

Butein Ameliorates Renal Concentrating Ability in Cisplatin-Induced Acute Renal Failure in Rats

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The present study examined whether the cisplatin-induced nephropathy could be ameliorated by administration of butein isolated from the stems of *Rhus verniciflua* STOCKS. The present study showed that polyuric profile was revealed in cisplatin-induced acute renal failure (ARF) rats associated with decreases in urinary sodium, potassium, chloride, and creatinine excretion, and osmolality. Among these renal functional parameters, urinary volume and osmolality were partially restored by administration of butein (10 mg/kg, i.p.), but electrolytes and creatinine excretion were not restored. Both solute-free water reabsorption and creatinine clearance were also significantly decreased in rats subjected to cisplatin. When butein was administered in rats with cisplatin-induced ARF for 4 d, solute-free water reabsorption was improved by 91% compared with that of cisplatin-induced ARF rats, but creatinine clearance was not restored. The expression levels of aquaporin 2 (AQP 2) in the inner, outer medulla, and cortex were significantly decreased in the kidney of ARF, which were partially reverted by administration of butein. In histological examination of the kidney, butein treatment partially prevented the lesions at tubules of renal cortex in cisplatin-induced ARF rats, while the lesions at glomeruli were not ameliorated. Taken together, butein ameliorates renal concentrating ability via up-regulation of renal AQP 2 water channel in rats with cisplatin-induced ARF without ameliorating effect on renal filtration defect.

Key words butein; cisplatin-nephrotoxicity; renal concentrating ability; aquaporin 2

Although cisplatin is used widely as an antineoplastic agent, its therapeutic usefulness may be limited by the potential toxicity that leads to acute renal failure (ARF). ARF is a syndrome defined as an acute reduction in renal function. ARF is commonly due to acute tubular necrosis with usually reversible loss of renal function incurred from ischemic or nephrotoxic insults. ARF induced by cisplatin in rats exhibits characteristic structural alterations in renal tubule epithelia in association with an impairment of urinary concentrating mechanism. ARF, when caused by cisplatin, is typically characterized by a nonoliguria, a severe reduction in glomerular filtration rate (GFR), a variable fall in renal blood flow, and a decrease in the urinary concentrating ability.¹⁻⁴ Certain pathophysiologic conditions associated with an altered urinary concentration have been related causally to an altered regulation of aquaporin (AQP) water channel in the kidney.⁵⁻⁸ Several recent studies have provided convincing of reducing expression of renal aquaporin 2 (AQP 2) in rats with cisplatin-induced ARF.^{9,10}

Butein (3,4,2',4'-tetrahydroxychalcone), a plant polyphenol, is one of the major active component of the stems of *Rhus verniciflua* STOCKS have been traditionally used for treatment of pain, parasite, and thrombotic disease in Far East Asia such as Korea, Japan, and China. The pharmacological actions of butein have previously been demonstrated in several studies. These beneficial effects include antioxidant and anti-inflammatory activities,¹¹⁻¹³ elicitation of endothelium-dependent vasodilation,¹⁴ induction of apoptosis,^{13,15,16} inhibition of protein kinase activity,^{17,18} and glutathione reductase,¹⁹ and inhibitory activity against HIV-1 protease.²⁰ In addition to these actions, it has been reported that butein ameliorates glomerulonephritis in rats.²¹ Recent studies have shown that ARF induced by either ischemia-reperfusion or nephrotoxic drugs was ameliorated by admin-

istration of polyphenolic compounds or flavonoids isolated from medicinal plants.²¹⁻²³

The present study, therefore, has two objectives. First, we investigated whether the renal functional parameters are restored by administration of butein in rats with cisplatin-induced ARF. Second, we also examined whether water channel (AQP 2) expression and histological changes in the kidney is restored as a consequence of administration of butein in rats with cisplatin-induced ARF.

MATERIALS AND METHODS

Chemicals Cisplatin [*cis*-diaminedichloroplatinum(II)] was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Butein was isolated from the stems of *Rhus verniciflua* STOCKS (Fig. 1). The structure of butein was identified by the comparison of spectral properties (MS, ¹H- and ¹³C-NMR) with those reported in the literature.¹³ All other unstated chemicals and reagents were analytical grade.

Experimental Animals The animal procedures were in strict accordance with the National Institutes of Health Guidelines for the Care and Use of laboratory Animals and were approved by the Institutional Animal Care and Utilization Committee. Male Sprague-Dawley rats (200-220 g)

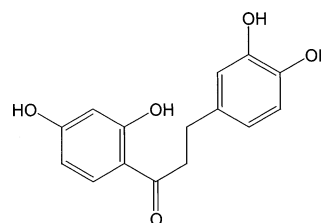


Fig. 1. Chemical Structure of Butein

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were purchased from Korean Experimental Animals Co. (Daejeon, Korea). The rats were housed in metabolic cages in the animal room with an automatic temperature (22 °C) and lighting (12 h light–dark cycle) control. To induce cisplatin-induced ARF, they received an injection of cisplatin (5 mg/kg, i.p.) and returned to the metabolic cage and were kept to recover for four days. Four groups of animals consisted of six rats each were investigated: control group administered with distilled water, cisplatin-induced ARF group, ARF group administered with butein (10 mg/kg, i.p.), and control group administered with butein (10 mg/kg, i.p.).

Renal Function Monitoring Each group of rats was maintained in separate metabolic cages, allowing quantitative urine collections and measurements of water intake. On days 0 (before cisplatin treatment), 1, 2, 3, and 4, 24 h urine samples were collected between 09:00 and 10:00 am. for the determination of the levels of creatinine, sodium ion, potassium ion, and osmolality. On the day of sacrifice, blood was collected immediately following decapitation for the measurement of sodium and potassium ion levels, osmolality, and creatinine concentrations in the plasma. Sodium and potassium ion concentrations of plasma and urine samples were measured using an electrolyte analyzer (NOVA 4, Biochemical, Waltham, MA, U.S.A.). Urine and plasma osmolalities were measured using an Advanced CRYOMATIC™ osmometer (Model 3C2, Needham Height, MA, U.S.A.). Creatinine concentrations of plasma and urine were measured by colorimetric methods using a spectrophotometer (Milton Roy, Rochester, NY, U.S.A.). Solute-free water reabsorption ($T^{\circ}H_2O$) was calculated by the following formula: $T^{\circ}H_2O = V \times (U_{osmol} / P_{osmol} - 1)$, where V is urine volume, U_{osmol} is urine osmolality, and P_{osmol} is plasma osmolality.

Preparation of Protein Extract from Kidney Kidneys were immediately isolated after decapitation of the rats, rapidly frozen in liquid N_2 , and stored at $-72^{\circ}C$ until assay. The inner medulla, outer medulla, and cortex from frozen kidney tissues were dissected and homogenized with Polytron homogenizer at 3000 rpm in a solution containing 250 mmol/l sucrose, 1 mmol/l ethylenediaminetetraacetic acid (EDTA), 0.1 mmol/l phenylmethylsulfonyl fluoride (PMSF, freshly prepared) and 20 mmol/l potassium phosphate buffer (pH 7.6). Large debris and nuclear fragments were removed by two low speed spins in succession ($1000 \times g$, 5 min; $10000 \times g$, 10 min) at $4^{\circ}C$. Supernatants from these low speed spins were ultracentrifuged at $100000 \times g$ for 1 h at $4^{\circ}C$. The resultant pellet was resuspended for Western blotting of AQP 2. The protein concentration was determined by the method of Bradford²⁵ with bovine serum albumin as a standard.

Western Blot Analysis Protein samples (10 μg ; outer medulla, 5 μg ; inner medulla, 50 μg ; cortex) obtained above were electrophoretically fractionated with a discontinuous system consisting of a 12.5% (w/v) polyacrylamide resolving gel and 5% (w/v) stacking gel, followed by transfer to a nitrocellulose membrane at current constant of 100 mA (with a starting voltage of 20 V). The membrane was washed in Tris–buffered saline (TBS, pH 7.6) containing 0.1% tween-20 (TBST), blocked with 5% (w/v) nonfat milk in TBST for 1 h, and incubated with anti-rabbit polyclonal AQP 2 antibody (Alomone Lab., Jerusalem, Israel) in 2% (w/v) non-fat milk/TBS for 1 h at room temperature. The membranes were

then incubated with a horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (1 : 1500) in 2% non-fat milk/TBS for 1 h. The bound antibody was detected by enhanced chemiluminescence (Amersham, Buckinghamshire, U.K.) procedure. The relative protein expression levels were determined by analysing the signals captured on Hyperfilm (Amersham, Buckinghamshire, U.K.) using an image analyzer (Imager III, Bioneer, Chungwon, Korea).

Histological Examination The kidneys isolated from all three groups were fixed in 10% (v/v) formalin in 50 mM phosphate buffer (pH 7.0) for 24 h at $4^{\circ}C$. The tissues were subsequently embedded in paraffin, sectioned (4 μm) and stained with hematoxylin and eosin for light microscopy (Olympus, Tokyo, Japan).

Statistical Analysis The statistical significance of differences between four groups means was determined using analysis of variance (ANOVA). Student's *t*-test was used to determine significance between two group means, if applicable, $p < 0.05$ was considered statistically significant.

RESULTS

Renal Functional Parameters The time courses of several renal functional parameters in rats with control, ARF, butein-treated ARF, and butein-treated control are shown in Fig. 2. Rats with ARF from 1 to 4 d showed a polyuric nature of the renal failure ($p < 0.01$ vs. control, Fig. 2A), which was partially restored by administration of butein ($p < 0.05$ vs.

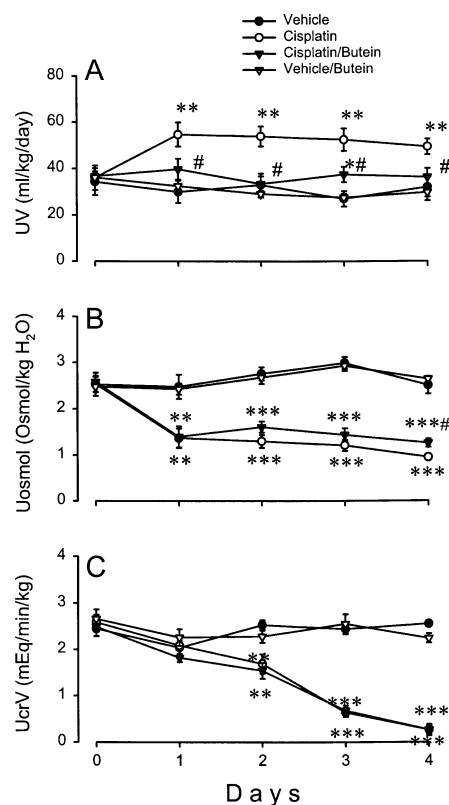


Fig. 2. Effects of Butein on Urinary Volume (A), Osmolality (B), and Creatinine Excretion (C) in Control Rats (Vehicle), Cisplatin-Induced ARF Rats (Cisplatin), Cisplatin-Induced ARF Rats Treated with Butein (Cisplatin/Butein), and Control Rats Treated with Butein (Vehicle/Butein)

Each data represents mean \pm S.E. of 6 experiments. ** $p < 0.01$, *** $p < 0.001$ compared with control; # $p < 0.05$ compared with ARF rats.

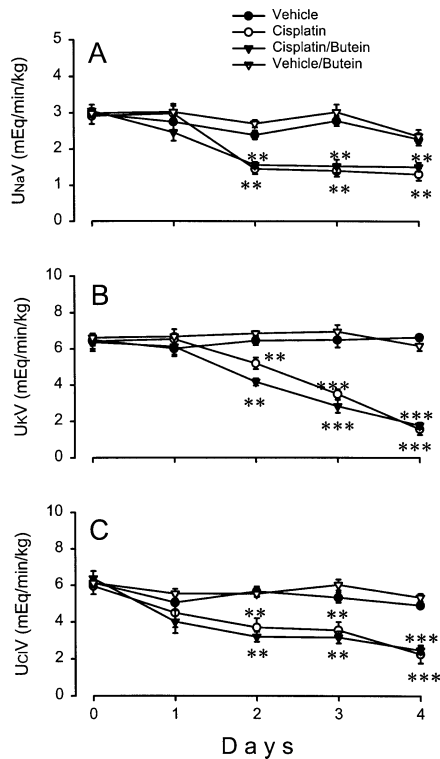


Fig. 3. Effects of Butein on Urinary Sodium (A), Potassium (B), and Chloride Excretion (C) in the Control Rats (Vehicle), Cisplatin-Induced ARF Rats (Cisplatin), Cisplatin-Induced ARF Rats Treated with Butein (Cisplatin/Butein), and Control Rats Treated with Butein (Vehicle/Butein)

Each data represents mean \pm S.E. of 6 experiments. ** $p < 0.01$, *** $p < 0.001$ compared with control.

ARF group, Fig. 2A). As expected, rats with ARF showed significant decrease in urine osmolality ($p < 0.001$ vs. control group, Fig. 2B). This decrease of urine osmolality in ARF rats was partially restored on day 4 by i.p. administration of butein ($p < 0.05$ vs. ARF group, Fig. 2B). The urinary creatinine excretion (UcrV) was decreased on days 2, 3, 4 by treatment with cisplatin ($p < 0.01$ vs. control group on day 2; $p < 0.001$ vs. control group on day 3, 4, Fig. 2C), which were not restored by administration of butein (Fig. 2C). The urinary sodium (UNaV), potassium (UKV), and chloride (UCIV) excretion were also significantly decreased in rats with cisplatin-induced ARF group compared with that of control group (Figs. 3A–C). As shown in Figs. 3A–C, the i.p. administration of butein did not restore these functional parameters. Next, we determined the plasma creatinine concentration (Pcr), creatinine clearance (Ccr), and solute-free water reabsorption (T^cH_2O) on day 4 in rats (Fig. 4). The Ccr was significantly decreased in ARF rats with increase of Pcr (* $p < 0.001$ vs. control, Figs. 4A, B, respectively). These same renal parameters were not restored by i.p. administration of butein for 4 d (Figs. 4A, B). The T^cH_2O also decreased in ARF rats ($p < 0.001$ vs. control group, Fig. 4C), but this parameter was partially restored by administration of butein ($p < 0.05$ vs. ARF group, $p < 0.01$ vs. control group, Fig. 4C). There were no any changes of renal functional parameters in the butein-treated control rats compared with those in control rats.

AQP 2 Expression The expression level of AQP 2 protein was determined in the renal inner medulla, outer

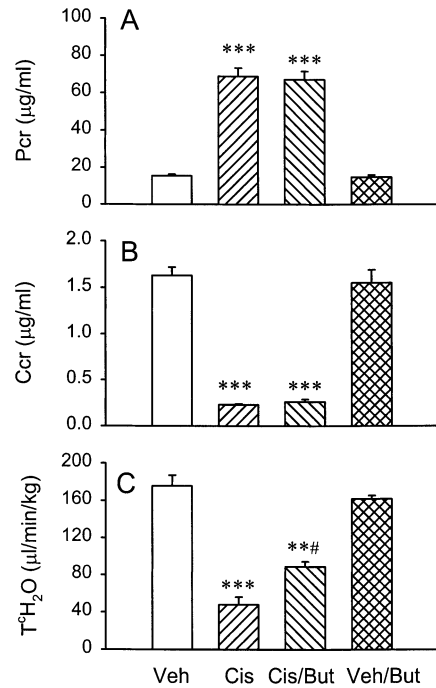


Fig. 4. Effect of Butein on Plasma Creatinine Concentration (A), Creatinine Clearance (B), and Solute-Free Water Reabsorption (C) in Control Rats (Vehicle), Cisplatin-Induced ARF Rats (Cisplatin), Cisplatin-Induced ARF Rats Treated with Butein (Cisplatin/Butein), and Control Rats Treated with Butein (Vehicle/Butein) on 4 d after Administration of Butein

Each data represents mean \pm S.E. of 6 experiments. ** $p < 0.01$, *** $p < 0.001$ compared with control; # $p < 0.05$ compared with ARF rats.

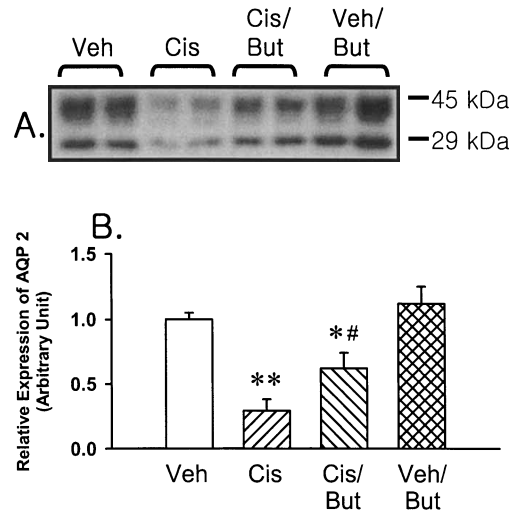


Fig. 5. Representative Western Blot (A) and Corresponding Densitometric Analyses (B) of AQP 2 Expression in the Renal Inner Medulla of Control Rats (Veh), Cisplatin-Induced ARF (Cis), Butein-Treated ARF Rats (Cis/But), and Butein-Treated Control Rats (Veh/But) on 4 d after Administration of Butein

Each data represents mean \pm S.E. of 4 experiments. * $p < 0.05$, ** $p < 0.01$, compared with control; # $p < 0.05$ compared ARF rats.

medulla, and cortex by Western blot analysis. The anti-AQP 2 antibody recognized 29 kDa and 35 to 50 kDa protein bands, corresponding to nonglycosylated and glycosylated AQP 2, respectively.¹² The expression levels of AQP 2 in the inner (Fig. 5), outer medulla (Fig. 6), and cortex (Fig. 7) were significantly decreased in the kidney of ARF rats ($p < 0.01$ vs. control group, respectively). This down-regulation in

AQP 2 water channel was partially reverted by administration of butein ($p < 0.05$ vs. ARF group, respectively, Figs. 5–7).

Histological Changes Histological examination of kidneys of rats subjected to cisplatin-induced ARF revealed the distinctive pattern of cisplatin-induced nephrotoxic injury, with clear signs of epithelial necrosis and desquamation affecting tubules of cortex (Fig. 8). By light microscopic examination of cisplatin-induced ARF rats, glomeruli, proximal tubules, and distal tubules were severely disrupted. However, the butein treatment partially prevented the lesions at proximal and distal tubules of renal cortex in cisplatin-induced ARF rats, while the lesions at glomeruli were not ameliorated (Fig. 8).

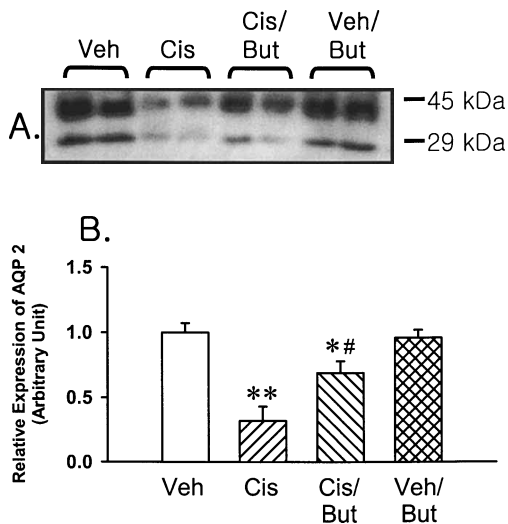


Fig. 6. Representative Western Blot (A) and Corresponding Densitometric Analyses (B) of AQP 2 Expression in the Renal Outer Medulla of Control (Veh), Cisplatin-Induced ARF (Cis), Butein-Treated ARF Rats (Cis/But), and Butein-Treated Control Rats (Veh/But) on 4 d after Administration of Butein

Each data represents mean \pm S.E. of 4 experiments. * $p < 0.05$, ** $p < 0.01$, compared with control; # $p < 0.05$ compared ARF rats.

DISCUSSION

The aim of this study was to investigate the effect of an administration of butein on renal function in the cisplatin-induced ARF rats. The present study showed that the urinary flow rate in cisplatin-induced ARF rats was significantly increased in association with decreases in urinary sodium, potassium, and chloride excretion and osmolality in urine. Rats with cisplatin-induced ARF showed an almost 2 times greater urinary volume than control group. These findings are not different from previously published data in which the marked polyuria has been identified in the cisplatin-induced ARF.^{1–4} Clearance study indicated that GFR was decreased following cisplatin-treatment in rats. Moreover, the urinary creatinine excretion was attenuated in rats with cisplatin-in-

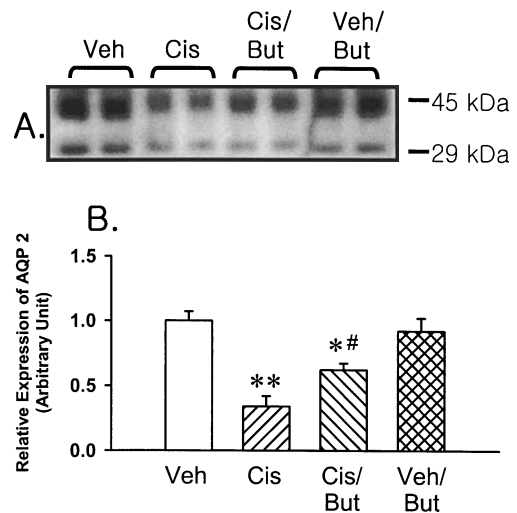


Fig. 7. Representative Western Blot (A) and Corresponding Densitometric Analyses (B) of AQP 2 Expression in the Renal Cortex of Control (Veh), Cisplatin-Induced ARF (Cis), Butein-Treated ARF Rats (Cis/But), and Butein-Treated Control Rats (Veh/But) on 4 d after Administration of Butein

Each data represents mean \pm S.E. of 4 experiments. * $p < 0.05$, ** $p < 0.01$, compared with control; # $p < 0.05$ compared ARF rats.

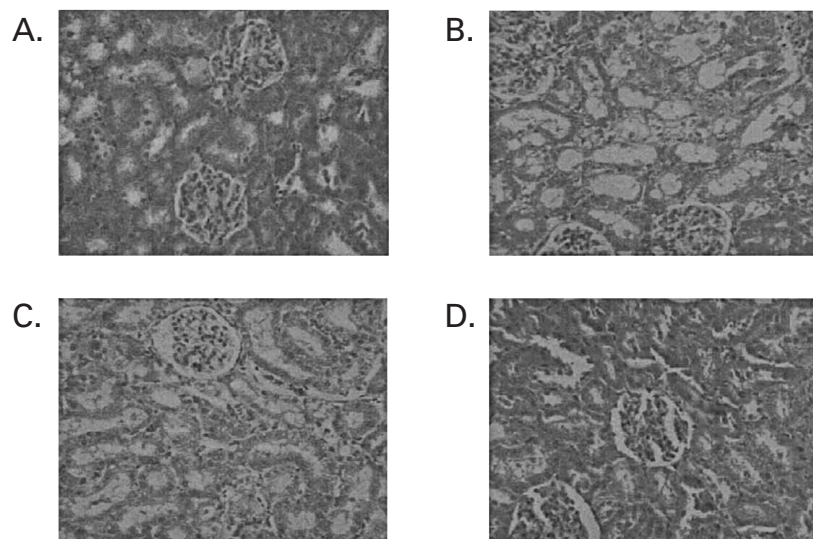


Fig. 8. Histological Examination of the Renal Cortex

Kidney obtained from control (A), cisplatin-induced ARF rats (B), ARF rats treated with butein (C), and control rats treated with butein (D) (Hematoxylin & Eosin, magnification $\times 300$).

duced ARF rats resulting in increase of plasma concentration of creatinine. In the present study, solute-free water reabsorption was significantly decreased in the rats subjected to cisplatin compared with control. Histological examination of kidney in ARF rats at 4 d following cisplatin-treatment revealed that glomeruli, proximal tubules, and distal tubules were severely disrupted and left with a denuded basement membrane. The time course studies reveal that the urinary concentrating ability is impaired as early as 24 h following cisplatin-injury. These data demonstrate that both urinary concentrating ability and GFR are attenuated in association with renal glomerular and tubular lesions in cisplatin-induced ARF rats. Water excretion is dependent on the balance between the GFR and the net rate of water reabsorption along the renal tubules.²⁶⁾ Therefore, increased urinary flow rate with cisplatin-induced renal injury could be associated with a failure of either the thick ascending limbs or collecting ducts, or both.³⁾ The data presented in this study also shows that butein preserves some of renal functional parameters following cisplatin-induced ARF. When butein was administered in ARF rats for 4 d, solute-free water reabsorption was improved by 91% compared with that of cisplatin-induced ARF rats. The decrease of urine osmolality in cisplatin-induced ARF rats was partially restored as the rats were administered with butein. Moreover, polyuria in cisplatin-induced ARF rats was significantly attenuated by administration of butein. On the other hand, creatinine clearance and the excretions of urinary electrolytes were not ameliorated by administration of butein. These data suggest that butein has an improving effect on urinary concentrating ability of kidney without improving effect on renal filtration function. Because the urinary concentrating mechanism is closely related with the expression of AQP 2 water channel,^{6–8,10)} the effect of butein on the expression of AQP 2 protein in the kidney of cisplatin-induced ARF rats was investigated in this study. The present study showed that the expression levels of AQP 2 in the renal inner, outer medulla, and cortex were significantly decreased in rats with cisplatin-induced ARF after 4 d, which were partially restored by administration of butein. These results suggest that the ameliorative effect of urinary concentrating ability by butein may be related to the up-regulation of the AQP 2 expression in the kidney of rats with cisplatin-induced ARF. Histological examination of renal cortex in the present study supports this suggestion. The kidney of cisplatin-induced ARF rats revealed that glomeruli, proximal tubules, and distal tubules were severely disrupted. Of these, the lesions at tubules of renal cortex were partially ameliorated by administration of butein, but lesions at glomeruli were not altered.

Nonetheless, the exact mechanism of this ameliorating effect on renal concentrating ability of butein is not completely clear, we suggest possibility as a mechanism of the pharmacological action of butein. It is possible that butein, as an antioxidant agent, may inhibit the cisplatin-induced reactive oxygen species (ROS) generation or scavenged the ROS before they reach the cell targets damaging the glomerular kidney function. As well known, ROS play a key role in cisplatin-induced renal injury^{27,28)} and butein has an antioxidant activity.^{11,29)} In the previous study, various antioxidant agents showed protective effect against renal injury induced by cisplatin in rats.^{30–32)}

In summary, butein, a plant polyphenol, ameliorates renal concentrating ability *via* up-regulation of renal AQP 2 water channel in rats with cisplatin-induced ARF.

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