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 miltiorrhizae Radix on excretion of urinary arachidonate metabolites was examined in both normal rats and those given adenine. Urinary excretion of prostaglandin E_2 (PGE_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) decreased while urinary thromboxane B_2 (TXB_2) excretion increased markedly with the progression of renal failure. Rats administered magnesium lithospermate B showed an increase of urinary PGE_2 excretion at the 6th and 12th days. Excretion of 6-keto-$PGF_{1\alpha}$ 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 PGE_2 and 6-keto-$PGF_{1\alpha}$ 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 TXB_2 excretion between the control and magnesium lithospermate B-treated groups throughout the experimental period.

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

We have demonstrated that administration of Salviae miltiorrhizae 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.[1-3] Salviae miltiorrhizae 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 Rhizoma, or by the Chinese medicinal prescription, Onpi-tô (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 miltiorrhizae Radix, and reported the details of its structure.[7] 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 E_2 and 6-keto-prostaglandin $F_{1\alpha}$ in rats with relatively mild renal failure induced by 6 d 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 on 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 6 d 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 A [^{125}I]prostaglandin E_2 radioimmunoassay (RIA) kit was obtained from New England Nuclear (Boston, MA, U.S.A.), and [^{3}H]6-keto-prostaglandin $F_{1\alpha}$ and [^{3}H]thromboxane B_2 RIA kits were purchased from Amersham Co. (Amersham, U.K.).

Magnesium Lithospermate B Magnesium lithospermate B was isolated and purified from the extract of roots of Salviae miltiorrhizae Radix (*Salvia miltiorrhiza* BUNGE) produced in China, as described previously.[7] The chemical structure of this compound is shown in Fig. 1.

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 in the literature.[15-17] 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: PGE_2, 95%; 6-keto-PGF_1, 93%; TXB_2, 93%. The eluate from the octadecyl column was evaporated under N_2, and 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–iso-octane–AcOH–H_2O = 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.

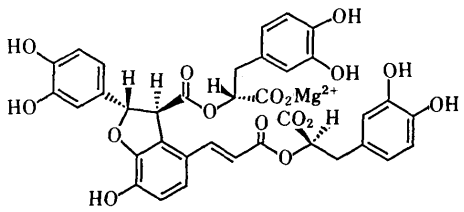

Fig. 1. Structural Formula of Magnesium Lithospermate B

Silica gel in the 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 by vol.) and then analyzed using a RIA kit. The 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. The final recoveries of the [^{125}I]PGE_2, [^{3}H]6-keto-$PGF_{1\alpha}$ and [^{3}H]TXB_2 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

PGE_2 Changes in PGE_2 excretion are shown in Fig. 2. The urinary excretion of PGE_2 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 PGE_2 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, PGE_2 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-$PGF_{1\alpha}$ Figure 3 shows the effect of magnesium lithospermate B on 6-keto-$PGF_{1\alpha}$ excretion. The urinary 6-keto-$PGF_{1\alpha}$ 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-$PGF_{1\alpha}$ 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-$PGF_{1\alpha}$ excretion in the magnesium lithospermate B-treated group on days 18 and 24 showed behavior similar to that of PGE_2, no significant changes being produced.

TXB_2 The amounts of urinary TXB_2 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. TXB_2 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 TXB_2 excretion in the magnesium lithospermate B-treated group was slightly decreased on days 12, 18 and 24

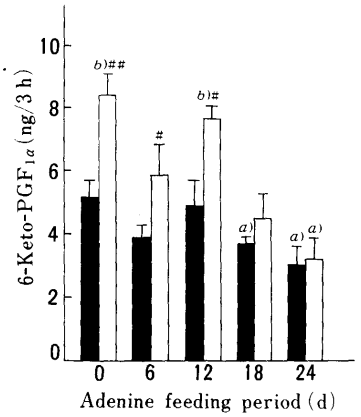

Fig. 3. Effect of Magnesium Lithospermate B on Urinary 6-Keto-Prostaglandin $F_{1\alpha}$ Excretion

■, control rats; □, magnesium lithospermate B-treated rats. Values are means ± S.E. of 6 rats. Statistical significance: *a*) $p<0.05$, *b*) $p<0.01$ *vs.* normal control rats, #$p<0.05$, ##$p<0.01$ *vs.* respective control rats.

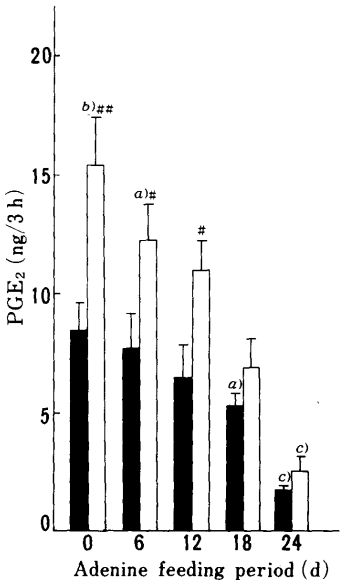

Fig. 2. Effect of Magnesium Lithospermate B on Urinary Prostaglandin E_2 Excretion

■, control rats; □, magnesium lithospermate B-treated rats. Values are means ± S.E. of 6 rats. Statistical significance: *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ *vs.* normal control rats, #$p<0.05$, ##$p<0.01$ *vs.* respective control rats.

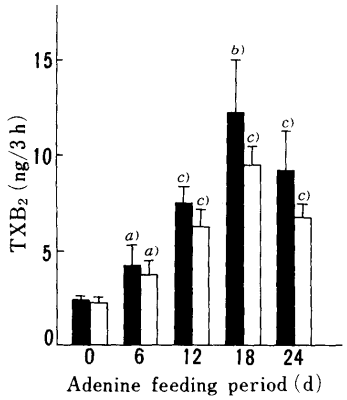

Fig. 4. Effect of Magnesium Lithospermate B on Urinary Thromboxane B_2 Excretion

■, control rats; □, magnesium lithospermate B-treated rats. Values are means ± S.E. of 6 rats. Statistical significance: *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ *vs.* normal control rats.

compared with the control value. There were no significant changes in urinary excretion of TXB_2 between the control and magnesium lithospermate B-treated groups. In normal rats, TXB_2 values remained nearly unchanged after the administration of magnesium lithospermate 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_2-metabolizing enzymes, and PGE_2 excreted into urine is considered to correspond chiefly to that produced in the renal medulla.[18] It is generally considered that the levels of 6-keto-$PGF_{1\alpha}$ and TXB_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.[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_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-$PGF_{1\alpha}$ also decreased along with the progression of renal failure. In contrast, excretion of TXB_2 increased significantly, showing a pattern of prostaglandin variation similar to that found in animals with ureteral obstruction, glycerol or mercuric chloride toxemia, immunologic renal injury or genetic systemic lupus erythematosus.[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 lithospermate B was given in order to determine the effect of this compound on urinary prostaglandins. The levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ 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 lithospermate 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 lithospermate 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, methylguanidine, guanidinosuccinic 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.[9,10,12] Thus, it is possible that magnesium lithospermate 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_2 and 6-keto-$PGF_{1\alpha}$ were achieved in normal rats rather than in rats administered adenine. However, there was no effect of magnesium lithospermate B on TXB_2 in rats given this compound at a dose of 10 mg/kg body weight, in contrast to its effect on PGE_2 and 6-keto-$PGF_{1\alpha}$ 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_2 and PGI_2, and that of vasoconstrictive prostanoids *i.e.*, TXB_2, in the kidney.

In the kidney, PGE_2 and PGI_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.[21] In contrast, it is known that TXB_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.[22-24] The experimental results on the 6th and 12th days of adenine administration suggest that magnesium lithospermate B has a protective action against renal failure. This speculation was also supported by the results of our previous studies,[3,8,25,26] in which we investigated the effects of a single intraperitoneal dose, continuous intraperitoneal administration and continuous oral administration of magnesium lithospermate 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 lithospermate B has been found to decrease blood urea nitrogen, creatinine and methylguanidine, which accumulate in the body during renal failure, and to improve uremic symptoms.

Magnesium lithospermate 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.[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 lithospermate 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

1) T. Yokozawa, H. Y. Chung, and H. Oura, *J. Med. Pharm. Soc. WAKAN-YAKU*, **2**, 446 (1985).
2) H. Y. Chung, T. Yokozawa, and H. Oura, *Chem. Pharm. Bull.*, **34**, 3818 (1986).
3) T. Yokozawa, H. Y. Chung, H. Oura, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **36**, 316 (1988).
4) H. Y. Chung, T. Yokozawa, and H. Oura, *Chem. Pharm. Bull.*, **36**, 274 (1988).
5) H. Y. Chung, T. Yokozawa, and H. Oura, *J. Med. Pharm. Soc. WAKAN-YAKU*, **4**, 59 (1987).
6) H. Oura and T. Yokozawa, *Kidney and Dialysis*, **26** (separate vol.), 38 (1989).
7) T. Tanaka, S. Morimoto, G. Nonaka, I. Nishioka, T. Yokozawa, H. Y. Chung, and H. Oura, *Chem. Pharm. Bull.*, **37**, 340 (1989).
8) T. Yokozawa, H. Y. Chung, T. W. Lee, H. Oura, T. Tanaka, G. Nonaka, I. Nishioka, and A. Hirai, *Chem. Pharm. Bull.*, **37**, 1568 (1989).
9) T. Yokozawa, P. D. Zheng, H. Oura, and F. Koizumi, *Nephron*, **44**, 230 (1986).
10) T. Yokozawa, H. Y. Chung, and H. Oura, *Jpn. J. Nephrol.*, **29**, 1129 (1987).

11) T. Yokozawa, H. Oura, and T. Nakada, *Jpn. J. Nephrol.*, **29**, 1145 (1987).
12) T. Koeda, K. Wakaki, F. Koizumi, T. Yokozawa, and H. Oura, *Jpn. J. Nephrol.*, **30**, 239 (1988).
13) T. Yokozawa, Z. L. Mo, and H. Oura, *Nephron*, **51**, 388 (1989).
14) T. Yokozawa, N. Fujitsuka, and H. Oura, *Nephron*, **52**, 347 (1989).
15) H. G. Morris, N. A. Sherman, and F. T. Shepperdson, *Prostaglandins*, **21**, 771 (1981).
16) A. Hirai, K. Tahara, Y. Tamura, H. Saito, T. Terano, and S. Yoshida, *Prostaglandins*, **30**, 749 (1985).
17) L. M. Demers and D. D. Derck, "Advances in Prostaglandin and Thromboxane Research," Vol. 6, ed. by B. Samuelsson, P. W. Ramwell, and R. Raoletti, Raven Press, New York, 1980, p. 193.
18) J. C. Frölich, T. W. Wilson, B. J. Sweetman, M. Smigel, A. S. Nies, K. Carr, J. T. Watson, and J. A. Oates, *J. Clin. Invest.*, **55**, 763 (1975).
19) R. M. Boyd, A. Nasjletti, P. M. Heerdt, and P. G. Baer, *Am. J. Physiol.*, **250**, F58 (1986).
20) Y. Shioda and K. Nishikawa, "Prostaglandin 6," ed. by K. Abe, S. Murota, and S. Yamamoto, Tokyo Kagaku Dojin, Tokyo, 1988, p. 207.
21) G. G. N. Serneri, G. Masotti, and S. Castellani, "Contributions to Nephrology," Vol. 49, ed. by G. M. Berlyne and S. Giovannetti, Karger, Basel, 1985, p. 156.
22) T. Okegawa, P. E. Jonas, K. DeSchryver, A. Kawasaki, and P. Needleman, *J. Clin. Invest.*, **71**, 81 (1983).
23) E. A. Lianos, G. A. Andres, and M. J. Dunn, *J. Clin. Invest.*, **72**, 1439 (1983).
24) A. Kawasaki and P. Needleman, *Circ. Res.*, **50**, 486 (1982).
25) T. W. Lee, T. Yokozawa, H. Oura, G. Nonaka, I. Nishioka, and A. Hirai, Abstracts of Papers, the 61th Annual Meeting of the Japanese Biochemical Society, Tokyo, October 1988, p. 766.
26) T. Yokozawa, T. W. Lee, H. Oura, G. Nonaka, I. Nishioka, and A. Hirai, Abstracts of Papers, the 31th Annual Meeting of the Japanese Society of Nephrology, Nara, October 1988, p. 239.
27) Y. Koshihara, T. Neichi, S. Murota, A. Lao, Y. Fujimoto, and T. Tatsuno, *FEBS Lett.*, **158**, 41 (1983).