Effect of Magnesium Lithospermate B on Urinary Excretion of Arachidonate Metabolites in Rats with Renal Failure

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The effect of magnesium lithospermate B isolated from Salviae miltiorrhiza Radix on excretion of urinary arachidonate metabolites was examined in both normal rats and those given adenine. Urinary excretion of prostaglandin E₂ (PGE₂) and 6-keto-prostaglandin F₁₆₂ (6-keto-PGF₁₆₂) decreased while urinary thromboxane B₂ (TXB₂) excretion increased markedly with the progression of renal failure. Rats administered magnesium lithospermate B showed an increase of urinary PGE₂ excretion at the 6th and 12th days. Excretion of 6-keto-PGF₁₆₂ also showed a significant increase on the 6th and 12th days in rats with renal failure induced by the administration of adenine. However, these effects were lower than the corresponding values in normal rats. In addition, urinary PGE₂ and 6-keto-PGF₁₆₂ excretions showed no appreciable difference in rats that exhibited progressive renal failure with continuation of the adenine administration period, as shown on the 18th and 24th days. There were no significant changes in TXB₂ excretion between the control and magnesium lithospermate B-treated groups throughout the experimental period.

Keywords: renal failure; magnesium lithospermate B; prostaglandin E₂; 6-keto-prostaglandin F₁₆₂; thromboxane B₂; rat

We have demonstrated that administration of Salviae miltiorrhiza Radix (a traditional Chinese medicinal herb known as "Dan shen") improves uremic symptoms, producing significant decreases in the serum levels of urea nitrogen, creatinine, methylguanidine and guanidinosuccinic acid and improvement in both hyperphosphatemia and the pattern of free amino acids in blood.¹⁶⁻⁻³⁵ Salviae miltiorrhiza Radix has also been shown to improve excretion by facilitating renal function, exhibiting a characteristic effect that is not produced by another medicinal herb, Rhei Rhiizoma, or by the Chinese medicinal prescription, Onpi-tó (Wen-pi-tang).⁴⁻⁶ In addition, the present authors recently isolated magnesium lithospermate B, a newly characterized substance that facilitates renal function, from an aqueous extract of Salviae miltiorrhiza Radix, and reported the details of its structure.⁷⁻⁻⁸ As part of an investigation into the mechanism of action of this compound, the effect of indomethacin was investigated. It was found that the renal function-facilitating action of magnesium lithospermate B was eliminated by indomethacin, suggesting that this compound acts on the prostaglandin system. Indeed, magnesium lithospermate B increased urinary excretions of prostaglandin E₂ and 6-keto-prostaglandin F₁₆₂ in rats with relatively mild renal failure induced by 6d of adenine ingestion.⁸ In the present paper, further studies were carried out to determine the prostaglandin levels in the urine of magnesium lithospermate B-treated rats as the period of adenine administration was lengthened, in order to ascertain which stage of renal failure this compound was effective in.

Materials and Methods

Animals and Treatment: Male rats of the JCL:Wistar strain, with a body weight of about 200 g, were used for the experiment and kept at a temperature of 23 ± 1 °C under a 12-h dark-light cycle. Animals with renal failure were prepared by feeding them an 18% casein diet containing 0.75% adenine for 6, 12, 18 or 24 d. The normal animals were fed on an 18% casein diet for 12 d. In rats with renal failure induced by adenine, renal impairment becomes aggravated as the period of adenine feeding is increased. It was previously confirmed by histological and biochemical procedures that renal failure was present after 6d of adenine ingestion.⁹⁻⁻¹⁴ On the 6th, 12th, 18th or 24th day of the experimental diet, magnesium lithospermate B was intraperitoneally administered to the rats. In a preliminary experiment, dose-dependency was observed following the administration of up to 20 mg/kg body weight magnesium lithospermate B. Therefore, a dose of magnesium lithospermate B of 10 mg/kg body weight was used in the present experiment. Control rats were treated with an equal volume of saline. At about 3 h after intraperitoneal administration of magnesium lithospermate B, the bladder was reflexly emptied by having each rat inhale ether for 3–5 s. The urine thus voided was discarded. During the next 3 h, the urine was collected, and collection was terminated after the bladder had again been emptied reflexly by ether inhalation. The blood urea nitrogen values of the rats used in this experiment reached a significantly increased level of 2.7 times (46.1 ± 3.5 mg/dl) those of normal rats on the 6th experimental day and 3.5 times (59.7 ± 3.9 mg/dl) those levels on the 12th experimental day. An abnormally high value of about 105 mg/dl was noted on day 24.

Chemicals: 6-keto-prostaglandin E₂ (RIA) kit was obtained from New England Nuclear (Boston, MA, U.S.A.), and [³H]-6-keto-prostaglandin F₁₆₂ and [³H]-thromboxane B₂ RIA kits were purchased from Amersham Co. (Amercham, U.K.).

Molecular Synthesis: Magnesium lithospermate B magnesium lithospermate B was isolated and purified from the extract of roots of Salviae miltiorrhiza Radix (Salvia miltiorrhiza Bunge) produced in China, as described previously.⁷⁻⁻⁸ The chemical structure of this compound is shown in Fig. 1.

Prostaglandin Assay: Prostaglandin E₂ (PGE₂), 6-keto-prostaglandin F₁₆₂ (6-keto-PGF₁₆₂), and thromboxane B₂ (TXB₂) in urine were measured by radioimmunassay as reported in the literature.¹²⁻⁻¹³ Prostaglandins in the urine sample were extracted with an octadecyl silica mini-column (Analytichem International, Harbor City, U.S.A.). Representative recoveries for the various compounds using this extraction procedure were estimated to be as follows: PGE₂, 95%; 6-keto-PGF₁₆₂, 93%; TXB₂, 93%. The eluate from the octadecyl column was evaporated under N₂ and the residue was redissolved in EtOAc, and separated on a Silica gel G plate (Whatman Chemical Separation Inc., Chilton, U.S.A.), using a solvent system of EtOAc-iso-octane-acOH-H₂O = 180:50:20:100 (by vol.). Prostaglandin standards were run in parallel with the samples and the positions of the standards were determined by exposure to iodine vapor.

Fig. 1. Structural Formula of Magnesium Lithospermate B

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Silica gel in the corresponding areas, containing PGE₂, 6-keto-PGF₁α, or TXB₂, was scraped off, and the metabolites were extracted with MeOH-ether (1:1 vol.) and then analyzed using a RIA kit. The recoveries of these metabolites by this extraction procedure were 85%, 82%, and 87%, respectively. The radioactivity was determined in an Aloka liquid scintillation spectrometer, model LSC-900, or an Aloka well gamma system, model ARC-500. The final recoveries of the [³H]PGE₂, [³H]6-keto-PGF₁α, and [³H]TXB₂ initially added to the urine samples were 81%, 76%, and 76%, respectively. Appropriate corrections for recovery rates were made in order to calculate the concentrations of prostaglandins.

Statistics The significance of differences between the control rats and those with renal failure, treated or nontreated with magnesium lithospermate B, was tested by using Student's t test.

Results

PGE₂ Changes in PGE₂ excretion are shown in Fig. 2. The urinary excretion of PGE₂ was maintained within the range of 8.47 ± 1.14 ng/3 h in normal rats, whereas in the adenine-administered rats, it decreased gradually with the progress of renal failure, becoming approximately 62% of the normal value by the 18th day after the start of administration. The level then decreased markedly and remained in the range of 1.74 ± 0.17 ng/3 h after the 24th day. When magnesium lithospermate B at 10 mg/kg body weight was intraperitoneally administered to normal rats, the amount of urinary PGE₂ was increased to 15.44 ng/3 h (an 82% change, p < 0.01). Significant changes produced by magnesium lithospermate B administration were also observed in rats given an adenine diet for 6 or 12 d: from 7.70 to 12.20 ng/3 h on the 6th day (a 58% change, p < 0.05) and from 6.43 to 10.92 ng/3 h on the 12th day (a 70% change, p < 0.05). On day 18, PGE₂ showed a tendency to increase from 5.24 to 6.87 ng/3 h (a 31% change) after treatment with the magnesium lithospermate B. A slight increase was observed on the 24th day.

6-Keto-PGF₁α Figure 3 shows the effect of magnesium lithospermate B on 6-keto-PGF₁α excretion. The urinary 6-keto-PGF₁α excretion in adenine-administered rats had significantly decreased by 29% and 42% of the level in normal rats by days 18 and 24, respectively, but the level of this prostaglandin showed no significant difference on the 6th and 12th days. In an examination of the effect of intraperitoneal administration of magnesium lithospermate B, a significant increase was observed in normal rats. The 6-keto-PGF₁α excretion was increased from 5.11 to 8.28 ng/3 h (a 62% change, p < 0.01). The levels in rats given adenine for 6 or 12 d were still as high as 5.82 and 7.58 ng/3 h, respectively. In contrast, urinary 6-keto-PGF₁α excretion in the magnesium lithospermate B-treated group on days 18 and 24 showed behavior similar to that of PGE₂, no significant changes being produced.

TXB₂ The amounts of urinary TXB₂ excreted by the rats fed on the adenine diet rose to 4.24—12.24 ng/3 h compared with the level of 2.42 ng/3 h for normal rats on days 6, 12, 18 and 24. TXB₂ values were 1.75 times greater than those of normal rats on the 6th experimental day, 3.10 times greater on the 12th experimental day and 5.06 times greater on the 18th experimental day. An abnormally high value of about 12.24 ± 2.96 ng/3 h was noted on day 18. By day 24, this had dropped to 379% of the normal level, but was similar to the value at days 12 and 18 (Fig. 4). However, urinary TXB₂ excretion in the magnesium lithospermate B-treated group was slightly decreased on days 12, 18 and 24.
compared with the control value. There were no significant changes in urinary excretion of TXB\textsubscript{2} between the control and magnesium lispersmate B-treated groups. In normal rats, TXB\textsubscript{2} values remained nearly unchanged after the administration of magnesium lispersmate B.

**Discussion**

Arachidonic acid metabolites produced in the kidney are excreted into urine and venous blood in the kidney, either as active metabolites or after further metabolism. The renal cortex is rich in PGE\textsubscript{2}-metabolizing enzymes, and PGE\textsubscript{2} excreted into urine is considered to correspond chiefly to that produced in the renal medulla.\textsuperscript{18} It is generally considered that the levels of 6-keto-PGF\textsubscript{1\alpha} and TXB\textsubscript{2} in urine mainly reflect their production levels in the kidney. This theory, however, has not yet been proved, and some researchers believe that these substances are derived from circulatory blood.\textsuperscript{19} In any case, although the use of urine for measuring arachidonic acid metabolites produced in the kidney involves some problems, it is a useful method for obtaining data from living animals.

In rats given an adenine diet, the urinary level of PGE\textsubscript{2} gradually became lower with the progression of renal failure, reaching a significant reduction of 38% at 18 d and one of 79% at 24 d in comparison with the control level. Although the range of variation was narrower, the level of 6-keto-PGF\textsubscript{1\alpha} also decreased along with the progression of renal failure. In contrast, excretion of TXB\textsubscript{2} increased significantly, showing a pattern of prostaglandin variation similar to that found in animals with ureteral obstruction, gynecol or mercuric chloride toxiemia, immunologic renal injury or genetic systemic lupus erythematosus.\textsuperscript{20}

On the other hand, in rats given an adenine diet for 6, 12, 18 or 24 d in order to induce renal failure, a single intraperitoneal administration of magnesium lispersmate B was given in order to determine the effect of this compound on urinary prostaglandins. The levels of PGE\textsubscript{2} and 6-keto-PGF\textsubscript{1\alpha} were significantly increased at 6 and 12 d, tended to be increased at 18 d and were still slightly increased at 24 d. These findings show that magnesium lispersmate B exerted its effect on the prostaglandin system in rats with mild or moderate renal failure, whereas its effect became weaker as renal failure progressed, and that magnesium lispersmate B has some effect on the production of the arachidonic acid cascade. The mechanism of these phenomena remains undefined. In rats fed on an adenine diet, the serum levels of urea nitrogen, creatinine, methylgluandine, guanindinosuccinic acid, etc. increased as the period of adenine feeding lengthens. Pathological studies of the kidneys in such animals have revealed lesions of the proximal tubules, a proportion of the distal tubules and the glomeruli. The glomerular filtration rate, renal plasma flow and renal blood flow also decreased progressively as the renal impairment increased due to prolonged administration of adenine.\textsuperscript{9,10,12} Thus, it is possible that magnesium lispersmate B might ameliorate the prostaglandin production, when remnant renal tissues are still functioning to some extent, as shown on the 6th and 12th days of adenine administration. In support of this speculation, significant increases in PGE\textsubscript{2} and 6-keto-PGF\textsubscript{1\alpha} were achieved in normal rats rather than in rats administered adenine. However, there was no effect of magnesium lispersmate B on TXB\textsubscript{2} in rats given this compound at a dose of 10 mg/kg body weight, in contrast to its effect on PGE\textsubscript{2} and 6-keto-PGF\textsubscript{1\alpha} excretion. These results suggest that there might be a qualitative and/or quantitative difference between the regulation of formation of vasodilative prostanoids i.e., PGE\textsubscript{2} and PGD\textsubscript{2}, and that of vasoconstrictive prostanoids i.e., TXB\textsubscript{2}, in the kidney.

In the kidney, PGE\textsubscript{2} and PGD\textsubscript{2} not only dilate renal blood vessels to increase renal blood flow but also relax mesangial cells, suppress immune function, and cause suppression of platelet aggregation.\textsuperscript{23} In contrast, it is known that TXB\textsubscript{2} acts on renal blood vessels to produce contraction, thereby decreasing renal blood flow, and causes contraction of mesangial cells, leading to proteinuria or decreased glomerular filtration.\textsuperscript{22-24} The experimental results on the 6th and 12th days of adenine administration suggest that magnesium lispersmate B has a protective action against renal failure. This speculation was also supported by the results of our previous studies,\textsuperscript{3,8,25,26} in which we investigated the effects of a single intraperitoneal dose, continuous intraperitoneal administration and continuous oral administration of magnesium lispersmate B. This compound increased the glomerular filtration rate, renal plasma flow and renal blood flow, which had been decreased because of renal failure, and facilitated excretion of urea, creatinine and sodium, suggesting an improvement in renal hemodynamics. In addition to the above findings, magnesium lispersmate B has been found to decrease urea nitrogen, creatinine and methylguandine, which accumulate in the body during renal failure, and to improve uremic symptoms.

Magnesium lispersmate B is a tetramer of caffeic acid, the monomer of which has been isolated from a Chinese plant, Artemisia rubripes NAKAI, and is reported to be a potent inhibitor of 5-lipoxygenase activity.\textsuperscript{27} It has been demonstrated that such inhibition of the lipoxygenase pathway causes a shift of arachidonic acid from the lipoxygenase to the cyclooxygenase pathway, which is thought to result in the increased formation of cyclooxygenase metabolites. However, the effect of magnesium lispersmate B on lipoxygenase activity as the period of adenine administration is lengthened has not yet been studied. Further investigation along these lines is therefore required.

**References**