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원 저

Effect of *Salviae Radix* on Impairment of Membrane Transport Function in Rabbits with Myoglobinuric Acute Renal Failure

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마이오글로빈뇨성 급성 신부전 토끼에서 신장 세포막 수송 기능 장애에 대한 丹參의 효과

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목 적 : Rhabdomyolysis에 의해 유발된 급성 신부전시 나타나는 신장세뇨관 세포에서 물질이동의 저해가 丹參 추출액에 의해 방지될 수 있는 지를 조사하였다.

방 법 : 토끼에 50% glycerol을 10 ㎖/㎏씩 대퇴근육내 주사한 후 뇨와 혈액을 채취하여 신기능을 측정하고, 신피질 절편을 분리하여 실험하였다.

결 과 : 토끼에 50% glycerol을 10 ㎖/㎏씩 대퇴근육내 주사한 결과 사구체여과율의 감소와 Na 배설분율의 증가가 나타남으로서 glycerol 주입이 rhabdomyolysis에 의해 급성신부전이 유발되었음을 보였다. Glycerol을 주사하기 전 7일 동안 丹參 추출액 (0.05%)을 0.3 g/㎏씩 경구 투여한 결과 glycerol에 의해 유발된 사구체여과율의 감소와 Na 배설분율의 증가가 유의하게 방지되었다. Glycerol만을 주사한 동물에서는 포도당과 인산의 요배설분율이 각각 현저하게 증가하였으나, 이러한 증가는 丹參 추출액에 의해 억제되었다. 급성신부전이 유발된 신장피질에서 분리한 brush-border membrane vescicles (BBMV)에서 포도당과 인산의 이동은 정상 신장과 비교하여 유의한 감소가 나타나고, microsomal fraction에서 측정한 Na+-K+-ATPase 활성도 억제되었다. 이러한 억제현상은 丹參 추출액을 전처치한 결과 방지되었다. 급성신부전이 유발된 신장피질 절편에서 유기 음이온인 p-aminohippurate 이동과 유기 양이온인 tetraethylammonium의 이동이 억제되었고, 이러한 변화는 丹參 추출액에 의해 방지되었다. Rhabdomyolysis에 의해 유발된 포도당과 인산의 배설분율의 증가는 항산화제로 잘 알려진 DPPD 전처치로 방지되었다.

결 론 : Rhabdomyolysis에 의한 급성신부전의 유발 과정에 반응성 산소기가 중요한 역할을 할 가능성을 보이고 있고, 丹參 추출액 전처치는 rhabdomyolysis에 의한 급성 신부전시 나타나는 근위세뇨관에서 물질의 재흡수 장애를 방지하고 있다. 丹參 추출액의 방지 효과는 항산화작용에 기인할 것으로 사료된다. *(J Korean Oriental Med 2000;21(3):119-128)*

Key Words: *Salviae Radix*, Rhabdomyolysis, Acute renal failure, Membrane transport function, Membrane vesicles, Organic ion uptake, Rabbit kidney

INTRODUCTION

Myoglobinuric acute renal failure induced by intramuscular or subcutaneous injection of 50% glycerol has been extensively used as a model of experimental acute renal failure in animals[1,2]. The

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injection of 50% glycerol causes rhabdomyolysis and subsequent leads to acute renal failure. Clinically, myoglobinuric acute renal failure are induced by traumatic and nontraumatic causes. The crush syndrome, strenuous physical exertion, and ischemic myopathy are responsible for traumatic myoglobinuric acute renal failure. Etiologies of nontraumatic rhabdomy olysis with acute renal failure involve a variety of diseases affecting muscle such as McArdles disease, alcohol overdose, heroin overdose, prolonged coma, and severe electrolyte abnormality including hypokalemia and hypophosphatemia[2].

A number of studies in vivo and in vitro provide evidences that the rhabdomyolysis-induced acute renal failure is mediated by iron-induced generation of reactive oxygen species. Paller[3] noted that the iron chelator deferoxamine therapy mitigated myoglobinuric acute renal failure. Shah and Walker[4] also demonstrated that deferoxamine and hydroxyl radical scavengers each protected against the glycerol acute renal failure model. Similar results were reported by others[5,6]. The typical morphological feature of heme protein-induced acute renal failure is proximal tubular necrosis[7]. Because of the large volumes of fluid entering and being reabsorbed from the proximal tubule and its many and variety transport mechanisms are dependent on metabolic energy[8], proximal reabsorption would be severely affected in rhabdomyolysis-induced acute renal failure.

Salviae Radix (SR) is a drug promoting blood circulation to remove blood stasis, removing heat from the blood and reliving restlessness. It is used to subdue kidney failure, hypertension, coronary disorder, cerebrovascular disorder[9]. Our previous studies showed that *Salviae Radix* extract (SRE) prevented reactive oxygen species-induced cell injury[10]. Therefore, the present study was carried out to determine whether SRE has protective effect against impairment in membrane transport function of the proximal tubule in rabbits with rhabdomyolysis-induced acute renal failure.

MATERIALS AND METHODS

Salviae Radix extract (SRE) preparation

2 kg of crushed crude drug was extracted with methyl alcohol under reflux for 4 hr three times and the total extractive was evaporated under reduced pressure to give 168 g. 50 g of methyl alcohol extract was suspended into water and extracted with n-hexane to remove fats. The remaining water suspension was extracted with butanol to obtain 6.8 g. The extract was dissolved in saline.

Induction of glycerol-induced acute renal failure

New Zealand White rabbits weighing 1.5-2.5 kg were housed in metabolic cages to collect urine. The animals were allowed 2 days to acclimate to the cages, and followed by a 24-hr basal period, during which urine and blood samples were collected. They were injected with 10 ml/kg of 50% glycerol into the muscles of both hind limb. In order to test the effect of SRE, rabbits were pretreated with SRE (0.3 g/day/kg body wt., orally) for 7 days before glycerol administration. Individual 24-hr urine samples were collected for 24 hr after the glycerol injection and blood samples were taken from ear vein.

Urine and blood analyses

Samples of urine and blood were analyzed for creatinine (Iatron Lab., Japan), glucose (Iatron Lab., Japan), phosphate[11], and Na^+ (flame photometer, Beckman). Glomerular filtration rate (GFR) was estimated from the creatinine clearance and fractional excretion of Na^+ was calculated in the standard fashion.

Accumulation of organic ions in renal cortical slices

The uptake of organic ions in cortical slices was performed as previously described[12]. Animals were sacrificed 24 hr following the administration of glycerol or saline. The kidneys were quickly removed and the renal artery was immediately perfused with an ice-cold isotonic saline solution containing 140 mM NaCl, 10 mM KCl and 1.5 mM $CaCl_2$, to remove as much blood as possible. Thin (0.4-0.5 mm thick) slices of renal cortex were prepared using a Stadie-Riggs microtome and were stored in an ice-cold modified Cross-Taggart medium containing 130 mM NaCl, 10 mM KCl, 1.5 mM $CaCl_2$, 5 mM Na acetate and 20 mM Tris/HCl (pH 7.8). Approximately 50 mg (wet wt.) of slices were then transferred into a 20 ml beaker containing 4 ml of the modified Cross-Taggart medium, and incubated with 14C-labeled substrates. The concentrations of substrates used were 75μM for p-aminohippurate (PAH) and 10μ M for tetraethylammonium (TEA). The incubation was carried out for 60 min in a Dubnoff metabolic shaker at 25℃ under a 100% oxygen atmosphere. After incubation, the slices were quickly removed from the beaker, blotted, weighed and solubilized in 1 N NaOH. Aliquots of the incubation medium and the solubilized tissue were pipetted into a scintillation vial containing Aquasol (New England Nuclear) and the radioactivity was determined using a liquid scintillation counter (Packard Tricarb 300C). The uptake of organic ions by renal slices was expressed as the slice to medium (S/M) ratio: the concentration of the compound in the tissue (mole/g wet tissue) divided by that in the medium (mole/ml medium).

Na^+-K^+-ATPase activity measurement

The microsomal Na^+-K^+-ATPase activity was measured as described previously[13]. The microsomal fraction was prepared from cortex of kidneys of control and glycerol-treated rabbits. The ATPase activity of the microsomal fraction was determined by measuring inorganic phosphate (Pi) released by ATP hydrolysis during incubation of microsome with an appropriate medium containing 3 mM ATP (Sigma) as the substrate. The total ATPase activity was determined in the presence of 100 mM Na^+, 20 mM K^+, 3 mM Mg^{2+}, 2 mM EDTA, and 40 mM imidazole (pH 7.4). The Mg^{2+}-ATPase activity was determined in the absence of K and in the presence of 1 mM ouabain. The difference between the total and the Mg^{2+}-ATPase activities was taken as a measure of the Na^+-K^+-ATPase activity. After a 5-min preincubation at 37℃, the reaction was initiated with the addition of the microsomal fraction. At the end of a 10-min incubation, the reaction was terminated by the addition of ice-cold 6% perchloric acid. The mixture was then centrifuged at 3,500 × g, and P*i* in the supernatant fraction was determined by the method of Fiske and SubbaRow[11].

Preparation of plasma membrane vesicles

Brush-border membrane vesicles (BBMV) were isolated by the Percoll-density gradient centrifugation and Mg^{2+}-precipitation method, as previously described[14,15]. The vesicles were suspended in the vesicle buffer, adjusted to yield a protein concentration of 6 mg/ml and stored at -70℃ until use. The composition of vesicle buffer is given in figure legends. Protein was determined according to Bradford[16] using γ-globulin as a standard.

Transport studies in membrane vesicles

The uptake of substrates by vesicles was measured by a rapid filtration technique. Briefly, the reaction was initiated by adding membrane vesicles to the incubation medium (a 1:10 dilution of membrane vesicle suspension) containing 50 μM of [^{14}C]-D-glucose or ^{32}P at 25℃. The composition of the incubation medium is

given in figure legends. At the designated times, 100㎕ aliquots were taken and quickly filtered under vacuum through Millipore filters (HAWP, 0.45㎛m pore size) which had been soaked overnight in distilled water. The filters were then washed with 5 ml of ice-cold stop solution comprising the identical composition to the incubation medium but without substrate, and dissolved in 1.0 ml of methoxyethanol. After addition of 10 ml of scintillation cocktail, the amount of radioactivity taken up by vesicles was determined by liquid scintillation spectrometry (Packard Tricarb 300C). Nonspecific binding of radioactive substrate to the plasma membrane was determined by incubating vesicles in transport buffer containing 0.1% deoxycholate and radiolabeled substrates. All uptake data were corrected for nonspecific binding.

Measurement of ATP content

ATP levels were measured on renal cortical tissues with a luciferin-luciferase assay. Tissues were homogenized with 2 ml of an ice-cold extracting solution containing 0.1% Triton X-100 and 0.1 M perchloric acid, and placed on ice for 5 min. The homogenate was centrifuged at 3,000×g for 10 min, and the supernatant was diluted with distilled water, and 45 ㎕ of 20 mg/ml luciferin-luciferase was added to 5 ㎕ of the diluted sample. Light emission was recorded at 20 sec. with a luminometer (MicroLumat LB96P, Berthold, Germany). Protein content was determined by the method of Bradford[16], and ATP was expressed as nmoles per mg protein.

Reagents

[^{14}C]-D-glucose, 32Phosphate, [^{14}C]-PAH, and [^{14}C]-TEA were purchased from the Amersham International (Amersham, UK). N,N' -diphenyl-p-phenylenediamine (DPPD) was purchased from Aldrich Chemical (Milwaukee WI). All other chemicals were of the highest commercial grade available.

Statistical analysis

The data are expressed as mean ± S.E. and the difference between two groups was evaluated using Student' s *t*-test. A probability level of 0.05 was used to establish significance.

RESULTS

Changes in GFR and Na^+ excretion

When animals were treated with glycerol, GFR decreased markedly to approximately 11% of the basal value (0.16 ± 0.04 vs. 1.45 ± 0.15 l/day/kg for the basal period). However, GFR was increased to 28% of the basal value in animals treated with glycerol after SRE pretreatment (0.43 ± 0.07 vs. 1.53 ± 0.08 l/day/kg for the basal period) (Fig. 1A). The average fractional excretion of Na^+ was markedly increased from 1.08 ± 0.22% to 8.44 ± 1.45% by the administration of glycerol alone, whereas it was increased from 1.05 ± 0.09% to 3.82 ± 0.22% by glycerol administration after SRE pretreatment (Fig. 1B). These results indicate that the administration of glycerol induces acute renal failure, which is attenuated by SRE pretreatment for 7 days.

Changes in fractional excretion of glucose and phosphate

In order to determine whether proximal tubular reabsorptive function is impaired in rabdomyolysis-induced acute renal failure, the urinary fractional excretion of glucose and phosphate, solutes that are reabsorbed via an active mechanism in the proximal tubule, was measured. The average fractional excretion of glucose in glycerol-treated animals was 3.04 ± 0.12%, which was more than 43-fold greater than the basal value (0.07 ± 0.02%). However, when animals

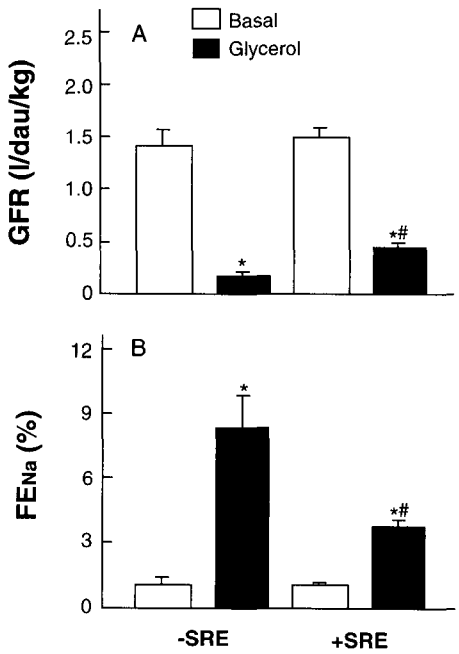

Fig. 1. Changes in glomerular filtration rate (GFR, A) and fractional excretion of Na^+ (FENa, B) 24 hr following administration of glycerol in rabbits with or without *Salviae Radix* extract (SRE). Acute renal failure was induced by intramuscular injection of glycerol (10 ml/kg), and SRE (0.3 mg/kg, orally) was pretreated for 7 days. Data are mean±S.E. of six experimen ts. * : P<0.05 compared with the respective basal value. # : P<0.05 compared with glycerol alone.

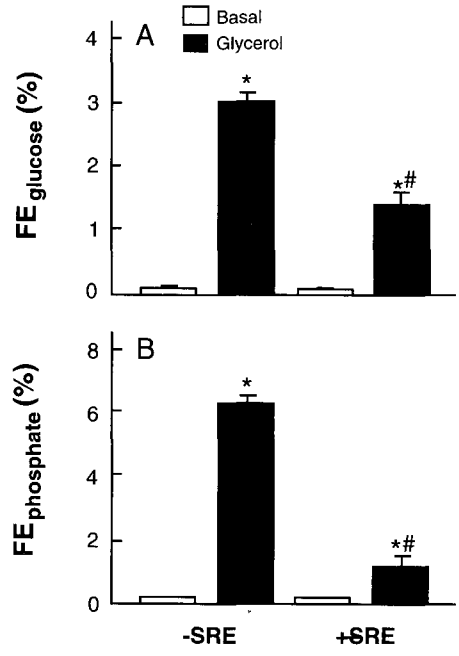

Fig. 2. Changes in fractional excretion of glucose (A) and inorganic phosphate (B) 24 hr following adminis tration of glycerol in rabbits with or without *Salviae Radix* extract (SRE). Experimental conditions were of the same as in Fig. 1. Data are mean±S.E. of six experiments. * : P<0.05 compared with the respec tive basal value. # : P<0.05 compared with glycerol alone.

were treated with glycerol after SRE pretreatment, it was 1.36 ± 0.22%, which was only 17-fold greater than the basal value (0.08 ± 0.01%) (Fig. 2A). Thus, the fractional excretion of glucose in SRE-pretreated animals was significantly lower than SRE-nontretreated animals. Similar protective effect was observed in the fractional excretion of phosphate (Fig. 2B). The average fractional phosphate excretion was increased more than 27-fold of the basal value by the administration of glycerol alone (6.34 ± 0.24 vs. 0.23 ± 0.01% for the basal period), but in animals treated with glycerol after SRE pretreatment it was increased only to 4.3-fold (1.21 ± 0.02 vs. 0.28 ± 0.02% for the basal period).

Changes in uptakes of glucose and phosphate by BBMV

Purified membrane vesicles possess considerable advantages over whole cell or tissue preparations to define alterations in transporter itself within the membrane[17]. In order to determine whether the administration of glycerol produced a direct impairment in proximal tubular transporters, uptakes of glucose and phosphate were measured in BBMV isolated from renal cortex of control and glycerol-treated animals with or

without SRE pretreatment. As shown in Fig. 3A, the glucose uptake in animals treated with glycerol alone decreased from 256.45 ± 10.55 pmole/mg protein/min to 165.42 ± 7.94 pmole/mg protein/min, which was significantly increased to 211.12 ±7.82 pmole/mg protein/min by SRE pretreatment. Likewise, the phosphate uptake in SRE-pretreated animals was also higher than that in the administration of glycerol alone (Fig. 3B). The phosphate uptake in the control was 156.91 ± 10.33 pmole/mg protein/min, and it was 110.62 ± 6.92 and 148.92 ± 5.48 pmole/mg protein/min in the glycerol alone and glycerol with SRE pretreatment, respectively. These results suggest that the administration of glycerol causes a direct impairment in membrane transport carrier proteins and its effect is prevented by SRE pretreatment.

Changes in microsomal Na^+-K^+-ATPase activity

In order to examine whether the administration of glycerol impaired Na^+-pump activity, we measured Na^+-

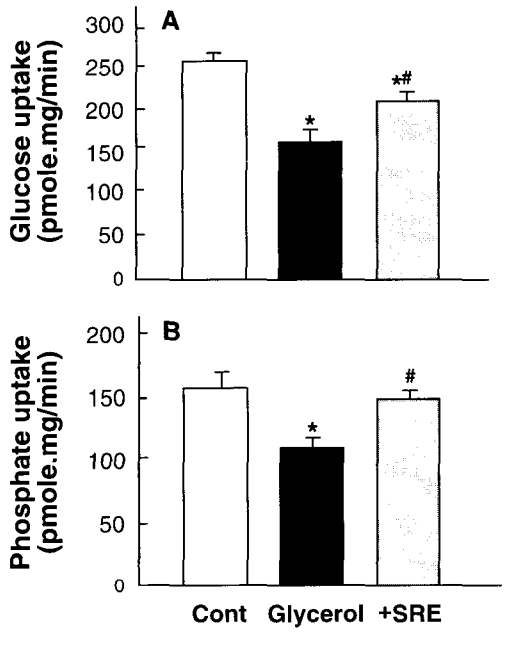

Fig. 3. Changes in glucose (A) and phosphate (B) uptake by brush-border membrane vesicles (BBMV) 24 hr following administration of glycerol in rabbits with or without *Salviae Radix* extract (SRE). Membrane vesicles were loaded with a buffer containing 100 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris (pH 7.5) and were incubated in a buffer containing 50 M [^{14}C]-D-glucose, 100 mM mannitol, 100 mM NaCl and 20 mM Hepes/Tris (pH 7.5) for 1 min at 25 ℃. Animals were treated as described in Fig. 1. Data are mean±S.E. of six experiments. * : P<0.05 compared with the control. # : P<0.05 compared with glycerol alone.

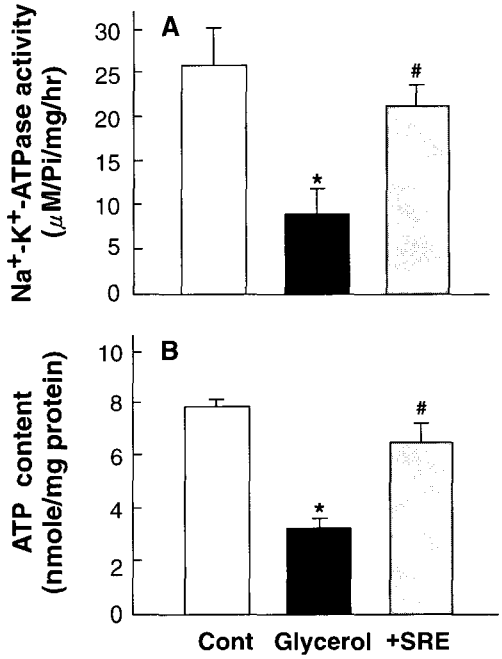

Fig. 4. Changes in Na^+-K^+-ATPase (A) and ATP content in renal cortical tissue activity 24 hr following administration of glycerol in rabbits with or without *Salviae Radix* extract (SRE). Data are mean±S.E. of six experiments. * : P<0.05 compared with the control. # : P<0.05 compared with glycerol alone.

K^+-ATPase activity in microsomes prepared from cortex of kidneys 24 hr after administration of glycerol. As shown in Fig. 4A, Na^+-K^+-ATPase activity in glycerol-treated rabbits were significantly lower than the control (8.93 ± 2.93 vs. 25.93 ±4 .35 μM Pi/mg protein/hr for the control), similarly to those reported by Westenfelder et al.[1] who observed that cortical and medullary Na^+-K^+-ATPase activities were significantly depressed in rats with glycerol-induced acute renal failure. Reduction of Na^+-K^+-ATPase activity in glycerol-treated rabbits was prevented by SRE pretreatment (21.45 ± 2.25 μM Pi/mg protein/hr).

Changes in cellular ATP levels

Since glucose and phosphate are reabsorbed by an active mechanism in the proximal tubules, reabsorption of these solutes should be affected by alterations of ATP content in the cytosol. To test the possibility, ATP levels were measured in the control and glycerol-treated animals. As shown in Fig. 4B, ATP content in control animals was 7.94 ± 0.22 nmole/mg protein and glycerol-treated animals was reduced to 3.21 ± 0.34 nmole/mg protein. However, ATP levels returned almost to the control levels in SRE-pretreated animals (6.57 ± 0.73 nmole/mg protein).

Organic ion uptake by renal cortical slices

Renal cortical accumulation of organic ions has been used as a sensitive indicator in the assessment of nephrotoxicity[18]. We, therefore, measured in the present study the uptake of organic anion PAH and organic cation TEA by renal cortical slices as a biochemical index for an in vitro evaluation of proximal tubular cell injury. As shown in Fig. 5A, the S/M ratio of PAH uptake in control slices were 14.26 ± 0.12, and it was inhibited by glycerol administ ration to 6.92 ± 0.66. However, it reversed to 10.23 ± 0.67 in animals treated with glycerol after SRE pretreatment. Similar results were observed in TEA uptake (Fig. 5B). The S/M ratio of the uptake was 17.35 ± 0.77 and 8.09 ± 0.57 in the control and glycerol-treated animals, respectively. The S/M ratio in glycerol-treated rabbits was lower than in SRE-pretreated rabbits (13.09 ± 0.75). Thus, the ability of cortical slices to accumulate organic ions was markedly decreased in rhabdomyolysis-induced acute renal failure, which was attenuated by SRE pretreatment.

Effect of antioxidant on alterations in membrane transport function

In the last series of experiments, it was determined whether the antioxidant could prevent glycerol-induced alterations in fractional excretion of glucose and phosphate. DPPD attenuated glycerol-induced increase in fractional excretion of glucose (Fig. 6A). Likewise, an increase in the average fractional excretion of phosphate in glycerol-treated animal was significantly attenuated by DPPD (Fig. 6B).

DISCUSSION

The present study demonstrates that at 24 hr after the administration of glycerol there is marked reduction in GFR along with an increase in fractional Na+ excretion (Fig. 1), indicating generation of rhabdomyolysis-induced acute renal failure. Our data also showed that glycerol injection caused an increase in fractional excretion of glucose and phosphate (Fig. 2). Since these solutes are reabsorbed in the proximal tubule, these results indicate that reabsorptive function of the proximal tubule is severely impaired in rhabdomyolysis-induced acute renal failure.

The rate-limiting step in the proximal reabsorption of glucose and phosphate appears to lie at the brush border membrane of the proximal tubule, and involves Na^+-dependent transport mechanism[19]. Therefore, reduction

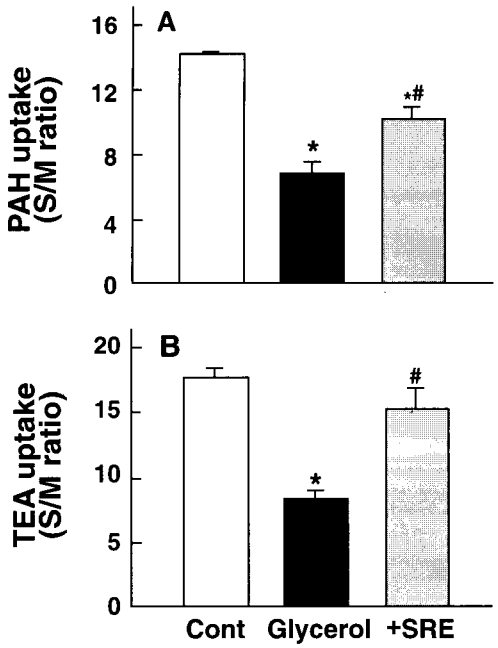

Fig. 5. Changes in PAH (A) and TEA (B) uptake by renal cortical slices 24 hr following administration of glycerol in rabbits with or without *Salviae Radix* extract (SRE). Animals were treated as described in Fig. 1. Data are mean±S.E. of six experiments. * : P<0.05 compared with the control. # : P<0.05 compared with glycerol alone.

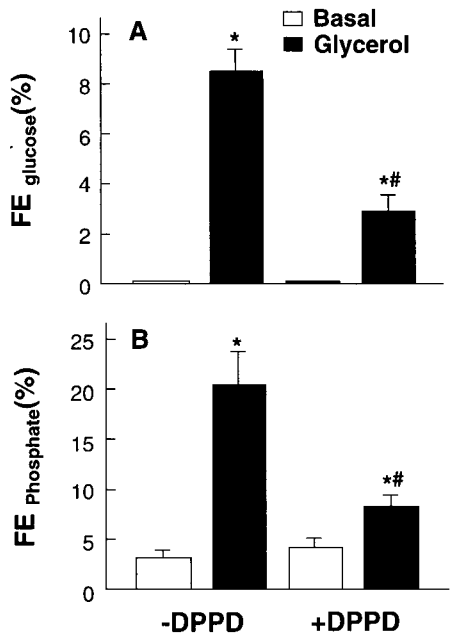

Fig. 6. Changes in fractional excretion of glucose (A) and inorganic phosphate (B) 24 hr following administration of glycerol in rabbits pretreated with or without DPPD. Acute renal failure was induced by intramuscular injection of glycerol (10 ml/kg), and DPPD (0.5 g/kg, i.p.) was pretreated 24 hr before the administration of glycerol. Data are mean±S.E. of six experiments. * : P<0.05 compared with the respective basal value. # : P<0.05 compared with glycerol alone.

in reabsorption of these solutes could be resulted from a direct impairment in transporters on the brush-border membrane and/or reduction in Na^+-pump activity in the basolateral membrane. Indeed, the present study shows that uptakes of these solutes by BBMV and the Na^+-K^+-ATPase activity were significantly inhibited in kidneys from rabbits with rhabdomyolysis-induced acute renal failure (Figs. 3, 4A). Reduction in uptakes by BBMV could be resulted from a decrease in the number of carrier for these solutes on the membrane and/or a decrease in turnover rate. In addition, inhibition of Na^+-K^+-ATPase activity would cause disruption of the Na^+ gradient, which is driving force for solutes transported by a Na^+-dependent mechanism, leading to reduction in reabsorption of solutes.

Alternatively, reduction in functional Na^+-pump activity could be attributed to the depletion of ATP in the cytosol. Indeed, the present study shows that ATP levels were reduced in rhabdomyolysis-induced acute renal failure (Fig. 4B).

The capacity of renal cortical slices to accumulate PAH and TEA was markedly attenuated following glycerol injection (Fig. 5). This reflects the impairment in PAH and TEA transport in the basolateral membrane

and thereby reduction in secretory capacity of organic ions in proximal tubules by glycerol injection. Taken together above data, these results indicate that both reabsorption and secretion of solutes through the proximal tubular membrane are impaired by glycerol injection. Similarly to our results, Preuss et al.[20] reported in rats that the slice uptake of PAH and TEA decreased to 60 and 59% of the control, respectively, at 3 hr after the glycerol injection.

The present study demonstrates that SRE pretreatment prevents significantly renal filtration failure and impaired tubular function induced by the administration of glycerol in rabbits. When rabbits received SRE for 7 days prior to glycerol injection, they were able to maintain their GFR significantly higher than rabbits given glycerol alone. The fractional excretion of Na^+ in SRE-pretreated animals decreased as compared with the administration of glycerol alone. SRE-induced protection against the increased fractional excretion of glucose and phosphate was accompanied by amelioration of both reduced uptakes of these solutes through brush-border membranes and depressed Na^+-pump activity. Similarly, the reduction in uptakes of organic ions, solutes that are secreted by active processes in the proximal tubule, was also prevented by SRE pretreatment.

The mechanism whereby SRE pretreatment provides its protective effect is not evident from the results of the present study. However, previous studies showed that SRE prevents the oxidant-induced renal cell injury[10]. Studies in vivo and in vitro have demonstrated that reactive oxygen species are responsible for rhabomyolysis-induced acute renal failire[7]. The present study also showed that rhabomyolysis-induced increase in the fractional excretion of glucose and phosphate is resulted from the oxidative stress as evidenced by the protective effect of an antioxidant DPPD (Fig. 6). Therefore, it is suggested that the antioxidant action of SRE may be responsible for the protection against rhabomyolysis-induced impairment of renal tubular transport function in rabbits, although the precise mechanism whereby SR extraction exerts antioxidant effect remains to be explored.

SUMMARY

This study was carried out to determine if *Salviae Radix* extract (SRE) exerts protective effect against alterations in membrane transport function in rabbits with rhabdomyo lysis-induced acute renal failure. Acute renal failure was induced by intramuscular administration of glycerol (50%, 10 ml/kg). GFR in the glycerol-injected animals was reduced to 11% of the basal value and the fractional Na^+ excretion was increased to 7.8-fold, indicating generation of acute renal failure. When animals received SRE pretreatment for 7 days prior to glycerol injection, such changes were significantly attenuated. The fractional excretion of glucose and phosphate was increased more than 43-fold and 27-fold, respectively, in rabbits treated with glycerol alone. However, they were increased to 17- and 4.3-fold, respectively, in SRE-pretreated rabbits, and these values were significantly lower than those in rabbits treated with glycerol alone. Uptakes of glucose and phosphate in purified isolated brush-border membrane, the Na^+-K^+-ATPase activity in microsomal fraction, and cellular ATP levels all were reduced in rabbits treated with glycerol alone. Such changes were prevented by SRE pretreatment. Uptakes of organic ions, PAH and TEA, in renal cortical slices were inhibited by the administration of glycerol, which was prevented by SRE pretreatment. Pretreatment of an antioxidant DPPD significantly attenuated the increase in the fractional excretion of glucose and phosphate induced by rhabdomyolysis.

These results indicate that rhabdomyolysis causes

impairment inreabsorption of solutes in the proximal tubule via the generation of reactive oxygen species, and SRE pretreatment may provide the protection against the rhabdomyolysis-induced impairment by its antioxidant action.

REFERENCES

1. Westenfelder C, Arevalo GJ, Crawford PW, Zerwer P, Baranowski RL, Birch FM, Earnest WR, Hamburger RK, Coleman RD and Kurtzman NA. Renal tubular function in glycerol-induced acute renal failure. Kid Int. 1980;18:432-444.
2. Dubrow A and Flamenbaum W. Acute renal failure associated with myoglobinuria and hemoglobinuria. In Acute Renal Failure (Brenner BM, Lazarus JM, eds). New York. Churchill Livingstone. 1988;251-278.
3. Pal ler MS. Hemoglobin- and myoglobin-induced acute renal failure in rats: role of iron in nephrotoxicity. Am J Physiol. 1988;255:539-544.
4. Shah SV and Walker PD. Evidence sugesting a role for hydroxyl radical in glycerol-induced acute renal failure. Am J Physiol. 1988;255:438-443.
5. Nath KA, Balla G, Vercollotti GM, Balla J, Jacob HS, Levitt MD and Rosenberg ME. Induction of heme oxygenase is a rapid and protective response in rhabdomyolysis in the rat. J Clin Invest. 1992;90:267-270.
6. Zager RA. Combined mannitol and defroxamine therapy for myoglobinuric renal injury and oxidant tubular stress. Mechanistic and therapeutic implications. J Clin Invest. 1992;90:711-719.
7. Zager RA. Rhabdomyolysis and myohemoglobinuric acute renal failure. Kid Int. 1996;49:314-326.
8. Kinter, L. B. and Short, B. G.. Anatomy and physiology of the kidney. A brief review. In Toxicology of the Kideny (JB. Hook and RS Goldstein, Ed.), 2nd ed. New York. Raven Press. 1993;1-36.
9. Lee, S. I. Herbal, Seoul, Co. Medical Herb. 1975;419-420.
10. Kim, S. B. and Jeong, J. C. Protective effect of Salviae Radix extraction in H2O2 induced renal cell injury. Korean Oriental Medical Society. 1998;19(1):38-48.
11. Fiske CH and SubbaRow Y. The colorimetric determination of phosphorus. J. Biol. Chem, 1925; 66:375-400.
12. Kim YK and Kim YH. Differential effect of Ca2+ on oxidant-induced lethal cell injury and alterations of membrane functional integrity in renal cortical slices. Toxicol Appl Pharmacol. 1996;141:607-616.
13. Kim YK, Byun HS, Kim YH, Woo JS and Lee SH. Effect of cisplatin on renal function in rabbits: Mechanism of reduced glucose reabsorption. Toxicol Appl Pharmacol. 1995;130:19-26.
14. Kim YK, Jung JS and Lee SH. Dicarboxylate transport in renal basolateral and brush-border membrane vesicles. Can J Physiol Pharmacol. 1992;70:106-112.
15. Kim YK, Jung JS and Lee SH. Inhibition of H+/organic cation antiport by carboxyl reagents in rabbit renal brush-border membrane vesicles. J Pharmacol Exp Ther, 1993;266:500-505.
16. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-254.
17. Sachs G, Jackson RJ and Rabon EC. Use of plasma membrane vesicles. Am J Physiol. 1980;238:151-164.
18. Hirsch FH. Differential effects of nephrotoxic agents on renal transport and metabolism by use of in vitro techniques. Environ Health Perspect. 1976;15:89-99.
19. Valtin H. Renal function: Mechanisms preserving fluid and solute balance in health. 2nd ed. Boston, Little, Brown and Company. 1983;65-85.
20. Preuss HG, Tourkantonis A, Hsu C-H, Shim, PS, Barzyk P, Tio F and Shreiner GE. Early events in various forms of experimenatal acute tubular necrosis in rats. Lab Invest. 1975;32:286-2924.