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A Comparative Study of Several Agents Alone and Combined in Protection of the Rodent Kidney from Warm Ischaemia: Methylprednisolone, Propranolol, Furosemide, Mannitol, and Adenosine Triphosphate-Magnesium Chloride

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Summary. The effect of 60 min of in situ warm ischaemia (37 °C) on renal function was investigated in the rat model. In addition, the effect of pretreatment with intravenously administered furosemide, mannitol, propranolol, methylprednisolone, and adenosine triphosphate-magnesium chloride (ATP-$MgCl_2$), singly or in combination, was studied. The ischaemic kidney was effectively protected by the administration of methylprednisolone alone but not by furosemide, mannitol, ATP-$MgCl_2$, or propranolol singly or in combination. These results demonstrate that rat kidneys subjected to 60 min of warm ischaemia can be safely protected with methylprednisolone pretreatment (3 mg/100 g of body weight) 30 min before warm ischaemia.

Key words: Ischaemic kidney, Kidney protection, Methylprednisolone, Renal function.

Methylprednisolone, propranolol, furosemide, and mannitol are often used during several phases of renal preservation and transplantation to prevent ischaemic damage to the organ. In cadaveric kidney transplantation, normothermic ischaemia has a particularly adverse effect on renal function. As a means of protecting the kidney from both this and subsequent preservational injury, the common practice is to administer pharmacological agents singly or in combination to donors and recipients as well as to use preservation fluid.

The problem of preventing post-transplantation oliguria has not been without controversy. Several clinical and experimental investigations showed that methylprednisolone, propranolol, adenosine triphosphate-magnesium chloride (ATP-$MgCl_2$), or potent diuretics (mannitol and furosemide) given prophylactically could ameliorate or even prevent renal failure [4, 5, 8, 16, 17, 21, 24]. Timing of administration of the drug, however, has been controversial [8, 21, 24].

In this study, an attempt was made to determine the most effective agent or combination of agents in the protection of rat kidneys from 60 min of in situ warm ischaemia.

Materials and Methods

Female Sprague-Dawley rats weighing 200 to 300 g were used. The animals had free access to food and water. Premedication consisted of 0.0015 mg of atropine intramuscularly per 100 g of body weight. Ether was used for anaesthesia, and 500 U/kg of heparin sodium was given via the external jugular vein [1], which was also used for drug injection and blood withdrawal. A heating lamp and pad were used in all animals.

Through a midline incision, a right nephrectomy was performed. The vascular pedicle and ureter of the remaining left kidney were occluded with a Silastic-covered microvascular bulldog clamp for 60 min.

During the period of ischaemia, the abdomen was temporarily closed. Serum creatinine and blood urea nitrogen (BUN) levels were determined on days 0, 3, and 10, and survival was recorded. Drugs and timing of their administration are described in Table 1.

The data on creatinine and BUN obtained from all groups on day 3 and day 10 after warm ischaemia were subjected to a one-way analysis of variance. The data were transformed to logs before analysis, and the mean values on day 3 and the differences between the values on days 10 and 3 were compared among the experimental groups. Animals that died before the third day after surgery were not included in the analysis. A multiple comparison procedure (Duncan's multiple range test) was used to test for differences among specific groups. Mean values not covered by any single bracket are significantly different.

Results (Figs. 1 and 2)

Log mean serum creatinine and BUN values on day 3 (Table 2) and the changes in values from day 10 to day 3 (Table 3)

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Table 1. Drugs and time of administration in an ischaemic renal rat model

	Treatment groups	No. of animals	Intravenous dose/100 g of body weight	Time before or after ischaemia
1	Controls	8	2 ml of normal saline	Before
2	Methylprednisolone	8	3 mg	30 min before
3	Propranolol	9	0.1 mg	10 min before
4	Propranolol	8	0.1 mg	30 min before
5	Furosemide	8	1 mg	10 min before
6	Mannitol	7	50 mg (25% solution)	10 min before
7	Methylprednisolone + propranolol	8	3 mg + 0.1 mg	30 min before
8	Methylprednisolone + mannitol + furosemide	7	3 mg + 50 mg + 1 mg	30 min before / 10 min before
9	Mannitol + furosemide	9	50 mg + 1 mg	10 min before
10	ATP-$MgCl_2$	6	20 μmol	20 min before
11	ATP-$MgCl_2$	7	20 μmol	Immediately after

ATP-$MgCl_2$ = adenosine triphosphate-magnesium chloride

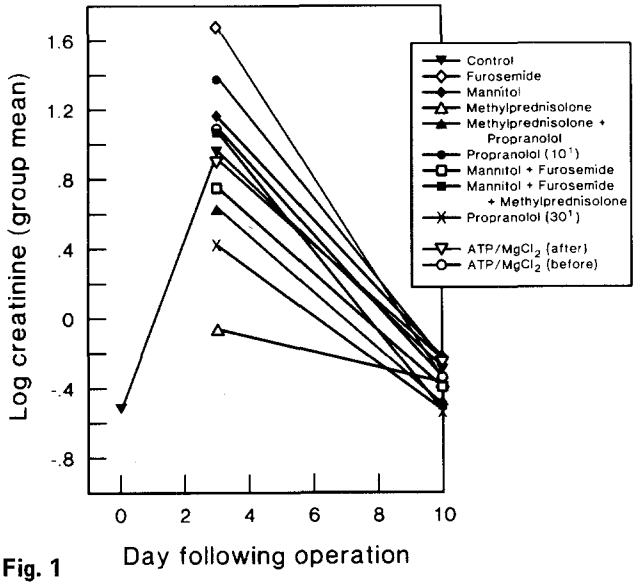

Fig. 1

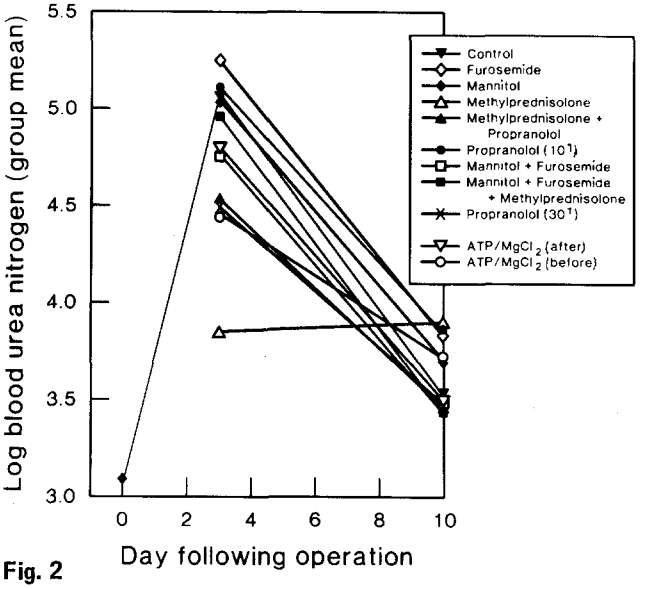

Fig. 2

Fig. 1. Log creatinine values (mean) for control and experimental groups on days 0, 3, and 10 after 60 min of warm ischaemia

Fig. 2. Log blood urea nitrogen values (mean) for control and experimental groups on days 0, 3, and 10 after 60 min of warm ischaemia

were compared between experimental groups. The level of creatinine, but not of BUN, returned to normal on day 10 in all animals, but top creatinine and BUN levels in the methylprednisolone pretreated group were normal on the third day.

The treatment with propranolol, furosemide, mannitol, ATP-$MgCl_2$, and combinations of these agents (groups 3 through 11) did not protect the kidney, which was ischaemically damaged. Serum creatinine and BUN values on the third day and for day 10 minus day 3 were not significantly different from those of the control kidneys (group 1). The mortality rate was especially high in the furosemide and the 10-min propranolol groups.

In contrast to these results, methylprednisolone (group 2) had a significant protective effect on the ischaemically damaged kidney ($P < 0.05$ in comparison with control value). The log serum creatinine value increased to log −0.06 on the third day and returned to upper normal levels by the tenth day. The log BUN value increased to 3.85 on the third day and was −0.05 on the tenth day. All animals survived.

Discussion

Of the agents used in these experiments, only methylprednisolone resulted in significant improvement of renal function in comparison with function in controls.

Numerous studies reported protection of an organ from the effects of warm ischaemia or cold preservation by the administration of methylprednisolone [14, 20]. The benefi-

Table 2. Group mean serum creatinine and blood urea nitrogen values (log-transformed data) on day 3[a]

Creatinine				Blood urea nitrogen			
Group		No. of animals surviving	Mean	Group		No. of animals surviving	Mean
5	Furosemide	7/8	1.68	5	Furosemide	7/8	5.24
3	Propranolol (10 min)	6/9	1.39	3	Propranolol (10 min)	6/9	5.11
6	Mannitol	7/7	1.16	1	*Controls*	8/8	5.07
11	ATP-$MgCl_2$ (after)	7/7	1.09	6	Mannitol	7/7	5.05
8	Methylprednisolone + mannitol + furosemide	6/7	1.07	8	Methylprednisolone + mannitol + furosemide	6/7	4.96
1	*Controls*	8/8	0.95	11	ATP-$MgCl_2$ (after)	7/7	4.82
10	ATP-$MgCl_2$ (before)	6/6	0.91	9	Mannitol + furosemide	9/9	4.76
9	Mannitol + furosemide	9/9	0.75	7	Methylprednisolone + propranolol	8/8	4.53
7	Methylprednisolone + propranolol	8/8	0.63	10	ATP-$MgCl_2$ (before)	5/6	4.47
4	Propranolol (30 min)	8/8	0.42	4	Propranolol (30 min)	8/8	4.44
2	Methylprednisolone	8/8	−0.06	2	Methylprednisolone	8/8	3.85
MSE = 0.41, $P < 0.001$[b]				MSE = 0.56, $P = 0.03$[b]			

[a] Brackets indicate multiple comparisons ($\alpha = 0.05$)
[b] Probability corresponding to overall F-statistic
ATP-$MgCl_2$ = adenosine triphosphate-magnesium chloride

Table 3. Group mean serum creatinine and blood urea nitrogen values (log-transformed data), day 10 minus day 3[a]

Creatinine				Blood urea nitrogen			
Group		No. of animals surviving	Mean	Group		No. of animals surviving	Mean
2	Methylprednisolone	8/8	−0.38	2	Methylprednisolone	8/8	0.05
7	Methylprednisolone + propranolol	6/8	−0.96	10	ATP-$MgCl_2$ (before)	5/6	−0.70
4	Propranolol (30 min)	7/8	−1.00	7	Methylprednisolone + propranolol	6/8	−0.97
10	ATP-$MgCl_2$ (before)	5/6	−1.07	8	Methylprednisolone + mannitol + furosemide	4/7	−1.04
9	Mannitol + furosemide	8/9	−1.12	3	Propranolol (10 min)	4/9	−1.10
1	*Controls*	7/8	−1.19	4	Propranolol (30 min)	7/8	−1.17
6	Mannitol	6/7	−1.24	6	Mannitol	6/7	−1.18
8	Methylprednisolone + mannitol + furosemide	4/7	−1.35	11	ATP-$MgCl_2$ (after)	7/7	−1.30
3	Propranolol (10 min)	4/9	−1.39	9	Mannitol + furosemide	8/9	−1.34
11	ATP-$MgCl_2$ (after)	7/7	−1.44	5	Furosemide	5/8	−1.37
5	Furosemide	5/8	−1.75	1	*Controls*	7/8	−1.54
MSE = 0.38, $P = 0.09$[b]				MSE = 0.46, $P = 0.004$[b]			

[a] Brackets indicate multiple comparisons ($\alpha = 0.05$)
[b] Probability corresponding to overall F-statistic
ATP-$MgCl_2$ = adenosine triphosphate-magnesium chloride

cial effect is most likely explained by the ability of methylprednisolone to stabilize lysosomal membranes, since lysosomes contain enzymes that activate the kinin system, disrupt vascular endothelium, and produce vasoconstriction. Starling and associates [20] showed that pretreatment of dogs with methylprednisolone and the addition of it to the perfusate resulted in decreased perfusate lysosomal enzyme concentration and a delay in the increase in peripheral vascular resistance.

Clinically, acute renal failure after transplantation was reduced in kidneys protected with methylprednisolone and furosemide [13]. Others demonstrated that methylprednisolone is beneficial when given 2 h before onset of ischaemia in dogs [24]. But Green and associates [8] could not dem-

onstrate any benefit when methylprednisolone was given 3 h before ischaemia in the rabbit model. This is in contradiction to our finding of life-sustaining renal function when methylprednisolone was administered 30 min before ischaemia in the rat model. The difference may result from the short half-life of methylprednisolone; a species difference also may contribute.

The administration of propranolol is associated with a significant reduction in arterial blood pressure and heart rate accompanied by a decrease in cardiac output. Also, propranolol, as a β-adrenergic inhibitor, attenuates the effect of nerve stimulation on renin release at both normal and low blood pressures [25]. Plasma concentrations of propranolol are high at 30 min and then decline logarithmically during the next 2 h. The renin disappears from the plasma during the 30 min after administration of the drug and then begins to increase again as the plasma concentrations of propranolol decrease [25]. Its effectiveness in improving graft survival and renal function after 30, 60, and 120 min of warm ischaemia in a canine kidney harvesting and preservation model has been shown [22, 23].

Chevalier and Finn [4] showed that propranolol attenuated intratubular obstruction after ischaemia and that a salutary effect was achieved not by alteration in renal haemodynamics but by reduction in the severity of intratubular obstruction in rats.

The precise mode of action of propranolol is unknown, but its effect is time-dependent. Creatinine levels and survival rates of the group in which propranolol was given 30 min before warm ischaemia were significantly better than those in the group in which propranolol was given 10 min before warm ischaemia.

Furosemide, by inhibiting the active reabsorption of chloride in the ascending loop of Henle, prevents the reabsorption of sodium that passively follows chloride. Its value in the prevention of renal failure is controversial. Lucas and associates [11] evaluated the effects of furosemide on renal function and haemodynamics in 54 critically ill surgical patients and showed that furosemide does not protect against renal failure by altering or increasing renal blood flow but may cause renal failure by producing hypovolemia.

Rikukawa and Lindsey [17] demonstrated that when furosemide was given to rats 10 min before the induction of 2 h of kidney ischaemia, survival improved from 16 to 50%. Green and associates [8] and Toledo-Pereyra and co-workers [24] saw no beneficial effect of furosemide given 5 to 20 min before ischaemia in rabbits and dogs.

In the present study, pretreatment of rats with furosemide did not improve postischaemic renal function. Rather, furosemide tended to be detrimental, since high mean creatinine and BUN values were recorded in this group.

Mannitol produces an osmotic diuresis by inhibiting sodium and water reabsorption and by producing an increase in blood flow and glomerular filtration rate [15]. Considerable experimental and clinical data support the possibility that prophylactic application of mannitol prevents the development of ischaemic acute renal failure. However, improved renal function was evident only when mannitol was given 5 to 15 min before the 60-min period of ischaemia in a rabbit model [5]. It was ineffective in dogs when given 20 min before 90 min of warm renal ischaemia [24]. The protective effect of mannitol is explained by a reduction in the size of ischaemically injured cells and the prevention of the "no reflow" phenomenon [8].

In our study, mannitol given 10 min before ischaemic insult was of no significant benefit in the protection of azotemia in the rat.

Adenosine triphosphate (ATP) plays an essential role in the metabolism of all living cells and is the energy transporter of the only freely available metabolic energy in the cell. With acute ischaemia, a rapid decrease occurs in the tissue content of ATP because of an inhibition of the oxidative phosphorylation.

Administration of ATP-$MgCl_2$ before, during, or after a prolonged period of severe shock was suggested to have a beneficial effect on the survival of rats and pigs [3, 9, 12, 19]. It was suggested [10] that ischaemic kidney and liver cells, but not normal cells, are permeable to externally added ATP and that exogenous ATP-$MgCl_2$ can restore cellular ATP levels after an ischaemic episode, but this suggestion is controversial.

Schloerb and associates [18] investigated the effect of intravenous administration of 6.25 μmol of ATP-$MgCl_2$ on survival and tissue ATP levels in haemorrhagic shock in rats; ATP-$MgCl_2$ did not enhance survival or tissue levels, and the administered ATP seemed to be rapidly degraded. Garvin and associates [7], however, evaluated the effect of ATP-$MgCl_2$ alone or in combination with dipyridamole administered intravenously before 30 min of warm ischaemia and followed by Collins C-4 flush and 24 h of cold storage preservation; ATP-$MgCl_2$ pretreatment alone or with dipyridamole did not improve kidney function, although cortical ATP levels were significantly greater at all time intervals in pretreated kidneys.

Collins and associates [6] demonstrated that the relationship between levels of total adenine nucleotide and function was of less predictive value after cold storage or continuous perfusion and that the derangement of adenine nucleotide metabolism did not seem to be the primary cause of renal damage after ischaemia.

In our model, ATP-$MgCl_2$ did not improve renal function when administered intravenously 20 min before or immediately after 60 min of warm ischaemia. This result confirms most of the recent reports but contradicts previous findings [16, 19].

Thus, in this as in other studies [14, 20, 24], methylprednisolone continues to be the most effective agent in preventing renal failure due to warm ischaemia. Tests of other agents have given controversial results, and the values seem not predictable. Similarly, ATP-$MgCl_2$ – although associated with increased levels of total adenine nucleotide [2] – is of no benefit.

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