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Renal Responses to Magnesium Lithospermate B

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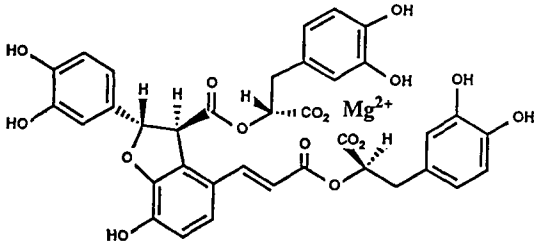
Abstract—Renal responses to magnesium lithospermate B isolated from *Salviae miltiorrhizae* radix were examined in normal rats. Urinary sodium, potassium, prostaglandin E_2 and kallikrein excretion was significantly increased after magnesium lithospermate B administration, whereas excretion of urinary 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 was unchanged. Rats administered with the drug also revealed a slight elevation of plasma renin activity and the levels of angiotensins I and II. Plasma aldosterone was decreased slightly. No significant changes were observed in angiotensin-converting enzyme or blood pressure.

The constituents of *Salviae miltiorrhizae* radix reported so far include naphthoquinone (phenanthrene quinone) derivatives, such as tanshinone I, tanshinone II-A, tanshinone II-B, cryptotanshinone, isotanshinone I, isotanshinone II, isocryptotanshinone, tanshinonic acid, hydroxytanshinone and miltirone (Shibata et al 1982). The present authors recently isolated magnesium lithospermate B, from an aqueous extract of the root, and reported the details of the structure of this substance that improves renal function (Tanaka et al 1989; Yokozawa et al 1989). In the present study, determinations of electrolytes, hormones and blood pressure were made in rats treated with magnesium lithospermate B to investigate the renal responses to this substance and to elucidate its characteristics.



Magnesium lithospermate B.

Materials and Methods

Animals and treatment

Male LWH: Wistar rats, 200–210 g, were placed in metabolic cages at 23 ± 1°C under a 12 h dark/light cycle. The animals had free access to an ordinary 18% casein diet, of the following composition (g/100 g): casein 18; α-corn starch 57·9; sucrose 15; soybean oil 2; salt mixture 4; vitamin mixture 1; cellulose powder 2; choline chloride 0·1. An aqueous solution of magnesium lithospermate B was administered orally at a dose of 10 mg kg^{-1} day^{-1}, for 18 successive days as drinking water, while control rats received tap-water.

Individual 24 h urine samples (collected on days 17–18) were collected for determination of electrolytes, prostaglandins and kallikrein. On day 18 of the experimental period, blood pressure was determined and then the rats were decapitated. The serum or plasma obtained was used for determination of hormone levels. Throughout the experiment there were no statistically significant differences in body weight between the control and magnesium lithospermate B-treated rats. The food intake of each rat was essentially proportional to weight change. Six rats were used for each experimental group. Values are expressed as means ± s.e.

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Chemicals

The [^{125}I]prostaglandin E_2 RIA kit used was from New England Nuclear, USA. [^{3}H]6-Keto-prostaglandin $F_{1\alpha}$ and [^{3}H]thromboxane B_2 RIA kits were purchased from Amersham Co., UK. Aprotinin and DL-Val-Leu-Arg p-nitroanilide were obtained from Sigma Chemical Co., USA.

Magnesium lithospermate B

Magnesium lithospermate B was isolated and purified from an extract of the roots of *Salviae miltiorrhizae* Bunge produced in China, as previously described (Tanaka et al 1989; Yokozawa et al 1989).

Determination of urinary electrolytes

Sodium and potassium were measured with an electrolyte measurement apparatus (AHS/Japan Corporation, Tokyo, Japan) based on the ion electrode method.

Urinary prostaglandin assay

Prostaglandin E_2 (PGE_2), 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane B_2 (TxB_2) were measured by radioimmunoassay (Demers & Derck 1980; Morris et al 1981; Hirai et al 1985).

Urinary kallikrein assay

The activity of kallikrein was assayed according to Amundsen et al (1979).

Blood hormone assay

Plasma renin activity was determined by a modification of the radioimmunoassay method developed by Katz & Smith

(1972) and Yun et al (1976). The plasma level of angiotensin I was estimated by a radioimmunoassay (Waite 1973), plasma angiotensin-converting enzyme activity by colorimetric analysis (Kasahara & Ashihara 1981), and the plasma level of angiotensin II and the serum level of aldosterone by radioimmunoassay (Iinuma et al 1977; Morimoto et al 1983).

Blood pressure determination

The systolic, diastolic and mean blood pressures of each conscious rat were determined by a tail-pulse pick-up method (Pfeffer et al 1971) and recorded with an MK-100 Automatic Sphygmotonograph (Muromachi Kikai Co. Ltd, Tokyo, Japan). The blood pressure was determined throughout the experiment.

Statistics

The significance of differences between the control and magnesium lithospermate B-treated groups was tested using Student's *t*-test. Differences at a P value less than 0·05 were considered to be statistically significant.

Results

Urine volume, urinary sodium and potassium excretion

Table 1 shows the effect of magnesium lithospermate B on urine volume and urinary electrolyte excretions. Electrolytes, but not urine volume, were increased significantly on day 18 following administration of the compound. Urinary sodium excretion was significantly increased from 2·63 to 2·98 mM/24 h in the group treated with the agent. Similarly, magnesium lithospermate B significantly increased urinary potassium excretion from 1·17 to 1·42 mM/24 h (a 21% change, $P < 0·001$). However, there were no significant differences in urine volume between the control and magnesium lithospermate B-treated groups.

Urinary excretion of prostaglandins and kallikrein

Changes in urinary excretion of prostaglandins and kallikrein following the administration of magnesium lithospermate B are summarized in Table 2. Urinary excretion of PGE_2 was increased from 26·30 to 38·48 ng/24 h (a 46% change, $P < 0·01$) in rats treated for 18 consecutive days. The administration of magnesium lithospermate B significantly increased the urinary excretion of kallikrein by 24% over the control value. However, there were no significant changes in urinary excretion of 6-keto-$PGF_{1\alpha}$ and TxB_2 between the control and lithospermate B-treated groups.

Table 1. Effect of magnesium lithospermate B on urine volume, and urinary excretion of sodium and potassium in rats.

Parameters	Control	Treated
Urine volume (mL/24 h)	12·28 ± 0·69	14·50 ± 0·94
Sodium excretion (mM/24 h)	2·63 ± 0·12	2·98 ± 0·01[a]
Potassium excretion (mM/24 h)	1·17 ± 0·01	1·42 ± 0·01[a]

Statistical significance: [a] $P < 0·001$ vs control rats.

Table 2. Effect of magnesium lithospermate B on urinary excretion of prostaglandins and kallikrein in rats.

Parameters	Control	Treated
PGE_2 excretion (ng/24 h)	26·30 ± 1·31	38·48 ± 2·88[a]
6-Keto-$PGF_{1\alpha}$ excretion (ng/24 h)	2·07 ± 0·23	1·84 ± 0·24
TxB_2 excretion (ng/24 h)	17·25 ± 2·48	18·69 ± 1·61
Kallikrein excretion (U/24 h)	2·22 ± 0·01	2·76 ± 0·13[b]

PGE_2 = prostaglandin E_2; 6-Keto-$PGF_{1\alpha}$ = 6-keto-prostaglandin $F_{1\alpha}$; TxB_2 = thromboxane B_2. Statistical significance: [a] $P < 0·01$, [b] $P < 0·001$.

Table 3. Effect of magnesium lithospermate B on hormone levels in the blood of rats.

Parameters	Control	Treated
Plasma renin activity (ng mL^{-1} h^{-1})	6·49 ± 0·91	8·21 ± 0·78
Plasma angiotensin I (ng mL^{-1})	2·99 ± 0·37	3·74 ± 0·49
Plasma angiotensin-converting enzyme activity (mIU mL^{-1})	38·46 ± 1·07	38·16 ± 0·77
Plasma angiotensin II (pg mL^{-1})	14·43 ± 4·14	22·72 ± 5·21
Serum aldosterone level (pg mL^{-1})	133·83 ± 18·40	104·42 ± 12·59

Table 4. Effect of magnesium lithospermate B on blood pressure of rats.

Parameters	Control	Treated
Systolic blood pressure (mmHg)	139·44 ± 3·10	137·86 ± 2·91
Diastolic blood pressure (mmHg)	100·69 ± 2·32	94·35 ± 1·95[a]
Mean blood pressure (mmHg)	113·81 ± 2·30	109·02 ± 1·68

Statistical significance: [a] $P < 0·05$ vs control rats.

Hormone levels in the blood

Table 3 shows blood levels of hormones in rats given magnesium lithospermate B for 18 consecutive days. The plasma renin activity was slightly higher in the treated than in control rats (8·21 vs 6·49 ng mL^{-1} h^{-1}; a 27% increase). Angiotensins I and II also tended to increase in treated rats (25 and 57% higher vs control, respectively). In contrast, the aldosterone level tended to be reduced from 133·83 to 104·42 pg mL^{-1} (a 22% change) after treatment. No remarkable change was observed in angiotensin-converting enzyme activity.

Blood pressure

As shown in Table 4, diastolic blood pressure was significantly lowered by 6% compared with the control upon oral administration of magnesium lithospermate B. A similar change was also observed in mean blood pressure, but this variation was not statistically significant. The systolic blood pressure remained unchanged up to the 18th day after administration of magnesium lithospermate B.

Discussion

The administration of magnesium lithospermate B increased the excretion of both sodium and potassium. The observed natriuresis and kaliuresis were significantly higher, 13 and 21%, respectively, than the control. The natriuresis was not accompanied by potassium retention, and thus a significant increase in potassium excretion was observed. This implies that the natriuretic effect of magnesium lithospermate B is not mediated via reduced aldosterone secretion. However,

we confirmed that in spite of the lower level of aldosterone, the urinary excretion of sodium and potassium was increased following magnesium lithospermate B. One possible mechanism for these phenomena could be a fall in peritubular oncotic pressure (Brenner et al 1969; Schnermann et al 1972). This would increase delivery of sodium to the distal nephron and collecting duct, which play a role in volume regulation and solute excretion (Stein & Reineck 1974; Jamison et al 1979). On the other hand, kallikrein has been found localized in these areas and is thought to be a modulator of sodium balance (Ørstavik et al 1976; Scicli & Carretero 1986). Renal kallikrein is synthesized primarily in the distal tubule and released into the tubular lumen, interstitial cells and vascular spaces (Scicli & Carretero 1986). Virtually all the kallikrein in urine comes from the kidney and its level is considered to reflect renal kallikrein production. Therefore, urinary kallikrein is commonly used to assess the renal kallikrein-kinin system. In the present study, urinary excretion of kallikrein was significantly increased by magnesium lithospermate B. These data, taken together with the preceding results on urinary electrolyte excretion, suggest that magnesium lithospermate B may act on the kallikrein-kinin system in rats.

Several lines of evidence seem to indicate a close relationship between the renal kallikrein-kinin and prostaglandin systems. Bradykinin is a potent stimulator of prostaglandin synthesis (McGiff et al 1972). In addition, the excretions of urinary kallikrein and prostaglandin have been shown to change in parallel under certain conditions, and prostaglandin production by rabbit isolated perfused kidney seems to be dependent on endogenously produced kinin (Colina-Chourio et al 1976; Nasjletti et al 1978). In the present study, urinary PGE_2 excretion was significantly increased in parallel with the increase of kallikrein. Although the degree of the increase in PGE_2 was not consistent with that in kallikrein, this result clearly indicated that magnesium lithospermate B activates the kallikrein-prostaglandin system. It has been shown previously that PGE_2 not only increases renal blood flow by dilating renal blood vessels, but also produces relaxation of mesangial cells, suppression of immune function and inhibition of platelet aggregation (Serneri et al 1985). From the results of the present study, it is presumed that magnesium lithospermate B modulates renal haemodynamics. In addition, studies on isolated perfused tubules have indicated that PGE_2 can cause direct tubular inhibition of sodium reabsorption (Stokes & Kokko 1977). Therefore, it is possible that increased synthesis of PGE_2 in magnesium lithospermate B-treated rats may be one of the factors responsible for increased electrolyte excretory function. On the other hand, there were no changes in the excretion of 6-keto-$PGF_{1\alpha}$ or TxB_2. This may be explained by the hypothesis of Needleman et al (1979) that various pools of fatty acid cyclo-oxygenase may be tightly coupled to different pools of arachidonate, which in turn are linked to specific membrane receptors for agonist peptides.

Possible interaction of the prostaglandin-kallikrein system with the renin-angiotensin-aldosterone system is of particular concern, since the juxtaglomerular apparatus constitutes the region responsible for renin production and the macula densa cells of the distal tubules are noted for their kallikrein production. These regions are closely linked both anatomically and functionally (Carretero & Scicli 1976). The rats of the treated group showed a moderate increase in plasma renin activity. Similar changes produced by magnesium lithospermate B administration were observed in the angiotensins I and II, whereas the level of aldosterone was lower than in controls. However, no changes in angiotensin-converting enzyme activity or mean blood pressure were seen. Although our data cannot explain the ultimate mechanisms responsible for the higher renin activity and levels of angiotensins I and II in magnesium lithospermate B-treated rats,our findings are consistent with the hypothesis that the renal kallikrein-kinin system is involved not only in regulation of electrolyte excretion but also renin activation. The mechanism of conversion of prorenin to renin in-vivo is controversial, although kallikrein may be involved in the conversion (Sealey et al 1978). Suzuki et al (1980) have reported that urinary kallikrein acts directly on the rat kidney to release renin, possibly via proteolytic conversion of prorenin to active renin, and that this release is completely abolished by trasylol. Cumming & Robson (1985) and Cumming et al (1989) have also shown that, in nephrotic syndrome, renal kallikrein is able to reach the systemic circulation via the lymphatics or renal veins, and that subsequent kinin generation influences capillary permeability.

In summary, the urinary electrolyte excretion-facilitating action of magnesium lithospermate B may be due in part to enhancement of the kallikrein-kinin and prostaglandin systems.

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