PHARMACOLOGICAL STUDIES ON EXPERIMENTAL NEPHRITIC RATS (2). ANTINEPHRITIC EFFECT OF VARIOUS DRUGS ON MASUGI'S NEPHRITIS

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Abstract—Masugi's nephritis was induced in rats by a single i.v. injection of anti-kidney serum from rabbits immunized with the homogenate of rat whole kidneys. The antinephritic effect of drugs was evaluated through determination of biochemical parameters such as contents of protein and enzymes excreted into the urine and serum cholesterol content by the preadministration and intermittent administration tests. In the preadministration test, of test drugs, betamethasone ($0.2 \text{ mg/kg} \pm 3 \text{ p.o.}$) and azathioprine ($25 \text{ mg/kg} \pm 3 \text{ p.o.}$) showed an antinephritic effect. Betamethasone in particular resulted in normalization of urine and serum parameters. In the intermittent test, all drugs tested were effective. Significant recovery effects were observed with betamethasone ($0.1 \text{ mg/kg} \pm 6 \text{ p.o.}$), prednisolone ($5 \text{ mg/kg} \pm 6 \text{ p.o.}$), azathioprine ($30 \text{ mg/kg} \pm 6 \text{ p.o.}$), tranexamic acid ($200 \text{ mg/kg} \pm 6 \text{ i.p.}$) and cetraxate ($100 \text{ mg/kg} \pm 6 \text{ i.p.}$) on urinary protein excretion and with all test drugs including indomethacin ($5 \text{ mg/kg} \pm 6 \text{ p.o.}$) and cyclophosphamide ($2.5 \text{ mg/kg} \pm 6 \text{ p.o.}$) on urinary enzyme excretions. Serum cholesterol levels reverted to normal levels after administration of betamethasone. Using this nephritic model, the antinephritic effect of drugs could be tentatively evaluated.

As one of experimental models of nephritis, renal injury produced by chemical materials such as mercuric chloride, uranium, potassium chromate, cantaridine and arsenic acid has hitherto been employed. Although these models produce results similar to cases of acute renal insufficiency, results are different in the case of glomerulonephritis. Since Masugi (1) succeeded in producing an experimental nephritic model by the injection of anti-kidney serum into rats or rabbits in 1934, pathologists and clinicians have concentrated on the studies of nephritic models based on immune mechanisms (2-5). We previously reported that in addition to protein, enzymes such as alkaline phosphatase and lactic dehydrogenase were markedly excreted into urine in Masugi's type of nephritis in rats (6, 7). The present study was designed to estimate the antinephritic effect of various drugs by employing the same Masugi model.

MATERIALS AND METHODS

Experimental nephritis

Experimental nephritis was produced in male Wistar rats weighing about 120 g by the i.v. injection of 0.7 ml/100 g of body weight of anti-rat kidney rabbit serum (anti-kidney serum) by the method described in a previous paper (6).

Evaluation of antinephritic effect of drugs

The antinephritic effect of drugs was tested in two ways. In the first experiment, each test drug was given to a group of 8 rats once a day for 3 consecutive days from the 2nd day before the injection of anti-kidney serum. Each drug was dissolved in 0.9% saline or suspended in saline solution of 1% gum arabic and given p.o. or i.p. in a volume of 1 ml/100 g of body weight. In addition to the above drug-treated nephritic (test drug) group, the nephritic (control) and non-nephritic (normal) groups were set in the experiment. The control group was given i.v. 0.7 ml/100 g of body weight of anti-kidney serum and 0.9% saline of the same volume instead of test drug. The normal group was given i.v. 0.7 ml/100 g of the serum only from normal rabbits instead of anti-kidney serum. The 24 hr urine samples from the 9th to the 10th days after the anti-kidney serum injection were collected and on the 10th day, blood samples were obtained. Though determination of biochemical parameters in the urine and serum (urine: protein content and alkaline phosphatase and lactic dehydrogenase activities; serum: cholesterol content) according to the methods (6) as previously described, the effect of each test drug was evaluated. This is referred to as a preadministration test.

In the second experiment, each test drug was given to a group of 8 rats once daily $\times 3$ before the anti-kidney serum injection. For the following 3 days, each test drug was withheld and then given again once daily $\times 3$ on the 4th, 5th and 6th days in the same doses as before. On the 10th day, the rats were sacrificed and several biochemical parameters in the urine and serum were determined as in the preadministration test. This is referred to as the intermittent administration test.

The efficacy of each test drug was expressed as a percent recovery as shown in Tables. Student's t-test was used for statistical evaluations.

Drugs

The following drugs were applied. Steroidal anti-inflammatory agents: Prednisolone (Toyo Jozo Co., Ltd.) and betamethasone (Toyo Jozo Co., Ltd.). Immunosuppressive agents: Azathioprine (Tanabe Seiyaku Co., Ltd.) and cyclophosphamide (endoxan, Shionogi Seiyaku Co., Ltd.). Antiplasmins: Tranexamic acid (Daiichi Seiyaku Co., Ltd.) and cetraxate (DV-1006, Daiichi Seiyaku Co., Ltd.) (8). A non-steroidal anti-inflammatory agent: Indomethacin (Merck-Banyu Co., Ltd.).

RESULTS

Antinephritic effects of drugs in preadministration test

Effects on the urinary excretion of protein: As shown in Table 1, the urinary excretion of protein (mg/24 hr urine) in the control group brought about a sharp increase of 1917.6% in comparison with the normal group. Betamethasone (0.2 mg/kg $\times 3$ p.o.), a steroidal anti-inflammatory agent, resulted in a significant recovery effect of about 30% on the urinary excretion of protein. However, prednisolone (10 mg/kg $\times 3$ p.o.), another steroid, was ineffective. Of two immunosuppressive agents, only azathioprine (25 mg/kg $\times 3$ p.o.) exhibited a recovery effect of about 70%, being the most potent of the drugs tested. With

TABLE 1. Effects of various drugs in the preadministration test on the urinary excretion of protein in experimental nephritic rats

Groups	Dose mg/kg × 3	mg/24 hr urine	% recovery
Normal group		11.4 ± 2.6	
Control group		230.0 ± 40.7	(1917.6)
Test drug groups			
Prednisolone	10 p.o.	194.3 ± 34.4	16.3
Betamethasone	0.2 p.o.	158.2 ± 20.1*	32.8
Azathioprine	25 p.o.	82.5 ± 12.3***	67.5
Cyclophosphamide	10 p.o.	207.4 ± 36.8	10.3
Tranexamic acid	200 i.p.	264.0 ± 42.6	-15.5
Cetraxate	100 i.p.	180.2 ± 37.8	22.8
Indomethacin	5 p.o.	174.4 ± 30.8	25.4

Results are mean ± S.E. obtained from 8 rats. % recovery = $\frac{C-T}{C-N} \times 100$ (C: control, T: test drug, N: normal). The number in parenthesis indicates percent increase of control to normal. Asterisk indicates a significant difference from control (***: $P < 0.001$, *: $P < 0.05$).

tranexamic acid and cetraxate, antiplasmin agents and indomethacin, a non-steroidal anti-inflammatory agent, there was no significant recovery.

Effects on the urinary excretions of alkaline phosphatase and lactic dehydrogenase: As indicated in Table 2, the urinary excretion of alkaline phosphatase (Bessey-Lowry unit/24 hr urine × 10²) in control group induced a marked increase of 1060.0% in comparison with normal group. Both betamethasone (0.2 mg/kg × 3 p.o.) and azathioprine (25 mg/kg × 3 p.o.) which were effective against the proteinuria also exhibited a significant recovery effect on the urinary excretion of this enzyme. Indomethacin (5 mg/kg × 3 p.o.) also exerted a significant recovery effect.

TABLE 2. Effects of various drugs in the preadministration test on the urinary excretions of alkaline phosphatase and lactic dehydrogenase in experimental nephritic rats

Groups	Dose mg/kg × 3	Alkaline phosphatase		Lactic dehydrogenase	
		Bessey-Lowry unit /24 hr urine × 10 ²	% recovery	Hill unit/24 hr urine × 10 ²	% recovery
Normal group		0.5 ± 0.1		14.1 ± 1.7	
Control group		5.8 ± 1.1	(1060.0)	99.9 ± 15.9	(608.6)
Test drug groups					
Prednisolone	10 p.o.	4.1 ± 0.8	32.1	66.7 ± 10.1	38.7
Betamethasone	0.2 p.o.	3.1 ± 0.4*	50.9	31.9 ± 5.3**	79.3
Azathioprine	25 p.o.	2.8 ± 0.3**	56.6	85.1 ± 15.3	17.2
Cyclophosphamide	10 p.o.	3.7 ± 0.7	39.6	62.4 ± 12.4	43.7
Tranexamic acid	200 i.p.	5.8 ± 1.4	0	72.2 ± 13.8	32.3
Cetraxate	100 i.p.	3.8 ± 0.6	37.7	99.9 ± 18.1	0
Indomethacin	5 p.o.	3.2 ± 0.2*	49.1	43.0 ± 8.0*	66.3

Asterisk indicates a significant difference from control (**: $P < 0.01$, *: $P < 0.05$). Other explanations as in Table 1.

The increase of urinary excretion of lactic dehydrogenase (Hill unit/24 hr urine $\times 10^3$) induced by nephritis was less than that of alkaline phosphatase, being 608.6% higher than normal level. Betamethasone (0.2 mg/kg $\times 3$ p.o.) and indomethacin (5 mg/kg $\times 3$ p.o.) induced a recovery effect of over 60% on the excretion of this enzyme.

Effects on serum cholesterol content: As shown in Table 3, the cholesterol content in serum (mg/dl) of the control group was 115.5% higher than that of normal group. None of test drugs showed a significant recovery effect. With betamethasone and azathioprine, there was only a slight tendency toward recovery.

Antinephritic effects of drugs in intermittent administration test

Effects on the urinary excretion of protein: As given in Table 4, the urinary excretion of protein (mg/24 hr urine) in the control group induced a marked increase of 1599.1% as compared with normal group. Prednisolone (5 mg/kg $\times 6$ p.o.) and betamethasone (0.1

TABLE 3. Effects of various drugs in the preadministration test on serum cholesterol level in experimental nephritic rats

Groups	Dose mg/kg $\times 3$	mg/dl	% recovery
Normal group		68.4 $\pm$ 4.2	
Control group		147.4 $\pm$ 21.5	(115.5)
Test drug groups			
Prednisolone	10 p.o.	144.9 $\pm$ 15.1	3.2
Betamethasone	0.2 p.o.	103.9 $\pm$ 16.8	55.1
Azathioprine	25 p.o.	104.3 $\pm$ 20.2	54.6
Cyclophosphamide	10 p.o.	136.9 $\pm$ 18.2	13.3
Tranexamic acid	200 i.p.	145.3 $\pm$ 21.0	2.7
Cetraxate	100 i.p.	134.9 $\pm$ 11.8	15.8
Indomethacin	5 p.o.	181.0 $\pm$ 14.3	-42.5

Explanations as in Table 1.

TABLE 4. Effects of various drugs in the intermittent administration test on the urinary excretion of protein in experimental nephritic rats

Groups	Dose mg/kg $\times 6$	mg/24 hr urine	% recovery
Normal group		11.1 $\pm$ 1.4	
Control group		188.6 $\pm$ 30.1	(1599.1)
Test drug groups			
Prednisolone	5 p.o.	68.3 $\pm$ 13.6***	67.8
Betamethasone	0.1 p.o.	63.7 $\pm$ 15.3***	70.4
Azathioprine	30 p.o.	102.3 $\pm$ 14.9*	48.6
Cyclophosphamide	2.5 p.o.	159.6 $\pm$ 20.8	16.3
Tranexamic acid	200 i.p.	75.8 $\pm$ 11.6**	63.5
Cetraxate	100 i.p.	110.1 $\pm$ 16.7*	44.2
Indomethacin	5 p.o.	185.3 $\pm$ 34.3	1.6

Asterisk indicates a significant difference from control (***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$). Other explanations as in Table 1.

mg/kg $\times$ 6 p.o.) resulted in a potent recovery effect of about 70% on the urinary excretion of protein. Tranexamic acid (200 mg/kg $\times$ 6 i.p.) was effective next to both steroids. Azathioprine (30 mg/kg $\times$ 6 p.o.) and cetraxate (100 mg/kg $\times$ 6 i.p.) had significant recovery effects of over 40%.

Effects on urinary excretions of alkaline phosphatase and lactic dehydrogenase: As seen in Table 5, the urinary excretion of alkaline phosphatase (Bessey-Lowry unit/24 hr urine $\times 10^2$) in control group caused a strong increase of 500.0% in comparison with normal group. With all test drugs, except for prednisolone, significant recovery effects were recognized on the excretion of this enzyme. Betamethasone (0.1 mg/kg $\times$ 6 p.o.), tranexamic acid (200 mg/kg $\times$ 6 i.p.) and cetraxate (100 mg/kg $\times$ 6 i.p.) in particular resulted in normalization of this parameter.

TABLE 5. Effects of various drugs in the intermittent administration test on the urinary excretions of alkaline phosphatase and lactic dehydrogenase in experimental nephritic rats

Groups	Dose mg/kg $\times$ 6	Alkaline phosphatase		Lactic dehydrogenase	
		Bessey-Lowry unit /24 hr urine $\times 10^2$	% recovery	Hill unit/24 hr urine $\times 10^2$	% recovery
Normal group		0.6 $\pm$ 0.4		11.4 $\pm$ 6.8	
Control group		3.6 $\pm$ 0.8	(500.0)	32.2 $\pm$ 11.8	(182.5)
Test drug groups					
Prednisolone	5 p.o.	3.2 $\pm$ 0.6	13.3	15.5 $\pm$ 3.0*	80.3
Betamethasone	0.1 p.o.	0.7 $\pm$ 0.7***	96.7	11.2 $\pm$ 4.4**	101.0
Azathioprine	30 p.o.	1.8 $\pm$ 0.2*	60.0	22.0 $\pm$ 7.2	49.0
Cyclophosphamide	2.5 p.o.	2.1 $\pm$ 0.6*	50.0	20.1 $\pm$ 9.4	58.2
Tranexamic acid	200 i.p.	0.5 $\pm$ 0.3***	103.3	25.0 $\pm$ 7.8	34.6
Cetraxate	100 i.p.	0.7 $\pm$ 0.8***	96.7	22.3 $\pm$ 11.3	47.6
Indomethacin	5 p.o.	1.2 $\pm$ 0.5**	80.0	25.4 $\pm$ 9.0	32.7

Asterisk indicates a significant difference from control (***: $P<0.001$, **: $P<0.01$, *: $P<0.05$). Other explanations as in Table 1.

TABLE 6. Effects of various drugs in the intermittent administration test on serum cholesterol level in experimental nephritic rats

Groups	Dose mg/kg $\times$ 6	mg/dl	% recovery
Normal group		70.8 $\pm$ 3.2	
Control group		155.2 $\pm$ 25.9	(119.2)
Test drug groups			
Prednisolone	5 p.o.	133.2 $\pm$ 14.2	26.1
Betamethasone	0.1 p.o.	67.8 $\pm$ 10.8**	103.6
Azathioprine	30 p.o.	110.3 $\pm$ 19.7	53.2
Cyclophosphamide	2.5 p.o.	153.7 $\pm$ 23.0	1.8
Tranexamic acid	200 i.p.	112.2 $\pm$ 13.3	50.9
Cetraxate	100 i.p.	95.3 $\pm$ 20.1	71.0
Indomethacin	5 p.o.	123.1 $\pm$ 13.8	38.0

Asterisk indicates a significant difference from control at $P<0.01$. Other explanations as in Table 1.

On the other hand, the urinary excretion of lactic dehydrogenase (Hill unit/24 hr urine $\times 10^2$) due to nephritis was only the increase of 182.5% against normal level. Both steroids, prednisolone (5 mg/kg $\times$ 6 p.o.) and betamethasone (0.1 mg/kg $\times$ 6 p.o.) showed recovery effects more than 80% on this enzyme excretion. Cyclophosphamide (2.5 mg/kg $\times$ 6 p.o.), azathioprine (30 mg/kg $\times$ 6 p.o.) and cetraxate (100 mg/kg $\times$ 6 i.p.) exhibited only a tendency of recovery effects.

Effects on serum cholesterol content: As illustrated in Table 6, the cholesterol content in serum (mg/dl) of control group was 119.2% more than that of normal group. Serum cholesterol levels returned to normal levels with betamethasone (0.1 mg/kg $\times$ 6 p.o.). Of other test drugs, azathioprine (30 mg/kg $\times$ 6 p.o.), tranexamic acid (200 mg/kg $\times$ 6 i.p.) and cetraxate (100 mg/kg $\times$ 6 i.p.) exhibited only the tendency of recovery effects.

DISCUSSION

Effects of drugs on parameters which clearly indicate the development of nephritis are required for correct evaluations of antinephritic effects. Of several biochemical parameters determined in the present experiments, the urinary excretions of protein and enzymes such as alkaline phosphatase and lactic dehydrogenase appear to be the most suitable for this purpose. Among the test drugs, betamethasone, a steroid, and azathioprine, an immunosuppressant, showed a potent antinephritic effect by inhibiting the changes in these parameters due to nephritis, in the preadministration test. This effect of both drugs may be partially due to the inhibition of the injury of renal tissue subsequent to antigen-antibody reaction after administration of anti-kidney serum.

On the other hand, following the intermittent administration, all drugs tested were effective against nephritis. Azathioprine was considerably effective in both tests, while cyclophosphamide had a moderate effect, in the intermittent administration test. Miyakawa et al. (9) investigated the effects of prednine and cyclophosphamide on the chemical composition of glomerular basement membrane by using the model of nephrotoxic nephritis in rats and reported that an immunosuppressant exhibited more therapeutic effects than did a steroid. Tranexamic acid and cetraxate, antiplasmin agents, showed a significant antinephritic action only through the intermittent administration test. Edward et al. (10) showed that plasmin activity in plasma and urine was elevated in various renal diseases. Kobayashi et al. (11) reported that antiplasmins were effective in treating chronic nephritic diseases. At present there are few drugs which have a marked efficiency in cases of nephritis and an antiplasmin agent may be worthy of application in the clinical field. Although indomethacin, a non-steroidal anti-inflammatory agent, inhibited enzyme excretions into urine, there were no effects on proteinuria. However, Vihert et al. (12) reported that this drug was considerably effective in reducing proteinuria and in improving the course of diffuse glomerulonephritis under experimental and clinical conditions. The discrepancy in the results is mainly attributed to the difference in experimental models.

In the present experiments, the effect of drugs on Masugi's nephritis in rats was tentatively evaluated through determination of several biochemical parameters in urine and

serum. On the other hand, Shibata et al. (4, 5) reported that a chronic nephritis which developed slowly could be induced in rats with a single i.v. administration of anti-serum obtained with the purified antigen of kidney. Possible effects of drugs on this chronic model are under investigation in our laboratory.

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