Notice of Retraction

The editorial committee has noticed that this publication as a Note in Biological and Pharmaceutical Bulletin contains data sets identical to those presented in papers, already published in another journal, from the same laboratory with the same corresponding author. Due to a violation of the editorial policies of the journal, this Note has been retracted by the committee.

The Editorial Committee of the Pharmaceutical Society of Japan (April 15, 2014)
Dietary Supplementation of L-Carnosine Prevents Ischemia/Reperfusion-Induced Renal Injury in Rats

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The effects of dietary supplementation of L-carnosine (β-alanyl-L-histidine) on ischemia/reperfusion-induced acute renal failure (ARF) in rats were examined. Ischemic ARF was induced by occlusion of the left renal artery and vein for 45 min followed by reperfusion, 2 weeks after contralateral nephrectomy. Renal functional parameters such as blood urea nitrogen, plasma creatinine, creatinine clearance, urine flow, urinary osmolality and fractional excretion of sodium were measured. Renal function in ARF rats markedly decreased at 1 d after reperfusion. Prior feeding of L-carnosine-containing diet (0.0001 w/w%) for 2 weeks attenuated the ischemia/reperfusion-induced renal dysfunction. Histopathological examination of the kidney of ARF rats revealed severe renal damages, such as tubular necrosis, proteinaceous casts in tubuli and medullary congestion, which were also significantly suppressed by the dietary supplementation of L-carnosine. These findings strongly suggest that L-carnosine supplementation is useful as a prophylactic treatment in the development of the ischemic ARF.

Key words L-carnosine; acute renal failure; ischemia; reperfusion

L-Carnosine (β-alanyl-L-histidine) is abundant in skeletal muscle of humans and many species of animals. Several studies have demonstrated that this peptide has antioxidative and free radical scavenging functions.1–5 It has recently been reported that L-carnosine exerts neuroprotective and cardioprotective effects in PC12 cells and cardiomyoblasts exposed to hypoxia/reoxygenation, respectively.6,7 It is accumulating evidence that antioxidative agents and substances can attenuate cardiovascular diseases such as hypertension and post-ischemic organ damage.8,9 Most recently, we found that an intravenous bolus injection of L-carnosine attenuated the post-ischemic renal injury in rats.10 Furthermore, dietary L-carnosine feeding to rats efficiently suppressed the development of hypertension induced by deoxycorticosterone acetate and salt.11 In addition, we observed also an inhibitory effect of L-carnosine on the neural activity of the renal sympathetic nerve,12 which is closely related to the antihypertensive effect of L-carnosine. In the present study, we asked whether dietary supplementation of L-carnosine could overcome the ischemia/reperfusion-induced renal injury in rats.

MATERIALS AND METHODS

Materials L-Carnosine-containing diet (0.0001 w/w% in commercial normal diet) was obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan). The concentration of L-carnosine was determined based on the previous study.11 All other reagents used were of analytical grade.

Animals and Experimental Design Male Sprague–Dawley rats (10 weeks of age, Japan SLC, Shizuoka, Japan) were used. Animals were housed in a light-controlled room with a 12 h light/dark cycle and were allowed ad libitum access to food and water. Experimental protocols and animal care methods in the experiments were approved by the Experimental Animal Committee at Osaka University of Pharmaceutical Sciences. 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.), and then separated into a sham-operated group (sham) and a normal diet group. After 2 weeks for recovery and the dietary supplementation, to induce ischemic acute renal failure, 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 to allow reperfusion. 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 1 d after the ischemia. At the end of urine collection for 5 h, blood samples were drawn from the thoracic aorta, and then the 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.

Histological Studies The excised kidneys were preserved in phosphate-buffered 10% formalin, embedded in paraffin wax, cut into thin sections (4 μm) according to conventional techniques. The sections were stained with hematoxylin and eosin. Histopathological changes were analyzed for tubular necrosis, proteinaceous casts, and medullary congestion, as suggested by Solez et al.12 Tubular necrosis and proteinaceous casts were graded as follows: no damage (0), mild (+1, unicellular, patchy isolated damage), moderate (+2, damage less than 25%), severe (+3, damage between 25 and 50%), and very severe (+4, more than 50% damage). The degree of medullary congestion was defined by: no congestion (0), mild (+1, vascular congestion with identification of erythrocytes by ×400 magnification), moderate (+2, vascular congestion with identification of erythrocytes by ×200 magnification), severe (+3, vascular congestion with identification of erythrocytes by ×100 magnification), and very severe (+4, vascular congestion with identification of erythrocytes by ×40 magnification). Evaluations were made in a blind manner.

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**Analytical Procedures**  
Blood urea nitrogen (BUN) 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. Creatinine clearance (Ccr, ml/min/kg) was calculated from the formula \( Ccr = \frac{Ucr \times UF}{Pcr} \), where Ucr and Pcr are creatinine concentration in urine and plasma, respectively, and UF is urine flow. Urinary osmolality (Uosm) was measured by freezing point depression (Fiske Associates, Ux-bridge, MA, U.S.A.). Urine and plasma sodium concentrations were determined using a flame photometer (205D; Hitachi, Ibaraki, Japan). Fractional excretion of sodium (FE_{Na}, %) was calculated from the formula \( FE_{Na} = \frac{U_{NaV}}{P_{Na}} \times \frac{Ccr}{P_{cr}} \times 100 \), where \( U_{NaV} \) is urinary excretion of sodium and \( P_{Na} \) is the plasma sodium concentration.

**Statistical Analysis**  
Values are expressed as the mean±S.E.M. Relevant data was processed by InStat (Graph-PAD Software for Science, San Diego, CA, U.S.A.). Statistical analysis for renal functional studies, we used one-way analysis of variance followed by Dunnett’s tests for multiple comparison. Histological data were analyzed using the Mann–Whitney test. For all comparisons, differences were considered significant at \( p<0.05 \).

**RESULTS**

**Renal Function at 1 d after the Ischemia/Reperfusion and Effect of Dietary L-carnosine Supplementation**  
As shown in Fig. 1, renal function of rats subjected to 45 min ischemia showed a marked deterioration when measured at 1 d after reperfusion. As compared with sham-operated rats, normal diet-fed ARF rats showed significant increases in BUN, Pcr, UF and FE_{Na}, and significant decreases in Ccr and Uosm. Dietary supplementation of L-carnosine for 2 weeks significantly attenuated the ischemia/reperfusion-induced renal dysfunction. L-Carnosine supplementation to sham-operated rats produced no significant effects in their renal functional parameters (data not shown).

**Histological Renal Damage at 1 d after the Ischemia/Reperfusion and Effect of Dietary L-carnosine Supplementation**  
Histopathological examination revealed severe lesions in the kidney of normal diet-fed ARF rats. These changes were characterized by proteinaceous casts in tubuli in the inner zone of medulla, medullary congestion and hemorrhage in the outer zone inner stripe of medulla, and tubular necrosis in the outer zone outer stripe of medulla (Figs. 2B, E, H). Dietary supplementation of L-carnosine for 2 weeks markedly improved the above lesions (Figs. 2C, F, I). These findings were confirmed by scoring the histopathological changes (Fig. 3). On the other hand, no histopathological changes were observed in the kidney of sham-operated animals (Figs. 2A, D, G)

**DISCUSSION**

In this study, we demonstrated that dietary supplementation of L-carnosine efficiently suppressed the ischemia/reperfusion-induced renal dysfunction and tissue injury. The content of L-carnosine in diet (0.0001 w/w%), which was determined based on the previous study,\(^\text{11}\) was extremely low. Although we did not determine a daily intake of diet on each
animal, a feeding of this diet (approximately 15—30 g/rat/d) is equal to a dosage of 15—30 μg of L-carnosine/rat/d. In our recent study,11) the development of mineralocorticoid-dependent hypertension was markedly attenuated by the feeding of the same diet. Since a small dose of L-carnosine (1 μg/rat, i.v.) could decrease the neural activity of renal sympathetic nerve, which is known to be closely related to the pathology of hypertension, we suggested that the antihypertensive effect of L-carnosine was mediated by its decreasing action on sympathetic nervous system in the kidney.

Ischemic ARF is a frequent clinical syndrome with high morbidity and mortality. Reperfusion of previously ischemic renal tissue initiates a complex cellular events that results in injury and the eventual death of renal cells due to a combination of apoptosis and necrosis.13) The molecular mechanisms underlying the ischemia/reperfusion-induced renal injury are poorly understood, but it has been reported that several causal factors (ATP depletion, reactive oxygen species, phospholipase activation, neutrophil infiltration, vasoactive peptides etc.) are contributive to the pathogenesis of this renal damage.14) Enhancement of renal sympathetic nervous activity and its consequent effect on norepinephrine overflow is involved in the renoprotective effect.15) We also found that ischemia/reperfusion-induced ARF was attenuated by a surgical or pharmacological blockade of renal sympathetic nerve, followed by a suppression of elevated renal venous norepinephrine levels.16) Similar suppressive effect on renal venous norepinephrine levels were also observed in rats given i.v. bolus injection of L-carnosine (1—10 μg/kg).10) Taken together, it is reasonable to consider that the beneficial effect of L-carnosine feeding on the ischemia/reperfusion-induced renal damage is closely related to the suppressive action on the renal sympathetic nerve activity, although further studies are needed to determine whether antioxidative characteristics of L-carnosine is involved in the renoprotective effect.

L-Carnosine (β-alanyl-L-histidine) is known to be hydrolyzed by an enzyme, carnosinase, which is abundantly present in serum.18) In the present study, we could not observe a significant elevation of plasma L-carnosine level in animals fed L-carnosine-containing diet (12.9±2.6 pmol/10 μl vs. 12.1±2.2 pmol/10 μl in normal-diet group). Therefore, L-histidine cleaved by carnosinase from L-carnosine or its decarboxylated metabolite L-histamine may be involved in the L-carnosine’s action. Yamano et al.19) found that the suppressive effect of L-carnosine on hyperglycemia induced by intracranial injection of 2-deoxy-D-glucose is attenuated by histamine H1 receptor antagonist thioperamide. They also observed a similar suppressive effect of L-histamine on the 2-deoxy-D-glucose-induced hyperglycemia. Most recently, we also obtained evidence that the decreasing action of L-carnosine (1 μg/rat, i.v.) on renal sympathetic nerve activity was due to its metabolite L-histidine cleaved by carnosinase, and mediated by the activation of histamine H1-receptor in the central nervous system (Kurata et al., unpublished observations).

Thus, one can speculate that the ameliorating effect of L-carnosine on the post-ischemic renal injury is mediated by the histamine/H1 receptor system. However, there is no available evidence regarding the conversion of administered L-carnosine into histamine. Further experiments are required to clarify whether histamine and H1 receptor are contributive to L-carnosine-induced beneficial effect on the ARF.

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