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Short communication

Proteasome participates in the pathogenesis of ischemic acute renal failure in rats

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Abstract

Acute renal failure was induced by occlusion of the left renal artery and vein for 45 min followed by reperfusion, 2 weeks after contralateral nephrectomy. Renal function parameters such as blood urea nitrogen, plasma creatinine, creatinine clearance, urine flow and urinary osmolality were measured to test the effectiveness of drugs. Renal function in untreated acute renal failure rats markedly decreased at 24 h after reperfusion. The administration of PSI, *N*-benzyloxycarbonyl-Ile-Glu(*O*-*t*-Bu)-Ala-leucinal, a proteasome inhibitor, at a dose of 1 mg/kg before the occlusion abolished the decreases in the renal function of acute renal failure rats. Calpeptin (1 mg/kg), a calpain inhibitor, attenuated the deterioration of renal function to the same extent as 0.1 mg/kg PSI, but no significant difference was observed between the untreated and calpeptin-treated acute renal failure groups. Histopathological examination of the kidney of untreated acute renal failure rats revealed severe lesions, such as tubular necrosis, proteinaceous casts in tubuli and medullary congestion, all of which were significantly suppressed by PSI (1 mg/kg) treatment. In contrast, calpeptin, at the same dose, was ineffective against the development of renal lesions. These results suggest that proteasome participates in the pathogenesis of ischemic acute renal failure. Thus, proteasome may be a potential target for the identification of agents that may be useful in the treatment of diseases whose etiology is dependent on ischemia/reperfusion.

Keywords: Proteasome; Calpain; Ischemia/reperfusion; Renal failure, acute

1. Introduction

Many observations indicate that regulated intracellular proteolysis is an important mechanism for controlling key reactions underlying both normal and pathological processes. Most cells contain two major nonlysosomal neutral proteinases in the cytosol. One is proteasome, a multicatalytic proteinase complex present in cells in both 20S (700 kDa) and 26S (2000 kDa) forms. The 20S proteasome functions as the proteolytic core of the 26S proteasome complex that degrades ubiquitin-conjugated proteins. This proteolytic pathway is involved in the processing and degradation of regulatory proteins that control cell cycle progression, gene transcription or antigen presentation (Coux et al., 1996); however, its pathophysiological role in vivo is still unclear. The other cytosolic neutral proteinase is calpain, a cysteine proteinase present in cells as a low Ca^{2+} sensitive μ-calpain or a high Ca^{2+} sensitive *m*-calpain. Calpain is known to be a mediator of hypoxic/ischemic injury in brain (Lee et al., 1991), liver (Bronk and Gores, 1993) and myocardium (Tolnadi and Korecky, 1986). This proteinase has also been implicated in hypoxic injury in renal proximal tubules (Edelstein et al., 1995, 1996). These observations led us to examine whether a calpain inhibitor has protective effects on ischemic acute renal failure in animal models. Our results were somewhat unexpected because calpeptin, a potent calpain inhibitor (Tsujinaka et al., 1988), at a dose of 1 mg/kg, did not have a significant protective effect against the deterioration of renal function in ischemic acute renal failure rats. We examined the effects of PSI, *N*-benzyloxycarbonyl-Ile-Glu(*O*-*t*-Bu)-Ala-leucinal, a potent proteasome inhibitor on renal functional and histological damage in ischemic acute renal failure, and the effects of the drug were compared with those seen with calpeptin.

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2. Materials and methods

2.1. Animals and experimental design

Male Sprague–Dawley rats (10 weeks of age, Japan SLC, Shizuoka) were used. Two weeks before the study (at 8 weeks of age), the right kidney was removed through a small flank incision under pentobarbital anesthesia (50 mg/kg, i.p.). After a 2-week recovery period, these rats were separated into five groups: (1) sham-operated control, (2) untreated ischemic acute renal failure, (3) ischemic acute renal failure pretreated with PSI (0.1 mg/kg, i.p.), (4) ischemic acute renal failure pretreated with PSI (1 mg/kg, i.p.), (5) ischemic acute renal failure pretreated with calpeptin (1 mg/kg, i.p.). To induce ischemic acute renal failure, the rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and the left kidney was exposed through a small flank incision. The left renal artery and vein were occluded with a nontraumatic clamp for 45 min. At the end of the ischemic period, the clamp was released for blood reperfusion. PSI, calpeptin or vehicle (35% ethanol, 35% polyethylene glycol 400 and 30% saline) in a volume of 1 ml/kg was injected intraperitoneally, 1 h before the occlusion. In sham-operated control rats, the kidney was treated identically, except for the clamping. Animals exposed to 45-min ischemia were housed in metabolic cages at 24 h after the ischemia. At the end of urine collection for 5 h, blood samples were drawn from the thoracic aorta, and then left kidneys were excised under pentobarbital anesthesia (50 mg/kg, i.p.). The plasma was separated by centrifugation. These samples were used for measurement of renal function parameters. Blood urea nitrogen and creatinine levels in plasma or urine were determined using the blood urea nitrogen-test-Wako and creatinine-test-Wako (Wako Pure Chemical Industries, Osaka, Japan), respectively. Urinary osmolality was measured by freezing point depression (Fiske, MA). The kidneys were preserved in phosphate-buffered 10% formalin, after which the kidneys were chopped into small pieces, embedded in paraffin wax, and cut at 3 μm and stained with hematoxylin and eosin. Histopathological changes were graded as no change (0), mild (1), severe (3) and very severe (4) based on the microscopical observation of each section.

2.2. Drugs

PSI (Peptide Institute, Osaka, Japan) and calpeptin (generous gift from Suntory, Osaka, Japan) were dissolved in 35% ethanol, 35% polyethylene glycol 400 and 30% saline just before administration. Other chemicals were obtained from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemical Industries (Osaka, Japan).

2.3. Statistical analysis

Values are expressed as means ± S.E.M. The data were analyzed for significant differences between the sham-operated and vehicle-administered acute renal failure groups using the Student's unpaired *t*-test. Statistical analysis for renal functional studies was performed using one-way analysis of variance followed by a Dunnett-type multiple comparison test. Histological data were analyzed using the Steel test. Differences were considered significant at $P < 0.05$.

3. Results

As shown in Table 1, the renal function of rats subjected to 45-min of ischemia showed a marked deterioration when measured 24 h after reperfusion. As compared with sham-operated rats, untreated acute renal failure rats showed significant increases in blood urea nitrogen, plasma creatinine and urine flow and significant decreases in creatinine clearance and urinary osmolality. The administration of PSI at a dose of 0.1 mg/kg tended to attenuate the deterioration of renal function, but no significant difference was observed between the untreated and 0.1 mg/kg PSI-treated acute renal failure groups. The preventive effect of PSI at the higher dose (1 mg/kg) was potent, and values of renal function parameters were similar to those seen in sham-operated control rats. In contrast, the effect of calpeptin (1 mg./kg) was much less potent than that of

Table 1
Effects of PSI and calpeptin on blood urea nitrogen (BUN), plasma creatinine (Pcr), creatinine clearance (Ccr), urine flow (UF) and urinary osmolality (UosM) after ischemia/reperfusion
Each value represents the mean ± S.E.M. ARF: acute renal failure.

Experimental group	BUN (mg/dl)	Pcr (mg/dl)	Ccr (ml/min/kg)	UF (μl/min/kg)	UosM (mosM/kg)
Sham ($n = 4$)	22.5 ± 1.1	0.66 ± 0.02	5.49 ± 0.52	34.8 ± 3.9	1380 ± 104
Untreated ARF ($n = 5$)	61.4 ± 7.0[a]	1.71 ± 0.36[a]	2.04 ± 0.42[a]	69.3 ± 5.9[a]	555 ± 64[a]
ARF + PSI 0.1 mg/kg ($n = 5$)	43.6 ± 3.3	1.18 ± 0.10	2.63 ± 0.32	57.6 ± 6.1	701 ± 78
ARF + PSI 1 mg/kg ($n = 5$)	24.1 ± 1.7[b]	0.55 ± 0.22[c]	5.66 ± 0.62[b]	37.6 ± 5.5[b]	1136 ± 163[b]
ARF + calpeptin 1 mg/kg ($n = 5$)	46.8 ± 10	1.21 ± 0.36	3.25 ± 0.66	56.7 ± 7.6	727 ± 106

[a] $P < 0.01$, compared with sham.
[b] $P < 0.01$.
[c] $P < 0.05$, compared with untreated ARF.

Table 2
Effects of PSI and calpeptin on histopathological changes of kidneys in ARF rats
Each value represents the mean ± S.E.M. ARF: acute renal failure. Grades: no change (0), mild (1), moderate (2), severe (3), very severe (4).

Experimental group	Tubular necrosis	Proteinaceous casts	Medullary congestion
Untreated ARF ($n = 5$)	3.40 ± 0.24	2.60 ± 0.24	3.20 ± 0.20
ARF + PSI 0.1 mg/kg ($n = 5$)	3.60 ± 0.24	2.00 ± 0.45	2.60 ± 0.40
ARF + PSI 1 mg/kg ($n = 5$)	1.20 ± 0.24[a]	0.80 ± 0.20[a]	1.20 ± 0.20[a]
ARF + calpeptin 1 mg/kg ($n = 5$)	3.20 ± 0.20	2.60 ± 0.24	3.00 ± 0.45

[a] $P < 0.01$, compared with untreated ARF.

the same dose of PSI and was the same extent as that of the lower dose of PSI (0.1 mg/kg).

Histopathological examination revealed severe lesions in the kidneys of untreated acute renal failure rats. These changes were characterized by tubular necrosis, proteinaceous casts in tubuli, and medullary congestion and hemorrhage. Pretreatment with 1 mg/kg PSI prevented the development of all the lesions. In contrast, 1 mg/kg calpeptin, as well as the lower dose of PSI, was ineffective against the development of renal lesions (Table 2).

4. Discussion

Our present results showed that PSI was capable of preventing renal function impairment as well as renal lesions in rats with ischemia/reperfusion-induced acute renal failure, thereby suggesting that this proteinase inhibitor has beneficial effects on ischemic acute renal failure.

The use of PSI in animal and cell culture models has revealed the physiological functions and pathophysiological roles of proteasome (Figueuredo-Pereira et al., 1994b; Traenckner et al., 1994; Girscavage et al., 1996; Okamoto et al., 1998; Takaoka et al., 1998). PSI is recognized as a potent and a cell-penetrating peptide aldehyde inhibitor of proteasome but it does have weak calpain-inhibiting activity (Figueuredo-Pereira et al., 1994b). Thus, one may point out that the preventive effect of PSI on ischemia/reperfusion-induced acute renal failure in rats is due to its inhibitory action on calpain. In the present study, we used a potent calpain inhibitor calpeptin to evaluate the involvement of calpain in the development of ischemic acute renal failure in rats and compared its potency with that of PSI. PSI prevented ischemic acute renal failure, whereas calpeptin had little effect at the same dose of 1 mg/kg (1 mg of PSI and calpeptin correspond to 1.62 and 2.76 μmol, respectively, values being calculated from their molecular weights). These results suggest that the ineffectiveness of calpeptin can be considered as a negative control that is required for determining the involvement of proteasome in the effect of a proteasome inhibitor with calpain-inhibiting activity, such as PSI. It therefore is reasonable to consider that the improvement of renal dysfunction and degeneration seen after the treatment of ischemic acute renal failure rats with PSI is not due to the inhibition of calpain, but must result from the inhibition of proteasome.

Calpain is known to be a mediator of hypoxic/ischemic injury in brain (Lee et al., 1991), liver (Bronk and Gores, 1993), myocardium (Tolnadi and Korecky, 1986) and renal proximal tubules, in which the increase in calpain activity precedes cell membrane damage and a calpain inhibitor provides cytoprotection against hypoxic injury (Edelstein et al., 1995, 1996). Despite the importance of calpain to cellular events in hypoxic/ischemic injury, we could not demonstrate a crucial role for it in the protection against ischemic acute renal failure in a rat model. Because calpeptin did not significantly improve ischemic acute renal failure at a single dose of 1 mg/kg, we can not exclude the possibility that calpain is involved in the pathogenesis of this type of acute renal failure. In higher doses, calpeptin may ameliorate the renal function impairment in ischemic acute renal failure. However, caution must be exercised when calpeptin is used in higher doses to interfere with calpain function, because higher concentrations of calpeptin can inhibit the enzymatic activity of purified proteasome (Figueuredo-Pereira et al., 1994a). Rather, the possibility that even the weak effect of 1 mg/kg calpeptin on ischemic acute renal failure in rats may be due to its inhibitory action on proteasome warrants consideration.

There is no circumstantial evidence regarding the change in proteasome activity during ischemia followed by reperfusion and the mechanism for the development of ischemic acute renal failure via a proteasome-dependent proteolytic pathway. Although it therefore remains unknown whether the inhibitory effect of PSI on proteasome is related to any of the different stimuli that induce ischemic acute renal failure, the stimuli probably include endothelin-1, which is an important deleterious mediator in the pathogenesis of postischemic acute renal failure (Firth and Ratcliffe, 1992; Chan et al., 1994; Gellai et al., 1994). A recent observation that PSI lessens the increased aortic endothelin-1 content in deoxycorticosterone acetate-salt hypertensive rats suggests that a proteasome-dependent proteolytic pathway plays an important role in the enhanced production of endothelin-1 in blood vessels in this model of hypertension

(Okamoto et al., 1998). Based on these findings, it may well be that the proteasome pathway is also involved in the enhanced production of renal endothelin-1 in ischemic acute renal failure. Further experiments are required to clarify the mechanism for the development of ischemic acute renal failure via a proteasome-dependent proteolytic pathway.

In summary, our results suggest that proteasome has an important role in the pathogenesis of ischemic acute renal failure in rats. In addition, intervention by inhibition of proteasome in this model offers beneficial effects, as evidenced by improvement of renal dysfunction and degeneration. Thus, proteasome may be a potential target for the identification of agents that may be useful in the treatment of diseases whose etiology is dependent on ischemia/reperfusion.

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