Protective effects of Daio-botampi-to and its three major components on rat kidney and renal proximal tubule cells subjected to ischemia (hypoxia)-reperfusion (reoxygenation)

Erbo DONG, Takako YOKOZAWA,* Zhong Wu LIU, Satoshi ODA, Yasuko MUTO, Masao HATTORI and Hiroshi WATANABE

Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University

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Abstract

The effects of Daio-botampi-to (大黄牡丹皮湯) and its three major components-Rhei Rhizoma, Moutan Cortex and Persicae Semen-in ischemic-reperfused rats were examined. When Daio-botampi-to and Rhei Rhizoma were given orally at a dose of 200 mg/kg body weight/day for 20 consecutive days prior to ischemia and reperfusion, the conversion of xanthine dehydrogenase (XDH) to xanthine oxidase (XOD) was inhibited. In addition, both were able to sustain or increase the activities of the antioxidation enzymes superoxide dismutase, catalase and glutathione peroxidase, while malondialdehyde levels in the serum and renal tissue were lower. Decreased levels of urea nitrogen and creatinine in serum demonstrated a protective action against the renal dysfunction caused by ischemia and recirculation. On the other hand, it was demonstrated that Daio-botampi-to and Rhei Rhizoma could affect proximal tubule cells cultured under hypoxia-reoxygenation, probably by preventing oxygen free radicals from attacking cellular membranes. Although Moutan Cortex and Persicae Semen did not produce the same effects, being blood circulation-facilitating agents, it is believed that their action may occur through another mechanism, i.e. improving impaired reflow of blood on recirculation, resulting in prolonged ischemia.

Key words Daio-botampi-to, Rhei Rhizoma, ischemia (hypoxia)-reperfusion (reoxygenation), proximal tubule cell.

Introduction

Sufficient ischemia and reperfusion can trigger burst production of reactive oxygen free radicals, which then attack tissues, causing irreversible injury to a number of organs including the heart, liver, brain, small intestine and kidney. There is evidence that the xanthine oxidase (XOD) system is the main source of these radicals during episodes of ischemia followed by reperfusion.[1] Since the kidney is an organ sensitive to oxidant stress,[2,3] and clinically, temporary hypoxia or ischemia of this organ is difficult to avoid, an approach by which toxic free radical generation could be inhibited by enhancing the endogenous antioxidant defense system, replenishing potential antioxidant and suppressing XOD is obviously necessary.

In this study, we investigated the effects of Daio-botampi-to (大黄牡丹皮湯) and its three components (Rhei Rhizoma, Moutan Cortex and Persicae Semen) on an animal model of renal ischemia. Composed of five ingredients, Daio-botampi-to which is usually classified as a laxative, functions by removing blood stasis and eliminating edema *et al.*[4] Since Rhei Rhizoma has potential antioxidative activity and improves renal function in a chronic renal failure animal model,[5,6] and Moutan Cortex and Persicae Semen are crude drugs for facilitating blood circulation and removing thrombus,[7,8] we infer that they would exert a positive effect on renal damage during recirculation after ischemia.

The aim of this investigation was to clarify how

*〒930-01 富山市杉谷2630
富山医科薬科大学和漢薬研究所細胞資源工学部門 横澤隆子
2630 Sugitani, Toyama 930-01, Japan

these agents affect the conversion of xanthine dehydrogenase (XDH)-XOD and alter the activity of the antioxidative enzyme complex, i.e. superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px), in pretreated ischemic kidneys. An *in vitro* experiment with epithelial tubule cells co-cultured with Daio-botampi-to and its major components under hypoxia-reoxygenation was also undertaken to evaluate their response to hypoxic injury.

Materials and Methods

Prescription and crude drugs : The prescription's extract was made according to the same standard (formula composition, dosage of each crude drug and production techniques but without any excipient) as that of the commercial product from Kotaro Pharmaceutical Co., Ltd., Osaka, Japan. The crude drug extract was treated in the same manner as the prescription. A voucher specimen is deposited in the laboratory of Kotaro Pharmaceutical Co.

Medium and reagents : Dulbecco's modified Eagle medium/nutrient mixture F-12 (D-MEM/F-12) and fetal calf serum (FCS) were purchased from Life Technologies, Inc. (Grand Island, NY, USA) and Cell Culture Laboratories (Cleveland, OH, USA), respectively. A commercial kit (lactate dehydrogenase CII-Test Wako) for assaying lactate dehydrogenase (LDH) was obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Animal experiments : Male LWH : Wistar rats with a body weight of 150-160 g were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan). They were kept in a wire-bottomed cage under a conventional lighting regimen with a dark night. The room temperature (about 25°C) and humidity (about 60 %) were controlled automatically. Laboratory pellet 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 animals were divided into 5 groups, avoiding any intergroup difference in body weight gain. One group was given water, while the other was given Daio-botampi-to, Rhei Rhizoma, Moutan Cortex or Persicae Semen orally at a dose of 200 mg/kg body weight/day for 20 consecutive days. Six rats were used for each experimental group. After sodium pentobarbital anesthesia by intraperitoneal administration at 60 mg/kg body weight, bilateral flank incisions were made and the right kidney was removed. The left renal pedicle was then dissected to expose the left renal artery. A nontraumatic vascular clamp was placed across the renal artery for 45 min. Afterwards the clamp was removed to allow blood to reperfuse or recirculate into the organ for 120 min. After blood samples had been obtained by cardiac puncture under anesthesia, the kidney was immediately immersed in liquid nitrogen and ground into a fine powder using a mortar and pestle. About 0.5 g of frozen powder was added to 5 ml of homogenizing buffer consisting of 50 mM of K^+-phosphate buffer, containing 10 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF) and 0.1 mM EDTA, pH 7.0, and centrifuged at 40,000×g for 15 min. The XDH and XOD activities in the supernatant were measured by a spectrophotometric method, which was based on measurement of the change in optical density at 295 nm due to the appearance of uric acid, according to the method of Parks *et al.*[9] In order to determine the other enzymes, the kidney was perfused, and then homogenized with a 4-fold volume of ice-cold physiological saline and the activities of enzymes in the homogenate were determined. The activity of SOD was measured according to the nitrous acid method described by Elstner and Heupel[10] and Oyanagui,[11] which is based on the inhibition of nitrite formation from hydroxylamine in the presence of superoxide (O_2^-) generators. Catalase activity was measured by following the decomposition of hydrogen peroxide (H_2O_2)[12] directly by the decrease in extinction at 240 nm. The difference in extinction (ΔE_{240}) per unit time was used as a measure of the catalase activity. GSH-Px activity was obtained by colorimetry of 2-nitro-5-thiobenzoic acid, a compound produced through the reaction between glutathione and 5,5'-dithiobis (2-nitrobenzoic acid).[13] Protein content was determined by the method of Lowry *et al.*,[14] with bovine serum albumin as a standard. Malondialdehyde (MDA) was measured according to the method of Uchiyama and Mihara.[15]

Blood samples were allowed to clot at 4°C, and then centrifuged. The sera obtained in this manner

were used for determination of blood chemical parameters. MDA was determined using the method of Naito and Yamanaka.[16] Urea nitrogen and creatinine (Cr) were determined using the commercial reagents BUN Kainos and CRE-EN Kainos (Kainos Laboratories, Inc., Tokyo, Japan).

Cultured cell experiments : Commercially available LLC-PK_1 cells were maintained at 37°C in a humidified atmosphere of 5 % CO_2 in air (routine conditions) on culture plates with 5 % FCS-supplemented D-MEM/F-12 medium. After confluence had been reached, the cells were seeded on fresh 96-well culture plates at 10^4 per well. Daio-botampi-to or its component was added to the culture 2 h later, and the plates were incubated under routine conditions for 48 h. In another experiment, the cells, after culture under routine conditions for 41 h, were exposed to hypoxia in an anaerobic chamber for 6 h and then returned to routine conditions for another 1 h. Leakage of lactate dehydrogenase (LDH) into the culture medium was assayed using a commercial kit. The level of lipid peroxidant released from cultured cells was estimated by measuring the amount of MDA as described by Yagi.[17] All assays were performed in 5 determinations.

Statistics : Data are presented as mean±S.E. Differences among groups were analyzed by Dunnett's test. Significance was accepted at $p<0.05$.

Results

Alterations of XDH and XOD activity

Table I lists the changes in XDH and XOD activities among normal, and ischemia-reperfusion control or drug-treated kidneys. Ischemia showed an obvious effect on the two types enzymes activity. In comparison with the normal group, a clear decline in the activity of either total or XDH or XOD in the control, Moutan Cortex and Persicae Semen groups was observed. However, in terms of total activity, the prescription and Rhei Rhizoma showed an equivalent or stronger effect than either of the normal groups. With regard to XDH, its activity in ischemic kidneys from animals pretreated with the prescription or Rhei Rhizoma was increased about 1.3-fold that of the ischemia control ($p<0.001$). However, there was little difference between the control and Moutan Cortex- or Persicae Semen-treated groups. Moreover, in the case of XOD, as shown in Table I, its activity in all groups except for the control was significantly reduced from the normal value. The increased total activity in the prescription and Rhei Rhizoma groups was due largely to the increment of XDH activity. These findings indicate that Daio-botampi-to and Rhei Rhizoma not only effectively inhibit the conversion of XDH to XOD but also stimulate the former's activity.

Activities of antioxidation enzyme complex

The activities of oxidase complex in pretreated kidney were variable. Rats given Daio-botampi-to and Rhei Rhizoma show high activities of the three enzymes against the corresponding control (Table II), SOD being increased by 13 % and 19 %, catalase by 7 % and 10 %, and GSH-Px by 43 % and 50 %, respectively, almost reaching the normal levels. In contrast, in the Moutan Cortex and Persicae Semen groups, SOD and catalase activities were reduced to even

Table I Effect of Daio-botampi-to and its main components on XDH-XOD activities.

Group	Total (10^{-3} OD/min)	XDH (10^{-3} OD/min)	XOD (10^{-3} OD/min)
Normal	3.83±0.17	2.50±0.20	1.33±0.14
Ischemic and reperfused			
Control	3.34±0.27[a]	2.27±0.16	1.08±0.15
Daio-botampi-to	3.94±0.38[d]	3.03±0.29[b,e]	0.91±0.17[c]
Rhei Rhizoma	3.83±0.29[d]	2.96±0.19[a,e]	0.86±0.09[c]
Moutan Cortex	3.26±0.20[a]	2.30±0.23	0.96±0.06[b]
Persicae Semen	3.23±0.26[a]	2.19±0.18	1.04±0.16[a]

Statistical significance : [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ *vs.* normal values, [d]$p<0.05$, [e]$p<0.001$ *vs.* control values with ischemia-reperfusion.

Table II Effects of Daio-botampi-to and its main components on oxygen species-scavenging enzymes in the left kidney after ischemia-reperfusion.

Group	SOD (U/mg protein)	Catalase (U/mg protein)	GSH-Px (U/mg protein)
Normal	24.33±0.87	295.2±7.0	152.3±5.0
Ischemic and reperfused			
Control	19.51±0.99[b]	234.2±4.2[b]	97.0±6.8[b]
Daio-botampi-to	22.14±0.89[a,d]	251.1±11.9[b]	138.8±5.9[e]
Rhei Rhizoma	23.28±1.18[e]	258.4±8.1[b,d]	145.5±9.8[e]
Moutan Cortex	18.99±1.30[b]	211.6±9.4[b,d]	103.1±8.6[b]
Persicae Semen	12.15±0.26[b,c]	213.4±13.4[b,c]	137.2±7.2[a,c]

Statistical significance : [a]$p<0.05$, [b]$p<0.001$ *vs.* normal values, [c]$p<0.05$, [d]$p<0.01$, [e]$p<0.001$ *vs.* control values with ischemia-reperfusion.

Table III Effects of Daio-botampi-to and its main components on MDA.

Group	Serum MDA (nmol/ml)	Kidney MDA (nmol/g)
Normal	1.69±0.12	28.9±3.0
Ischemic and reperfused		
Control	2.38±0.12[b]	57.7±6.2[b]
Daio-botampi-to	1.99±0.10[a,d]	46.2±4.9[b,c]
Rhei Rhizoma	1.99±0.08[a,d]	31.1±3.0[d]
Moutan Cortex	2.21±0.21[b]	55.5±4.1[b]
Persicae Semen	2.01±0.08[a,c]	55.4±3.3[b]

Statistical significance : [a]$p < 0.01$, [b]$p < 0.001$ *vs.* normal values, [c]$p<0.01$, [d]$p<0.001$ *vs.* control values with ischemia-reperfusion.

Table IV Effects of Daio-botampi-to and its main components on urea nitrogen and Cr in serum.

Group	Urea nitrogen (mg/dl)	Cr (mg/dl)
Normal	16.2±0.3	0.41±0.02
Ischemic and reperfused		
Control	23.1±1.1[b]	0.53±0.04[b]
Daio-botampi-to	19.1±1.2[b,d]	0.47±0.03[a,c]
Rhei Rhizoma	18.3±0.5[a,d]	0.45±0.01[d]
Moutan Cortex	20.1±0.6[b,d]	0.47±0.01[a,c]
Persicae Semen	19.3±0.4[b,d]	0.49±0.02[b]

Statistical significance : [a]$p < 0.01$, [b]$p < 0.001$ *vs.* normal values, [c]$p<0.01$, [d]$p<0.001$ *vs.* control values with ischemia-reperfusion.

lower than those of the control. However, the activity of GSH-Px was increased by 6 % and 41 %, respectively.

MDA in serum and renal tissue

There were slight differences between each treated and control group in the serum MDA levels, which were decreased by 16 % in the prescription-, Rhei Rhizoma- and Persicae Semen-treated groups but only by 7 % in the Moutan Cortex group. However, in ischemic kidneys the level was increased by nearly 100 % as compared with intact kidney. This increment was effectively suppressed by either Daio-botampi-to or Rhei Rhizoma with marked significance, although the values were still higher than those in normal rats, whereas the other two compounds had no effect, as shown in Table III.

Blood urea nitrogen and Cr

A decrease of urea nitrogen in serum was clearly observed in all 4 treated groups, among which there were obvious differences from the ischemia control (Table IV). A similar trend was also found in Cr content, excluding the Persicae Semen-treated group.

LDH leakage from cultured cells

During routine incubation for 48 h, a certain amount of LDH was released from the cells, being about 119.3 ± 3.6 mIU/ml, as shown in Table V. Upon coculture with different concentrations of Daio-botampi-to and Rhei Rhizoma, this leakage was significantly suppressed at 12.5 to 125 μg/ml, respectively. Hypoxia for 6 h and then routine culture for 1 h resulted in marked leakage of the enzyme (176.9 ± 4.6 mIU/ml). Pretreatment of the cells with Daio-botampi-to or Rhei Rhizoma limited this leakage marked, only 25 μg/ml appearing in the medium. On

Table V Effect of Daio-botampi-to and its main components on LDH leakage from LLC-PK$_1$ cells.

Group	Concentration (μg/ml)	LDH activity (mIU/ml)	
		Routine	Hypoxia/reoxygenation
Control	–	119.4±3.6	176.9±4.6
Daio-botampi-to	2.5	115.6±3.9	171.3±7.2
	12.5	102.9±7.0^b	168.4±3.9
	25	95.8±9.9^c	161.7±6.7^a
	50	94.0±5.1^c	152.1±7.3^c
	125	79.9±2.8^c	148.1±8.5^c
Rhei Rhizoma	2.5	116.6±4.9	171.9±6.9
	12.5	104.1±4.4^b	169.9±5.4
	25	95.3±3.7^c	161.4±6.8^b
	50	91.9±6.2^c	160.7±4.5^b
	125	89.5±6.9^c	149.2±7.9^c
Moutan Cortex	2.5	117.5±3.9	175.3±4.2
	12.5	118.8±2.5	170.9±8.7
	25	115.9±3.0	168.7±3.8
	50	100.3±6.1^c	163.8±5.2^a
	125	97.6±4.7^c	163.4±6.9^a
Persicae Semen	2.5	119.3±2.4	176.4±7.3
	12.5	119.2±3.1	174.5±4.3
	25	117.2±4.5	168.9±7.2
	50	113.2±4.7	167.8±7.1
	125	108.4±4.6^b	164.2±3.7^a

Statistical significance : $^a p<0.05$, $^b p<0.01$, $^c p<0.001$ *vs.* control values.

Table VI Effect of Daio-botampi-to and its main components on MDA leakage from LLC-PK$_1$ cells.

Group	Concentration (μg/ml)	MDA (nmol/well)	
		Routine	Hypoxia/reoxygenation
Control	–	0.129±0.009	0.166±0.014
Daio-botampi-to	2.5	0.118±0.009	0.139±0.008^a
	12.5	0.110±0.006^b	0.129±0.012^c
	25	0.089±0.003^c	0.126±0.009^c
	50	0.083±0.006^c	0.124±0.005^c
	125	0.080±0.009^c	0.114±0.017^c
Rhei Rhizoma	2.5	0.122±0.008	0.153±0.011
	12.5	0.118±0.006	0.136±0.010^b
	25	0.100±0.005^c	0.119±0.005^c
	50	0.084±0.011^c	0.113±0.008^c
	125	0.069±0.008^c	0.103±0.011^c
Moutan Cortex	2.5	0.122±0.012	0.163±0.013
	12.5	0.121±0.009	0.159±0.012
	25	0.112±0.012	0.154±0.026
	50	0.104±0.005	0.147±0.021
	125	0.095±0.006	0.126±0.006^a
Persicae Semen	2.5	0.124±0.010	0.163±0.012
	12.5	0.121±0.012	0.156±0.014
	25	0.119±0.007	0.145±0.014
	50	0.115±0.008	0.143±0.013
	125	0.114±0.011	0.135±0.017^a

Statistical significance : $^a p<0.05$, $^b p<0.01$, $^c p<0.001$ *vs.* control values.

the other hand, little change was observed in either the Moutan Cortex- or Persicae Semen-treated group at an identical concentration, but these was significant suppression at 50 or 125 μg / ml, respectively (Table V).

MDA level released into culture medium

In routine culture medium, 0.129 nmol of MDA was detected in the control group. The level was reduced in all pretreated groups, although this was significant only in the Daio-botampi-to and Rhei Rhizoma groups (Table VI). Moreover, hypoxic incubation led to the formation of a large amount of MDA, being 1.3-fold that in routine culture. The inhibition of this event evident in the 4 treated groups bore a resemblance to that of LDH, i.e. only the cells pretreated with Daio-botampi-to and Rhei Rhizoma demonstrated a positive effect.

Discussion

Studies have confirmed that ischemia and reperfusion can cause functional and structural damage to renal tissue, due largely to excessive toxic free radicals generated during the procedure by the following major factors : 1) XOD reaction, including conversion of XDH to XOD, and 2) degradation of enzymatic or non-enzymatic scavenging systems.[18-23] Although an improvement has been shown to be effected by certain antioxidants such as SOD and dimethylthiourea, they have limitations for actual medical application.[24,25] Recently, therefore, much attention has been paid to natural-origin antioxidants because of their less severe side effects and abundant availability.

In the present study, we first examined the alterations of XDH and XOD activities in both normal and

ischemia-reperfused kidneys (after 45 min of ischemia and then 120 min of reperfusion) from rats untreated or pretreated with Daio-botampi-to and three major components prior to ischemia. It was found that Daio-botampi-to and Rhei Rhizoma significantly prevented the conversion of XDH to XOD in the tissue during recirculation after ischemia ($p<0.001$, respectively), although neither Moutan Cortex nor Persicae Semen produced such an effect, suggesting that Rhei Rhizoma contributed mainly to the prescription. Although the main mechanism responsible still remains unclear, this is of critical significance because, as postulated, during ischemia or hypoxia XDH is converted very rapidly to the O_2^--producing oxidase.[26] Hypoxanthine, which accumulates in ischemic tissues as result of ATP catabolism, is an oxidizable substrate for the enzyme. The reducible substrate, molecular oxygen, is provided during reperfusion, and with it comes a burst of O_2^- radical production. The O_2^- may subsequently undergo dismutation to form H_2O_2, a very rapid reaction that is increased several-fold in the presence of the enzyme SOD. H_2O_2, which is relatively nontoxic, can interact with O_2^- in the presence of metal chelates to form the highly reactive hydroxyl radical (·OH), leading to chain lipid peroxidation.[27 29] The observed increase of MDA in the ischemic kidney confirmed this (Table III). As a result, the increments of urea nitrogen and Cr in serum indicated that such peroxidation consequently gave rise to renal dysfunction.

On the other hand, it has been proven that under normal physiological conditions XOD-XDH exists in kidney tissue in a reduced form, and the activity of either XOD or XDH could also be affected by ischemia-reperfusion. Although evidence obtained here demonstrates sufficiently that Daio-botampi-to and its major ingredient, Rhei Rhizoma, could effectively increase XDH activity by inhibiting XOD, this does not seem enough to explain the whole mechanism responsible for ameliorating renal dysfunction resulting from ischemia, since it was demonstrated that another crucial function, endogenous scavenging, could not be ignored.

The tissue is normally protected against the noxious effects of free radicals by a number of antioxidants and enzymes, so-called scavenger substances. One of them, SOD, catalyses the dismutation of O_2^- to H_2O_2, and two others, catalase and GSH-Px, catalyse the conversion of H_2O_2 directly to H_2O, i.e. without the formation of ·OH. It has been reported that hypoxanthine, which is the end product of the anoxic degradation of ATP, accumulates in renal tissue during ischemia. During recirculation (and reoxygenation) of the kidney, the hypoxanthine is rapidly oxidized, and free radicals may be formed in amounts exceeding the capacity of the normal scavenger system.[30, 31] Pretreatment with Daio-botampi-to or Rhei Rhizoma not only delayed the oxidation of hypoxanthine but also stimulated the activities of antioxidant enzyme pathways such as $O_2^-\rightarrow$SOD$\rightarrow H_2O_2\rightarrow$catalase (or GSH-Px)$\rightarrow H_2O$, as well as probably postponing or halting the oxidation of hypoxanthine, although this requires further confirmation.[32] Although neither Moutan Cortex nor Persicae Semen had any function, they were able to exert their effect through selective elevation of GSH-Px activity.

Since the damage caused by peroxidation is specifically membrane damage, it is necessary to evaluate the mode of action of the prescription or its three components at the cellular level. In this study, epithelial proximal tubule cell lines were chosen as a model because cells of this kind are one of the major targets of oxygen free radical attack.[33] As mentioned in Methods, the cells were co-cultured with various concentrations of samples under dual conditions : routine (95 % air, 5 % CO_2) and hypoxia. The results showed that in comparison with the corresponding control groups, exposure to hypoxia-reoxygenation culture made the cells release LDH on a large scale, indicating the occurrence of cellular membrane insult. Additionally, the MDA level in the medium was largely increased by 29 % of the intact control, indicating the causality involved in LDH leakage. This suggests that the main feature of cell damage would be membrane injury caused by oxidative stress.

Based on the above findings, it can be inferred that proximal tubule cell insult is certainly one of the causes of acute renal failure induced by ischemia-reperfusion. Therefore, protection of membranes from oxygen free radical attack or repair of the damage would possibly provide a therapeutic approach for treatment of renal failure. Daio-botampi-to

extract at very low concentration (12.5 μg/ml) in the culture medium attenuated significantly the leakage of LDH and almost completely abolished the lipid peroxidant formed as a response to hypoxia/reoxygenation. In similar experiments with three ingredients, it was noted with interest that the most potent one was Rhei Rhizoma, whereas the other two acted weakly, which is consistent with our *in vivo* findings above. Lipid peroxidation is known to occur in biological membranes with potentially injurious consequences such as increment of membrane permeability, causing LDH leakage. Therefore, membrane protection through antiperoxidation is suggested to be the mechanism of action of the drugs.

It was observed that two components in the prescription - Moutan Cortex and Persicae Semen - failed to show effects similar to those of the other two. As a whole, the effectiveness of the prescription is due to each component it contains. Because both Moutan Cortex and Persicae Semen can facilitate systemic blood circulation and remove thrombus,[7,8] it is predicted that they may increase or improve the impaired reflow of blood on recirculation, resulting in prolonged ischemia.

It is concluded that Daio-botampi-to could ameliorate renal function when administered to rats prior to renal ischemia-reperfusion by inhibiting the conversion of XDH to XOD and increasing the activities of antiperoxidant enzymes in the tissue. In addition, this prescription could also exert a protective effect on proximal tubule cells. The precise mechanism is obviously attributable to its antiperoxidation function. Results obtained using its three major components showed that only Rhei Rhizoma had similar actions to Daio-botampi-to, suggesting that it plays a crucial role in the prescription.

和文抄録

大黄牡丹皮湯並びにその主構成生薬の大黄，牡丹皮，桃仁の効果を腎の虚血-再灌流ラットを用い検討した。虚血-再灌流前に大黄牡丹皮湯あるいは大黄を 200 mg/kg 体重/日，20 日間経口投与したところ，xanthine dehydrogenase の xanthine oxidase への変換を抑制，血清並びに腎組織中の malondialdehyde レベルが低下したが，抗酸化酵素の superoxide dismutase, catalase, glutathione peroxidase 活性はいずれも上昇した。血清中の尿素窒素，creatinine レベルの低下も認めたが，これらは虚血-再灌流によって生じる腎機能不全に対する保護作用によるものと考えられた。一方，大黄牡丹皮湯と大黄は培養近位尿細管細胞を用いた低酸素-再酸素化条件下による細胞傷害をも阻止した。しかし，駆瘀血薬の牡丹皮，桃仁にはこのような効果は認められず，これら生薬は異なった作用機序で作動していることが示唆された。

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