Potentiating Effect of Converting Enzyme Inhibitor Captopril to the Renal Responses of Magnesium Lithospermate B in Rats with Adenine-Induced Renal Failure

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The renal responses of magnesium lithospermate B were investigated in the presence or absence of pretreatment with the converting enzyme (kinase II) inhibitor, captopril, in rats with adenine-induced renal failure. Magnesium lithospermate B (10 mg/kg body weight) caused a marked increase in the levels of the renal functional parameters (glomerular filtration rate, renal plasma flow and renal blood flow), accompanied by significant increases in urinary excretions of prostaglandin E2 (PGE2), kallikrein, sodium and creatinine. The administration of magnesium lithospermate B in combination with captopril (2 mg/kg body weight, 2 times) caused a further increase in renal functional parameters, urinary sodium and creatinine excretions. However, the kallikrein activity was similar to the control level. There were no significant changes between urinary PGE2 following magnesium lithospermate B alone, or in combination with captopril. In addition, angiotensin converting enzyme activity did not change following the administration of magnesium lithospermate B alone, but was significantly decreased in rats given captopril, both alone and in combination with magnesium lithospermate B. The captopril administration group (captopril alone or in combination with magnesium lithospermate B) showed a significant decrease in blood pressure. From these results, it seems that the combination of magnesium lithospermate B and captopril induces a further increase in renal function by improving the renal circulatory state.

Keywords renal failure; magnesium lithospermate B; renal response; captopril; rat

Various conservative therapies are available for chronic renal failure, such as a low protein-high calorie diet, essential amino acid therapy, and administration of activated charcoal or lactulose.1–3) We have been studying the actions of crude drugs in rats with experimental renal failure for several years as part of our research on drug therapy. Through such studies, we have shown that the administration of Salviae Miltiorrhizae Radix (a traditional Chinese medicinal herb known as "Dan Shen") is effective in improving renal function parameters, producing significant increases in the glomerular filtration rate, renal plasma flow and renal blood flow.4) In addition, magnesium lithospermate B was isolated and identified as a biologically active component which improved renal function.5) Studies on the mechanism of action of this compound, which is a new constituent of Salviae Miltiorrhizae Radix, are currently in progress in our laboratory.6–10) In the previous paper, pretreatment with a kallikrein inhibitor (aprotinin) blunted magnesium lithospermate B-evoked renal responses as well as urinary excretions of prostaglandin E2 (PGE2) and kallikrein, which indicated that the kallikrein–kinin system is involved in renal responses to magnesium lithospermate B through activation of the prostaglandin system.8) The present study is designed to confirm the precise mechanism by which magnesium lithospermate B improves renal function through the activation of the kinin–kallikrein and prostaglandin systems in kidney. For this purpose, the experiment was performed in the presence or absence of captopril, which has kinin-potentiating activity.

Materials and Methods

Animals and Treatment Male rats of the Wistar strain (SLC Ltd., Hamamatsu, Japan), 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, Crea Japan Inc., Tokyo, Japan). They were then fed ad libitum on an 18% casein diet containing 0.75% adenine. It was previously confirmed that renal failure was present after 6 d of adenine ingestion.11–18) On the 6th day of the experimental diet, four groups of studies were performed. In group 1, intraperitoneal administration of the vehicle saline for captopril was performed, followed 3 h later by intraperitoneal injection of saline (0.5 ml/kg body weight). In group 2, captopril was dissolved in saline immediately before use and injected at a dose of 2 mg/kg body weight into the rats, followed 3 h later by intraperitoneal injection of the vehicle for magnesium lithospermate B and captopril (2 mg/kg). In group 3, 3 h after administration of the vehicle for captopril the rats received magnesium lithospermate B (10 mg/kg body weight) dissolved in saline and the vehicle for captopril. In group 4, 3 h after captopril (2 mg/kg) administration, magnesium lithospermate B (10 mg/kg) and captopril (2 mg/kg) were given. Renal function was determined at about 6 h after intraperitoneal administration of magnesium lithospermate B and/or captopril. For determination of sodium, potassium, creatinine, PGE2, and kallikrein, urine was collected 3–6 h after treatment. Both the angiotensin converting enzyme (ACE) activity in plasma and blood pressure were determined in rats that had 6 h of administration of the agent. Six rats were used for each experimental group. Values are expressed as means ± S.E.

Chemicals Captopril was obtained from Squibb (Princeton, NJ, U.S.A.). [125I]Prostaglandin E2, RIA kit was provided by New England Nuclear (Boston, MA, U.S.A). DL-Val–Leu–Arg p-nitroanilide was obtained from Sigma Chemical Co., U.S.A.

Magnesium Lithospermate B Magnesium lithospermate B was isolated and purified from an extract of roots of Salviae Miltiorrhizae Radix (Salvia Miltiorrhiza Bunge) produced in China, as described previously.19) This compound gave a tan amorphous powder, [x]D20 +147.7° (c=0.7, MeOH). The purity was checked by measuring its optical rotation and by thin-layer

Fig. 1. Structural Formula of Magnesium Lithospermate B

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chromatography [Kieselgel 60 F 254, benzene-ethyl formate-formic acid (2:7:1), Rf 0.3] and high-performance liquid chromatography [Cosmosil SC-18, 22% CH₃CN in 20 mm H₃PO₄, 4 22.0 min]. The chemical structure is shown in Fig. 1.

**Determination of Renal Function** The glomerular filtration rate (GFR) and renal plasma flow (RPF) were measured by means of a renal clearance test using single intravenous administration of sodium thiosulfate or sodium para-aminohippurate, respectively, as an indicator. 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 urethra was then voided. During the next 30 min urine was collected, and the collection was terminated at the same time the bladder 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. Thiocysulfate and para-aminohippurate concentrations in the plasma and urine were determined by titrimetry and colorimetry, respectively. Renal blood flow (RBF) was calculated on the basis of RPF and the hematocrit value (Ht) using the equation shown below. Ht was determined with a hematocrit measurement apparatus, model KH-120A (Kubota Co., Tokyo, Japan).

\[
\text{RPF} = \frac{\text{RBF}}{1 - \text{Ht}} \quad \text{(ml/min)}
\]

**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.

**Determination of Urinary Creatinine** Creatinine was measured by high-performance liquid chromatography using a step-gradient system. Urinary PGE₂ assay PGE₂ was measured by radioimmunoassay as reported elsewhere.

**Urinary Kalikrein Assay** The activity of kalikrein was measured according to the method of Amundsen et al.

**Blood ACE Assay** The assay of ACE (peptidylpeptide hydrolase, EC 3.4.15.1) was estimated by colorimetric analysis.

**Blood Pressure Determination** The systolic, mean and diastolic blood pressures of each conscious rat were determined by a tail-pulse pick-up method and recorded with an MK-100 Automatic Sphygmontograph (Muromachi Kikai Co., Ltd., Tokyo, Japan). Blood pressure was determined repeatedly throughout the experiment.

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

**Results**

**Renal Function** The GFR in rats given adenine significantly decreased (by 48%) compared to the level in normal rats. The RPF and RBF in the adenine-treated rats exhibited no significant differences when compared with those in normal rats. Intraperitoneal administration of magnesium lithospermate B increased the GFR in rats with renal failure. As shown in Table I, administration of magnesium lithospermate B significantly increased the GFR by about 68%, from 3.07 to 5.16 ml/min/kg. The combination of magnesium lithospermate B and captopril further increased the GFR to 6.29 ml/min/kg (a 105% change, p < 0.05). Treatment with captopril alone also induced a significantly increased GFR. A significant increase in RPF and RBF was observed in response to administration of 10 mg/kg body weight of magnesium lithospermate B. Treatment with magnesium lithospermate B alone caused 42% and 34% increases in RPF and RBF as compared with the control rats, respectively (19.61 vs. 13.82 ml/min/kg and 34.78 vs. 25.97 ml/min/kg). After combined magnesium lithospermate B and captopril treatment, RPF and RBF were further augmented to 26.93 and 46.95 ml/min/kg, respectively (95% and 81% increases). Captopril alone also significantly increased RPF and RBF.

**Urine Volume, Urinary Electrolyte and Creatinine Excretions** As shown in Table II, urine volume, urinary electrolyte and creatinine excretions decreased on day 6 following adenine administration. Urine volume increased significantly, from 2.90 to 3.70 ml/3 h in the group treated with magnesium lithospermate B. Urinary sodium and creatinine excretions also exhibited a significant increase when compared with the respective control values. The combination of magnesium lithospermate B and captopril showed a further increase in urine volume, urinary sodium and creatinine excretions. In contrast, there were no significant differences in urinary potassium excretion between the control and magnesium lithospermate B-treated groups. The administration of magnesium lithospermate B and captopril combined also showed no significant increase. Further, a slight increase in urine volume, urinary sodium

**Table I. Effect of Magnesium Lithospermate B on Renal Functional Parameters with and without Captopril Pretreatment**

<table>
<thead>
<tr>
<th>Group</th>
<th>Captopril</th>
<th>GFR (ml/min/kg)</th>
<th>RPF (ml/min/kg)</th>
<th>RBF (ml/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td>-</td>
<td>5.90 ± 0.28</td>
<td>16.85 ± 1.11</td>
<td>29.51 ± 1.27</td>
</tr>
<tr>
<td>Renal failure rats</td>
<td>Control</td>
<td>3.07 ± 0.20</td>
<td>13.82 ± 1.73</td>
<td>25.97 ± 3.16</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5.22 ± 0.92</td>
<td>22.66 ± 2.64</td>
<td>44.63 ± 6.24</td>
</tr>
<tr>
<td></td>
<td>Magnesium lithospermate B</td>
<td>5.16 ± 0.40</td>
<td>19.61 ± 1.81</td>
<td>34.78 ± 3.47</td>
</tr>
<tr>
<td></td>
<td>Magnesium lithospermate B</td>
<td>6.29 ± 1.13</td>
<td>26.93 ± 3.87</td>
<td>46.95 ± 6.74</td>
</tr>
</tbody>
</table>

GFR, glomerular filtration rate; RPF, renal plasma flow; RBF, renal blood flow. Statistical significance: a) p < 0.05, b) p < 0.01 vs. normal rats, c) p < 0.05, d) p < 0.01, e) p < 0.001 vs. renal failure control rats.

**Table II. Effect of Magnesium Lithospermate B on Urine Volume, Urinary Electrolyte and Creatinine Excretions with and without Captopril Pretreatment**

<table>
<thead>
<tr>
<th>Group</th>
<th>Captopril</th>
<th>Urine volume (ml/3 h)</th>
<th>Na (µmol/3 h)</th>
<th>K (µmol/3 h)</th>
<th>Cr (µg/3 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td>-</td>
<td>3.38 ± 0.10</td>
<td>367 ± 29</td>
<td>155 ± 17</td>
<td>944 ± 23</td>
</tr>
<tr>
<td>Renal failure rats</td>
<td>Control</td>
<td>2.90 ± 0.09</td>
<td>290 ± 34</td>
<td>121 ± 13</td>
<td>756 ± 34</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.35 ± 0.30</td>
<td>335 ± 58</td>
<td>127 ± 21</td>
<td>850 ± 61</td>
</tr>
<tr>
<td></td>
<td>Magnesium lithospermate B</td>
<td>3.70 ± 0.26</td>
<td>407 ± 18</td>
<td>150 ± 13</td>
<td>1002 ± 19</td>
</tr>
<tr>
<td></td>
<td>Magnesium lithospermate B</td>
<td>3.80 ± 0.13</td>
<td>486 ± 36</td>
<td>157 ± 21</td>
<td>1069 ± 64</td>
</tr>
</tbody>
</table>

Statistical significance: a) p < 0.05, b) p < 0.01, c) p < 0.001 vs. normal rats, d) p < 0.01, e) p < 0.001 vs. renal failure control rats.
and creatinine excretions was observed in response to administration of captopril alone. No remarkable change was observed in potassium excretion.

**Urinary PGE₂ and Kallikrein Excretions** The changes in urinary excretion of PGE₂ and kallikrein following administration of magnesium lithospermate B and/or pretreatment with captopril are summarized in Table III. The urinary excretion of PGE₂ was decreased by 20% of the level in normal rats on the 6th day after adenine administration. A significant increase in PGE₂ was observed in response to administration of 10 mg/kg body weight of magnesium lithospermate B. Similar changes were obtained in rats following administration of magnesium lithospermate B and captopril. Captopril alone caused a 54% increase in PGE₂ excretion, but this variation was not statistically significant. On the other hand, the kallikrein activity in rats fed the adenine diet decreased to 8.4 mU/3h, compared to a level of 68.92 mU/3h for the normal rats. Magnesium lithospermate B induced a marked enhancement of the urinary kallikrein (a 59% change, p < 0.05). However, there were no significant changes in urinary kallikrein following magnesium lithospermate B administration in the group pretreated with captopril or administration of captopril alone.

**ACE Activity and Blood Pressure** As shown in Table IV, the ACE activity in adenine-treated rats was slightly higher when compared to the activity in normal rats. Intraperitoneal administration of captopril (captopril alone and in combination with magnesium lithospermate B) significantly decreased the ACE activity in rats with renal failure. However, magnesium lithospermate B alone did not change the ACE activity in renal failure. Furthermore, the systolic, mean and diastolic blood pressures in rats fed on the adenine diet were significantly higher than those for the normal rats; however, the captopril administration group (captopril alone and in combination with magnesium lithospermate B) induced a significant decrease in blood pressure. The levels of mean and diastolic blood pressure decreased also after treatment with magnesium lithospermate B alone, while the systolic blood pressure remained nearly unchanged.

**Discussion** Several lines of evidence seem to indicate a close relationship between the renal prostaglandin and kallikrein–kinin systems: bradykinin is a potent stimulator of prostaglandin synthesis.²⁶ Urinary prostaglandin and kallikrein excretions have been shown to change in parallel in some experimental conditions, and prostaglandin production by the isolated perfused rabbit kidney seems to be dependent on endogenously produced kinin. Urinary kallikrein decreases in many forms of hypertension and renal disease both in man and laboratory animals.²⁷–³⁰ Furthermore, inhibition of the kallikrein–kinin system by aprotinin has been found to reduce the aldosterone-induced increase in urinary PGE₂ excretion,³¹ suggesting that enhanced prostaglandin production after administration of aldosterone might be mediated by the kallikrein–kinin system. In the preceding paper, it was reported that aprotinin, an inhibitor of kallikrein which therefore suppresses kinin generation, abolishes renal hemodynamic and prostaglandin-excretory responses to magnesium lithospermate B. This suggested that magnesium lithospermate B could act at least partly through activation of the kallikrein–kinin system in the kidney. The same results were demonstrated by the present experiment. In rats with renal failure, the urinary excretion of PGE₂ (it has been shown by Frölich et al.³²) that PGE₂ in urine is mostly derived from the kidney), GFR, RPF and RBF increased significantly after magnesium lithospermate B administration, in proportion to the increase in kallikrein activity. In the present study, the extent to which endogenous kinin contributes to the manifestation of the action of magnesium lithospermate B was also investigated using captopril.
In rats with renal failure, captopril administration prior to magnesium lithospermate B caused a further increase in GFR, RPP and RBF in comparison to the increased levels of those administered with magnesium lithospermate B alone. On the other hand, the kininreleasing activity was similar to the control level in the group given both captopril and magnesium lithospermate B. In this regard, Vinci et al.\textsuperscript{33} reported that kininreleasing activity was decreased, while urinary kinin excretion was markedly increased by captopril in both sodium-replete and sodium-deplete patients; they discussed the possible differences in the effects of captopril on magnesium lithospermate B and magnesium lithospermate B. In this regard, Vinci et al.\textsuperscript{33} reported that urinary kinin excretion was markedly increased by captopril in both sodium-replete and sodium-deplete patients; they discussed the possible differences in the effects of captopril on magnesium lithospermate B and magnesium lithospermate B. In this regard, Vinci et al.\textsuperscript{33} reported that urinary kinin excretion was markedly increased by captopril in both sodium-replete and sodium-deplete patients; they discussed the possible differences in the effects of captopril on magnesium lithospermate B and magnesium lithospermate B.

According to the report of Marks et al.,\textsuperscript{35} the converting enzyme inhibitor, captopril, inhibits the physiological activity of the renin–angiotensin system by inhibiting the production of angiotensin II as well as by decreasing the production and release of aldosterone. Meanwhile, captopril also acts on the kinin-degrading enzyme, kininase II, enhancing the action of endogenous kinin. Since ACE activity decreased significantly after captopril administration in this study, it is clear that the decomposition of kinin into inactive peptides is suppressed. In the group given both captopril and magnesium lithospermate B, blood pressure and ACE activity significantly decreased, suggesting a decrease in angiotensin II. Inhibition of angiotensin II formation is probably the major mechanism by which captopril reduces blood pressure. However, other mechanisms may be involved. Bradykinin, like angiotensin II, also stimulates membrane phospholipase A\textsubscript{2} in various tissues, and thereby stimulates the production of degradative compounds such as PGE\textsubscript{2}.\textsuperscript{36,37} As mentioned above, the significant increase of renal functional parameters is associated with a significant increase of PGE\textsubscript{2} in rats pretreated with captopril compared to those given magnesium lithospermate B alone, and it is suggested that PGE\textsubscript{2} may be involved in the regulation of renal function by captopril. There is also interaction between the renal prostaglandins and renin–angiotensin system. McGiff et al.\textsuperscript{38} reported that the intrarenal arterial infusion of angiotensin II was stimulated the release of prostaglandins from the kidney, counteracting the vasoconstricting effects of angiotensin II. On the other hand, several studies have demonstrated that the effect of kinin on renal function is associated with prostaglandin release. Bradykinin-induced natriuresis is also associated with an increase in renal PGE\textsubscript{2} synthesis.\textsuperscript{36,39} Additionally, Kauker has reported that kinin exerts a direct action on urinary tubules to accelerate the excretion of sodium.\textsuperscript{40} Therefore, a significant increase in renal function and urinary PGE\textsubscript{2} excretion, which occurs in response to captopril, is thus unlikely to result from the effect of captopril on the renin–angiotensin–aldosterone system, but might be related to a change in kinin metabolism following captopril pretreatment. In the present experiment, using a combination of captopril and magnesium lithospermate B, urinary excretion of sodium also increased, suggesting that endogenous kinin facilitates the excretion of sodium from the kidney. These effects were also noted after administration of captopril alone, indicating its involvement in the maintenance of renal function and hemodynamics and blood pressure regulation. However, the combination of magnesium lithospermate B and captopril was found to have an additive effect on improving the condition of renal failure.

The exact location in the kidney where the action of magnesium lithospermate B takes place remains to be elucidated in the future. Scisci et al.\textsuperscript{41} found in an experiment using the stop flow method that kinin was produced in the distal uriniferous tubules. Using the micropuncture method, Farman et al.\textsuperscript{42} demonstrated that most PGE\textsubscript{2} in urine was produced in the distal uriniferous tubules. Taking their data into account, the distal uriniferous tubules are most likely to be the site of the action of magnesium lithospermate B. Further studies with specific glomerular kinin inhibitors and kinin receptor blockers will help to clarify the involvement of this system in the renal effect of magnesium lithospermate B.

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References


