Novel pharmacological potential of Rhei Rhizoma against peroxynitrite-induced oxidative renal injury

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Abstract

This study was focused on the protective role of Rhei Rhizoma extract from peroxynitrite ($ONOO^-$)-induced renal damages. The employed *in vivo* $ONOO^-$-generation model of lipopolysaccharide plus ischemia-reperfusion resulted in the elevations of plasma 3-nitrotyrosine level and renal myeloperoxidase (MPO) activity as the indicators of *in vivo* $ONOO^-$ generation. However, the administration of Rhei Rhizoma extract at doses of 30 and 60 mg/kg body weight/day for 30 days significantly decreased the concentrations of 3-nitrotyrosine and MPO activity. In addition, the rats given Rhei Rhizoma extract inhibited xanthine oxidase (XOD) activity but not inducible nitric oxide synthase activity. It suggests that Rhei Rhizoma extract might play the role as a superoxide anion scavenger through the inhibition of XOD activity as well as direct $ONOO^-$ scavenger. The protective role of Rhei Rhizoma extract was also shown in the increase of antioxidant, glutathione, and the reduction in the lipid peroxidation in renal mitochondria. Moreover, the *in vivo* $ONOO^-$ generation system resulted in the renal functional impairment assessed by the increase in plasma levels of urea nitrogen and creatinine, whereas the rats administered Rhei Rhizoma extract decreased the levels significantly, implying the alleviation of renal dysfunction induced by $ONOO^-$. This study suggests that Rhei Rhizoma would play the role as an effective therapeutic potential for the oxidative stress-induced renal failure.

Key words Rhei Rhizoma, peroxynitrite, oxidative stress, renal failure.

Introduction

The generation and toxicity of reactive oxygen and nitrogen species have been closely associated with *in vivo* damages including proteins, DNA and biological molecules, which eventually cause a variety of pathological events.[1,2] In recent years, the biochemical and physiological properties of peroxynitrite ($ONOO^-$), which formed from the diffusion-controlled interaction between nitric oxide (NO) and superoxide anion (O_2^-), have been well established as a strong oxidizing radical in various human diseases and animal model of diseases.[3-6] $ONOO^-$-induced tyrosine nitration alters the structure and function of proteins, and $ONOO^-$ and its spontaneous decomposition to hydroxyl radical (·OH) inflicts damage on biological molecules.[7,8] Therefore, the new approach to prevent effectively and safely various diseases including renal failure through the attenuation of $ONOO^-$-induced oxidative damages attracted much attention, and in the light of this fact Chinese traditional medicines as natural antioxidative sources without low toxicity and side effects have great potential.

We previously demonstrated that the Chinese prescription Wen-Pi-Tang extract, which is clinically used for renal failure, contributed to the modulation of $ONOO^-$ formation and played beneficial roles against $ONOO^-$-mediated oxidative injury and renal dysfunction *in vivo*.[9] Moreover, we have previously found that the most active crude drug of Wen-Pi-Tang in improving metabolism of uremic toxins under condition of renal failure is Rhei Rhizoma whose effective roles reduce the blood levels of urea nitrogen, creatinine (Cr), methylguanidine (MG) and guanidinosuccinic acid caused after the induction of renal failure, indicating that Rhei Rhizoma extract ameliorated the severity of renal disorders.[10,11] However, the protective effect of Rhei Rhizoma extract against $ONOO^-$-induced oxidative damage in kidney is an

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unknown pharmacological action, although several tannin compounds composed of Rhei Rhizoma exert the scavenging activity of $ONOO^-$ formation under the *in vitro* system.[12]

Therefore, we investigated in the present study whether Rhei Rhizoma extract protects the protein nitration and renal injury associated with excessive $ONOO^-$ under the lipopolysaccharide (LPS) plus ischemic-reperfused animal model.

Materials and Methods

Reagents : LPS (from *Escherichia coli* serotype 055:B5), 3-nitro-L-tyrosine, hexadecyltrimethylammonium bromide (HETAB) and 3,3',5,5'-tetramethylbenzidine, pepstatin A, chymostatin, aprotinin, phenylmethylsulfonyl fluoride (PMSF), flavin adenine dinucleotide (FAD), tetrahydrobiopterin, dithiothreitol (DTT), β-nicotinamide-adenine dinucleotide phosphate (reduced, NADPH), lactate dehydrogenase (LDH) and sodium pyruvate were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of Rhei Rhizoma extract : Roots of *Rheum officinale* BAILLON produced in China were finely powdered and extracted at 100℃ with water, as described previously.[13] The filtrate was concentrated under reduced pressure to obtain brown residue, resulting in a yield of about 33%, by weight, of the original preparation.

Animals and treatment : The "Guidelines for Animal Experimentation" approved by Toyama Medical and Pharmaceutical University were followed in these experiments. Male Wistar rats at the age of 5 weeks (120-130 g) were obtained from Japan SLC, Inc. (Hamamatsu, Japan). The rats were kept in wire-bottomed cages and exposed to a constant 12 h light-dark cycle. The room temperature and humidity were maintained automatically at about 25℃ and 60%, respectively. Laboratory commercial chow (CLEA Japan Inc., Tokyo, Japan; comprising 24.0% protein, 3.5% lipid and 60.5% carbohydrate) and water were given *ad libitum*. Following several days of adaptation, the rats were divided into four groups, avoiding any intergroup differences in body weight. Water was given to two groups (sham and control), while Rhei Rhizoma extract was dissolved in water and given orally to rats at a dose of 30 or 60 mg/kg body weight/day using a stomach tube. Our preliminary study demonstrated that the administration period played an important role in the protection against the damage caused by LPS plus ischemia-reperfusion, in that the protective activity was greater with a longer administration period. Therefore, in the present study, the extract of Rhei Rhizoma was administered for 30 consecutive days. After 30 days, the rats were intraperitoneally anesthetized with 50 mg/kg sodium pentobarbital. Using aseptic technique, bilateral flank incisions were made, and the renal artery and vein of the kidney were occluded with microvascular clamps for 60 min, and then they were subjected to reperfusion for 350 min. At 50 min after the ischemia, the rats received an intravenous LPS injection (5 mg/kg body weight). The sham group underwent sham surgery (incisions were made to expose the kidneys, but the renal pedicles were not clamped). After 6 hrs of the LPS challenge, blood was collected from cardiac puncture and centrifuged immediately to prepare plasma. Subsequently, the renal arteries of each rat were perfused with ice-cold perfusion buffer comprising 50 mM sodium phosphate, 10 mM EDTA-2Na and 120 mM NaCl, the kidneys were removed, quickly frozen and kept at -80℃ until analysis.

Measurement of plasma 3-nitrotyrosine level : The plasma concentration of 3-nitrotyrosine was determined by HPLC, following the method of both van der Vliet *et al.*[14] and Kaur *et al.*[15] with slight modifications. Briefly, the blood samples were centrifuged for 15 min at 17,000 × g, and then the resulting plasma was incubated with proteinase K (1 U/10 mg protein) for 18 h at 55℃. The samples were centrifuged for 15 min at 17,000 × g and passed through a 10,000-Da molecular mass cut-off filter. The samples were loaded onto a reversed-phase column (Nucleosil 5 μC-18, 250 × 46 mm) at 25℃ and eluted with 50 mM KH_2PO_4-H_3PO_4 (pH 3.01) containing 10% MeOH (v/v) at a flow rate of 0.8 ml/min. Detection of the amino acid derivatives was accomplished by monitoring ultraviolet absorbance at 365 nm. The peaks were identified by comparing their retention times with those of authentic standards added to additional samples and quantified according to their peak areas relative to known amounts of the external standards.

Determination of enzyme activities in renal tissue : The renal myeloperoxidase (MPO) activity was determined according to the tetramethylbenzidine method as

described by Suzuki *et al.*[16] and Laight *et al.*[17] Kidney tissue was homogenized in 5 volumes of a solution containing 0.5% HETAB (w/v) dissolved in 50 mM potassium phosphate buffer (pH 6.0), and then centrifuged for 30 min at 20,000 × g at 4℃. This supernatant was incubated for 2 h at 60℃, and then centrifuged again at 4,000 × g for 12 min. An aliquot of supernatant (40 μl) was removed and added to a reaction mixture (160 μl) containing 1.6 mM 3,3',5,5'-tetramethylbenzidine and 3 mM hydrogen peroxide diluted in 80 mM phosphate buffer (pH 5.4) in a 96-well microplate. The rate of change in absorbance was measured spectrophotometrically at 650 nm and the activity was expressed as absorbance/min/100 mg protein. Inducible nitric oxide synthase (iNOS) activity, an initiator of NO production, was measured using the method described by Suh *et al.*[18] The kidney was homogenized in cold 40 mM Tris-HCl (pH 8.0) containing 5 μg/ml pepstatin A, 1 μg/ml chymostatin, 5 μg/ml aprotinin and 100 μM PMSF. The homogenate was centrifuged at 12,000 × g for 5 min at 4℃ to discard nuclei and cell debris, and aliquots of supernatant fraction were used in the experiments described below. Triplicate aliquot (10-20 μg protein) of each supernatant was incubated in 20 mM Tris-HCl (pH 7.9) containing 4 μM FAD, 4 μM tetrahydrobiopterin, 3 mM DTT, 2 mM L-arginine and 2 mM NADPH in a 96-well plate for 180 min at 37℃. Residual NADPH was oxidized enzymatically with 10 units/ml LDH and 5 mM sodium pyruvate in a final volume of 130 μl, and was quantified by the Griess assay. Xanthine oxidase (XOD) activity was evaluated by the method of Parks *et al.*[19] with slight modifications. Briefly, the kidney was homogenized in 9 volumes of solution containing 10 mM DTT, 1 mM PMSF, 0.1 mM EDTA-2K dissolved in 50 mM potassium phosphate buffer (pH 7.0), and then centrifuged at 800 × g for 20 min at 4℃. The supernatant was centrifuged again at 105,000 × g for 1 h at 4℃, and an aliquot of supernatant (0.2 ml) was added to a reaction mixture (1.8 ml) containing 50 μM xanthine and 100 μM EDTA-2K dissolved in potassium phosphate buffer (pH 7.8). After 30 min of incubation at 37℃, 20% trichloroacetic acid was added to a reaction mixture, and XOD activity was determined by measuring the absorbance at 295 nm. Protein levels were determined by the micro-biuret method with bovine serum albumin as the standard.[20]

Measurement of oxidative damage in renal mitochondria : The renal mitochondria were prepared essentially following the procedure of Jung and Pergande.[21] Briefly, renal tissue was homogenized in 3 volumes of ice-cold preparation medium containing 210 mM mannitol, 70 mM sucrose, 0.5 mM EDTA and 10 mM Tris-HCl at pH 7.4. The homogenate was centrifuged for 10 min at 800 × g in a refrigerated centrifuge. The resulting supernatant was further centrifuged for 5 min at 12,000 × g and 4℃. The pellet was then resuspended in preparation medium to a concentration of about 10 μg mitochondrial protein/ml and stored on ice. Throughout the isolation procedure, the tissue and all solutions were kept at 0-4℃. Glutathione (GSH) was measured by the method of Floreani *et al.*,[22] using *o*-phthalaldehyde as the fluorescent reagent. The level of lipid peroxidant was estimated as thiobarbituric acid (TBA)-reactive substance according to the method of Uchiyama and Mihara.[23]

Determination of urea nitrogen and Cr in plasma : Plasma urea nitrogen and Cr were determined using the commercial reagents BUN Kainos and CRE-EN Kainos (Kainos Laboratories, Inc., Tokyo, Japan), respectively.

Data analysis : The results are expressed as the mean ± S.E. values in each group. Statistical significance for the comparison of four groups was evaluated using Dunnett's test. In all cases, levels for statistically significant difference were set at $p<0.05$.

Results

Plasma concentration of 3-nitrotyrosine

Figure 1 shows the plasma concentration of 3-nitrotyrosine as a marker of $ONOO^-$ formation *in vivo*. The plasma 3-nitrotyrosine level of the control group subjected to LPS plus ischemia-reperfusion was elevated markedly to 309 pmol/ml, whereas in the sham-operated group, there was no detectable level. However, the oral administration of Rhei Rhizoma extract at doses of 30 and 60 mg/kg body weight/day for 30 days significantly reduced the level of 3-nitrotyrosine from 309 pmol/ml to 247 and 84 pmol/ml (20 and 73% decreases), respectively.

Renal MPO activity

Figure 2 exhibits the renal MPO activity related to tyrosine nitration. In rats subjected to LPS plus ischemia-reperfusion, the renal MPO activity was sig-

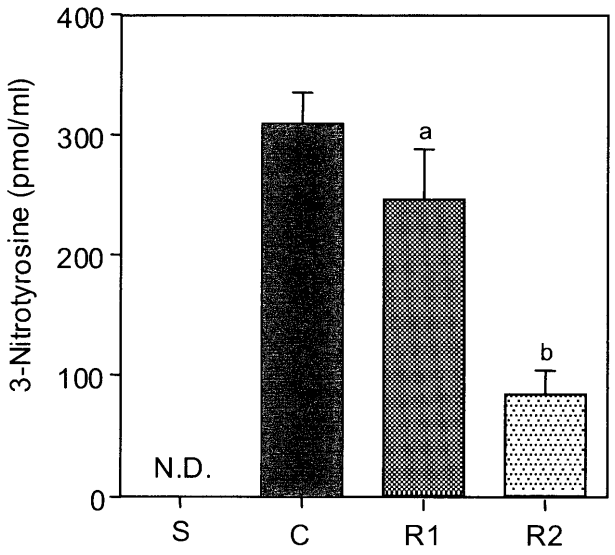

Fig. 1 Effect of Rhei Rhizoma extract on plasma 3-nitrotyrosine level. S, sham treatment; C, LPS plus ischemia-reperfusion; R1, LPS plus ischemia-reperfusion after Rhei Rhizoma extract (30 mg/kg B.W./day); R2, LPS plus ischemia-reperfusion after Rhei Rhizoma extract (60 mg/kg B.W./day). N.D., not detectable. [a]$p<0.05$, [b]$p<0.001$ vs. LPS plus ischemic-reperfused control values.

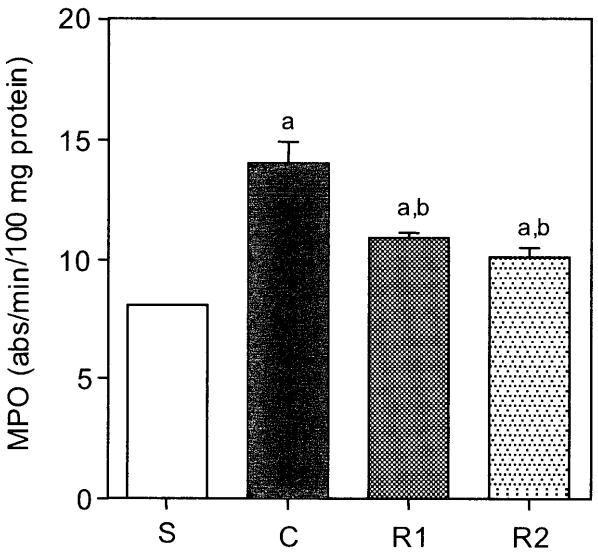

Fig. 2 Effect of Rhei Rhizoma extract on renal MPO activity. S, sham treatment; C, LPS plus ischemia-reperfusion; R1, LPS plus ischemia-reperfusion after Rhei Rhizoma extract (30 mg/kg B.W./day); R2, LPS plus ischemia-reperfusion after Rhei Rhizoma extract (60 mg/kg B.W./day). [a]$p<0.001$ vs. sham treatment values, [b]$p<0.001$ vs. LPS plus ischemic-reperfused control values.

nificantly increased to 14.04 abs/min/100 mg protein, as compared with the value of sham-operated rats, 7.95 abs/min/100 mg protein. In contrast, the oral administration of Rhei Rhizoma extract at 30 and 60 mg showed significant decreases to 10.93 and 10.08 abs/min/100 mg protein, respectively.

iNOS and XOD activities in renal tissue

The activities of iNOS and XOD in renal tissue are shown in Table I. While iNOS activity of sham-operation group was 0.71 pmol/mg protein/min, that of LPS plus ischemic-reperfused control group showed a significant increase to 2.28 pmol/mg protein/min. The administration of Rhei Rhizoma extract inhibited the renal iNOS activity slightly, however, the statistical significance has not been shown. Although the renal XOD activity did not exhibit the significant changes between the sham-operated rats and LPS plus ischemic-reperfused control rats, the administration of Rhei Rhizoma extract at 60 mg dose led to the decrease in the activity of XOD even to the lower value than sham-treatment.

Oxidative damage in renal mitochondria

As shown in Table II, the renal mitochondrial GSH level in LPS plus ischemic-reperfused control rats decreased significantly from 3.35 nmol/mg protein/min to 2.30 nmol/mg protein/min (31% decrease), whereas the oral administration of Rhei Rhizoma extract at doses of 30 and 60 mg showed significant increases to 2.83 and 2.87 nmol/mg protein/min (23 and 25% increases), respectively. The TBA-reactive substance level in renal mitochondria was slightly elevated in LPS plus ischemic-reperfused control rats compared with the sham-operated rats without showing statistical significance. On the other hand, the administration of Rhei Rhizoma extract at 60 mg dose resulted in the significant decrease in the level in comparison to the LPS plus ischemic-reperfused control group.

Urea nitrogen and Cr in plasma

Table III shows the effect of Rhei Rhizoma extract on parameters of renal function. The plasma urea nitrogen and Cr levels of LPS plus ischemia-reperfusion control rats showed the 5.1- and 6.1-fold increases, respectively, in comparison with those of the sham group. With the administration of Rhei Rhizoma extract at doses of 30 and 60 mg/kg body weight/day for 30 days prior to LPS plus ischemia-reperfusion, the urea nitrogen level was decreased significantly from 79.3 mg/dl to 68.0 and 60.9 mg/dl, respectively. Cr level was also decreased with significance from 1.77 mg/dl to 1.65 and 1.54 mg/dl, respectively.

Table I Effect of Rhei Rhizoma extract on renal iNOS and XOD activities.

Group	iNOS (pmol/mg protein/min)	XOD (pmol/mg protein/min)
Sham treatment	0.71 ± 0.31	74.8 ± 6.3
LPS plus ischemic-reperfused		
Control	2.28 ± 0.16[a]	98.2 ± 15.6
Rhei Rhizoma extract (30 mg/kg B.W./day)	2.13 ± 0.18[a]	76.5 ± 13.7
Rhei Rhizoma extract (60 mg/kg B.W./day)	2.02 ± 0.07[a]	66.2 ± 17.4[b]

[a]$p<0.001$ vs. sham treatment values, [b]$p<0.05$ vs. LPS plus ischemic-reperfused control values.

Table II Effect of Rhei Rhizoma extract against oxidative damages of renal mitochondria.

Group	GSH (nmol/mg protein/min)	TBA-reactive substance (nmol/mg protein/min)
Sham treatment	3.35 ± 0.04	2.10 ± 0.05
LPS plus ischemic-reperfused		
Control	2.30 ± 0.20[a]	2.31 ± 0.03
Rhei Rhizoma extract (30 mg/kg B.W./day)	2.83 ± 0.10[a,c]	2.17 ± 0.21
Rhei Rhizoma extract (60 mg/kg B.W./day)	2.87 ± 0.10[a,c]	1.97 ± 0.28[b]

[a]$p<0.001$ vs. sham treatment values, [b]$p<0.05$, [c]$p<0.001$ vs. LPS plus ischemic-reperfused control values.

Table III Effect of Rhei Rhizoma extract on plasma urea nitrogen and Cr levels.

Group	Urea nitrogen (mg/dl)	Cr (mg/dl)
Sham treatment	15.6 ± 0.2	0.29 ± 0.01
LPS plus ischemic-reperfused		
Control	79.3 ± 4.8[a]	1.77 ± 0.06[a]
Rhei Rhizoma extract (30 mg/kg B.W./day)	68.0 ± 0.8[a,b]	1.65 ± 0.04[a,b]
Rhei Rhizoma extract (60 mg/kg B.W./day)	60.9 ± 1.1[a,b]	1.54 ± 0.04[a,b]

[a]$p<0.001$ vs. sham treatment values, [b]$p<0.001$ vs. LPS plus ischemic-reperfused control values.

Discussion

Rhei Rhizoma is well known to exert various pharmacological properties, purgative, antibacterial, astringent, stomachic, antitumor, anti-inflammation and cholagogic effects. Our previous study demonstrated that Rhei Rhizoma extract ameliorated the severity of renal failure through the decrease in uremic toxins such as urea nitrogen, Cr and MG.[10,11] In addition, the biological activities of Rhei Rhizoma such as improvement of nitrogen metabolism have been also reported.[24,25] Furthermore, Rhei Rhizoma is one of the most frequently prescribed herbs in Chinese traditional medicine for a variety of diseases such as constipation, blood-stasis syndrome, diarrhea, hypertension, mental and renal disorders.[26] Among the prescriptions, Wen-Pi-Tang attracted much attention in that it attenuates renal injury induced by oxidative stress.[9,27,28] Furthermore, the protective activity of Wen-Pi-Tang from renal failure is mainly attributed to Rhei Rhizoma.[10,11] On the basis of these evidences, the present study is focused on the novel pharmacological effect of Rhei Rhizoma on $ONOO^-$-induced renal damages under *in vivo* system.

We employed the LPS plus ischemia-reperfusion rat model as the *in vivo* $ONOO^-$-generation system. From our previous observation, we could confirm that the LPS plus ischemia-reperfusion process in the kidney causes the generations of $ONOO^-$ and its metabolites, and depletion in the antioxidative status.[9] Furthermore, renal photomicrographs subjected to the process were observed to cause acute renal injury in that the process results in the sclerosis of renal distal tubules through the expansion of mesangial regions.[29] Therefore, the LPS plus ischemia-reperfusion animal model is clearly useful to evaluate the effect of promising therapeutic agents from $ONOO^-$-

induced renal oxidative damages.

As a maker of $ONOO^-$ formation *in vivo* and a stable end-product of $ONOO^-$ oxidation, the nitrotyrosine level was estimated under the LPS plus ischemia-reperfusion. The nitration of target proteins can alter the structure or function of cellular molecules, leading to cell damage and dysfunction. Actually, the expression of 3-nitrotyrosine related to cell or tissue damage in pathological conditions has been demonstrated in the lesions such as atherosclerotic plaque, ischemia-reperfusion and acute renal failure.[30,31] The recent observations of 3-nitrotyrosine have been extensively assumed to arise from interaction of secondary oxidants spontaneously derived from $ONOO^-$ and reaction of nitrogen dioxide *via* MPO-catalyzed oxidation of NO_2^-, indicating two alternative enzymatic and non-enzymatic pathways for 3-nitrotyrosine production.[32,33] Our present results showed that the plasma 3-nitrotyrosine level and renal MPO activity cause significant increases in rats subjected to LPS plus ischemia-reperfusion, whereas the oral administration of Rhei Rhizoma extract reduced significantly plasma concentration of 3-nitrotyrosine and renal MPO activity (Figs. 1 and 2). From these results, protective activity of Rhei Rhizoma extract from nitration of tyrosine residues was mainly caused from direct inhibition of $ONOO^-$ formation and reduction of MPO activity.

$ONOO^-$ is generated from NO and O_2^- that have been associated with the activities of iNOS and XOD, respectively. The LPS plus ischemia-reperfusion process led to the significant elevation in the activity of iNOS but not in that of XOD (Table I). Several studies suggested that $ONOO^-$ can feed back, and down-regulate XOD activity and O_2^- generation that occurred during reperfusion following the postischemic burst of $ONOO^-$ generation, indicating the modulation of XOD function by $ONOO^-$ or NO.[34-36] The present study also supported the feed back role of $ONOO^-$ and down-regulation of XOD activity in the $ONOO^-$-generation *in vivo* model. On the other hand, the oral administration of Rhei Rhizoma extract inhibited the XOD activity markedly but not iNOS activity. It implies that Rhei Rhizoma extract would play the role as a scavenger of $ONOO^-$ precursor, O_2^-, through the inhibition of XOD activity as well as a direct $ONOO^-$ scavenger.

Against the oxidative cellular damages induced by $ONOO^-$, mitochondria is the most crucial locus for the intracellular formation and reaction of $ONOO^-$, and these interactions are recognized to contribute closely to the biological and pathological effects of $ONOO^-$.[37,38] However, the roles of cytochrome *c* oxidase, GSH, glutathione peroxidase (GSH-Px) and ubiquinol as cellular and mitochondrial $ONOO^-$ detoxification system can inhibit effectively $ONOO^-$-induced damage.[39-41] Therefore, we investigated effect of Rhei Rhizoma extract against $ONOO^-$-generated oxidative stress by the measurement of mitochondrial GSH level. Our results proved the fact that the decreased GSH level in renal mitochondria of LPS plus ischemic-reperfused rats resulted in the elevation by the administration of Rhei Rhizoma extract (Table II). Lizasoain *et al.*[42] and Castro *et al.*[39] reported that $ONOO^-$ caused the depletion of antioxidant defense mechanism in biological system and GSH protects significantly mitochondrial damage by $ONOO^-$-mediated oxidative stress. Moreover, renal GSH is well known to protect renal failure as an important cellular antioxidant, ·OH scavenger and cofactor for GSH-Px.[43] On the basis of these reports, the present result proposed that Rhei Rhizoma extract may alleviate mitochondrial oxidative damage and improve renal failure by $ONOO^-$ through the elevation of antioxidative defense status in biological system. Furthermore, the administration of Rhei Rhizoma extract reduced significantly the lipid peroxidation in renal mitochondria. It suggests that the protective effect of Rhei Rhizoma on renal mitochondrial damage by $ONOO^-$ is attributed to the reduction in the lipid peroxidation related to the attenuation of oxidative damages and/or increase in the antioxidant defense system.

The reduction of renal function during the development of renal failure indicates indirectly accumulation of uremic toxins in the body and influences on several biochemical or physiological functions of the kidney.[44] In the present study, the elevated plasma urea nitrogen and Cr levels as parameters of renal injury by LPS plus ischemia-reperfusion process were decreased by the oral administration of Rhei Rhizoma extract prior to this process, indicating that Rhei Rhizoma extract can prevent the renal disorder induced by $ONOO^-$ formation (Table III). In accordance with the present result, we previously also reported the protective effect of Rhei Rhizoma on renal dysfunction in *in vivo* renal failure model.[10,11] Taken together, we propose clearly the

beneficial effect of Rhei Rhizoma extract in alleviating renal functional impairment under $ONOO^-$-mediated renal injury.

The present study demonstrated a novel pharmacological action of Rhei Rhizoma extract that may prevent renal injury and disorder through reduction of protein nitration, elevation of mitochondrial detoxification system and alleviation of renal functional impairment against $ONOO^-$-induced oxidative damage. Thus, Rhei Rhizoma would be expected to provide effective therapeutic potential for the oxidative stress-induced renal failure.

和文抄録

本研究は，パーオキシナイトライト（$ONOO^-$）による腎障害に対する大黄エキスの保護効果について調べた。本実験で用いた *in vivo* $ONOO^-$ 生成のリポポリサッカライド＋虚血－再灌流モデルでは，血漿中の3－ニトロチロシンレベルと腎組織中のミエロペルオキシダーゼ（MPO）活性が上昇した。しかし，大黄エキスを30あるいは60 mg/kg 体重/日を30日間経口投与した群では，3－ニトロチロシンレベルとMPO活性が有意に低下し，キサンチンオキシダーゼ（XOD）活性も低下したが，誘導型一酸化窒素合成酵素活性は変化しなかった。これらの結果から，大黄エキスは直接的な $ONOO^-$ 消去作用と，XOD活性の阻害による O_2^- 消去作用を有することが示唆された。大黄エキスはまた，腎ミトコンドリアの抗酸化物質のグルタチオンレベルを増加し，脂質過酸化レベルが逆に低下していることを確認した。また *in vivo* $ONOO^-$ 生成群では，血漿尿素窒素とクレアチニンレベルが増加し，腎機能が低下していたが，大黄エキス投与群ではこれらはいずれも有意に低下し，腎機能の改善作用がみられた。以上より，大黄エキスは酸化ストレスによる腎障害に対し効果的な治療の可能性が示唆された。

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