Role of Oriental medicines in the treatment of acute renal failure : Carthami Flos, Rhei Rhizoma and Astragali Radix

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Abstract

Rats with renal failure induced by injection of glycerol show increased levels of urea nitrogen and creatinine (Cr) in serum and decreased creatinine clearance (Ccr). Fractional excretion of sodium (FE_{Na}) increases with the increase in urinary volume, while urinary osmotic pressure decreases. In glycerol-treated rats, Carthami Flos, Rhei Rhizoma and Astragali Radix all caused a decrease in urea nitrogen, Cr and FE_{Na}. In addition, Carthami Flos increased the urinary osmotic pressure, Rhei Rhizoma decreased the urinary volume, and Astragali Radix increased the Ccr, suggesting that these drugs ameliorate the abnormalities of acute renal failure through different modes of action.

Key words acute renal failure, glycerol, Carthami Flos, Rhei Rhizoma, Astragali Radix, rat.

Introduction

Although mortality due to acute renal failure has decreased in recent years, owing to advances in the control of water, electrolytes and nutrition and early introduction of dialysis, it is still substantial. On the other hand, it is undeniable that proper treatment at an early stage after onset of renal failure is highly likely to improve or normalize renal function. Therefore, various experimental studies have been carried out to examine the onset, maintenance mechanisms and aggravating factors of acute renal failure. However, few effective prophylactic or therapeutic drugs are currently available.

In 1974, the first case reports describing treatment of renal failure using a combination of both Chinese and Western medicine began to appear, stimulating clinical research on the prevention and treatment of this condition through Chinese medicine.[1] With this approach, renal failure is basically treated using drugs which activate the blood and body fluid energy at onset, cathartic and diuretic drugs at the hypouretic stage, and revitalizing drugs at the polyuretic stage or recovery stage.[1,2]

In the present study, three representative Oriental medicines, i.e., Carthami Flos as a drug activating the blood and body fluid energy, Rhei Rhizoma as a cathartic drug, and Astragali Radix as a revitalizing drug, were administered to rats with glycerol-induced acute renal failure in order to investigate their actions.

Materials and Methods

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

Crude drugs : The crude drugs used in this experiment were Carthami Flos (*Carthamus tinctorius* L.), Rhei Rhizoma (*Rheum officinale* BAILLON) and Astragali Radix (*Astragalus membranaceus* BUNGE). One hundred grams of each crude drug was boiled gently in 1,000 ml of water for 60 min. The extract was then concentrated under reduced pressure to leave a residue. The yields of Carthami Flos, Rhei

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Rhizoma and Astragali Radix were 31 %, 22 % and 27 %, respectively.

Experimental design : After deprivation of water for 6 h, rats received an injection of 50 % glycerol into the muscles of the rear limbs at 10 ml/kg body weight, and each crude drug (10 or 20 mg/kg body weight/day) was administered intraperitoneally once every 8 h for 2 days. 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.[3] Osmolarity was measured with an osmometer (OSA-21 ; Nikkiso Co. Ltd., Tokyo, Japan) using the cryoscopic method.[4] 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 :

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

$$FE_{Na}\ (\%) = (urinary\ Na/serum\ Na)/(urinary\ Cr/serum\ Cr) \times 100$$

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

Results

Data on biological parameters after administration of Carthami Flos are summarized in Table I. The blood urea nitrogen level in normal rats was 21.9 mg/dl, whereas in rats with glycerol-induced acute renal failure it increased significantly to about 3.3 times the normal value. Serum Cr, urine volume and FE_{Na} were also increased to 4.1, 3.5 and 4.4 times the normal values, respectively, while Ccr and urine osmolarity were decreased by 70 % and 73 % of the respective levels in normal rats.

Although urine volume and Ccr changes showed no significant differences between the control and Carthami Flos-treated groups, the level of blood urea nitrogen in rats given Carthami Flos intraperitoneally at 20 mg showed a significant decrease from 71.2 to 46.3 mg/dl (a 35 % change, $p<0.001$), as shown in Table I. Similarly, FE_{Na} was also decreased in the 20 mg Carthami Flos group, from 3.22 to 2.13 % (a 34 % change, $p<0.01$). In contrast, urine osmolarity, which was 451 mOsm/l in controls, increased significantly to 614 mOsm/l in rats given 20 mg of Carthami Flos. The Cr value was decreased to some extent in rats given 20 mg of Carthami Flos, but the change was not significant.

Table II shows the effects of Rhei Rhizoma on biological parameters in rats with glycerol-induced acute renal failure. The level of urea nitrogen was 71.2 mg/dl in the controls, whereas the value was significantly lower, by 33 %, in rats given 20 mg of Rhei Rhizoma. The level of urea nitrogen was also considerably (but not significantly) decreased in rats given 10 mg of Rhei Rhizoma. Similar changes produced by Rhei Rhizoma administration were observed in FE_{Na},

Table I Effects of Carthami Flos on biological parameters in glycerol-induced acute renal failure.

Parameter	Control	Carthami Flos		Normal
		10 mg	20 mg	
Blood urea nitrogen, mg/dl	71.2 ± 7.3^{b}	68.4 ± 8.0^{b}	$46.3\pm9.9^{a,d}$	21.9 ± 1.2
s-Cr, mg/dl	1.35 ± 0.17^{b}	1.46 ± 0.21^{b}	1.08 ± 0.29^{b}	0.33 ± 0.01
Urine volume, ml/day	41.1 ± 3.9^{b}	41.1 ± 6.2^{b}	33.3 ± 4.3^{b}	11.9 ± 1.1
Ccr, ml/kg B.W./min	0.96 ± 0.09^{b}	0.84 ± 0.11^{b}	1.11 ± 0.27^{b}	3.23 ± 0.75
FE_{Na}, %	3.22 ± 0.60^{b}	2.93 ± 0.30^{b}	$2.13\pm0.39^{a,c}$	0.74 ± 0.17
Urine osmolarity, mOsm/L	451 ± 50^{b}	420 ± 48^{b}	$614\pm104^{b,c}$	1641 ± 63

Statistical significance : $^{a}p<0.01$, $^{b}p<0.001$ vs. normal rats, $^{c}p<0.01$, $^{d}p<0.001$ vs. control rats with renal failure.

Table II Effects of Rhei Rhizoma on biological parameters in glycerol-induced acute renal failure.

Parameter	Control	Rhei Rhizoma		Normal
		10 mg	20 mg	
Blood urea nitrogen, mg/dl	71.2±7.3[b]	58.1±11.2[b]	47.8±4.8[a,d]	21.9±1.2
s-Cr, mg/dl	1.35±0.17[b]	1.13±0.16[b]	1.06±0.23[b]	0.33±0.01
Urine volume, ml/day	41.1±3.9[b]	40.1±1.9[b]	30.8±2.6[b,e]	11.9±1.1
Ccr, ml/kg B.W./min	0.96±0.09[b]	1.09±0.13[b]	0.96±0.32[b]	3.23±0.75
FE_{Na}, %	3.22±0.60[b]	2.70±0.30[b]	2.42±0.40[b,c]	0.74±0.17
Urine osmolarity, mOsm/L	451±50[b]	487±70[b]	437±36[b]	1641±63

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

Table III Effects of Astragali Radix on biological parameters in glycerol-induced acute renal failure.

Parameter	Control	Astragali Radix		Normal
		10 mg	20 mg	
Blood urea nitrogen, mg/dl	71.2±7.3[b]	53.4±9.5[b,d]	34.2±3.8[e]	21.9±1.2
s-Cr, mg/dl	1.35±0.17[b]	0.99±0.15[b,d]	0.68±0.06[a,e]	0.33±0.01
Urine volume, ml/day	41.1±3.9[b]	35.8±3.6[b]	42.7±2.2[b]	11.9±1.1
Ccr, ml/kg B.W./min	0.96±0.09[b]	1.56±0.29[b,c]	2.42±0.40[a,e]	3.23±0.75
FE_{Na}, %	3.22±0.60[b]	2.93±0.52[b]	1.38±0.24[e]	0.74±0.17
Urine osmolarity, mOsm/L	451±50[b]	510±48[b]	469±28[b]	1641±63

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

which was 16 % lower in the 10 mg group and 25 % lower in the 20 mg group than in the control group. Although the dose of 10 mg produced no differences in urine volume between the control and Rhei Rhizoma-treated groups, a further increase in the dose to 20 mg significantly decreased the urine volume from 41.1 to 30.8 ml/day (a 25 % change, $p<0.001$). However, the Cr, Ccr and urine osmolarity in rats given Rhei Rhizoma exhibited no significant differences in comparison with the controls.

As shown in Table III, administration of Astragali Radix to rats with glycerol-induced acute renal failure resulted in a decrease of urea nitrogen ; there was a 25 % decrease in the 10-mg group, and a 52 % decrease in the 20-mg group, both being statistically significant. The Cr values were also markedly and significantly decreased in rats given this crude drug, the value being 27 % lower in the 10-mg group and 50 % lower in the 20-mg group in comparison with the control group. Although the FE_{Na} value tended to be decreased in rats given 10 mg, further increase in the dose to 20 mg decreased the FE_{Na} value significantly from 3.22 to 1.38 % (a 57 % change, $p<0.001$), as shown in Table III. On the other hand, administration of Astragali Radix caused a 63 % increase in the Ccr value from 0.96 to 1.56 ml/kg body weight/min in the 10-mg group and a 152 % increase from 0.96 to 2.42 ml/kg body weight/min in the 20-mg group. However, rats given Astragali Radix showed no appreciable changes in urine volume or urine osmolarity.

Discussion

Glycerol-treated rats have a tendency to be both ischemic and nephrotoxic, and the renal failure induced in this way is regarded as the animal model most closely resembling human acute renal failure, showing strong involvement of the ischemic factor at onset.[5,6] However, it is generally considered that the

degree of renal failure correlates with the duration of dehydration. The more rigorous the dehydration, the more severe the effect on renal function. Thiel *et al.*[7], Hishida *et al.*[8] and Ishikawa[9] reported that in the rat, the degree of dehydration appeared to be a risk factor for the development of glycerol-induced acute renal failure. Our experiment demonstrated that glycerol injection increases urea nitrogen and Cr in serum. As the urine volume increased, FE_{Na} increased, although Ccr and urinary osmotic pressure decreased. Since the water abstention period was only 6 h, the induced acute renal failure was relatively mild.

Among the three drugs examined in the present study, Astragali Radix caused a marked decrease in urea nitrogen and Cr. Although there were no changes in urinary volume or osmotic pressure, an increase in Ccr and a significant decrease in FE_{Na} were found. On the other hand, Carthami Flos given at a high dose caused a decrease in urea nitrogen and Cr, with a significant decrease in FE_{Na}, similarly to Astragali Radix. However, unlike the other two crude drugs, Carthami Flos increased the urinary osmotic pressure. The changes in urea nitrogen, Cr and FE_{Na} induced by Rhei Rhizoma were similar to those induced by Carthami Flos. Rhei Rhizoma also decreased urinary volume significantly, although unlike Carthami Flos it had no effect on urinary osmotic pressure.

As is clear from the differences in the effects of the three drugs in glycerol-treated rats, Astragali Radix markedly improves Ccr, whereas Carthami Flos affects mainly urinary osmotic pressure. In addition, the former crude drug produced a marked improvement of hyperazotemia. In clinical acute renal failure, hyperazotemia is inevitable, and is common in the polyuretic or recovery stage, accompanied by a decrease in renal function, particularly uriniferous function.[10] In this connection, the improvement of this condition by Astragali Radix (increased Ccr and decreased FE_{Na}) is noteworthy, providing experimental evidence of the efficacy of Chinese drug therapy. It should also be noted that, unlike the other two crude drugs, Carthami Flos increased the urinary osmotic pressure, also improving the levels of urea nitrogen, Cr and FE_{Na}. In other words, Astragali Radix and Carthami Flos had common effects on urea nitrogen, Cr and FE_{Na}, although differing in potency, while the former caused changes in Ccr as opposed to the changes in urinary osmotic pressure caused by the latter. This suggests that Astragali Radix acts on glomeruli, whereas Carthami Flos acts on tubules.

On the other hand, Rhei Rhizoma, which has been shown experimentally and clinically to have beneficial effects on chronic renal failure,[11-15] was proved to have effects on urea nitrogen, Cr and FE_{Na}, as in the case of Astragali Radix and Carthami Flos. However, unlike these two, Rhei Rhizoma inhibited polyuria. Since the improvement in hyperazotemia was not accompanied by an increase in Ccr, this improvement was considered to be due to the anti-catabolic action of Rhei Rhizoma, a feature we have reported previously.[16,17]

All three crude drugs used in the present study reversed the increase in the FE_{Na} level under the conditions of acute renal failure (calculated from Na and Cr in the blood and urine). This indicates that the function of tubules and glomeruli was oriented toward improvement, providing experimental evidence that Carthami Flos, Rhei Rhizoma and Astragali Radix all ameliorate the condition of renal failure.

和文抄録

グリセロールの注射は血清尿素窒素，Cr を上昇させ，Ccr の低下をひき起こした。また尿量の増加とともにナトリウム排泄率 (FE_{Na}) が上昇したが，尿浸透圧は低下していた。これに対し紅花，大黄，黄耆投与群ではいずれも血清尿素窒素，Cr，FE_{Na} が低下したが，加えて紅花では尿浸透圧の上昇，大黄では尿量の低下，黄耆では Ccr が上昇し，これら和漢薬は異なった様式で急性腎不全状態を改善していることが示唆された。

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