Contribution of Prostaglandins to the Renal Responses to Magnesium Lithospermate B Isolated from Salviae Miltiorrhizae Radix

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The involvement of prostanoids in the improvement of adenine-induced renal failure in rats by magnesium lithospermate B was studied. After intraperitoneal administration of magnesium lithospermate B to renal failure rats, the levels of glomerular filtration rate, renal plasma flow and renal blood flow were increased. Urinary excretions of prostaglandin E_2 (PGE_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) in renal failure rats were increased by the administration of magnesium lithospermate B, while that of thromboxane B_2 had no effect. Pretreatment with indomethacin abolished the improving effect of magnesium lithospermate B on renal function concomitantly with markedly suppressed urinary excretion of prostanoids. These results suggest that the increased formation of PGE_2 and 6-keto-$PGF_{1\alpha}$ might contribute to the improvement of adenine-induced renal failure in rats by magnesium lithospermate B.

Keywords renal failure; magnesium lithospermate B; prostaglandin E_2; 6-keto-prostaglandin $F_{1\alpha}$; thromboxane B_2; rat

Introduction

We have so far investigated the effects of various oriental drugs and prescriptions on experimental rats with renal failure, as a part of a research project on pharmacotherapy for renal failure. Through such investigations, we have demonstrated that Salviae Miltiorrhizae Radix (Chinese crude drug named "dan shen") improves metabolism under conditions of renal failure.[1,2] In addition, the renal function-improving action of the extract has also been suggested by evidence of increased glomerular filtration rate, renal plasma flow and renal blood flow.[3,4] Thus, isolation and purification of the biologically active components of Salviae Miltiorrhizae Radix have been intensively performed, guided by the use of an index of renal function.[5] Magnesium lithospermate B was isolated and identified as a major biologically active component to improve renal failure.[6] However, the mechanism of the improving effect of magnesium lithospermate B on renal failure has not been determined yet.

It is well known that renal function is regulated by various neuro and humoral factors.[7] Among them, several prostaglandins and related compounds, which are metabolites of arachidonic acid, have been proposed to play an important role in the regulation of hemodynamics in the kidney, thereby modulating renal function.[8]

Therefore, in the present study, the effect of the administration of magnesium lithospermate B on renal function and urinary excretion of prostanoids in rats with renal failure induced by adenine was investigated in order to get further insight into the mechanism of the improvement of renal failure by magnesium lithospermate B.

Materials and Methods

Animals and Treatment Male rats of the LWH: Wistar strain, with a body weight of 200—210 g, were placed in metabolic cages and kept at a temperature of 23 ± 1 °C under a 12-h dark-light cycle. They were allowed an adaptation period of several days, during which they were fed on a commercial feed (type CE-2, CLEA Japan Inc., Tokyo, Japan). They were then fed *ad libitum* on an 18% casein diet containing 0.75% adenine. In rats with renal failure induced by adenine, renal impairment becomes aggravated as the period of adenine feeding increases. It was previously confirmed by histological and biochemical studies that renal failure was present after 6 d of ingestion of adenine.[9-13] Magnesium lithospermate B dissolved in saline was administered intraperitoneally to rats on an adenine diet for 6 d. Control rats were treated with an equal volume of saline. The blood urea nitrogen level in adenine-treated rats in this experiment was significantly increased (2.7 times higher than that of normal rats). Six rats were used for each experimental group. Values are expressed as means $\pm$ S.E.

Chemicals Prostaglandin E_2[^{125}I] radioimmunoassay (RIA) kit was provided by New England Nuclear (Boston, MA, U.S.A.). 6-Keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2[^{3}H] RIA kits were purchased from Amersham Co. (Amersham, U.K.).

Purification of Magnesium Lithospermate B from Salviae Miltiorrhizae Radix Commercially available Salviae Miltiorrhizae Radix (1.0 kg) produced in China was extracted twice with water (1.5 l) at 80 °C. After removal of the insolubles 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 of the column with water, elution with 50% aqueous methanol yielded polyphenols (62 g), which were chromatographed over Sephadex LH-20 (5.0 cm i.d. × 42 cm) with water containing increasing amounts of ethanol to afford three fractions; 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 separately rechromatographed over a Sephadex LH-20 column using water as the eluent to furnish compound **2** (1.98 g) and a further portion of compound **1** (4.3 g), respectively. Compound **1** was identified as magnesium lithospermate B on the basis of chemical and spectroscopic data as reported previously.[6]

Examination of Renal Function Glomerular filtration rate (GFR), renal plasma flow (RPF), hematocrit (Ht) and renal blood flow (RBF) were determined at about 6 h after intraperitoneal administration of the magnesium lithospermate B. GFR and RPF were measured by means of a renal clearance test using a single intravenous administration of sodium thiosulfate or sodium *para*-aminohippurate, respectively, as an indicator.[14,15] At 25 min after intravenous administration of either of these agents, the bladder was reflexly emptied by having each rat inhale ether for 3—5 s. The urine thus voided was discarded. During the next 30 min, the urine was collected, and collection was terminated after the bladders had again been emptied reflexly by ether inhalation. Blood samples were taken from conscious rats by heart puncture in the middle of the period used for the clearance test. Thiosulfate and *para*-aminohippurate concentrations in plasma and urine were determined by titrimetry and colorimetry, respectively. RBF was calculated on the basis of RPF and Ht using the equation shown below. Ht was determined with a hematocrit measurement apparatus, model KH-120A (Kubota Co., Ltd., Tokyo, Japan).

$$RBF = \frac{RPF}{1 - Ht} (ml/min)$$

Prostaglandin Assay Prostaglandin E_2 (PGE_2), 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane B_2 (TXB_2) in urine were measured by radioimmunoassay as reported elsewhere.[16-18] Urine was collected at

3—6 h after intraperitoneal administration of the magnesium lithospermate B. Prostaglandins in urine sample were extracted with an octadecyl silica mini column (Analytichem International, Harbor City, U.S.A.). Representative recoveries for various compounds by this extraction procedure were estimated to be as follows: PGE_2, 95%; 6-keto-$PGF_{1\alpha}$, 93%; TXB_2, 93%. The eluate from octadecyl column was evaporated under N_2; the residue was redissolved in EtOAc and separated on a Silica gel G plate (Whatman Chemical Separation Inc., Clifton, U.S.A.), using a solvent system of EtOAc : isooctane : AcOH : H_2O = 180 : 50 : 20 : 100 (v/v/v/v), upper layer. Prostaglandin standards were run in parallel with the samples and the positions of standards were determined by exposure of the plate to iodine vapor. Silica gel of corresponding areas containing PGE_2, 6-keto-$PGF_{1\alpha}$ or TXB_2 was scraped off, and the metabolites were extracted with MeOH–ether (1 : 1, v/v) and analyzed by using a RIA kit. Recoveries of these metabolites by this extraction procedure were 85, 82, and 82%, respectively. The radioactivity was determined in an Aloka liquid scintillation spectrometer, model LSC-900, or an Aloka Well Gamma System, model ARC-500. Finally, recovery of the [^{125}I]PGE_2, [^{3}H]6-keto-$PGF_{1\alpha}$ or [^{3}H]TXB_2 initially added to urine samples was 81, 76, or 76%, respectively. Appropriate corrections for recovery rates were made in calculating the concentration of prostaglandins.

Statistics The significance of differences between the normal and renal failure rats treated or non-treated with magnesium lithospermate B was tested by applying Student's t test.

Results

Renal Function GFR in normal rats was 5.65±0.27 ml/min/kg. This value decreased as kidney damage occurred following adenine administration. In the present study, GFR in rats given adenine significantly decreased by 51% of the level in normal rats. RPF and RBF in adenine-treated rats showed no significant difference when compared with those in normal rats (Table I). Intraperitoneal administration of magnesium lithospermate B (10 mg/kg body weight) increased GFR in renal failure rats. No further increase in GFR was observed when 20 mg/kg body weight magnesium lithospermate B was administered. However, pretreatment with indomethacin at a dose level of 10 mg/kg body weight abolished the increase in GFR, though indomethacin alone did not change the GFR in renal failure. Intraperitoneal administration of magnesium lithospermate B also increased RPF in renal failure rats. As shown in Table I, the administration of magnesium lithospermate B caused an increase from 18.31 to 24.17 ml/min/kg at the 5 mg level (a 32% change, $p<0.05$) and from 18.31 to 27.01 ml/min/kg at the 10 mg level (a 48% change, $p<0.05$). No further increase in RPF was observed when 20 mg/kg body weight was administered. A significant increase in RBF was observed in response to the administration of 10 mg/kg body weight. The intraperitoneal administration of 5 and 10 mg of magnesium lithospermate B caused 29% and 42% increases in RBF as compared with the control rats, respectively. No further increase in RBF was observed when 20 mg/kg body weight of magnesium lithospermate B was administered. The pretreatment with indomethacin also abolished the increases in RPF and RBF following magnesium lithospermate B administration in renal failure rats. The pretreatment with indomethacin itself did not change RPF or RBF in renal failure rats.

Urinary Prostaglandin Excretion Changes in urinary excretion of prostaglandins following the administration of magnesium lithospermate B and/or the pretreatment with indomethacin are shown in Table II. Urinary excretion of PGE_2 and 6-keto-$PGF_{1\alpha}$ was decreased by 15% and 24% of the level in normal rats on day 6 following adenine administration, respectively, while that of TXB_2 was increased by 37% of the level in normal rats. Administration of magnesium lithospermate B increased the urinary excretion of PGE_2 in renal failure rats from 7.09 to 9.60 ng/3 h at the dosage of 10 mg/kg body weight and to 10.25 ng/3 h at 20 mg/kg body weight. Urinary excretion of 6-keto-

TABLE I. Renal Responses to Magnesium Lithospermate B without and with Indomethacin Pretreatment

Group	Indomethacin	GFR (ml/min/kg)	RPF (ml/min/kg)	RBF (ml/min/kg)
Normal rat	−	5.65±0.27	20.83±2.73	35.31±5.03
Renal failure rat				
Control	−	2.76±0.22[a]	18.31±1.57	33.30±4.27
Control	+	3.06±0.23[a]	20.02±0.90	37.28±2.39
Magnesium lithospermate B, 5 mg	−	3.67±0.40[b]	24.17±1.46[b]	42.96±2.43[b]
Magnesium lithospermate B, 10 mg	−	3.91±0.29[b]	27.01±3.55[b]	47.26±6.16[b]
Magnesium lithospermate B, 20 mg	−	3.89±0.59[b]	26.86±2.92[b]	47.73±8.02[b]
Magnesium lithospermate B, 20 mg	+	2.92±0.36[a]	21.01±2.50	39.94±4.89

GFR = glomerular filtration rate; RPF = renal plasma flow; RBF = renal blood flow. Indomethacin was given intraperitoneally 30 min before magnesium lithospermate B. Statistical significance: *a*) $p<0.05$ *vs.* normal rat, *b*) $p<0.05$ *vs.* renal failure.

TABLE II. Effect of Magnesium Lithospermate B on Urinary Prostaglandin Excretion without and with Indomethacin Pretreatment

Group	Indomethacin	PGE_2 (ng/3 h)	6-Keto-$PGF_{1\alpha}$ (ng/3 h)	TXB_2 (ng/3 h)
Normal rat	−	8.30±2.47	5.75±1.56	2.82±0.26
Renal failure rat				
Control	−	7.09±0.30	4.39±0.31	3.86±1.02
Control	+	0.63±0.36[b,e]	1.10±0.13[b,e]	0.72±0.10[b,e]
Magnesium lithospermate B, 10 mg	−	9.60±0.91[c]	6.18±1.24	3.44±0.31
Magnesium lithospermate B, 20 mg	−	10.25±1.06[d]	7.03±1.26[c]	3.93±0.35[a]
Magnesium lithospermate B, 20 mg	+	0.80±0.48[b,e]	1.06±0.14[b,e]	0.79±0.06[b,e]

PGE_2 = prostaglandin E_2; 6-Keto-$PGF_{1\alpha}$ = 6-keto-prostaglandin $F_{1\alpha}$; TXB_2 = thromboxane B_2. Indomethacin was given intraperitoneally 30 min before magnesium lithospermate B. Statistical significance: *a*) $p<0.05$, *b*) $p<0.001$ *vs.* normal rat, *c*) $p<0.05$, *d*) $p<0.01$, *e*) $p<0.001$ *vs.* renal failure control rat.

$PGF_{1\alpha}$ was also increased by the administration of magnesium lithospermate B. As shown in Table II, the administration of magnesium lithospermate B at a dose level of 10 mg/kg body weight caused an increase in urinary excretion of 6-keto-$PGF_{1\alpha}$, though the effect was statistically not significant. The administration of 20 mg of magnesium lithospermate B significantly increased urinary excretion of 6-keto-$PGF_{1\alpha}$ by 60% of the control value. However, there were no significant changes in urinary excretion of TXB_2 between the control and magnesium lithospermate B-treated groups either at the dosage of 10 or 20 mg. Pretreatment with indomethacin markedly suppressed urinary excretion of PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 in renal failure rats given magnesium lithospermate B. Similar results were obtained when renal failure rats were given indomethacin alone.

Discussion

Recently we isolated magnesium lithospermate B from the water extract of Salviae Miltiorrhizae Radix as a major biologically active component to improve renal failure in rats.[6] In the present study, intraperitoneal administration of magnesium lithospermate B at doses of 5 to 20 mg caused an improvement of renal function in rats with adenine-induced renal failure. A markedly reduced GFR following adenine ingestion was improved by the administration of magnesium lithospermate B at 20 mg/kg body weight. In addition, RPF and RBF were also increased in renal failure rats. These changes in renal function seem to contribute to the improvement of uremic symptoms in rats.

Several prostaglandins and related compounds have been proposed to play an important role in the regulation of hemodynamics in the kidney. Among them, PGE_2 and PGI_2 are said to have vasodilating effects on mesangial cells of the glomeruli and the small vessel system of the kidney, thereby regulating renal function.[8] Accordingly, we investigated whether these arachidonic acid metabolites might be involved in the improvement of adenine-induced renal failure in rats by magnesium lithospermate B. First of all, the effect of indomethacin, a potent cyclooxygenase inhibitor on renal function in uremic rats was studied during the treatment with magnesium lithospermate B.

The improving effect of magnesium lithospermate B on GFR, RPF and RBF in renal failure rats was completely abolished by pretreatment with indomethacin. These results suggest that cyclooxygenase metabolites of arachidonic acid might play an important role in the improvement of renal failure by the treatment with magnesium lithospermate B. Then we investigated the effect of the administration of magnesium lithospermate B on urinary excretion of prostanoids, *e.g.*, PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2. A notable finding was that intraperitoneal administration of magnesium lithospermate B to renal failure rats increased urinary excretion of PGE_2 and 6-keto-$PGF_{1\alpha}$, while that of TXB_2 had no effect.

The mechanism of increased urinary excretion of PGE_2 and 6-keto-$PGF_{1\alpha}$ has still not been determined. However, the present results suggest that the formation of vasodilating prostaglandins *i.e.*, PGE_2 and PGI_2 (an active form of 6-keto-$PGF_{1\alpha}$) might be increased by the administration of magnesium lithospermate B, which is thought to have a beneficial effect on impaired renal function. On the other hand, there was no effect of magnesium lithospermate B on TXB_2 (biosynthesis of which occurs upon obstruction of the ureter or pathological changes, resulting in platelet aggregation and vasoconstriction[19-21]) in rats given this substance at 10 and 20 mg/kg body weight, in contrast to its effect on PGE_2 and 6-keto-$PGF_{1\alpha}$. These results suggest that there might be a qualitative and/or quantitative difference in the regulation of the formation of vasodilating prostanoids, *i.e.*, PGE_2 and PGI_2, and vasoconstricting prostanoid, *i.e.*, TXB_2, in the kidney. According to Okahara *et al.*,[22,23] who investigated the release of PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 from the kidney in response to various stimuli, the levels of metabolites of arachidonic acid were increased after infusion of arachidonic acid. After bradykinin infusion, 6-keto-$PGF_{1\alpha}$ was clearly increased but TXB_2 was not. It thus seems that magnesium lithospermate B could have an action mechanism different from that described above.

In the present study, pretreatment of renal failure rats with a potent cyclooxygenase inhibitor, indomethacin, abolished the improving effect of magnesium lithospermate B on renal function concomitantly with markedly suppressed urinary excretion of PGE_2 and 6-keto-$PGF_{1\alpha}$. Pretreatment with indomethacin itself had no significant effect on renal function in renal failure rats, though urinary excretion of prostanoids was markedly decreased. From these findings, it seems possible that the increased formation of PGE_2 and 6-keto-$PGF_{1\alpha}$ might contribute to the improvement of adenine-induced renal failure in rats by magnesium lithospermate B. In addition, it is considered that renal function is regulated by several possible mechanisms besides prostaglandins, including changes in renal perfusion pressure and hemodynamics, and changes in neurons (mainly, sympathetic neurons) and humoral factors (such as renin, angiotensin, aldosterone, vasopressin and catecholamine) that affect renal circulation.

Magnesium lithospermate B is a tetramer of caffeic acid, which was isolated from a Chinese plant *Artemisia rubripes* NAKAI and reported to be a potent inhibitor of lipoxygenase activity.[24] It was demonstrated that the inhibition of the lipoxygenase pathway caused a shift of arachidonic acid metabolism from the lipoxygenase to the cyclooxygenase pathway, which is thought to result in the increased formation of cyclooxygenase metabolites. However, the effect of magnesium lithospermate B on lipoxygenase activity has not been studied yet. Further investigation along this line is required.

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