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Benidipine Inhibits Apoptosis During Ischaemic Acute Renal Failure in Rats

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Abstract

We have investigated the effects of benidipine (hydrochloride), a calcium antagonist, against ischaemic acute renal failure in rats. Using histological examination, we studied whether the inhibition of apoptosis was associated with the protective effects of benidipine on the ischaemic renal injury.

Acute renal failure was induced by the unilateral clamping of the left renal artery for 60 min, followed by reperfusion and contralateral nephrectomy. Drugs were given intravenously 5 min before the unilateral clamping. Prophylactic administrations of benidipine ($10\,\mu g\,kg^{-1}$, i.v.) significantly ameliorated the development of renal failure as estimated by the measurements of serum creatinine and blood urea nitrogen 24 h after the reperfusion. Amlodipine (besilate, 100 and $300\,\mu g\,kg^{-1}$, i.v.) tended to attenuate renal dysfunction. Lisinopril (300 and $1000\,\mu g\,kg^{-1}$, i.v.), an angiotensin converting enzyme inhibitor, was ineffective in this acute renal failure model. Histological examination using the terminal transferase-mediated dUTP-biotin nick end-labelling (TUNEL) method to detect apoptotic cells revealed that the TUNEL-positive tubular epithelium was prominent in the renal cortex 24 h after the reperfusion. The TUNEL-positive cells were significantly reduced by pretreatment with benidipine.

The results demonstrate that benidipine can ameliorate the ischaemic acute renal failure in rats and suggest that the renoprotective effect of benidipine was at least partly attributable to the reduction of apoptosis in tubular epithelial cells.

Ischaemic acute renal failure is a frequent clinical syndrome with high morbidity and mortality. Many vascular and cellular factors have been implicated in the pathophysiology of ischaemic acute renal failure (Bonventre 1993). Although the cellular mechanism leading to ischaemic cell damage has not been clarified fully, several calcium antagonists have beneficial effects on ischaemic acute renal failure in a clinical situation as well as in experimental animals (Schrier & Burke 1991; Epstein 1998). Those observations support the importance of calcium in the pathogenesis of injury in renal cells, indicating that calcium blockade may contribute to preventing cell injury (Weinberg 1984; Schrier et al 1987, 1991).

Apoptosis, known as programmed cell death, has been shown in various mammalian cells. Schumer et al (1992) found cells with apoptotic morphology in renal ischaemia-reperfusion injury in rats. Recently, it has been reported that pretreatment with verapamil, a calcium antagonist, inhibited the induction of apoptosis in the tubular cells after renal ischaemia-reperfusion (Raafat et al 1997). Apoptosis in renal ischaemia-reperfusion injury might be correlated with an alteration in calcium homeostasis. However, the regulation and physiologic significance of the apoptosis remain unclear.

Benidipine (hydrochloride) is a 1,4-dihydropyridine calcium antagonist with slow-onset and

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long-lasting vasodilating effects (Karasawa & Kubo 1988; Karasawa et al 1988). Benidipine is useful clinically in the treatment of hypertension and angina pectoris (Fuji et al 1988; Yamada et al 1990). Karasawa & Kubo (1990) reported that benidipine has beneficial effects on ischaemia-induced acute renal failure in rats. We have studied the effects of benidipine against ischaemic acute renal failure in rats and compared them with those of amlodipine, a calcium antagonist, and lisinopril, an angiotensin converting enzyme (ACE) inhibitor. Moreover, we examined whether the inhibition of apoptosis was associated with the protective effects of benidipine on the ischaemic renal injury by histological examination using the terminal transferase-mediated dUTP-biotin nick end-labelling (TUNEL) method.

Materials and Methods

Animals

Male Wistar rats (230–280 g; Japan Shizuoka Laboratory Animal Center, Inc., Hamamatsu, Japan) were used. The rats were kept at $23 \pm 1^{\circ}C$ under a 12-h light–dark cycle, with free access to tap water and commercial chow (FR-2, Funabashi Farm, Chiba, Japan). All rats received humane care in compliance with the Guiding Principles for the Care and Use of Laboratory Animals formulated by the Japanese Pharmacological Society. The protocol was approved by the Bioethical Committee of Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd.

Drugs

Benidipine hydrochloride (Kyowa Hakko Kogyo, Tokyo, Japan) was dissolved in physiological saline containing 0·05% Tween 80. Amlodipine besilate, extracted from Amlodin tablets (Sumitomo Pharmaceuticals, Osaka, Japan), was dissolved in physiological saline containing 1% ethanol and 20% polyethyleneglycol-400. Lisinopril (Sigma Chemical, St Louis, MO) was dissolved in physiological saline. The concentration of the drug was adjusted to yield an injection volume of 0·1 mL/100 g body weight. The doses of benidipine and amlodipine were selected in reference to the report of Nomura et al (1994). The selected dose of lisinopril ($1000\ \mu g\ kg^{-1}$) was reported to inhibit ACE activity (Krishan et al 1998). Other chemicals were of reagent grade and were from commercial sources.

Surgical procedure and induction of ischaemia

The ischaemia-induced acute renal failure was produced by the method of Karasawa & Kubo (1990). Rats were anaesthetized with sodium pentobarbital ($50\ mg\ kg^{-1}$, i.p., Nembutal, Dainabot, Osaka, Japan). Under anaesthesia, the left kidney was exposed by a flank incision, and the renal artery was freed from perirenal fat. Temporary renal ischaemia was induced by clamping the left renal artery for 60 min. Immediately after reperfusion, the opposite (right) kidney was removed. Afterwards, the incisions were closed, and the rat was allowed to recover from anaesthesia. In sham-operated rats, the kidneys were treated identically except for clamping of the left renal artery. Drugs or the vehicles were injected into the tail vein 5 min before renal ischaemia. Twenty-four hours after reperfusion, blood samples for the measurements of serum creatinine and blood urea nitrogen were obtained from the abdominal aorta under ether anaesthesia. In the rats treated with benidipine, the blood samples were collected 6 and 24 h after the reperfusion. The left kidney was excised for histopathological examination. Creatinine and blood urea nitrogen were measured by an autoanalyser (AU600, Olympus, Tokyo, Japan).

Histological examination

The kidney was immediately cut into transversal sections around the renal pelvis at a thickness of approximately 5 mm and immersed in 10 vol% buffered formalin solution for fixation. After fixation, paraffin sections were made by a standard method. Specimens from all rats were stained with haematoxylin and eosin, and periodic acid-Schiff for light microscopic observation. Furthermore, specimens from the vehicle- and the benidipine-treated group at each time point were stained by the TUNEL method (Apop Tag Plus In Situ Apoptosis Detection kit, Oncor, Inc., MD). Specimens were observed under a light microscope (BH-2, Olympus). Histopathological scoring was performed from 0 (−) to 2 (+++) referring to well-established criteria (Conger et al 1994). The lesions in preparations stained with haematoxylin and eosin and periodic acid-Schiff were graded according to the percentage of the renal section (100%) occupied by the lesions. The scores for the lesions were as follows: −, 0%; ±, less than 25%; +, 25 ~ 50%; ++, 50 ~ 75%; +++, more than 75%. The TUNEL-positive cells found in preparations stained by the TUNEL method were graded according to the percentage of the renal section (100%) occupied by positive cells. The scores for

the cells were as follows: −, 0%; ±, less than 10%; +, 10 ~ 25%; ++, 25 ~ 50%; +++, more than 50%.

Statistical analysis

All values are expressed as means ± s.e.m. All statistics were performed using computer and statistical analysis software (SAS, version 6.12, SAS Institute, Inc., Cary, NC). Statistical analysis was performed using the Student's *t*- or Aspin-Welch test for comparison between two groups, or using one-way analysis of variance followed by Dunnett's test, or the Kruskal-Wallis test followed by Steel's test for multiple comparison. For histologic data examination, Wilcoxon's rank sum test was used. $P < 0.05$ was considered significant.

Results

Effects of benidipine, amlodipine and lisinopril on creatinine and blood urea nitrogen in rats subjected to renal ischaemia and reperfusion

Renal artery occlusion followed by reperfusion for 24 h significantly increased creatinine and blood urea nitrogen in vehicle-treated rats compared with sham-operated rats. The mean values of creatinine and blood urea nitrogen in the vehicle-treated rats were 3·0–3·3 and 95–108 mg dL^{-1}, respectively (Tables 1–4).

Table 1. Effects of benidipine on creatinine and blood urea nitrogen of rats following ischaemic renal failure (24 h).

Treatment	Dose (μg kg^{-1}, i.v.)	Creatinine (mg dL^{-1})	Blood urea nitrogen (mg dL^{-1})
Normal	–	0·25 ± 0·01**	12·93 ± 0·42***
Sham	–	0·42 ± 0·00**	19·92 ± 0·38***
Vehicle	–	2·97 ± 0·46	95·34 ± 8·99
Benidipine	3	1·62 ± 0·55	68·20 ± 14·58
	10	0·99 ± 0·14**	53·69 ± 7·06*

Values are expressed as the means ± s.e.m. of six animals. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the values in the vehicle group.

Table 2. Effects of amlodipine on creatinine and blood urea nitrogen of rats following ischaemic renal failure (24 h).

Treatment	Dose (μg kg^{-1}, i.v.)	Creatinine (mg dL^{-1})	Blood urea nitrogen (mg dL^{-1})
Normal	–	0·26 ± 0·00***	13·19 ± 0·32***
Sham	–	0·47 ± 0·01**	17·63 ± 0·40***
Vehicle	–	3·06 ± 0·39	98·04 ± 7·42
Amlodipine	100	2·88 ± 0·41	93·14 ± 9·94
	300	1·93 ± 0·44	70·04 ± 9·08

Values are expressed as the means ± s.e.m. of six animals. **$P < 0.01$, ***$P < 0.001$ compared with the values in the vehicle group.

Table 3. Effects of lisinopril on creatinine and blood urea nitrogen of rats following ischaemic renal failure (24 h).

Treatment	Dose (μg kg^{-1}, i.v.)	Creatinine (mg dL^{-1})	Blood urea nitrogen (mg dL^{-1})
Normal	–	0·29 ± 0·01***	13·97 ± 0·08***
Sham	–	0·46 ± 0·01***	17·95 ± 0·41***
Vehicle	–	3·23 ± 0·27	99·79 ± 4·48
Lisinopril	300	2·69 ± 0·54	92·84 ± 7·60
	1000	3·82 ± 0·30	112·01 ± 2·29

Values are expressed as the means ± s.e.m. of six animals. ***$P < 0.001$ compared with the values in the vehicle group.

Rats given 10 μg kg^{-1} (i.v.) benidipine before renal clamping had lower creatinine and blood urea nitrogen values compared with the vehicle-treated rats (Table 1). Amlodipine (300 μg kg^{-1}, i.v.) tended to attenuate elevations in creatinine and blood urea nitrogen, although the effects were not statistically significant (Table 2). Lisinopril (300 and 1000 μg kg^{-1}, i.v.) was ineffective in this acute renal failure model (Table 3).

Effect of benidipine on the time course of the change in rats subjected to renal ischaemia and reperfusion

Creatinine and blood urea nitrogen significantly increased from 6 h after the reperfusion (Table 4). The values at 24 h were higher than the values at 6 h. Benidipine attenuated the elevations in creatinine and blood urea nitrogen from 6 h after the reperfusion, and the effects at 24 h were statistically significant.

Effect of benidipine on renal ischaemia and reperfusion injury as assessed by histological examination

In the normal and the sham group at each time point, histopathological lesions were scarcely observed (Figure 1A). In the vehicle group, 6 h after reperfusion, tubular dilatation together with brush-border loss was widely observed in the renal cortex. Necrosis of the tubular epithelial cells (eosinophilic change and pyknotic nuclei) was sporadically observed in the renal cortex. Some of them were desquamated into the dilated tubular lumen. Furthermore, protein cast was focally observed in the dilated tubular lumen (Figure 1B). Twenty-four hours after reperfusion, these lesions were extended into the cortex, and in particular, necrosis of the tubular epithelial cells and desqua-

Table 4. Effects of benidipine on creatinine and blood urea nitrogen of rats at 6 and 24 h after induction of acute renal failure.

Treatment	Time after reperfusion (h)	Creatinine ($mg\,dL^{-1}$)	Blood urea nitrogen ($mg\,dL^{-1}$)
Normal	6	0·24 ± 0·01***	12·61 ± 0·52***
	24	0·24 ± 0·01***	14·00 ± 0·58***
Sham	6	0·42 ± 0·01***	25·72 ± 1·16***
	24	0·47 ± 0·01***	21·13 ± 0·76***
Vehicle	6	1·07 ± 0·03	40·46 ± 1·60
	24	3·28 ± 0·32	107·83 ± 6·29
Benidipine $10\,\mu g\,kg^{-1}$	6	0·84 ± 0·05**	40·33 ± 1·39
	24	1·27 ± 0·15***	65·85 ± 7·24**

Values are expressed as the means ± s.e.m. of six animals. $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$ compared with the values in the vehicle group.

mation of those cells into the tubular lumen were prominently observed (Figure 1D). In the benidipine group, all lesions seemed to be improved at each time point compared with the vehicle group (Figure 1C, E; Table 5).

Effect of benidipine on renal apoptosis during ischaemia-reperfusion assessed by histological examination using the TUNEL method

In the vehicle group, 6 h after reperfusion, the TUNEL-preparations revealed that the positive cell nuclei of the tubular epithelia were sporadically observed in the outer medulla. In the cortex, the positive tubular cells and cell debris in the tubular lumen were scattered. Twenty-four hours after the reperfusion, the findings described above prominently increased in the inner cortex (Figure 2A). In the benidipine group, the positive cells tended to be reduced at 6 h and were significantly reduced at 24 h compared with the vehicle group (Figure 2B; Table 5).

Discussion

Temporary occlusion of the renal artery followed by reperfusion reproducibly causes acute renal failure in experimental rats (Arendshorst et al 1975). Previous studies have shown that, in the ischaemic acute renal failure models, calcium antagonists including benidipine inhibit the renal injury (decreases in creatinine clearance and elevations of creatinine and blood urea nitrogen) (Goldfarb et al 1983; Garthof et al 1987; Rose et al 1987; Karasawa & Kubo 1990; Shudo et al 1994). In this study, the efficacy of benidipine was evaluated by measuring creatinine and blood urea nitrogen, and also by analysis of histological changes including apoptosis.

We have evaluated the effects of benidipine on the renal injury induced by ischaemia-reperfusion at 6 and 24 h after reperfusion. Nakajima et al (1996) reported that apoptotic cells were found in renal tissue as early as 3 h after reperfusion and the number of the apoptotic cells reached a peak at 12–24 h, whereas the tubular damage reached a peak at 24 h and tubular cell regeneration was found at the same time. We therefore focused on the effects of benidipine on the early (6 h) and the severe (24 h) phases of ischaemia-reperfusion injuries including apoptotic changes. In this study, serum creatinine and urea nitrogen levels, as indices of renal dysfunction, were increased by ischaemia-reperfusion. The changes at 24 h were greater than the changes at 6 h. In addition, we detected necrotic and apoptotic changes at 6 h after reperfusion, and these areas increased at 24 h. Thus, it seems that the time points (6 and 24 h) were appropriate for examining the effects of the drugs.

We compared the protective effects of benidipine on the renal injury induced by ischaemia-reperfusion with those of amlodipine or lisinopril at 24 h after reperfusion. The beneficial role of calcium antagonists against ischaemic acute renal failure have been reported in animals and man (Neumayer & Kunzendorf 1991; Schrier & Burke 1991; Epstein 1998). In this study, benidipine (3 or $10\,\mu g\,kg^{-1}$, i.v.) and amlodipine ($300\,\mu g\,kg^{-1}$, i.v.) lowered the creatinine and blood urea nitrogen levels elevated by the renal damage. The effect of $3\,\mu g\,kg^{-1}$ benidipine was comparable with that of $300\,\mu g\,kg^{-1}$ amlodipine. Moriyama & Karasawa (1994) reported that the EC50 value of benidipine for vasorelaxation was approximately one-fortieth that of amlodipine in canine coronary arteries precontracted with KCl. Moreover, the duration of the hypotensive action of benidipine at $10\,\mu g\,kg^{-1}$ (i.v.) was almost the same as that of amlodipine at $1500\,\mu g\,kg^{-1}$ (i.v.) in anaesthetized dogs. These observations indicate that benidipine is a more potent calcium antagonist than amlodipine. Thus, the difference in calcium blocking potency could explain the difference in the renoprotective effects

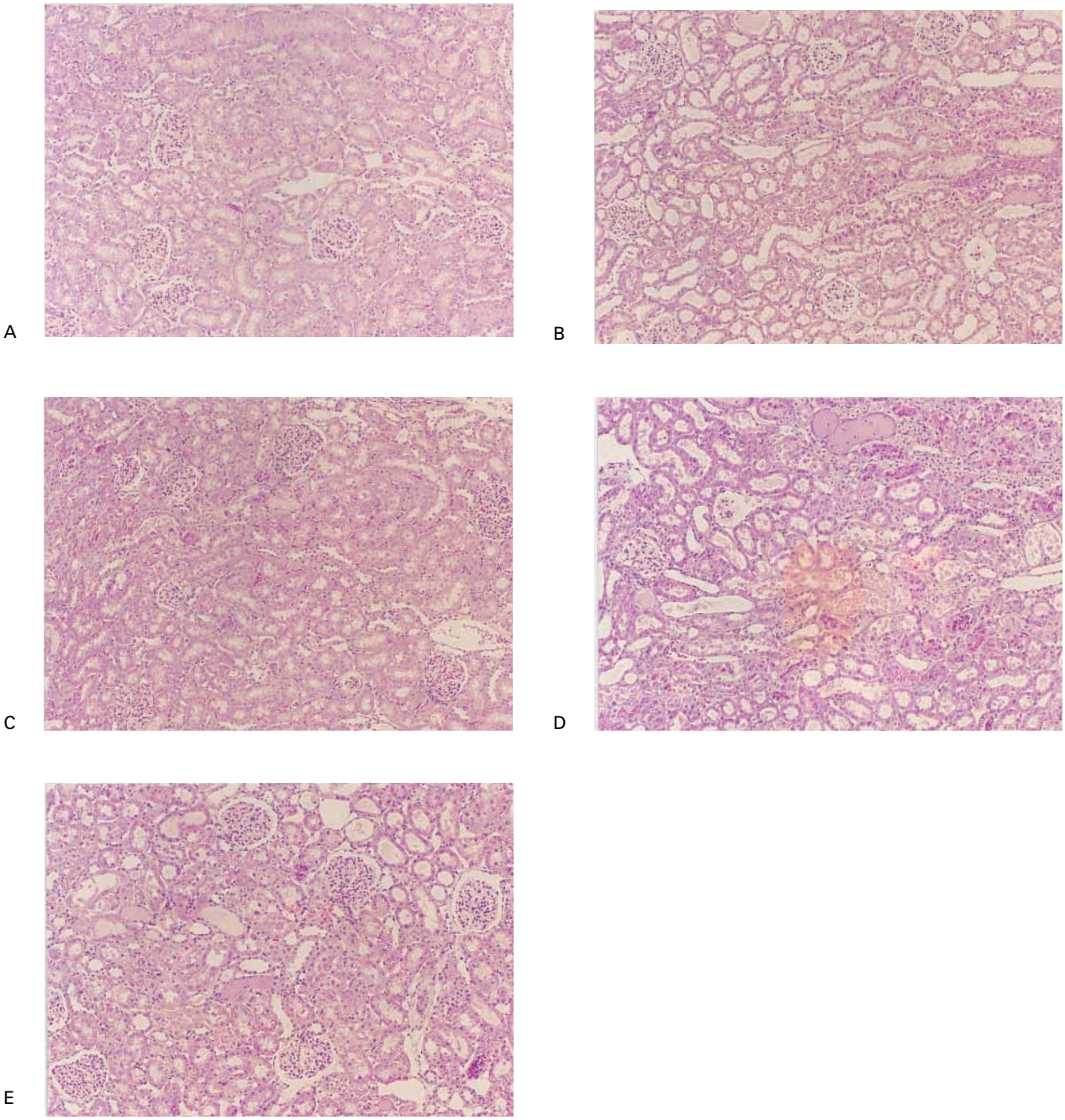

Figure 1. Light microphotographs of the renal cortex. A, the normal group. B, the vehicle group at 6-h reperfusion. Tubular dilatation and focal tubular necrosis are observed. C, the benidipine group at 6-h reperfusion. Slight tubular necrosis is seen. D, the vehicle group at 24-h reperfusion. Tubular necrosis and desquamation into the lumen are obvious. E, the benidipine group at 24-h reperfusion. Tubular lesions are less prominent compared with the vehicle group. Magnification $\times 100$.

of benidipine and amlodipine. In contrast, lisinopril (300 or 1000 $\mu g\,kg^{-1}$, i.v.) had no effect on the elevation of creatinine and blood urea nitrogen in the present model. It is likely that the severe calcium overload, which could lead to cell death, occurred after the reperfusion of an ischaemic kidney. Some studies have shown that the administration of ACE inhibitors before renal artery clamping was associated with reduced severity of post-ischaemic renal failure (Long et al 1993; Krishan et al 1998). The precise explanation for the lack of efficacy is uncertain. The discrepancy may be due to the differences in models and experimental protocol.

In recent years, apoptosis has been increasingly recognized as an important cause of cell death after

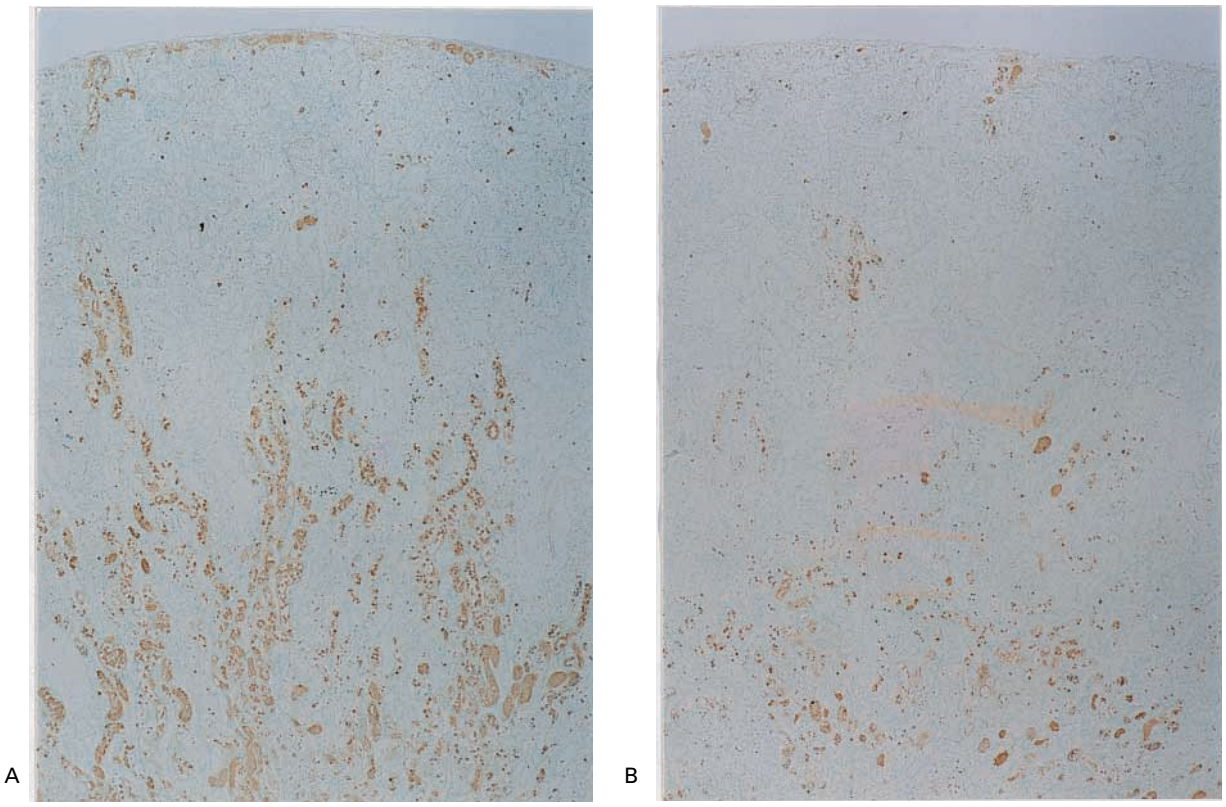

Figure 2. Light microphotographs of the renal cortex. A, the vehicle group at 24-h reperfusion. TUNEL-positive cells are obvious in the inner cortex. B, the benidipine group at 24-h reperfusion. TUNEL-positive cells are less prominent compared with the vehicle group. Magnification ×40.

Table 5. Effects of benidipine on histopathological scores of rats at 6 and 24 h after induction of acute renal failure.

Treatment	Time after reperfusion	Histopathological findings				
		Tubular necrosis	Tubular dilatation	Brush border loss	Protein casts	TUNEL positive cell
Vehicle	6 h	1·0 ± 0·0	1·0 ± 0·0	0·7 ± 0·1	0·6 ± 0·1	1·1 ± 0·1
	24 h	1·4 ± 0·1	1·4 ± 0·1	1·4 ± 0·1	0·8 ± 0·1	1·4 ± 0·1
Benidipine 10 $\mu g\,kg^{-1}$	6 h	0·7 ± 0·1*	0·6 ± 0·1**	0·5 ± 0·0	0·5 ± 0·0	0·8 ± 0·1
	24 h	0·9 ± 0·2*	0·7 ± 0·1**	0·9 ± 0·2*	0·6 ± 0·1	0·9 ± 0·2*

Findings observed in specimens stained with haematoxylin and eosin and periodic acid-Schiff (brush border loss) were graded as follows, according to the percentage of the renal section (100%) occupied by the lesions: −, 0%; ±, less than 25%; +, 25 ~ 50%; ++, 50 ~ 75%; +++, more than 75%. The positive cells found in the kidney specimens by the TUNEL method were graded as follows: according to the percentage of the renal section (100%) occupied by positive cells: −, 0%; ±, less than 10%; +, 10 ~ 25%; ++, 25 ~ 50%; +++, more than 50%. Histopathology scoring was from 0 (−) to 2 (+++) as described in methods. Values are expressed as the means ± s.e.m. of six animals. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ compared with the values in the vehicle group at each time.

ischaemia with or without reperfusion. Schumer et al (1992) reported that apoptosis is correlated with renal ischaemia-reperfusion injury. Shimizu & Yamanaka (1993) demonstrated that apoptosis was involved in the repair process of ischaemic tubular necrosis. Nakajima et al (1996) showed that renal ischaemia-reperfusion caused apoptotic changes in morphology and biochemical parameters, and they suggested that reduction of apoptotic cell death contributed to attenuation of tubular damage in ischaemic acute renal failure. Raafat et al (1997) showed that the pretreatment of verapamil, a calcium antagonist, inhibited those apoptotic changes 24 h after reperfusion. However, in that study, the effects of the calcium antagonist on the location of apoptotic morphology in the kidney and its time-

course changes remained unclear, and thus the results were qualitative. Therefore, in this study we used the TUNEL method to detect apoptotic cells histologically in the renal ischaemia-reperfusion injury and quantified the degree of inhibitory effects of benidipine on apoptosis. As a result, TUNEL-positive cells were detected in the tubular epithelial cells in the cortex and the outer medulla 6 h after the reperfusion. In particular, the apoptotic cells in the outer medulla did not show necrotic findings such as increased eosinophilia and pyknotic nuclei. These observations demonstrated that DNA fragmentation occurred in an early phase of renal ischaemia-reperfusion injury, in agreement with a previous report (Nakajima et al 1996). Twenty-four hours after reperfusion, the apoptotic tubular cells prominently increased in the inner cortex. Most apoptotic cells seemed to be located in the tubular epithelia showing the necrotic finding 24 h after reperfusion. In addition, we found that benidipine significantly decreased apoptotic cells as well as necrotic cells in the kidney subjected to the ischaemia-reperfusion. These results suggest that the renal ischaemia-reperfusion injury consists not only of tubular necrosis but also of tubular apoptosis and that the reduction of apoptosis is associated with the renoprotective effect of benidipine.

Several lines of evidence indicate that excessively increased cytosolic calcium (Ca-overload) is a primary factor responsible for the pathogenesis of cell injury in renal cells (Weinberg 1984; Schrier et al 1987). Moreover, intracellular calcium has been suggested to have a primary role in the regulation of endonuclease and protease activation as well as gene expression during apoptosis of renal cells in kidney ischaemia-reperfusion injury (Raafat et al 1997). Karasawa & Kubo (1990) reported that renal calcium content progressively increased after the start of reperfusion of an ischaemic kidney and reached the peak level after 24-h reperfusion, and that benidipine prevented the elevation of renal calcium levels. The preventive effect of benidipine on Ca-overload probably plays a major role in the renal protective action of benidipine.

Elevation of intracellular calcium levels in acute renal failure is known to be associated with the generation of oxygen-derived free radicals (Paller 1994). The increase in free radicals has been suggested to be involved in the pathogenesis of renal ischaemia-reperfusion injury. Additionally, free radicals have been reported to induce apoptosis in renal tubular epithelial cells (Ueda & Shah 1992). Previous studies have shown that some calcium antagonists reduced not only cytosolic calcium levels but also free radicals (Green et al 1989). It is uncertain if the reduction of free radicals was dependent on the calcium levels. However, the inhibitory effects of calcium antagonists on the generation of free radicals is considered to be related to the attenuation of apoptosis. Benidipine inhibited the increase in lipid peroxide, observed after reperfusion of an ischaemic kidney (Karasawa & Kubo 1990). Thus, the reduction of free radicals may in part account for the inhibitory effect of benidipine on apoptosis in this study. However, the exact mechanism remains unknown, and further investigations are necessary.

The importance of the vascular endothelium has been suggested in the regulation of renal circulation after renal ischaemia (Schrier & Burke 1991; Bankir et al 1998). The abnormality of the endothelial function was considered to contribute to the post-ischaemic vasoconstriction in the renal vasculature (Bankir et al 1998). Benidipine has been found to reduce vascular endothelial damage in various experimental models such as splanchnic artery occlusion and reperfusion (Karasawa et al 1991), myocardial ischaemia and reperfusion (Yao & Karasawa 1994), and endothelial injury induced by sodium citrate (Sato et al 1992). The protective effects of benidipine on endothelial injury seem to be independent of the calcium channel blockade in vascular smooth muscle cells. The endothelial protection may therefore be another mechanism of the renoprotective effect of benidipine in this study.

In conclusion, we have demonstrated that benidipine can protect the kidney from ischaemic injury in a rat model of acute renal failure. This suggests that the protective effect of benidipine against the ischaemic acute renal failure was at least partly attributable to the reduction of apoptosis in the tubular epithelium.

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References

Arendshorst, W. J., Finn, W. F., Gottschalk, C. W. (1975) Pathogenesis of acute renal failure following temporary renal ischemia in the rat. Circ. Res. 37: 558–568

Bankir, L., Kriz, W., Goligorsky, M., Nambi, P., Thomson, S., Blantz, R. C. (1998) Vascular contributions to pathogenesis of acute renal failure. Renal Failure 20: 663–677

Bonventre, J. V. (1993) Mechanisms of ischemic acute renal failure. Kidney Int. 43: 1160–1178

Conger, J. D., Schultz, M. F., Miller, F., Robimette, J. B. (1994) Responses to hemorrhagic arterial pressure reduction in different ischaemic renal failure models. Kidney Int. 46: 318–323

Epstein, M. (1998) Calcium antagonists and renal disease. Kidney Int. 54: 1771–1784

Fuji, Y., Suzuki, H., Katsumata, H., Nakajima, S., Saruta, T. (1988) Hormonal and renal responses to oral once-daily calcium entry blocker in normotensive and hypertensive persons. J. Cardiovasc. Pharmacol. 11: 438–443

Garthof, B., Hirth, C., Federmann, A., Kazda, S., Stash, J. P. (1987) Renal effects of 1,4-dihydropyridines in animal models of hypertension and renal failure. J. Cardiovasc. Pharmacol. 9: S8–S13

Goldfarb, D., Iaina, A., Serban, I., Gavendo, S., Kapuler, S., Eliahou, H. E. (1983) Beneficial effect of verapamil in ischemic acute renal failure in the rat. Proc. Soc. Exp. Biol. Med. 172: 389–392

Green, C. J., Gower, J. D., Healing, G., Cotterill, L. A., Fuller, B. J., Simpkin, S. (1989) The importance of iron, calcium and free radicals in reperfusion injury: an overview of studies in ischaemic rabbit kidneys. Free Radical Res. Commun. 7: 255–264

Karasawa, A., Kubo, K. (1988) Calcium antagonistic effects and the in vitro duration of actions of KW-3049, a new 1,4-dihydropyridine derivative, in isolated canine coronary arteries. Jpn. J. Pharmacol. 47: 35–44

Karasawa, A., Kubo, K. (1990) Protection by benidipine hydrochloride (KW-3049), a calcium antagonist, of ischemic kidney in rats via inhibitions of Ca-overload, ATP-decline and lipid peroxidation. Jpn. J. Pharmacol. 52: 553–562

Karasawa, A., Kubo, K., Shuto, K., Oka, T., Nakamizo, N. (1988) Vasodilating effects of the new calcium antagonist benidipine hydrochloride in anesthetized dogs and cats. Arzneim. Forsch. 38: 1707–1712

Karasawa, A., Rochester, J. A., Ma, X. L., Lefer, A. M. (1991) Protection of endothelial damage and systemic shock by benidipine, a calcium antagonist, in rats subjected to splanchnic ischemia and reperfusion. Circ. Shock 33: 135–141

Krishan, P., Sharma, A., Singh, M. (1998) Effect of angiotensin converting enzyme inhibitors on ischemia-reperfusion-induced renal injury in rats. Pharmacol. Res. 37: 23–29

Long, G. W., Misra, D. C., Juleff. R., Blossom, G., Czaka, P. F., Glover, J. L. (1993) Protective effects of enalaprilat against postischaemic renal failure. J. Surg. Res. 54: 254–257

Moriyama, T., Karasawa, A. (1994) Cardiovascular effects of benidipine and amlodipine in isolated tissues and anesthetized dogs. Biol. Pharm. Bull. 17: 1468–1471

Nakajima, T., Miyaji, T., Kato, A., Ikegaya, N., Yamamoto, T., Hishida, A. (1996) Uninephrectomy reduces apoptotic cell death and enhances renal tubular cell regeneration in ischemic ARF in rats. Am. J. Physiol. 271: F846–F853

Neumayer, H. H., Kunzendorf, U. (1991) Renal protection with the calcium antagonists. J. Cardiovasc. Pharmacol. 18: S11–S18

Nomura, H., Kusaka, H., Karasawa, A. (1994) Effects of benidipine HCl on renal functions in anesthetized spontaneously hypertensive rats. Jpn. Pharmacol. Ther. 22: 3453–3457 (in Japanese)

Paller, M. S. (1994) The cell biology of reperfusion injury in the kidney. J. Invest. Med 42: 632–639

Raafat, A. M., Murray, M. T., McGuire, T., DeFrain, M., Franko, A. P., Zafar, R. S., Palmer, K., Diebel, L., Dulchavsky, S. A. (1997) Calcium blockade reduces renal apoptosis during ischemia reperfusion. Shock 8: 186–192

Rose, H., Philipson, J., Pushett, J. B. (1987) Effect of nitrendipine in a rat model of ischemic acute renal failure. J. Cardiovasc. Pharmacol. 9: S57–S59

Sato, H., Karasawa, A., Kubo, K. (1992) Protective effects of benidipine on rat endothelial injury induced by sodium citrate. The Clinical Report 26: 4397–4402 (abstract in English)

Schrier, R. W., Burke, T. J. (1991) Role of calcium-channel blockers in preventing acute and chronic renal injury. J. Cardiovasc. Pharmacol. 18: S38–S43

Schrier, R. W., Arnold, P. E., Putten, V. V., Burke, T. J. (1987) Cellular calcium in ischemic acute renal failure: role of calcium entry blockers. Kidney Int. 32: 313–321

Schumer, M., Colombel, M. C., Sawczuk, I. S., Gobe, G., Connor, J., O'Toole, K. M., Olsson, C. A., Wise, G. J., Buttyan, R. (1992) Morphologic, biochemical, and molecular evidence of apoptosis during the reperfusion phase after brief periods of renal ischemia. Am. J. Pathol. 140: 831–838

Shimizu, A., Yamanaka, N. (1993) Apoptosis and cell desquamation in repair process of ischemia tubular necrosis. Virchows Arch. [B] 64: 171–180

Shudo, C., Masuda, Y., Sugita, H., Tanaka, S., Tomita, K. (1994) Effects of efonidipine hydrochloride (NZ-105), a new calcium antagonist, against acute renal failure in rats. Gen. Pharmacol. 25: 1451–1458

Ueda, N., Shah, S. V. (1992) Endonuclease-induced DNA damage and cell death in oxidant injury to renal tubular epithelial cells. J. Clin. Invest. 90: 2593–2597

Weinberg, J. M. (1984) Calcium as a mediator of renal tubular cell injury. Semin. Nephrol. 4: 174–191

Yamada, K., Yasui, S., Kishida, H., Hiejima, K., Murakami, E., Takekoshi, N. (1990) Clinical evaluation of benidipine hydrochloride (KW-3049) on angina pectoris. A double-blind comparative study with nifedipine. Ther. Res. 11: 1637–1656 (abstract in English)

Yao, K., Karasawa, A. (1994) Protective effects of benidipine against myocardial damage following ischemia and reperfusion in the isolated perfused rat heart. Biol. Pharm. Bull. 17: 517–521