Evidence suggesting a role for magnesium lithospermate B, an active component isolated from Dan Shen, in glycerol-induced acute renal failure rats

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Abstract

In order to evaluate the effectiveness of magnesium lithospermate B in alleviating acute renal failure, we used a rat model of acute tubular necrosis and designed two experimental methods. In experiment 1, normal rats were administered magnesium lithospermate B (20 mg/kg body weight/day) for 20 days before injection of glycerol. After deprivation of water for 18 h, the rats received an injection of 50 % glycerol into the muscle of the rear limb at 10 ml/kg body weight. The rats were given magnesium lithospermate B continually for 2 days orally, and then sacrificed by decapitation. In experiment 2, we treated rats with acute renal failure induced by the above method by intraperitoneal administration of magnesium lithospermate B (10 or 20 mg/kg body weight/day) once every 8 h for 2 days, after the rats had been deprived of water for 6 or 12 h. The markedly elevated levels of urea nitrogen and creatinine in blood and the reduced creatinine clearance related to progression of renal failure caused by injection of the nephrotoxic drug following changes in the dehydration period were significantly improved by oral and intraperitoneal administration of magnesium lithospermate B. Urine volume and urinary osmolarity, which were markedly lowered with the advance of renal failure in parallel with the various periods of dehydration, were obviously increased, and this compound appeared promising for suppressing the increased excretion of urinary glucose and fractional excretion of sodium due to alteration of tubule function following injection of glycerol.

Key words acute renal failure, glycerol, magnesium lithospermate B, Dan Shen, rat.

Introduction

Acute renal failure is characterized by rapid decline of the glomerular filtration rate and retention of nitrogenous waste products. This syndrome occurs in approximately 5 % of all hospital admissions. In some clinical settings such as intensive care units, acute renal failure occurs in up to 30 % of patients.[1] This condition can be divided into three categories : prerenal failure, postrenal failure and acute tubule necrosis. The latter is commonly encountered by a large number of nephrologists, since it appears in about three-quarters of all cases of acute renal failure.[2] Although its pathogenesis, especially the initial causative factor, is still uncertain, most workers consider that renal ischemia, intratubular obstruction, back - leakage of glomerular filtration across the damaged renal tubular epithelium and markedly impaired glomerular filtration are responsible. Among these, renal ischemia plays an essential role.[3]

Although many of the manifestations of acute renal failure can be controlled by conservative therapy, such as maintaining a stable water-electrolyte balance, minimizing catabolism, the use of antihypertensive agents and administration of ATP-$MgCl_2$ as

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an energy source to improve the function of the damaged tubule and vascular cells,[1] more effective methods are urgently needed. For acute tubular necrosis, dialysis is essential for removal of the etiological factors. However, prolonged maintenance dialysis places a great burden on the patient from both mental and physical aspects, and social problems including financial issues have now arisen due to the increase in the number of dialysis patients. Under these circumstances, it is very important to find an effective therapy for acute renal failure.

We have been studying the actions of crude drugs in rats with experimental renal failure, as part of a research program on drug therapy. Through such studies, we have demonstrated that administration of Salviae Miltiorrhizae Radix (a traditional Chinese medicinal herb known as Dan Shen) improves blood circulation and relieves blood stasis.[4-6] The present authors have also isolated magnesium lithospermate B from an aqueous extract of Salviae Miltiorrhizae Radix, and reported the details of its structure and activities.[7 16] In the present study, as part of a research project on pharmacotherapy for renal failure, we investigated the effect of magnesium lithospermate B on various parameters of renal function. For this purpose, we designed two experiments to study the preservative and improving action of magnesium lithospermate B on renal function.

Materials and Methods

Animals : Male Wistar rats were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan).

Purification of magnesium lithospermate B from Salviae Miltiorrhizae Radix : As reported previously,[7] commercially available Salviae Miltiorrhizae Radix (*Salviae Miltiorrhiza* BUNGE) (1.0 kg) produced in China was extracted twice with water (1.5 liters) at 80°C. After removal of insoluble matter by filtration, the filtrate was concentrated under reduced pressure (40°C) and subjected to MCI-gel CHP-20P (7.5 cm i.d. ×35 cm) column chromatography. After washing the column with water, elution with 50 % aqueous methanol yielded polyphenols (62 g), which were chromatographed using a Sephadex LH-20 column (5.0 cm i.d.× 42 cm) with water containing increasing amounts of ethanol to afford three fractions : I (4.8 g), II (0.35 g) and III (5.9 g), and compound 1 (7.56 g). Fractions I and III were rechromatographed separately on a Sephadex LH-20 column using water as the eluent to yield compound 2 (1.98 g) and a further fraction of compound 1 (4.3 g). Compound 1 was identified as magnesium lithospermate B on the basis of its ^{13}C nuclear magnetic resonance spectrum, infrared spectrum, negative fast-atom bombardment mass spectrum, proton nuclear magnetic resonance spectrum, energy-dispersive X-ray analysis and other data. Its chemical structure is shown in Fig. 1.

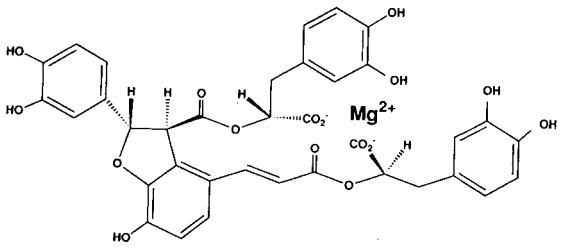

Fig. 1 Structural formula of magnesium lithospermate B.

Experimental design : In order to observe the action of magnesium lithospermate B in preventing or delaying acute renal failure in rats, we used an experimental protocol in which the rats were administered magnesium lithospermate B (20 mg/kg body weight/day) orally for 20 days before injection of glycerol. After deprivation of water for 18 h, the rats were injected with 50 % glycerol into the muscles of the rear limbs at 10 ml/kg body weight, and urine specimens were collected for 1-2 days thereafter. The rats were given magnesium lithospermate B continually *per os* for 2 days after glycerol injection, then sacrificed by decapitation to obtain their blood (experiment 1). In experiment 2, after deprivation of water for 6 or 12 h, rats received an intramuscular injection of 50 % glycerol into a hind limb, and magnesium lithospermate B (10 or 20 mg/kg body weight/day) was administered intraperitoneally once every 8 h for 2 days. Urine specimens were collected for 1-2 days after glycerol injection. After urine sampling, the rats were sacrificed by decapitation to obtain their blood.

Analyses : Urea nitrogen and creatinine (Cr) were

determined using commercial reagents (BUN Kainos and CRE-EN Kainos obtained from Kainos Laboratories, Inc., Tokyo, Japan). Sodium (Na) was measured with an electrolyte analyzer (AHS/Japan Corporation, Tokyo, Japan) using a hydrogen electrode.[17] Osmolarity was measured with an osmometer (OSA-21 ; Nikkiso Co. Ltd., Tokyo, Japan) using the cryoscopic method. Glucose was determined using the method of Momose *et al.*[18] The creatinine clearance (Ccr) was calculated on the basis of urinary Cr, serum Cr, urine volume and body weight, and fractional excretion of sodium (FE_{Na}) was calculated on the basis of urinary Na, serum Na, urinary Cr and serum Cr, using the equation shown below.

Ccr (ml/kg body weight/min) = {urinary Cr (mg/dl) × urine volume (ml)/serum Cr (mg/dl)} × {1,000/body weight (g)} × {1/1,440 (min)}

FE_{Na} (%) = (urinary Na/serum Na) / (urinary Cr/serum Cr) × 100

Statistics : Statistical analysis was performed by Dunnett's test.

Results

The results of experiment 1 are summarized in Table I. The blood urea nitrogen level of normal rats was 18.3 mg/dl ; whereas in glycerol-induced acute renal failure it increased significantly to about 13.7 times the normal value. The serum Cr, FE_{Na} and urinary glucose levels were also increased to 17.0, 35.3 and 5.0 times the normal ones, respectively, while Ccr and urine osmolarity were decreased by 99 % and 71 % of the respective levels in normal rats. Administration of magnesium lithospermate B caused a 74 % increase in Ccr from 0.057 to 0.099 ml/kg body weight/min. Oral administration of magnesium lithospermate B before injection of glycerol also increased the urine osmolarity of rats with renal failure. Although the urine volume was also increased to some extent in the magnesium lithospermate B group, the change was relatively slight. On the other hand, FE_{Na} fell from 20.83 to 12.52 % (a 40 % change) and urinary glucose from 135.9 to 97.2 mg/day (a 28 % change).

Table II shows the results obtained from experiment 2. In rats deprived of water for 6 h, the blood urea nitrogen level increased to reach 32.6 mg/dl in the controls, whereas the corresponding values for the rats given 10 and 20 mg of magnesium lithospermate B were decreased to 17.1 and 16.3 mg/dl, respectively. The Cr value was also lowered significantly in rats given magnesium lithospermate B. Although the urine volume change showed no significant differences between the control and magnesium lithospermate B-treated groups, the Ccr value was evidently increased from 3.59 to 4.60 ml/kg body weight/min at the 10-mg level (a 28 % change, $p < 0.05$) and from 3.59 to 4.98 ml/kg body weight/min at the 20-mg level (a 39 % change, $p < 0.01$). Similarly, the urine osmolarity in rats given the agent orally at 10 mg showed a significant increase from 763 to 918 mOsm/l (a 20 % change, $p < 0.01$). A further increase in the dose to 20 mg produced a further increase of the urine osmolarity to a level 23 % higher than in control rats,

Table I Biological parameters in rats with glycerol-induced acute renal failure, including those given magnesium lithospermate B.

Parameter	Normal	Control	Magnesium lithospermate B
			20 mg
Blood urea nitrogen, mg/dl	18.3±1.6	251.5±15.9**	254.4±16.0**
s-Cr, mg/dl	0.38±0.02	6.46±0.07**	5.91±0.38**
Urine volume, ml/day	21.1±1.8	16.1±3.6	22.6±4.2
Ccr, ml/kg B.W./min	5.56±0.48	0.057±0.009**	0.099±0.014**,a
FE_{Na}, %	0.59±0.05	20.83±4.18*	12.52±2.53*
Urine osmolarity, mOsm/l	1198±90	347±24**	427±25**,a
Urinary glucose, mg/day	27.3±1.4	135.9±13.7**	97.2±9.5**,a

Statistical significance : *$p < 0.05$, **$p < 0.001$ vs. normal rats, a$p < 0.05$ vs. control rats with renal failure.

Table II Biological parameters in rats with glycerol-induced acute renal failure, including those given magnesium lithospermate B.

Parameter	Control	Magnesium lithospermate B	
		10 mg	20 mg
Deprivation of water for 6 h			
Blood urea nitrogen, mg/dl	32.6±3.2	17.1±0.7[c]	16.3±1.1[c]
s-Cr, mg/dl	0.57±0.05	0.43±0.02[a]	0.38±0.01[b]
Urine volume, ml/day	34.8±2.8	33.1±1.2	28.4±2.4
Ccr, ml/kg B.W./min	3.59±0.27	4.60±0.21[a]	4.98±0.21[b]
FE_{Na}, %	2.43±0.38	1.14±0.17[b]	0.96±0.10[b]
Urine osmolarity, mOsm/l	763±32	918±20[b]	940±56[a]
Deprivation of water for 12 h			
Blood urea nitrogen, mg/dl	77.9±9.7	54.8±4.6	44.6±2.2[b]
s-Cr, mg/dl	1.68±0.28	1.11±0.18	0.91±0.04[a]
Urine volume, ml/day	18.8±1.6	19.1±2.2	24.9±2.1[a]
Ccr, ml/kg B.W./min	1.58±0.35	2.48±0.33	4.41±0.33[c]
FE_{Na}, %	3.07±0.51	1.49±0.27[a]	1.04±0.10[b]
Urine osmolarity, mOsm/l	607±63	863±26[b]	874±62[b]

Statistical significance : [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ vs. control rats with renal failure.

whereas FE_{Na} was decreased significantly at either the 10- or 20-mg dosage level, as shown in Table II.

When rats were deprived of water for 12 h, the blood urea nitrogen level in controls was 77.9 mg/dl, whereas it was 54.8 mg/dl in rats given magnesium lithospermate B at 10 mg/kg body weight/day. A further increase in the dose to 20 mg produced a further decrease in the blood urea nitrogen level to 44.6 mg/dl (Table II). The Cr level in the renal failure control group was 1.68 mg/dl, and this was reduced to 1.11 mg/dl in the rats given magnesium lithospermate B at 10 mg / kg body weight / day. Although this decrease was not statistically significant, a significant decrease to 0.91 mg/dl was achieved in the 20 mg group. On the other hand, urine output in control rats with renal failure was 18.8 ml/day, and in rats given 20 mg of magnesium lithospermate B, this increased significantly to approximately 32 % of the control value. The Ccr and urine osmolarity in rats given 10 mg of magnesium lithospermate B were also increased to 1.57 and 1.42 times the level in control rats, respectively, while FE_{Na} was decreased by 51 %. These effects were more prominent in rats given 20 mg of the compound than in those given 10 mg.

Discussion

Hypertonic glycerol-induced myoglobinuric acute renal failure is a widely applied and well characterized model of experimental acute renal failure. The disease which follows intramuscular injection of glycerol in the rat mimics the acute renal failure seen in crush syndrome in humans, because its primary cause and renal histological consequences such as tubule injury are similar to those seen clinically.[19, 20]

In the rat model of glycerol-induced acute renal failure, it is generally considered that the degree of renal failure maximally correlates with the duration of dehydration. The more rigorous the dehydration, the more severe the effect on renal function. Thiel *et al.*,[21] Hishida *et al.*[22] and Ishikawa[23] reported that in the rat, the degree of dehydration appeared to be a risk factor for the development of glycerol-induced acute renal failure. If previous dehydration had occurred, the reduction of renal blood flow and vasoconstriction after glycerol injection were more severe and prolonged. Similar results were obtained in our present study in rats injected with glycerol. As shown in

Tables I and II, a further increase in the time of dehydration produced a further increase in urea nitrogen and Cr in blood and a decrease of Ccr. Furthermore, the rats which underwent more severe dehydration exhibited more evident reduction of urine volume and urinary osmolarity. On the other hand, increases of FE_{Na} and urinary glucose were observed in the rats injected with glycerol. Thus, the findings of the present study indicated that alterations of renal parameters in rats injected with glycerol were closely correlated with the period of dehydration.

Because of the high morbidity of acute renal failure, and its potentially serious consequences, prophylactic therapy is of high importance. Preventive administration of traditional Chinese medicine which improves blood circulation and relieves blood stasis is empirically considered to decrease the incidence or severity of acute renal failure. Therefore, in the present study, we evaluated the protective effect of magnesium lithospermate B, an active component of Dan Shen, which is a representative agent among a group of traditional medicines used for improving blood flow and removing sticky blood,[24-26] against acute renal failure induced by glycerol in rats. Although the rats showed more severe renal dysfunction following more rigorous dehydration (deprivation of water for 18 h), the present data demonstrated that oral administration of magnesium lithospermate B prior to glycerol injection was highly effective in improving glomerular and renal tubular function. As shown in Table I, the serum Cr level tended to decrease upon pretreatment with magnesium lithospermate B in comparison with the corresponding control value. In contrast, Ccr, an effective index of the glomerular filtration rate, was evidently increased in comparison with the control. Urinary glucose was significantly and markedly decreased by oral administration of magnesium lithospermate B prior to injection of glycerol. Similarly, magnesium lithospermate B significantly increased urinary osmolarity of rats with acute renal failure induced by glycerol injection in comparison with the corresponding control values, showing excellent protection of renal function.

In a previous study, we found that magnesium lithospermate B inhibits the progression of renal failure when administered either intraperitoneally or orally, resulting in improvement of renal blood flow and function, and relief from uremia.[8,12,14] The present data also indicated that the markedly reduced Ccr and increased blood urea nitrogen and serum Cr related to progression of renal failure were significantly improved by intraperitoneal administration of magnesium lithospermate B, as shown in Table II. Although rats subjected to more severe dehydration showed more evident reduction of urine volume and osmolarity, the therapeutic action of magnesium lithospermate B was the same, irrespective of whether water was withheld for 6 or 12 h. Urine volume and urinary osmolarity were significantly increased in comparison with control values. However, the increase of urine volume in rats deprived of water for 6 h, similar to the polyuria stage of acute renal failure seen clinically, was reversed by intraperitoneal administration of magnesium lithospermate B, displaying a bimodal action on tubule function. Moreover, the increases in FE_{Na} and urinary glucose following injection of glycerol with various periods of dehydration were similarly inhibited by the active component isolated from Salviae Miltiorrhizae Radix, reflecting amelioration of renal failure (Table II). Not only oral administration of magnesium lithospermate B prior to injection of glycerol, but also intraperitoneal administration of this compound, produced renal protective effects. However, its effects on various parameters of renal function might be connected with the period of dehydration.

Renal vasoconstriction is suspected to play a major role in the pathogenesis of this model of acute renal failure, which is characterized by a considerable reduction in renal blood flow and the glomerular filtration rate.[27,28] This rapid vasoconstriction appears to include both preglomerular and postglomerular arterioles and leads to severe renal parenchymal ischemia.[29] Most importantly, this reduction in renal blood flow is correlated with the severity of renal failure. Tubular ischemia with glomerular lesions also suggests postglomerular vasoconstriction, since the tubular vascularization arises from the glomerular efferent arteriole.[30] The sequelae of tubular ischemia may be responsible for proximal and distal duct dysfunction. These include intratubular obstruction due to casts, cellular debris caused by extensive ne-

crosis of the epithelial cells of the proximal convoluted tubules, a decrease in Na and glucose reabsorption in the thick ascending limb of Henle associated with a decrease in Na and glucose absorption in the proximal tubules, since ischemic injury of the kidney disturbs the integrity of the epithelial cytoskeleton in the proximal tubules, and the inability of the kidney to regulate the excretion of free water and to concentrate the glomerular filtrate due to changes in inner medullary anatomy and medullary blood flow following postglomerular vasoconstriction.[31]

The mechanism of myoglobin-induced vasoconstriction has been widely and extensively investigated. Studies by Oken *et al.*[32] and Flamenbaum *et al.*[33] attempted to evaluate whether the renin-angiotensin system plays a role in the genesis of myoglobinuric acute renal failure, but failed to reveal any evidence of efficacy. On the other hand, Michielo *et al.*[34] and Wardle[35] evaluated the role of prostaglandins in the regulation of renal hemodynamics in rats administered glycerol, and showed that a deficiency of renal prostaglandin production is responsible for the resulting acute renal failure. Prostaglandin E_2 has a vasodilative effect and is cytoprotective for tubule cells, and thus would help to protect the vascular endothelium. This effect of magnesium lithospermate B on renal failure is consistent with our previous finding that prostaglandin E_2 was increased by magnesium lithospermate B, thus attenuating the severity of adenine-induced renal failure.[8,9] The results of the present study as well as those of previous research, which showed rapid improvement of renal hemodynamics 6 h after administration of magnesium lithospermate B,[8,9] imply that activation and acceleration of prostaglandin E_2 are probably involved in the action of magnesium lithospermate B.

From the present experimental evidence, we conclude that magnesium lithospermate B has a conspicuously protective action in rats injured by nephrotoxic agents and comprehensively improves renal function in rats with acute renal failure, not only the glomeruli but also the renal tubules. It is expected that magnesium lithospermate B would have broad applications in areas covered by a variety of medicines, including low-dose dopamine, trichlormethiazide, furosemide, ATP-$MgCl_2$, calcium channel blockers and amino acid infusion, which have already been tried for treatment of ischemic and nephrotoxic acute renal failure, but which have been either ineffective or offered only partial relief.[1] In addition, magnesium lithospermate B produced no side effects, even when the compound was administered repeatedly. An acute toxicity study of oral magnesium lithospermate B in terms of LD_{50} by the up and down method showed that it was very safe ($>3{,}000$ mg/kg in 6-week-old male ddy mice weighing 31-35 g).[13] In contrast, furosemide and trichlormethiazide caused several abnormalities of serum parameters, particularly electrolyte disturbance and an increase of serum glutamate oxaloacetate transaminase and blood urea nitrogen, when administered repeatedly.[36,37] Thus, magnesium lithospermate B may have some advantages over other therapeutic agents in ameliorating the renal damage in glycerol-treated rats.

In summary, a dramatic improvement of renal function, and amelioration of electrolyte disturbance and renal damage (tubular necrosis) were observed in rats with glycerol-induced acute renal failure pretreated and treated with magnesium lithospermate B. This shows that the compound may be a useful therapeutic medicine for acute renal failure. However, the exact mechanism of action of magnesium lithospermate B is still unclear, and more detailed clarification of these experimental phenomena is desirable.

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和文抄録

急性腎不全による死亡率は依然として高く，現在でもより有効な治療法を求めて病態解明への努力がなされている。本実験ではグリセロール誘発急性腎不全ラットを用い，magnesium lithospermate B の作用を検討した。グリセロールの注射は血清尿素窒素，Cr を上昇させ，Ccr の低下を引き起こした。また尿量の減少とともに尿浸透圧が低下したが，ナトリウム排泄率(FE_{Na})，グルコ

ース排泄量は上昇していた。これに対しmagnesium lithospermate B投与群では血清尿素窒素，Crが低下，Ccrが上昇，尿量の増加とともに尿浸透圧が上昇し，FENa，グルコース排泄量の低下作用を有していた。これらmagnesium lithospermate Bによる作用はグリセロール処理前に，あるいはグリセロール処理後に投与した場合，いずれにおいても認められ，腎循環系に好影響をもたらすことが示された。

References

1) Brady, H.R. and Brenner, B.M. : Acute renal failure. In "Harrison's Principles of Internal Medicine" (Ed. by Isselbacher, K.J., Braunwald, E., Wilson, J.D., Martin, J.B., Fauci, A.S. and Kasper, D.L.), McGraw-Hill, Inc., New York, pp.1265-1274, 1994.
2) Balslov, J.T. and Jorgensen, H.E. : A survey of 499 patients with acute anuric renal insufficiency. *Am. J. Med.* **34**, 753-764, 1963.
3) Patel, R., McKenzie, J.K. and McQueen, E.G. : Tamm-Horsfall urinary mucoprotein and tubular obstruction by casts in acute renal failure. *Lancet* **February 29**, 457-461, 1964.
4) Chung, H.Y., Yokozawa, T. and Oura, H. : Renal effect of aqueous extract from Salviae Miltiorrhizae Radix in normal rats. *Chem. Pharm. Bull.* **35**, 2465-2469, 1987.
5) Chung, H.Y., Yokozawa, T. and Oura, H. : Renal function-improving effect of Salviae Miltiorrhizae Radix extract. *J. Med. Pharm. Soc. WAKAN-YAKU* **4**, 59-64, 1987.
6) Chung, H.Y., Yokozawa, T. and Oura, H. : Acute effect of extract from Salviae Miltiorrhizae Radix on renal function in renal failure rats. *Chem. Pharm. Bull.* **36**, 274-278, 1988.
7) Yokozawa, T., Chung, H.Y., Oura, H., Nonaka, G. and Nishioka, I. : Isolation of a renal function-facilitating constituent from the Oriental drug, Salviae Miltiorrhizae Radix. *Jpn. J. Nephrol.* **31**, 1091-1098, 1989.
8) Yokozawa, T., Chung, H.Y., Lee, T.W., Oura, H., Tanaka, T., Nonaka, G., Nishioka, I. and Hirai, A. : Contribution of prostaglandins to the renal responses to magnesium lithospermate B isolated from Salviae Miltiorrhizae Radix. *Chem. Pharm. Bull.* **37**, 1568-1571, 1989.
9) Yokozawa, T., Chung, H.Y., Lee, T.W., Oura, H., Nonaka, G. and Nishioka, I.: Magnesium lithospermate B improves renal function via the kallikrein-prostaglandin system in rats with renal failure. *Jpn. J. Nephrol.* **32**, 893-898, 1990.
10) Yokozawa, T., Chung, H.Y., Lee, T.W., Oura, H., Nonaka, G. and Nishioka, I. : Potentiating effect of converting enzyme inhibitor captopril to the renal responses of magnesium lithospermate B in rats with adenine-induced renal failure. *Chem. Pharm. Bull.* **39**, 732-736, 1991.
11) Yokozawa, T., Lee, T.W., Oura, H., Nonaka, G. and Nishioka, I. : Effect of magnesium lithospermate B in rats with sodium-induced hypertension and renal failure. *Nephron* **60**, 460-465, 1992.
12) Yokozawa, T., Lee, T.W., Oura, H., Nonaka, G., Nishioka, I. and Hattori, M. : Effect of magnesium lithospermate B on renal and urinary kallikrein activities in rats with adenine-induced renal failure. *Jpn. J. Nephrol.* **35**, 337-342, 1993.
13) Yokozawa, T., Lee, T.W., Chung, H.Y., Oura, H., Nonaka, G. and Nishioka, I. : Depressor effect of magnesium lithospermate B, a component of Salviae Miltiorrhizae Radix, in spontaneously hypertensive rats. *Phytother. Res.* **8**, 271-275, 1994.
14) Yokozawa, T., Zhou, J.J., Hattori, M., Inaba, S., Okada, T., Oura, H., Nonaka, G. and Nishioka, I. : Effects of a Dan Shen component, magnesium lithospermate B, in nephrectomized rats. *Jpn. J. Nephrol.* **37**, 105-111, 1995.
15) Chung, H.Y., Kim, J.S., Chung, H.Y., Yokozawa, T. and Oura, H. : The promoting action of magnesium lithospermate B on the kinin-prostaglandin E_2 system in the kidney. *Pharmacol. Toxicol.* **76**, 240-244, 1995.
16) Yokozawa, T., Chung, H.Y., Dong, E. and Oura, H. : Confirmation that magnesium lithospermate B has a hydroxyl radical-scavenging action. *Exp. Toxic. Pathol.* **47**, 341-344, 1995.
17) Rechnitz, G.A. : Ion-selective electrodes. *Chem. Eng. News* **45**, 146-158, 1967.
18) Momose, T., Yano, Y. and Ohashi, K. : Organic analysis. XLIV. A new deproteinizing agent for determination of blood sugar. *Chem. Pharm. Bull.* **11**, 968-972, 1963.
19) Oken, D.E., Arce, M.L. and Wilson, D.R. : Glycerol-induced hemoglobinuric acute renal failure in the rat. *J. Clin. Invest.* **45**, 724-735, 1966.
20) Stein, J.H., Lifschitz, M.D. and Barnes, L.D. : Current concepts on the pathophysiology of acute renal failure. *Am. J. Physiol.* **234**, F171-F181, 1978.
21) Thiel, G., Wilson, D.R., Arce, M.L. and Oken, D.E. : Glycerol induced hemoglobinuric acute renal failure in the rat. *Nephron* **4**, 276-297, 1967.
22) Hishida, A., Kato, A. and Yamada, M. : Myoglobinuria and acute renal failure. *Jpn. J. Clin. Pathol.* **39**, 110-114, 1991.
23) Ishikawa, I. : Myohemoglobin-induced acute renal failure. *Kidney and Dialysis* **31**, 146-150, 1991.
24) Wang, C.S., Chen, C.S., Yang, T.T. and Lou, F.C. : *In vitro* Radix Salviae Miltiorrhizae action on blood anticoagulation and fibrinogenolysis. *Chin. Med.* **4**, 123-126, 1978.
25) Microcirculation Research Group, Department of Pathophysiology, Shanghai First Medical College : An experimental study of microcirculatory disturbances treated with Radix Salviae Miltiorrhizae. *Chin. Med. J.* **4**, 191-195, 1978.
26) Cheng, W.Z. : Pharmacological effect of Salviae Miltiorrhizae. B. *Acta Pharmaceutica Sinica* **19**, 876-880, 1984.
27) Lopez-Farre, A., Gomez-Garre, D., Bernabeu, F., Cajal, S.R., Perez-Rodrigo, P., Braquet, P. and Lopez-Novoa, J.M. : Platelet-activating factor mediates glycerol-induced acute renal failure in rats. *Clin. Sci.* **79**, 551-558, 1990.
28) Hsu, C.H., Kurtz, T.W. and Waldinger, T.P. : Cardiac output and renal blood flow in glycerol-induced acute renal failure in the rat. *Circ. Res.* **40**, 178-182, 1977.
29) Venkatachalam, M.A., Rennke, H.G. and Sandstrom, D.J. : The vascular basis for acute renal failure in the rat. *Circ. Res.* **38**, 267-279, 1976.
30) Dworkin, L.D. and Brenner, B.M. : The renal circulations. In "The Kidney" (Ed. by Brenner, B.M. and Rector, F.C.), W.B. Saunders Company, Philadelphia, pp.164-204, 1991.
31) Brezis, M., Rosen, S. and Epstein, F.H. : Acute renal failure. In "The Kidney" (Ed. by Brenner, B.M. and Rector, F.C.), W.B. Saunders Company, Philadelphia, pp.993-1061, 1991.
32) Oken, D.E., Cotes, S.C., Flamenbaum, W., Powell-Jackson, J.D. and Lever, A.F. : Active and passive immunization to angiotensin in experimental acute renal failure. *Kidney Int.* **7**, 12-18, 1975.
33) Flamenbaum, W., Kotchen, T.A. and Oken, D.E. : Effect of renin immunization on mercuric chloride and glycerol-induced renal failure. *Kidney Int.* **1**, 406-412, 1972.

34) Michiels, C., Arnould, T., Knott, I., Dieu, M. and Remacle, J. : Stimulation of prostaglandin synthesis by human endothelial cells exposed to hypoxia. *Am. J. Physiol.* **264**, C866-C874, 1993.
35) Wardle, E.N. : Acute renal failure and multiorgan failure. *Nephron* **66**, 380-385, 1994.
36) Nicholls, M.G. : Interaction of diuretics and electrolytes in congestive heart failure. *Am. J. Cardiol.* **65**, 17E-21E, 1990.
37) Kusaka, H. and Karasawa, A. : Effects of repeated administration of KW-3902, a novel adenosine A_1-receptor antagonist, on its pharmacological actions. *Jpn. J. Pharmacol.* **63**, 513-519, 1993.