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EFFECTS OF ASPIRIN, PREDNISOLONE AND INDOMETHACIN ON NEPHROTOXIC SERUM NEPHRITIS IN THE RAT

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1 The effects of aspirin, prednisolone, and indomethacin on nephrotoxic serum nephritis in rats was studied. The nephritis was induced by a single intravenous injection of nephrotoxic serum (NTS, rabbit anti-serum against the water-soluble renal antigen of the rat). The injection of NTS induced the heterologous phase of proteinuria (within a day after NTS injection) and then the autologous phase (5 to 7 days after NTS injection). The effect of drugs given before the NTS (i.e. prophylactically) or after the NTS (i.e. therapeutically) was investigated.

2 Aspirin, which was given orally at doses of 150 and 250 mg/kg daily from the day before NTS injection, suppressed the development of proteinuria in both the heterologous and the autologous phase, and lowered the serum cholesterol level towards the normal level. Aspirin (250 mg/kg daily, orally) had no significant effect against the established proteinuria in the autologous phase.

3 Prednisolone, which was given orally at doses of 3 and 5 mg/kg daily from the day before NTS injection, elevated the proteinuria in the heterologous phase, while inhibiting the development of proteinuria in the autologous phase. Prednisolone (5 mg/kg daily, orally) was ineffective against established proteinuria in the autologous phase.

4 Indomethacin (3 mg/kg daily, orally) did not exert any significant effect on proteinuria in either the heterologous or the autologous phase.

Introduction

Many steroidal and nonsteroidal anti-inflammatory drugs have been studied for their anti-nephritic effects in various experimental models (Hackel, Portfolio & Kinney, 1950; Heymann, Hunter & Hackel, 1962; Lim & Spargo, 1973, Feenstra, Lee, Greben, Arends & Hoedemaeker, 1975; Suzuki, Ito & Nagamatsu, 1978). However, so far as we know, there have been few reports as to the beneficial effects of aspirin on experimental nephritis.

In the present study, the anti-nephritic effects of aspirin were examined in the rats with nephrotoxic serum nephritis induced by a single intravenous injection of nephrotoxic serum (NTS, rabbit anti-serum against water-soluble renal antigen of the rat) and compared with those of prednisolone and indomethacin.

Methods

Animals

Male rats of the Wistar strain weighing 200–230 g at the beginning of the experiment were used.

Preparation of water-soluble renal antigens

Water-soluble renal antigens were prepared by the method of Shibata, Nagasawa, Takuma, Naruse & Miyakawa (1966). Perfused renal cortical tissue of the rat was ground with 0.9% w/v NaCl solution (saline) and buffered at pH 8.2 with 0.1 M sodium borate. Fifty mg of crystalline trypsin was added to about 10 g (wet weight) of cortical tissue and the mixture was incubated for 3 h at 37°C. The mixture was then heated at 60°C for 30 min to inactivate the trypsin and was centrifuged at 52,000 *g* for 35 min and the supernatant fluid was dialysed and lyophilized. This is referred to as TR-KCH.

Preparation of nephrotoxic serum

TR-KCH was injected into the femoral muscle of the rabbits (3 mg TR-KCH/rabbit with complete Freund's adjuvant) once a week for 5 weeks. Ten days after the last injection, the rabbits were bled through the carotid artery, and the sera were collected. The pooled sera were decomplemented by heating at 56°C for 30 min.

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This anti-serum against TR-KCH was designated nephrotoxic serum (NTS).

Induction of nephrotoxic serum nephritis

Nephrotoxic serum nephritis was induced in rats by a single intravenous injection of NTS. Nephrotoxic serum nephritis can be divided into two phases which are dependent on different pathogenic mechanisms (Unanue & Dixon, 1967). The heterologous phase, which is induced within a day after NTS injection, results from the interaction of the heterologous γ-globulin contained in NTS with glomerular antigens. While, the autologous phase, which develops 5 to 7 days after NTS injection, is dependent on the immunological response of the host to the heterologous γ-globulin. The injection of NTS at a dose of 0.5 ml/rat induced the heterologous phase proteinuria (on the average, 50 to 100 mg/24 h) and the autologous phase proteinuria (on the average, >150 mg/24 h).

Measurement of urinary protein

Rats were placed in individual metabolism cages with free access to food and water, and the urine excreted was collected for a period of 24 h; 3 ml of 5% TCA (trichloro-acetic acid) was added to 0.5 ml of urine and the aggregated protein was precipitated by centrifugation (3,000 rev/min for 6 min). The precipitated protein was determined by the biuret method.

Determination of serum cholesterol

Total serum cholesterol content was determined according to the method of Klose, Greif & Hagen (1975).

Assessment of renal function

Glomerular filtration rate (GFR) and renal plasma flow (RPF) were measured simultaneously on the basis of renal clearance of inulin and *p*-amino hippuric acid (PAH), respectively, by the method of Yamamoto, Yamazoe & Ueda (1962). Immediately after NTS injection (0.5 ml/rat), an aqueous solution of inulin (150 mg/ml water, 0.5 ml/100 g body weight) was given subcutaneously into one side of the gluteal area of the rat, and an oil emulsion of PAH (80 mg/ml emulsion of cotton seed oil and gum arabic, 0.5 ml/100 g body weight) was given subcutaneously into the other side of the gluteal area of the same rat under ether anaesthesia. At the same time, the rat was given orally 5 ml of water per 100 g of body weight. Thirty min after inulin and PAH administration, the bladder urine was excreted by pressing the pubic area; then the animal was placed in a metabolism cage and the urine excreted for 1 h was collected. The blood was taken from the tail at the beginning and the end of the period of urine collection.

The inulin concentrations in urine and blood were determined following the method described by Schreiner (1950), and the PAH concentrations were estimated by the method of Smith, Finkelstein, Aliminosa, Crawford & Graber (1945).

Drugs

Drugs used in this study were aspirin (Ono Yakuhin Co., Ltd), prednisolone (Nakarai Chemicals Co., Ltd), and indomethacin (Sumitomo Chemical Co., Ltd). All drugs were suspended in 5% solution of gum arabic and all were administered orally to rats in a volume of 0.5 ml per 100 g body weight.

Statistical analysis

Statistical analyses were performed according to Student's *t* test.

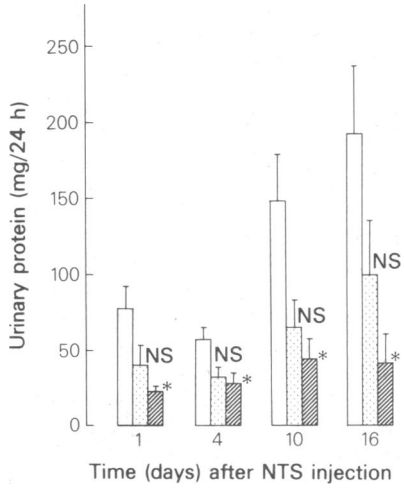

Figure 1 Effect of aspirin (prophylactic administration) on proteinuria in nephrotoxic serum (NTS) nephritis in the rat. Doses of aspirin (150 and 250 mg/kg) were administered orally once a day from the day before the NTS injection. The mean value of urinary protein in each group of rats is shown by the height of each column: Open columns = control (vehicle), $n=20$; stippled columns = aspirin (150 mg/kg daily), $n=10$; hatched columns = aspirin (250 mg/kg daily), $n=10$. Vertical lines show s.e.mean. Significantly different from control: $^*P<0.05$. NS: not significant.

Results

Effects of aspirin on nephrotoxic serum nephritis

Prophylactic administration Aspirin (150 and 250 mg/kg daily) was administered to rats from one day before NTS injection to 16 days after NTS injection (for 18 days). Urinary protein excreted over 24 h was determined on the 1st, 4th, 10th and 16th day after NTS injection. On the 17th day after NTS injection, animals were bled under ether anaesthesia for evaluation of the serum cholesterol level, and the kidneys were excised.

As shown in Figure 1, the treatment with aspirin (150 and 250 mg/kg daily) decreased urinary protein in both the heterologous and the autologous phase. The difference between the mean values for the urinary protein excreted in rats treated with 250 mg/kg of aspirin and in control rats was statistically significant ($P<0.05$) at all points of urinary protein determination, but the difference between control and animals receiving 150 mg/kg of aspirin was not statistically significant at the 5% level.

The mean kidney weight in rats with nephrotoxic serum nephritis was not significantly different from that of normal rats. In rats treated with aspirin (150 and 250 mg/kg daily), the kidney weight was equal to that of normal rats.

The serum level of total cholesterol in rats with nephrotoxic serum nephritis (92 ± 12.5 mg/dl) was significantly higher than that of normal rats (55 ± 1.63 mg/dl). Aspirin treatment lowered the cholesterol level dose-dependently towards the normal level, but the effect was not statistically significant at the 5% level.

Therapeutic administration Aspirin (250 mg/kg daily) was administered to rats with established proteinuria from the 15th day after NTS injection for 10 days. The protein content of urine excreted over a 24 h period was determined one day before and 2 and 10 days after the initiation of the aspirin treatment.

Aspirin slightly reduced the degree of proteinuria, but its effect was not statistically significant at the 5% level.

Effect of prednisolone on nephrotoxic serum nephritis

Prophylactic administration Prednisolone (3 mg/kg daily) was administered to rats from one day before the NTS injection to 29 days after the NTS injection (for 31 days). The urine excreted over a 24 h period was collected on the 1st, 4th, 7th, 10th, 14th, 21st and 28th day after the NTS injection. On the 30th day after NTS injection, rats were bled under ether anaesthesia and the kidneys were excised.

Five mg/kg of prednisolone was also administered to rats from one day before the NTS injection, and the protein content of urine excreted over a 24 h period was determined only on the 1st day after NTS injection. However, further experiments could not be carried out with these rats because 5 mg/kg of prednisolone made the animals susceptible to infection.

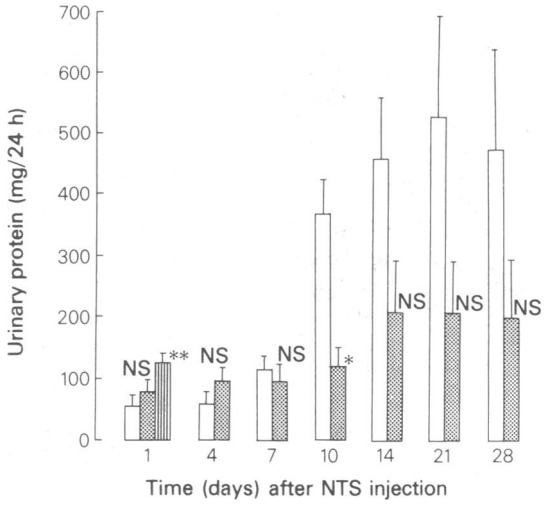

Figure 2 Effect of prednisolone (prophylactic administration) on proteinuria in nephrotoxic serum (NTS) nephritis in the rat. Doses of prednisolone (3 and 5 mg/kg) were administered orally once a day from the day before the NTS injection. The mean value of urinary protein in each group of rats is shown by the height of each column: Open columns = control (vehicle); $n=8$; stippled columns = prednisolone (3 mg/kg daily), $n=7$; striped column = prednisolone (5 mg/kg daily), $n=7$. Vertical lines show s.e.mean. Significantly different from control: $^*P<0.05$; $^{**}P<0.01$; NS: not significant.

As shown in Figure 2, rats treated with prednisolone (3 mg/kg daily) excreted more urinary protein than control rats in the heterologous phase (1st and 4th day following NTS injection), but this difference was not statistically significant at the 5% level. This tendency became more marked in rats receiving 5 mg/kg of prednisolone (statistically significant at the 1% level). On the other hand, the administration of prednisolone (3 mg/kg daily) suppressed the development of proteinuria in the autologous phase. This effect was statistically significant at the 10th day after NTS injection ($P<0.05$), but not significant at the 14th, 21st and 28th day.

The mean kidney weight in rats with nephrotoxic serum nephritis (0.44 g/100 g body weight) was significantly higher than that in normal rats (0.34 g/100 g body weight) at the 5% level. The treatment with prednisolone (3 mg/kg daily) suppressed the increase of kidney weight, but this suppression was not statistically significant at the 5% level.

The serum cholesterol level in rats with nephrotoxic serum nephritis (137 ± 32.6 mg/dl) was lowered by prednisolone treatment to 92 ± 29.6 mg/dl. This effect was not statistically significant at the 5% level.

Therapeutic administration Prednisolone (3 mg/kg daily) was administered to rats with established proteinuria from the 15th day after NTS injection (for 14 days). The urine excreted over a period of 24 h was obtained one day before and 7 and 10 days after the initiation of treatment.

The treatment with prednisolone for 14 days did not exert a significant effect on established proteinuria.

Effects of indomethacin on nephrotoxic serum nephritis

Prophylactic administration Indomethacin (3 mg/kg daily) was administered to rats from one day before the NTS injection to 29 days after the NTS injection (for 31 days). The urine excreted over a period of 24 h was collected on the 1st, 4th, 7th, 10th, 14th, 21st and 28th day after the NTS injection. On the 30th day after NTS injection, rats were bled under ether anaesthesia and the kidney was excised. The results are shown in Figure 3. The amount of urinary protein in both the heterologous and the autologous phase was not reduced by the treatment with indomethacin (3 mg/kg daily).

Treatment with indomethacin did not suppress the increase in kidney weight and elevation of serum cholesterol level.

Therapeutic administration Indomethacin (3 mg/kg daily) was administered to rats with established proteinuria in the autologous phase from 15 days after NTS injection (for 10 days). The urine excreted over a period of 24 h was collected one day before and 2 and 10 days after the start of indomethacin treatment.

Treatment with indomethacin (3 mg/kg daily) for 10 days did not significantly reduce the degree of proteinuria.

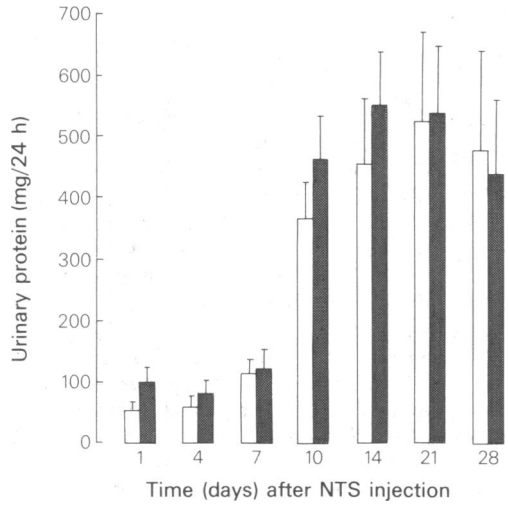

Figure 3 Effect of indomethacin (prophylactic administration) on proteinuria in nephrotoxic serum (NTS) nephritis in the rat. Indomethacin (3 mg/kg) was administered orally once a day from the day before the NTS injection. The mean value of urinary protein in each group of rats is shown by the height of each column: Open columns = controls (vehicle), $n = 8$; shaded columns = indomethacin (3 mg/kg daily), $n = 7$. Vertical lines show s.e.mean.

Effects of drugs on renal function

Test drugs (aspirin, 250 mg/kg; prednisolone, 5 mg/kg; indomethacin, 3 mg/kg) were administered orally at 24 and 3 h before the assessment of renal function (GFR and RPF). The results are summarized in Table 1. The mean values for GFR and RPF were essentially similar between control and aspirin-treated rats. On the other hand, in rats given prednisolone, the mean values for both GFR and RPF were significantly higher than those in the control group (for GFR, $P < 0.01$; for RPF, $P < 0.05$). In rats given indomethacin, the mean GFR was significantly higher than that in the control group ($P < 0.05$).

Table 1 Effects of drugs on renal function of the rat injected with nephrotoxic serum

Drug†	*Dose*	n	*GFR* (ml/min per 100 g body wt.)	*RPF* (ml/min per 100 g body wt.)
Vehicle (control)	–	6	0.79 ±0.04	2.21 ±0.17
Aspirin	250 mg/kg	6	0.90 ±0.07	2.15 ±0.32
Prednisolone	5 mg/kg	7	1.02** ±0.06	3.37* ±0.33
Indomethacin	3 mg/kg	7	0.93* ±0.05	2.89 ±0.32

†Drugs were administered one day and 3 h before the start of assessment of renal function
GFR: glomerular filtration rate; RPF: renal plasma flow.
Values are means ± s.e. Significantly different from control: *$P < 0.05$; **$P < 0.01$.

Discussion

The anti-nephritic effects of aspirin, prednisolone and indomethacin were examined in rats with nephrotoxic serum nephritis. Pretreatment with aspirin (150 and 250 mg/kg daily, orally) clearly prevented the development of proteinuria in both the heterologous and the autologous phase of rat nephrotoxic serum nephritis (Figure 1). There have been few reports as to the favourable effects of aspirin on experimental nephritis. Sternberg, Peyroux, Grochulski, Engler, Feret, Moisy, Lagrue & Jayle (1974) found that a large dose of lysine acetyl salicylate (500 mg/kg, orally) suppressed the proteinuria in the autologous phase, but significantly aggravated it in the heterologous phase of rat nephrotoxic serum nephritis. This disagreement between their results and ours as to the effect of aspirin on the heterologous phase of proteinuria may be due to different experimental conditions. Sternberg *et al.* prepared anti-rat kidney γ-globulins from rabbits immunized with insoluble rat kidney homogenate, whereas, we prepared NTS from rabbits immunized with water-soluble renal antigen.

One possibility is that aspirin might have altered the renal haemodynamics and consequently reduced the protein excretion in urine. To verify this, the effects of aspirin on GFR and RPF were estimated in the rat injected with NTS. In this experiment, aspirin showed no effect on GFR and RPF at a dose of 250 mg/kg daily, a dose which suppressed the proteinuria significantly. This result suggests that aspirin did not reduce the development of proteinuria by altering the renal haemodynamics but by preventing some of the pathological processes. This suggestion was also supported by the fact that administration of aspirin (250 mg/kg daily) showed only a slight effect on established proteinuria. Elevation of serum cholesterol level suggesting nephrotic disorder was observed in rats with nephrotoxic serum nephritis. The treatment with aspirin reduced this elevation, but its effect was not statistically significant.

The manner of action of prednisolone was distinct from that of aspirin. Treatment with prednisolone (3 and 5 mg/kg daily, orally) starting the day before NTS injection, enhanced rather than suppressed the development of proteinuria in the heterologous phase, although it significantly suppressed it in the autologous phase (Figure 2). It is not clear why prednisolone (3 and 5 mg/kg daily) aggravated the proteinuria in the heterologous phase. Similar effects have also been reported for ACTH and cortisone (Hackel *et al.*, 1950). GFR and RPF in the rat injected with NTS were significantly increased by prednisolone (Table 1). Part of the effect on proteinuria in the heterologous phase may be attributed to its influence on renal haemodynamics.

Prednisolone might have prevented the development of the autologous phase of proteinuria not by suppressing the inflammatory process itself but by preventing the formation of antibody against rabbit immunoglobulins fixed to glomerular capillaries since prednisolone could not reduce the urinary protein in the heterologous phase. This speculation is supported by the results showing that steroids prevented the development of proteinuria in autologous immune complex nephritis induced by an injection of antigens (Heymann *et al.*, 1962) but could not suppress the proteinuria in heterologous immune complex nephritis which was induced by an injection of preformed heterologous antibody directed against antigens present in the brushborder of proximal tubles of the rat kidney (Feenstra *et al.*, 1975).

Prednisolone also reduced the elevation of serum cholesterol concentration and the increase in kidney weight at the 30th day after NTS injection but these effects were not statistically significant at the 5% level.

The effects of indomethacin on experimental nephritis in the rat reported hitherto are not always consistent (Kupor, Lawance & McPhaul, 1976; Suzuki *et al.*, 1978; Sessa, Cioffi, Conte, Saruggia, Di Belgiojoso & Donati, 1978). In our experiments, prophylactic administration of indomethacin (3 mg/kg daily) could not prevent the development of proteinuria in either the heterologous phase or the autologous phase. For indomethacin, the daily dose of 3 mg/kg was the highest dose which did not affect the body weight gain of the rat.

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