Comparison of Acute Hemodynamic Effects of MC-838, a New Angiotensin-Converting Enzyme Inhibitor, with Captopril in Anesthetized Dogs

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Abstract—Effects of a new angiotensin-converting enzyme inhibitor, N-[3-(N-cyclohexanecarbonyl-D-alanylthio)-2-methylpropanoyl]-L-proline calcium (MC-838), on the systemic and coronary circulation were evaluated in anesthetized dogs, and the effects were compared with those of captopril. Administration of MC-838 (0.1, 0.3, 1.0 and 3.0 mg/kg, i.v.) produced a gradual and dose-dependent decline in aortic pressure associated with no marked changes in coronary blood flow, heart rate and LVdP/dt. Captopril (0.01, 0.03, 0.1 and 0.3 mg/kg, i.v.) also caused a dose-related decrease in aortic pressure, but the significant hypotension appeared more rapidly than that of MC-838. Both MC-838 and captopril inhibited selectively the pressor response to angiotensin I in a dose-related manner. The doses of MC-838 and captopril to lower mean aortic pressure by 10 mmHg from the pre-drug value were 2.8 mg/kg and 0.03 mg/kg, respectively; those of these drugs to cause 50% inhibition of angiotensin I-pressor response were 1.0 mg/kg and 0.04 mg/kg, respectively. When administration of MC-838 (3.0 mg/kg) was repeated three times at a 30 min-interval, the second and third injections caused no additional hypotension, while each of the repeated injections of captopril (0.3 mg/kg) produced significant hypotension. These results indicate that MC-838 inhibits angiotensin I-conversion and decreases systemic blood pressure more slowly and persistently than captopril in anesthetized dogs.

It is well-known that several angiotensin-converting enzyme inhibitors are effective in the treatment of patients with hypertension and/or congestive heart failure (1). However, the potency and time course of the action of the compounds have been shown not to be necessarily identical in experimental (2, 3) and clinical situations (4).

MC-838, N-[3-(N-cyclohexanecarbonyl-D-alanylthio)-2-methylpropanoyl]-L-proline calcium (Fig. 1), is a new angiotensin-converting enzyme inhibitor which has recently been synthesized in Japan. It has been demonstrated that oral administration of the inhibitor produces antihypertensive actions in experimentally and spontaneously hypertensive rats in preliminary studies, and it causes significant hypotension in normal volunteers (5). However, effects of MC-838 on the systemic and coronary circulation have not fully investigated. Thus, this study was designed to evaluate acute hemodynamic effects of MC-838 and to compare them with those of captopril as a standard drug.

![Chemical structure of MC-838](image-url)
For this purpose, effects of intravenous administration of MC-838 and captopril on coronary and systemic hemodynamics were estimated in the anesthetized dog.

**Materials and Methods**

Mongrel dogs of either sex weighing 11 to 25 kg were anesthetized with pentobarbital Na (25 mg/kg, i.v.) and were ventilated by an artificial respirator (Harvard, Model 607). A catheter filled with heparinized physiological saline (0.9% NaCl) was inserted through the left carotid artery into the aortic root and connected to a pressure transducer (Statham, P231D) to measure the aortic pressure. A catheter-tip manometer (Millar, PC-350) was introduced through the right femoral artery into the left ventricle to measure the left ventricular pressure. Left thoracotomy was performed through the fifth intercostal space. The pericardium was opened, and the heart was suspended in a pericardial cradle. The proximal portion of the left circumflex coronary artery (LCX) was carefully dissected free from the surrounding tissues. An electromagnetic flow probe (Statham, SP7515) was placed around the LCX, and it was connected to an electromagnetic flowmeter (Statham, SP2204) for the measurement of coronary blood flow. The zero reference was checked by temporary occlusions of the LCX just distal to the probe with a snare occluder.

Mean coronary blood flow was obtained by an electronic-capacitance filter with a 2-second time constant. Left ventricular dP/dt (LVdP/dt) was derived from differentiating the signal of left ventricular pressure using an electronic differentiator (Nihon Kohden, ED-601G). Heart rate was counted continuously with a cardiotachometer (Nihon Kohden, AT-600G) triggered by the left ventricular pressure pulse. Data were monitored continuously on an 8-channel pen recorder (Nihon Kohden, WT-685G).

**Experimental protocol:** Experiments were started at least 30 min after instrumentation. After control measurement, MC-838 (0.1, 0.3, 1.0 or 3.0 mg/kg, n=3 to 5) or captopril (0.01, 0.03, 0.1 or 0.3 mg/kg, n=4 to 7) was administered intravenously. To evaluate the extent of inhibition of angiotensin-converting enzyme, 50 ng/kg, i.v., of angiotensin I (Peptide Institute Protein Research Foundation), 20 ng/kg, i.v., of angiotensin II (Peptide Institute Protein Research Foundation) and 200 ng/kg, i.v., of dl-norepinephrine HCl (Sankyo Co., Ltd.) were injected before and 10 min after the administration of MC-838 or captopril. The percent inhibition of pressor response to angiotensin I was calculated as the difference between angiotensin I-induced increases in aortic pressure before and after treatment of the inhibitor divided by the control value. Additionally, a maximum dose of MC-838 (3 mg/kg, i.v., n=5) or captopril (0.3 mg/kg, i.v., n=7) was administered repeatedly at a 30 min-interval in order to obtain the hemodynamic changes when angiotensin-converting enzyme would be maximally inhibited.

All drugs were dissolved in 0.9% NaCl and injected in a bolus manner into the left jugular vein via a polyethylene catheter.

Coronary vascular resistance was calculated as the quotient of mean aortic pressure and mean coronary blood flow. Time sequence data were analyzed by two-way analysis of variance, and paired data were analyzed with the paired t-test. The level for statistical significance was P<0.05. All results were expressed as means±standard error.

**Results**

1. **Effects of intravenous administration of MC-838 and captopril on hemodynamic parameters:** As shown in Fig. 2, administration of MC-838 3 mg/kg, i.v., produced a gradual decline in aortic pressure without marked changes in coronary blood flow, heart rate and LVdP/dt. A pressor response to angiotensin I (50 ng/kg, i.v.) but not angiotensin II (20 ng/kg, i.v.) was apparently attenuated by MC-838 (Fig. 2). Significant hypotension was noted from 3 min after the administration of MC-838 (Table 1) by 9% of the control value. In the case of MC-838, administration of captopril decreased aortic pressure by 9% of the control value at 10 min. As in the case of MC-838, administration of captopril decreased aortic pressure by 9% of the control value at 5 min, which was not accompanied by significant changes in coronary blood flow, heart rate and LVdP/dt except coronary vascular resistance (Table 1). Significant
Fig. 2. Effect of MC-838 (3.0 mg/kg, i.v.) on hemodynamic parameters and responses to angiotensin I (Al, 50 ng/kg, i.v.) and angiotensin II (All, 20 ng/kg, i.v.). Note that administration of MC-838 produces a gradual decline in aortic pressure (AoP) without affecting coronary blood flow (CBF), left ventricular dP/dt (LVdP/dt) and heart rate (HR), and it almost abolishes the pressor response to angiotensin I despite no change in angiotensin II-induced response. MCBF=mean coronary blood flow.

Table 1. Effects of MC-838 and captopril on hemodynamic variables

<table>
<thead>
<tr>
<th></th>
<th>MAoP mmHg</th>
<th>MCBF ml/min</th>
<th>CVR mmHg/ml/min</th>
<th>HR beats/min</th>
<th>LVdP/dt mmHg/sec</th>
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</thead>
<tbody>
<tr>
<td><strong>MC-838, 3.0 mg/kg, i.v., n=5</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Control</td>
<td>113±5</td>
<td>28.0±4.7</td>
<td>4.44±0.64</td>
<td>137±7</td>
<td>2375±212</td>
</tr>
<tr>
<td>1 min</td>
<td>111±5</td>
<td>28.2±4.7</td>
<td>4.32±0.63</td>
<td>137±6</td>
<td>2417±221</td>
</tr>
<tr>
<td>3 min</td>
<td>106±5**</td>
<td>27.8±4.7</td>
<td>4.23±0.62</td>
<td>137±6</td>
<td>2354±227</td>
</tr>
<tr>
<td>5 min</td>
<td>105±5**</td>
<td>27.6±4.9</td>
<td>4.25±0.67</td>
<td>138±7</td>
<td>2349±225</td>
</tr>
<tr>
<td>10 min</td>
<td>103±5**</td>
<td>27.0±4.9*</td>
<td>4.27±0.70</td>
<td>137±7</td>
<td>2339±214</td>
</tr>
<tr>
<td><strong>Captopril, 0.1 mg/kg, i.v., n=5</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>124±8</td>
<td>31.4±1.8</td>
<td>4.05±0.46</td>
<td>149±8</td>
<td>2698±143</td>
</tr>
<tr>
<td>1 min</td>
<td>118±8*</td>
<td>32.2±1.4</td>
<td>3.72±0.35</td>
<td>152±9</td>
<td>2780±133</td>
</tr>
<tr>
<td>3 min</td>
<td>115±7**</td>
<td>32.0±1.3</td>
<td>3.63±0.32**</td>
<td>154±10</td>
<td>2795±153</td>
</tr>
<tr>
<td>5 min</td>
<td>113±7**</td>
<td>31.4±1.0</td>
<td>3.65±0.33*</td>
<td>154±10</td>
<td>2774±180</td>
</tr>
<tr>
<td>10 min</td>
<td>114±9**</td>
<td>31.0±0.6</td>
<td>3.70±0.30*</td>
<td>154±11</td>
<td>2772±219</td>
</tr>
</tbody>
</table>

MAoP=mean aortic pressure, MCBF=mean coronary blood flow, CVR=coronary vascular resistance, HR=heart rate, LVdP/dt=left ventricular dP/dt. *P<0.05, **P<0.01, when compared with the respective control.
hypotensive effect of captopril, however, was more rapidly revealed than that of MC-838 (Table 1).

Effects of MC-838 and captopril on responses of aortic pressure to angiotensin I, angiotensin II and norepinephrine are shown in Fig. 3. The pressor response to angiotensin I reduced after administration of MC-838 or captopril in a dose-related manner, while those to angiotensin II tended to increase and those to norepinephrine did not alter significantly when compared with the respective control value obtained before MC-838 or captopril (Fig. 3).

The extent of hypotension induced by both inhibitors was dose-dependently increased, and it was closely related to that of inhibition of angiotensin I-induced pressor response, as shown in Fig. 4. When calculated by using the method of least squares in linear regression, the doses of MC-838 and captopril to cause a decrease in mean aortic pressure by 10 mmHg from the pre-drug value were 2.8 mg/kg and 0.03 mg/kg, respectively; those of these drugs to cause 50% inhibition of angiotensin I-induced pressor response were 1.0 mg/kg and 0.04 mg/kg, respectively. The decreases in aortic pressure and percent inhibition of angiotensin I-pressor response produced by either inhibitor were significantly correlated (r=0.845, P<0.001, n=14; r=0.515, P<0.02, n=21, respectively).

2. Effects of repeated administration of MC-838 or captopril: Time course of mean aortic pressure after repeated administration of MC-838 (3.0 mg/kg, i.v.) and captopril (0.3 mg/kg, i.v.) is shown in Fig. 5. The first injection of MC-838 decreased significantly aortic pressure to 99±5 mmHg from the control of 113±5 mmHg. The second and third injections of MC-838, however, caused no additional decrease in aortic pressure (Fig. 5). Percent inhibitions of angiotensin I-pressor response after the first, second and third injections of MC-838 were 68±3%, 69±1% and 76±8%, respectively. On the other hand, significant decreases in aortic pressure were seen even after the second and third injections of captopril to a level similar to that after the first administration (Fig. 5), indicating that a partial recovery from the maximum hypotensive effect of captopril was.
certainly occurred as early as 30 min after the injection. In addition, the maximum decrease in aortic pressure was seen within 10 min after captopril, while it was seen more than 10 min after MC-838. Percent inhibitions of the pressor response induced by angiotensin I after the first, second and third injections of captopril were 82±3%, 83±4% and 79±4%, respectively. These results clearly indicate that the hypotensive effect of MC-838 outlasts that of captopril which occurs more rapidly than MC-838.

Discussion
The present experiments showed that intravenous administration of MC-838 produces a gradual decline in systemic blood pressure in a dose-related manner in anes-
thetized dogs. Additionally, we confirmed that captopril administration exerts the hypotensive effect in this experimental model as already has been demonstrated by several investigators (6–8). Both of these inhibitors did not change coronary blood flow. A decrease in aortic pressure induced by MC-838 was not associated with a significant change in myocardial contractility as measured by LVdP/dt, suggesting that MC-838 has no cardiac effect. Recently, captopril has been shown to lack a direct effect on cardiac performance in dogs (8–10). Thus, it seems likely that the hypotensive effect of MC-838 would be ascribable mainly to vasodilation of systemic resistance vessels, similar to what occurs in the case of captopril.

MC-838 inhibited the pressor effects of angiotensin I in a dose-related manner, but not those of norepinephrine. In contrast to the case of angiotensin I, the pressor response to exogenous angiotensin II was significantly enhanced by the highest dose of MC-838. This observation can possibly be accounted for by a reduction in the level of circulating endogenous angiotensin II which is considered to cause an increase in the number of available angiotensin II receptors to result in enhancement of the responsiveness to exogenous angiotensin II (11). Effects of MC-838 on the responses to angiotensin I, angiotensin II and norepinephrine were qualitatively similar to those of captopril, which are in accord with previous studies (6, 12). These findings indicate that MC-838, like captopril, may selectively inhibit conversion of angiotensin I to angiotensin II in the systemic vasculature and thereby leads to vasodilation through reducing the circulating level of endogenous angiotensin II. Results that significant correlation between the hypotension and the inhibition of angiotensin I-response was seen after MC-838 administration as shown in Fig. 4 may support this view.

Although it was not possible to determine the duration of the hypotensive action of MC-838 in this study, it seems reasonable that the effect of MC-838 on blood pressure appears longer in duration than that of captopril since an evident recovery from the maximum hypotension was seen 30 min after the administration of a large dose of captopril but not MC-838, as noted in Fig. 5. Also, Table 1 and Fig. 5 show that the effect of MC-838 is slower in onset than captopril.

If the blood pressure lowering effect of these inhibitors might only depend on blockade of the same angiotensin-converting enzyme, a given degree of inhibition of angiotensin I-pressor response could be expected to produce a definite decrease in blood pressure. However, the hypotensive action and inhibitory effect of angiotensin I-conversion of MC-838 were approximately 90 times and 25 times, respectively, less potent than those of captopril, implying that the degree of captopril-induced hypotension would be greater than MC-838 when compared at the same degree of inhibition of angiotensin conversion. These findings suggest that the extent of reduction in blood pressure induced by an angiotensin-converting enzyme inhibitor can not necessarily be determined by the potency of the inhibitory effect on the pressor response to exogenously given angiotensin I. Recently, it has been reported that there are temporal dissociations between the decline in blood pressure and angiotensin I-pressor response after captopril or other converting enzyme inhibitors administration (13–15). Cohen and Kurz (2) have suggested that the converting enzyme located in certain critical tissues such as the kidney, aorta and lung may have a more important role in the antihypertensive action of captopril and MK-421 in spontaneously hypertensive rats than the serum enzyme which would be largely responsible for hydrolysis of the exogenous angiotensin I. In this view, differences in hemodynamic effects between MC-838 and captopril may be partly explained by the possible difference in inhibitory efficacy on the converting enzymes existing in various sites.

The inhibitor of angiotensin-converting enzyme is considered to cause augmentation of the kinin level through kininase II inhibition (16) since this enzyme and kininase II are the same enzyme (17). In addition, it has been demonstrated that captopril facilitates synthesis of prostacyclin (18, 19) and suppresses sympathetic nerve trans-
mission (20, 21). However, it is not known whether MC-838 has such effects. The role of the above effects other than inhibition of the renin-angiotensin system in the acute hemodynamic changes after captopril or MC-838 administration remains to be determined. Additionally, it may be necessary to consider the contribution of metabolite(s) of MC-838 in its hypotensive action, because MC-838 contains a captopril-moiety in its chemical structure.

In summary, the present experiments demonstrated that intravenous administration of MC-838 produces inhibition of angiotensin I-conversion and decreases systemic blood pressure which appears more gradually and persistently when compared with captopril. However, extrapolations from the conclusions to clinical situations should be limited since findings in this study were obtained in the normotensively anesthetized dog.

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References


