collagen-induced platelet aggregation in blood collected from the renal vein was examined on the 20th and the 50th days after injection of anti-glomerular basement membrane serum (Fig. 4). The increase in platelet aggregation in anti-GBM nephritic

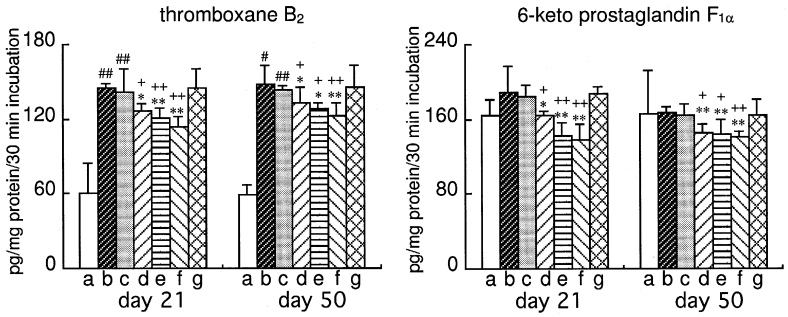


Fig. 5. Effect of a single administration of lipo-prostaglandin E_1 on glomerular thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$ production of rats with crescentic-type anti-glomerular basement membrane nephritis. Columns (a) control; columns (b) anti-GBM; columns (c) anti-GBM + vehicle; columns (d) anti-GBM + lipo-PGE_1 (20 μg/kg × 2 per day); columns (e) anti-GBM + lipo-PGE_1 (40 μg/kg × 2 per day); columns (f) anti-GBM + lipo-PGE_1 (80 μg/kg × 2 per day); columns (g) anti-GBM + azathioprine (20 mg/kg per day). Each column denotes the mean with S.D. for 5 rats. #$P < 0.05$, ##$P < 0.01$ vs. control. ∗ $P < 0.05$, ∗∗ $P < 0.01$ vs. anti-GBM. + $P < 0.05$, ++ $P < 0.01$ vs. anti-GBM + vehicle.

rats on the 20th and the 50th days was markedly inhibited by a single i.v. administration of lipo-prostaglandin E_1 (20 and 80 μg/kg). The decrease in renal tissue blood flow in anti-GBM nephritic animals on both days was markedly reversed by lipo-prostaglandin E_1 (20 and 80 μg/kg). Vehicle had no effect on the platelet aggregation and the renal tissue blood flow.

3.2.2. Effects of a single administration of lipo-prostaglandin E_1 on glomerular thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$ production (Fig. 5)

The effects of lipo-prostaglandin E_1 on glomerular thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$ production were examined at 4 h after the single i.v. administration of test drugs to nephritic rats on the 20th and the 50th day after injection of anti-glomerular basement membrane serum (Fig. 5). Glomerular thromboxane B_2 production in the anti-GBM group was about 2.5-fold higher than that of the control group on the 20th and the 50th day. In contrast, glomerular 6-keto prostaglandin $F_{1\alpha}$ production in anti-GBM nephritic animals was the same as that of control animals. At 4 h after drug treatment, lipo-prostaglandin E_1 at 20, 40 and 80 μg/kg dose dependently inhibited the increase in glomerular thromboxane B_2 production by 16 to 36% on the 20th and the 50th day. In addition, lipo-prostaglandin E_1 at three doses also caused decreases of 11% to 27% in 6-keto prostaglandin $F_{1\alpha}$. Vehicle and azathioprine (20 mg/kg) had no effects on glomerular prostaglandin production.

4. Discussion

In the present study, we evaluated the antinephritic effect of lipo-prostaglandin E_1, prostaglandin E_1 incorporated in lipid microspheres, using an experimental model of nephritis, namely anti-glomerular basement membrane nephritis in rats.

Lipo-prostaglandin E_1 significantly inhibited the development of proteinuria and glomerular histopathological changes when we started drug administration in the autologous phase, when glomerulonephritis was fully developed. The antinephritic effect of lipo-prostaglandin E_1 was more potent than that of azathioprine. Additionally, we demonstrated that a single i.v. administration of lipo-prostaglandin E_1 markedly reversed (1) the decrease in renal tissue blood flow, (2) the increase in platelet aggregation and (3) the increase in glomerular thromboxane B_2 production. Regarding the effect of prostaglandins on arachidonic acid metabolism, Kelley et al. (1986) reported that daily i.d. administration of prostaglandin E_1 suppressed the kidney cortex thromboxane B_2 synthesis in MRL-lpr mice. Friedlander et al. (1983) reported that prostaglandin E_1 (0.1–100 μM) stimulated cyclic AMP generation both in isolated glomeruli and in cultured glomerular cells. Moskowitz et al. (1983) reported that cyclic AMP inhibited *Naja naja* snake phospholipase A_2 activity in vitro. Therefore, it seems reasonable to consider that lipo-prostaglandin E_1 may reduce arachidonic acid metabolism by suppressing the activation of phospholipase A_2 via the increase in cyclic AMP, although the precise mechanism by which lipo-prostaglandin E_1 decreased thromboxane B_2 production remains unclear.

Furthermore, lipo-prostaglandin E_1 significantly reduced the elevation of antibody titer and the deposition of both rabbit immunoglobulin G and rat immunoglobulin G on glomerular basement membrane. Because azathioprine markedly inhibited the elevation of antibody titer and reduced only the deposition of rat immunoglobulin G, we consider that a reduced antibody titer may lead to less deposition of glomerular rat immunoglobulin G. However, the decrease in glomerular rabbit immunoglobulin G was not related to the decrease in auto-antibody levels. Mattana and Singhal (1992) reported that a thromboxane A_2 analogue enhanced immune complex uptake by cultured mesangial cells. Nagamatsu et al. (1994) also examined the effect of thromboxane A_2 receptor antagonists on the clearance of glomerular aggregated bovine serum albumin and suggested that thromboxane A_2 acts by delaying the disposal of macromolecules deposited in the glomeruli in vivo. Furthermore, they demonstrated that prostaglandin E_1 accelerated the clearance of glomerular aggregated bovine serum albumin (Nagamatsu and Suzuki, 1989b). Therefore, the decrease in glomerular rabbit immunoglobulin G deposition may be due to an increase in the ability to eliminate glomerular fixed hetero-antibody (rabbit immunoglobulin G) by lipo-prostaglandin E_1 itself and/or by reducing thromboxane A_2 generation.

In the present study, lipo-prostaglandin E_1 administered from the autologous phase had a beneficial effect on glomerulonephritis. These results suggest that lipo-prostaglandin E_1 may be a useful agent for the treatment of glomerulonephritis. It acts via inhibition of platelet aggregation, restoration of decreased renal tissue blood flow and amelioration of the abnormal production of arachidonic acid metabolites, thus decreasing the effect of glomerular rabbit immunoglobulin G.

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