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EFFECT OF MAGNESIUM LITHOSPERMATE B ON URINARY PROSTAGLANDINS IN RATS WITH RENAL FAILURE

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ABSTRACT.—Urinary excretion of prostaglandin E_2 showed a significant increase following administration of magnesium lithospermate B [**1**] in rats with renal failure. In contrast, that of thromboxane B_2 showed a significant decrease, indicating the improvement of renal failure.

The root of *Salvia miltiorrhiza* Bunge (Labiatae), a Chinese crude drug known as "Dan shen," has been traditionally used to improve blood circulation, relieve blood stasis, eliminate swelling, etc. In addition, it has recently been reported to show vasodilatory, hypotensive, anticoagulant, and antibacterial activities and to have a beneficial effect in patients with chronic renal failure (1,2).

In our laboratory, a series of experiments have been conducted on the action of this herb in rats with induced renal failure in an attempt to develop an effective medicinal therapy. These studies have revealed that an aqueous extract of *S. miltiorrhiza* root is effective for improving 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. *S. miltiorrhiza* root has also been shown to facilitate renal function (3–5). We recently isolated magnesium lithospermate B [**1**], a newly characterized substance that facilitates renal function, from an aqueous extract of *S. miltiorrhiza* root and reported the details of its structure (6). As part of an investigation into the mechanism of action of this compound, the effect of indomethacin was also investigated. It was found that the renal-function-facilitating action of magnesium lithospermate B was eliminated by indomethacin, suggesting that this compound may act on the prostaglandin system (7). In the present paper, further studies have been carried out to determine the prostaglandin levels in the urine of magnesium-lithospermate-B-treated rats.

The amounts of urinary excretion of prostaglandins after the administration of magnesium lithospermate B are shown in Table 1. In renal failure, the urinary excretion of prostaglandin E_2 (PGE_2) was decreased by 66% of the normal level. The administration of magnesium lithospermate B significantly increased the urinary excretion of PGE_2 from 29.6 to 72.5 ng/24 h. The urinary 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$)

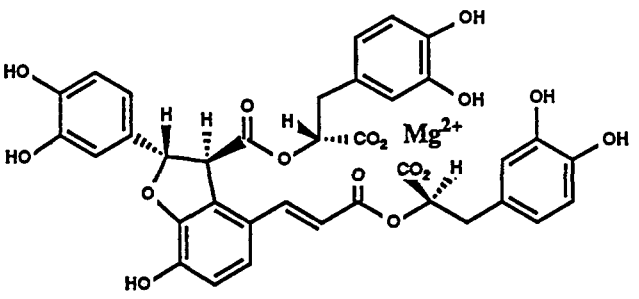

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TABLE 1. Effect of Magnesium Lithospermate B [1] on Urinary Excretion of Prostaglandins.[a]

Group	PGE_2 (ng/24 h)	6-Keto-$PGF_{1\alpha}$ (ng/24 h)	TXB_2 (ng/24 h)
Normal rat	86.5 ± 8.8	27.7 ± 2.3	9.3 ± 0.7
Renal failure rat			
control	29.6 ± 6.4[b]	22.9 ± 3.3	36.2 ± 3.4[c]
magnesium lithospermate B [1]	72.5 ± 13.4[d]	20.7 ± 2.0	23.3 ± 4.7[b,e]

[a] PGE_2 = prostaglandin E_2; 6-Keto-$PGF_{1\alpha}$ = 6-keto-prostaglandin $F_{1\alpha}$; TXB_2 = thromboxane B_2.
[b] $P < 0.01$ vs. normal rat.
[c] $P < 0.001$ vs. normal rat.
[d] $P < 0.01$ vs. renal failure control rat.
[e] $P < 0.05$ vs. renal failure control rat.

excretion in adenine-induced renal failure rats showed no appreciable changes at the 11–12th day compared with the normal values. In addition, there were no significant differences between the control and magnesium-lithospermate-B-treated groups. In contrast, the amount of urinary thromboxane B_2 (TXB_2) excreted by the rats fed on the adenine diet rose to 36.2 ng/24 h compared with the level of 9.3 ng/24 h for normal rats at the 11–12th day. After magnesium lithospermate B administration, TXB_2 was significantly decreased by 36%.

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 (8). 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 (9). 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 with renal failure, as shown in Table 1, the urinary level of PGE_2 showed a significant reduction of 66% at 12 days in comparison with the normal level. Although the range of variation was narrower, the level of 6-keto-$PGF_{1\alpha}$ also decreased with the progression of renal failure. In contrast, excretion of TXB_2 increased significantly, showing a pattern of prostaglandin variation, in part similar to that found in animals with ureteral obstruction, glycerol or adriamycin toxemia, immunologic renal injury, or genetic systemic lupus erythematosus (10–14). On the other hand, in renal failure rats given magnesium lithospermate B, it was noted that the urinary excretion of PGE_2 was increased in comparison with the control value, this increase being attributed to an enhanced level of prostaglandin production in renal tissue. Despite the fact that the level of urinary excretion of PGE_2 was increased in rats given magnesium lithospermate B, the level of 6-keto-$PGF_{1\alpha}$ did not change significantly. There was a significant decrease of magnesium lithospermate B on TXB_2 in rats given this substance at 10 mg/kg body weight per day from the 7th to the 12th day, in contrast to its effect on PGE_2. These results suggest that there may be a qualitative and/or quantitative difference between the regulation of formation of cyclooxygenase metabolites of arachidonic acid in the kidney. According to Okahara and co-workers (15, 16), 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 in-

creased but TXB_2 was not. It thus seems that magnesium lithospermate B could have a mechanism of action different from that described above.

In the kidney, PGE_2 and PGI_2 (an active form of 6-keto-$PGF_{1\alpha}$) 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 (17). 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 (18–20). From the results of the present study, it seems possible that prostaglandins may contribute to the improvement of adenine-induced renal failure seen in rats given magnesium lithospermate B. Magnesium lithospermate B appears to be act as a protective against renal failure.

EXPERIMENTAL

ANIMALS AND TREATMENTS.—Male rats of the LWH:Wistar strain with a body wt of 200–210 g were placed in metabolic cages and kept at a temperature of 23 ± 1° 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, Tokyo, Japan). They were then fed ad libitum on an 18% casein diet containing 0.75% adenine, which produced experimental renal failure in the animals (21–26). In rats with renal failure induced by adenine, renal impairment becomes aggravated as the period of adenine feeding increases. It has been previously confirmed by histological and biochemical procedures that renal failure is present after 6 days of ingestion of adenine. During the adenine feeding period, magnesium lithospermate B dissolved in saline was administered intraperitoneally from the 7th to the 12th day to rats that had mild to moderate renal impairment. In a preliminary experiment, a dose-dependent recovery from renal failure due to adenine ingestion was observed after the administration of magnesium lithospermate B up to 20 mg/kg body wt once a day. Therefore, a dose of magnesium lithospermate B of 10 mg/kg body wt was used in the present experiment. Control rats were treated with an equal volume of saline. Individual 24-h urine samples (collected from the 11th to the 12th day) were collected for the determination of prostaglandins. There were no statistically significant differences between the control and magnesium-lithospermate-B-treated groups with regard to body wt. The food intake of the two groups was essentially proportional to body wt change throughout the experimental period. Six rats were used for each experimental group. Values were expressed as means ± SE.

CHEMICALS.—The prostaglandin E_2 [^{125}I] RIA kit was a product of New England Nuclear, Boston, [^{3}H] 6-Keto-prostaglandin $F_{1\alpha}$ and [^{3}H] thromboxane B_2 kits for radioimmunoassay were purchased from Amersham, Amersham, UK.

MAGNESIUM LITHOSPERMATE B.—Magnesium lithospermate B [**1**] was isolated and purified from roots of *S. miltiorrhiza* grown in China, as described previously (6).

PROSTAGLANDIN ASSAY.—PGE_2, 6-keto-$PGF_{1\alpha}$, and TXB_2 in urine were measured by radioimmunoassay as reported in the literature (27–29). Prostaglandins in the urine sample were extracted with an octadecyl silica mini-column (Analytichem International, Harbor City, CA). Representative recoveries for the various compounds using this extraction procedure were estimated to be: PGE_2 95%; 6-keto-$PGF_{1\alpha}$ 93%; TXB_2 93%. The eluate from the octadecyl column was evaporated under N_2, redissolved in EtOAc, and separated on a Si gel G plate (Whatman Chemical Separation, Clifton, New Jersey), using a solvent system containing EtOAc–iso-octane–HOAc–H_2O (180:50:20:100). Prostaglandin standards were run parallel to the samples, and the positions of the standards were determined by exposure to iodine vapor. Si 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) and analyzed using a radioimmunoassay 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 normal and renal failure rats treated or non-treated with magnesium lithospermate B [**1**] was tested by applying Student's *t*-test.

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