Altered Prostaglandin Metabolism Induced by Angiotensin-Converting Enzyme Inhibitors in Broncho-Alveolar Lavage Fluid of the Guinea Pig

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ABSTRACT—The aim of this study was to investigate if prostaglandin (PG) metabolism is altered by angiotensin-converting enzyme (ACE) inhibitors as determined in the broncho-alveolar lavage fluid (BALF) of the guinea pig. Enalapril or imidapril was orally administered once a day for 2 weeks to Hartley male guinea pigs. Twenty-four hours after the last treatment, BALF was collected and the concentrations of PGI2, thromboxane A2 (TXA2) and PGE2 were measured by enzyme immunoassay. Enalapril significantly (P < 0.05) increased the TXA2 content, which was inhibited by indomethacin treatment and significantly (P < 0.05) decreased the PGI2 content. Imidapril, however, did not affect TXA2 or PGI2 generation. These findings suggest that altered PG metabolism may be associated with coughing as a side effect of enalapril.

Keywords: Imidapril, Angiotensin-converting enzyme inhibitor, Broncho-alveolar lavage fluid, Thromboxane A2, Prostacyclin

Angiotensin-converting enzyme (ACE) inhibitors are effective antihypertensive agents with other beneficial effects such as prevention of heart failure and ventricular hypertrophy (1–4). However, ACE inhibitors have been reported to cause coughing as one of their side effects with incidences of 1–33% (5–10). The mechanism of coughing induced by the ACE inhibitors remains unclear, although it has been speculated that cough may be caused by accumulation of bradykinin (9, 10) which stimulates the release of tachykinins including substance P and neurokinin A (11). Tachykinins stimulate the c-fibers whose activation causes coughing (11). Moreover bradykinin can induce the release of prostaglandins (PGs) including thromboxane A2 (TXA2), PGF2α, and PGE2, which can cause bronchoconstriction; PGE2 stimulates the c-fibers (12, 13). Sulindac and indomethacin, which inhibit cyclooxygenase, and picotamide, which has blocking action of thromboxane and inhibits thromboxane synthesis, reduced coughing induced by the ACE inhibitors in clinical trials (14–16). These reports suggest that some PGs may mediate coughing induced by the ACE inhibitors.

imidapril, (4S)-1-methyl-3-{(2S)-2[1-(1S)-1-ethoxycarbonyl-3-phenylpropyl)amino]propionyl}-2-oxoimidazolidine-4-carboxylic acid hydrochloride, belongs to a new class of N-carboxyalkyl dipeptide ACE inhibitors (17, 18). The antihypertensive effect of imidapril in the spontaneously hypertensive rat was comparable to that of enalapril, and about 4 times that of captopril. Furthermore, imidapril has a long-lasting antihypertensive effect as compared with enalapril and captopril (18, 19). In clinical studies in Japan (19) and Europe, imidapril caused coughing only in about 0.9% of patients. In contrast, cough was caused in 15–25% of patients by captopril and 10–33% by enalapril (5–10).

In the present study, using the guinea pig broncho-alveolar lavage fluid (BALF), we decided to investigate if prostaglandin metabolism is altered by enalapril. Imidapril with a minimum coughing effect was included as a reference drug.

MATERIALS AND METHODS

Sixty-six Hartley male guinea pigs (250–300 g; SLC, Hamamatsu) were used. Imidapril (15 and 60 mg/kg) or enalapril (15 and 60 mg/kg; Sigma, St. Louis, MO, USA) were dissolved in 0.5% carboxymethylcellulose (CMC), was orally administered once a day for 2 weeks. In a control group, 0.5% CMC was administered in the same manner. In a separate experiment, enalapril (60 mg/kg) was orally administered once a day for 2 weeks and simultaneously for the last 3 days, indomethacin (60
mg/kg, Sigma), which was also dissolved in 0.5% CMC, was injected intraperitonealy once a day. Six animals were injected with indomethacin alone.

Twenty-four hours after the last treatment, under sodium pentobarbital anaesthesia, a tracheotomy was performed, and a catheter was inserted into the trachea for sampling BALF. Lungs were lavaged 5 times with 5 ml of saline at 37°C through the catheter, and about 3-4 ml of BALF was collected. BALF with 30 μM added indomethacin was centrifuged at 3000 rpm for 10 min at 4°C. The supernatant was removed and stored at -80°C for determination of PG contents. 6-Keto-PGF₁α, a stable metabolite of PGI₂; TXB₂, a stable metabolite of TXA₂; and PGE₂ contents were determined by enzyme immunoassays (Biotrak, 6-keto-PGF₁α, TXB₂, PGE₂ EIA System; Amersham, Buckinghamshire, UK).

In a separate experiment, under sodium pentobarbital anaesthesia, a catheter was inserted into the carotid artery and the blood pressure (BP) and heart rate (HR) were monitored in animals treated with enalapril and imidapril for 2 weeks. In addition, to demonstrate ACE inhibition by enalapril or imidapril, a dose-response curve for angiotensin I was constructed. Angiotensin I was injected intravenously through the cannulated jugular vein, increasing the dose in a stepwise manner. BP and HR were allowed to return to the baseline levels between each increment of angiotensin I.

Data are presented as values of the mean±S.E. Statistical analyses was made with one-way ANOVA for comparisons among groups. When a significant value was reached, Dunnett’s multiple comparison was performed. A P value <0.05 was considered significant.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>MBP (mmHg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% CMC</td>
<td>85.5±4.5</td>
<td>57.0±4.6</td>
<td>72.6±4.6</td>
<td>332±6</td>
<td></td>
</tr>
<tr>
<td>Enalapril 15</td>
<td>76.5±6.0</td>
<td>47.0±4.1</td>
<td>62.8±4.8</td>
<td>325±12</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>75.0±2.4</td>
<td>49.0±2.2</td>
<td>64.0±2.2</td>
<td>317±7</td>
<td></td>
</tr>
<tr>
<td>Imidapril 15</td>
<td>87.0±4.1</td>
<td>59.0±2.6</td>
<td>74.0±3.4</td>
<td>333±9</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>64.5±4.0*</td>
<td>40.0±3.3*</td>
<td>53.0±3.1*</td>
<td>312±3</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as values of the mean±S.E. (n=5). SBP: systolic blood pressure, DBP: diastolic blood pressure, MBP: mean blood pressure, HR: heart rate. *P<0.05 vs values from animals treated with 5% CMC.

![Fig. 1. Changes in mean arterial blood pressure (Δ-MAP) from baseline in response to angiotensin I. It can be seen that the responses to angiotensin I in animals treated with enalapril and imidapril were weaker as compared with those in untreated animals. Open circles represent animals treated with 0.5% CMC; closed squares and closed triangles are animals treated with enalapril at doses of 15 and 60 mg/kg, respectively; open squares and open triangles represent animals treated with imidapril at doses of 15 and 60 mg/kg, respectively. Data are presented as values of the mean±S.E. (n=5).](image-url)
Fig. 2. TXB₂ content in BALF. Data are presented as values of the mean±S.E. Numbers indicate group size. IDM: indomethacin was injected intraperitoneally. *P<0.05 vs control group.

Fig. 3. 6-Keto-PGF₁α content in BALF. Data are presented as values of the mean±S.E. Numbers indicate group size. IDM: indomethacin was injected intraperitoneally. *P<0.05 vs control group.
RESULTS

Blood pressure and heart rate

Mean arterial blood pressure (MAP) in animals treated with imidapril at a dose of 60 mg/kg was significantly (P < 0.05) reduced (by 20 mmHg); MAP in animals treated with enalapril (60 mg/kg) was reduced by 10 mmHg. There was no significant difference in HR between the animals treated with enalapril, imidapril and the control group (Table 1). Systolic blood pressure in animals treated with a combination of enalapril and indomethacin was significantly (P < 0.05) reduced as compared with animals treated with CMC, which was not significantly different from the reduction induced by enalapril alone. When enalapril and imidapril were investigated directly against the pressor effect of angiotensin I, the BP dose-response curve for angiotensin I was shifted well to the right, with imidapril being about 4 times more potent than enalapril (Fig. 1).

PGI2, TXA2, PGE2 contents in BALF

Enalapril, at a dose of 60 mg/kg per day, significantly (P < 0.05) increased TXB2 content, which was reduced by indomethacin treatment, but imidapril did not significantly increase TXB2 (Fig. 2). Enalapril significantly (P < 0.05) reduced 6-keto-PGF1α content in BALF in a dose-dependent manner as compared with the value obtained from the control group. PGI2 generation was not affected by imidapril treatment (Fig. 3). PGE2 contents were not affected by either enalapril or imidapril treatment.

DISCUSSION

In this study using BALF, we have investigated the effects of enalapril and a new ACE inhibitor, imidapril, on PGs generation in the guinea pig. Enalapril significantly decreased PGI2 generation but increased TXA2 generation, which was inhibited by indomethacin, thus being cyclo-oxygenase dependent. Such effects were not observed with the new ACE inhibitor imidapril. MAP was significantly reduced by imidapril, but less significantly by enalapril. However, HR was not affected by these two drugs. When angiotensin I was injected intravenously and MAP was monitored, the ACE inhibition by imidapril was 4 times greater than that of enalapril.

Doses of enalapril and imidapril used in this study were higher than those used in rats. In this study, drug doses were decided based on changes in BP after oral administration and inhibitory action on BP increased by i.v. angiotensin I. Our results suggest that the doses of enalapril and imidapril to inhibit ACE action in guinea pigs are higher than those used in rats and thus, the doses used in this study may be suitable for evaluating the effects of the ACE inhibitors in BALF.

In clinical trials, sulindac and indomethacin which inhibit cyclo-oxygenase, and picotamide, which has a blocking action on thromboxane and inhibits thromboxane synthesis, is reported to reduce coughing induced by ACE inhibitors (14-16). In line with these observations, in this study, indomethacin reduced TXA2 generation, which was increased by enalapril. It has been reported that inhalation of a TXA2 agonist did not directly induced cough in the guinea pig, and capsaicin-induced cough was enhanced by ACE inhibitors treatment, which was inhibited by a TXA2 synthetase inhibitor (20). Furthermore, TXA2 is known to cause bronchial contraction and hyper-responsiveness to platelet-activating factor (21), allergen (22) and ozone (23) and has a chemokinetic activity (24). These observations suggest that TXA2 or perhaps another arachidonic acid metabolite may somehow mediate coughing induced by the ACE inhibitors. This notion is supported by the fact that imidapril, which has not been associated with coughing, did not increase TXA2 generation in BALF. Since PGE2 is known (12, 13) to stimulate c-fibers, which controls coughing, we determined its concentration in BALF, but found that it was not increased by any of the two ACE inhibitors.

In summary, in the model used in this study, enalapril reduced PGI2 generation but increased TXA2 generation; none of these effects were induced by the new ACE inhibitor imidapril. Furthermore, when tested as ACE inhibitors on BP, imidapril was strikingly more potent than enalapril. In clinical reports, coughing is associated with the therapeutic use of enalapril but as yet not with imidapril. However, to attribute coughing to the stimulation of TXA2 generation (or inhibition of PGI2 generation) requires further studies.

Acknowledgments

Imidapril, (4S)-1-methyl-3-{(2S)-2[N-(1S)-1-ethoxycarbonyl-3-phenylpropyl]amino}propionyl}-2-oxoimidazolidine-4-carboxylic acid hydrochloride, was a gift from Tanabe Seiyaku Co., Ltd., Japan. The authors thank Dr. A. Saniabadi for editing the manuscript and Mr. Y. Toshima, Mr. K. Kosuge and Dr. M. Ishiye for technical assistant.

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