Effect of Amezinium Metilsulfate on the Finger Skin Vasoconstrictor Response to Cold Stimulation and Venoconstrictor Response to Noradrenaline

Kazuhiro Harada, MD; Masami Ohmori, MD; Akio Fujimura, MD; Kyoichi Ohashi, MD*

Orthostatic hypotension can be caused by an inadequate vasoconstrictor response. The effects of amezinium on vasoconstrictor response to sympathetic stimulation and to exogenous noradrenaline were investigated and compared with those of midodrine. In 8 healthy men, the following experiments were performed after a single oral dose of 10 mg of amezinium, 2 mg of midodrine or a placebo. First, finger-tip blood flow (FTBF) was recorded using a laser Doppler flowmeter before and during the contralateral hand cooling and a reduction ratio of FTBF was calculated as an index of the vasoconstrictor response. Second, dose-response curves to increasing doses (1–512 ng/min) of noradrenaline infused locally to the dorsal hand vein were determined using a linear variable differential transformer. The reduction ratio of FTBF was significantly increased (p<0.05) by amezinium [placebo, 75.9±9.8(mean±SD%); amezinium, 85.1±7.9%; midodrine, 78.1±9.3%]. The infusion rate of noradrenaline producing a half-maximum venoconstriction was significantly decreased (p<0.05) by amezinium (placebo, 40.6±33.9 ng/min; amezinium, 21.0±21.3 ng/min; midodrine, 33.2±31.5 ng/min). These findings indicate that amezinium increases the vasoconstrictor response to sympathetic stimulation and to noradrenaline in normal subjects, and this mechanism might contribute to the improvement by amezinium of the symptoms of orthostatic hypotension. (Jpn Circ J 1998; 62: 824–828)

Key Words: Amezinium; Dorsal hand vein method; Laser Doppler, Midodrine; Vasoconstrictor response
Effect of Amezinium on Vasoconstrictor Response


Table 1 Blood Pressure and Pulse Rate in the Supine Position and 1 min After Standing Up After the Oral Intake of a Placebo, Amezinium and Midodrine (Mean ± SD, n=8)

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Amezinium</th>
<th>Midodrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>113±10</td>
<td>117±11</td>
<td>115±11</td>
</tr>
<tr>
<td>Standing</td>
<td>122±13</td>
<td>128±15</td>
<td>127±15</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>67±6</td>
<td>69±9</td>
<td>68±8</td>
</tr>
<tr>
<td>Standing</td>
<td>71±8</td>
<td>74±12</td>
<td>72±10</td>
</tr>
<tr>
<td>PR (beats/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>62±8</td>
<td>61±7</td>
<td>62±7</td>
</tr>
<tr>
<td>Standing</td>
<td>79±16</td>
<td>74±12</td>
<td>77±13</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; PR, pulse rate.

Finger-Tip Blood Flow (FTBF) Measurement

The subjects were comfortably seated with their right arm resting on a table at the heart level. Cutaneous FTBF was recorded from the pad of the right middle finger with a laser Doppler flowmeter (Peri Flux PF3, Perimed, Sweden) with an integrating probe with 7 efferent fibers. Laser Doppler flow was expressed in arbitrary units. The recording of FTBF was started after the subjects rested for more than 10 min, and when the value of FTBF stabilized, cold stimulation was performed by immersing the left hand in ice water (1–3°C) for 15 sec. FTBF rapidly decreased with a latent time of a few seconds and gradually returned to the level before the stimulation.

Dorsal Hand Vein Method

The LVDT used (Schaevits type 025MHR, Pennsauken, NJ, USA) has a freely movable core that rests over the center of the vein to be studied. There is a linear relationship between the vertical movement of the core and the change in voltage output. During the study, the subject was in the supine position with an arm placed on a support sloping upward at an angle of about 30 degrees from the horizontal. In this position, emptying of the superficial hand vein occurred. A 25-gauge needle was inserted into a dorsal hand vein and a continuous infusion of physiological saline was started. The core was located over the center of the vein about 10 mm downstream from the tip of needle. The voltage output was recorded on a strip chart recorder (Linearorder Mark VII; Graphtec, Yokohama, Japan). Ten minutes after the insertion of the needle, increasing doses (from 1 to 512 ng/min) of noradrenaline (Nor-Adrenalin®, Linearcorder Mark VII; Graphtec, Yokohama, Japan). Ten

Study Protocol

Subjects were instructed to refrain from caffeine-containing and alcoholic beverages from 12 h before the start of the study. No breakfast was taken on the study days. The study was carried out as a single, blind, placebo-controlled, 3 period cross-over study with an interval of 1–2 weeks. The subjects received a single oral dose of 10 mg of amezinium metilsulfate (amezinium) (Rismic®, Dainippon, Osaka, Japan), 2 mg of midodrine hydrochloride (midodrine) (Metligine®, Taisyo, Tokyo, Japan),

which are the recommended starting doses in Japan for these drugs11,12 or a placebo. Two and half hours after amezinium, around the tmax of the drug,13 1.5 h after midodrine, around the tmax of its active metabolite, dimethoxyphenylaminoethanol,14 or 2 h after placebo, the finger-tip skin vasocoecstror response to cold stimulation was assessed, followed by assessment of the vasoconstrictor response to noradrenaline infusion. The measurements started around 10.30 h. Between the preceding assessments (ie around 2 h and 50 min after amezinium, 1 hour and 50 min after midodrine, and 2 h and 20 min after the placebo) blood pressure and pulse rate were measured both in the supine position and 1 min after standing up, using a semiautomated sphygmomanometer (BP-103II, Nihon Colin, Komaki, Japan). The experiments were performed in a room with the ambient temperature controlled at 24–26°C.

Table 2 Emax and ED50 in Response to Noradrenaline Infusion After the Oral Dosing With Placebo, Amezinium or Midodrine

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Amezinium</th>
<th>Midodrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emax (%)</td>
<td>100.8±9.9</td>
<td>99.2±11.4</td>
<td>102.6±12.0</td>
</tr>
<tr>
<td>ED50 (ng/min)</td>
<td>40.6±33.9</td>
<td>21.0±21.3*</td>
<td>33.2±31.5</td>
</tr>
</tbody>
</table>

Mean±SD, n=8. Emax, maximum vasoconstrictory effect; ED50, infusion rate producing a half-maximum response. *p<0.05 vs placebo.

Data Evaluation FTBF: Data were expressed in arbitrary units. Effects of contralateral hand cooling on FTBF were quantitated using the following equation:

\[
\text{Reduction Ratio (RR)} = \frac{[\text{FTBF}_{\text{bef}} - \text{FTBF}_{\text{min}}]/\text{FTBF}_{\text{bef}}] \times 100\%
\]

where \(\text{FTBF}_{\text{bef}}\) is the blood flow recorded just before the cold stimulation and \(\text{FTBF}_{\text{min}}\) is the minimum blood flow recorded during the cold stimulation.

Dorsal Hand Vein Constriction: The individual dose-response curve was analyzed by the following equation:

\[
\text{Effect} = \frac{\text{E}_{\text{max}} \times e^x}{(\text{ED}_{50} + e^x)} \times 100\%
\]

where x is the infusion rate of noradrenaline, \(\text{E}_{\text{max}}\) is the maximum vasoconstrictor effect, and \(\text{ED}_{50}\) is the infusion rate producing a half-maximum response. The data were fitted to this equation by the least-squares method, providing estimates of \(\text{ED}_{50}\) and \(\text{E}_{\text{max}}\).

Data are expressed as mean ± SD. Statistical analysis for the differences between the drugs and placebo were performed by analysis of variance (ANOVA) followed by Scheffe’s multi-range test. A probability value of p<0.05 was considered as significant.

Results

Blood Pressure and Pulse Rate

Blood pressure and pulse rate in the supine position and 1 min after standing up are shown in Table 1. No significant differences were observed between placebo and drug treatments in any of these parameters.

FTBF Measurements

There were no significant differences in the FTBF
before the cold stimulation between placebo and drug treatments (198±17 units for placebo, 201±20 units for amezinium, and 191±18 units for midodrine). The minimum FTBF during the cold stimulation after amezinium, but not midodrine, treatment was significantly less (p<0.05) than that after placebo (48±12 units for placebo, 31±13 units for amezinium, and 42±11 units for midodrine). The RR of FTBF after the cold stimulation for amezinium, but not for midodrine, was significantly (p<0.05) increased (75.9±9.8% for placebo, 85.1±7.9% for amezinium and 78.1±9.3% for midodrine) (Fig 1).

Venoconstrictor Response to Noradrenaline

Dose-response curves for the effect of noradrenaline infusion on dorsal hand vein constriction after the 3 treatments are shown in Fig 2. The values of the E_{max} for the 3 treatments were comparable. However, after amezinium but not after midodrine treatment, ED_{50} was significantly (p<0.05) decreased compared with that of placebo (Table 2).

Discussion

Drugs used for the treatment of orthostatic hypotension include fludrocortisone acetate, a synthetic mineralcorticoid, inhibitors of prostaglandin synthesis, such as indomethacin, phenylpropanolamine, a sympathomimetic agent, and caffeine, [α]-Blockers and disopyramide are effective for vasovagal syncope, a common cause of faintness. In Japan, amezinium and midodrine both of which are vasoconstricting agents, are widely used for various type of orthostatic hypotension because of their excellent safety and good efficacy.

Orthostatic hypotension is induced by a variety of disorders, including inadequate vasoconstrictor response to sympathetic stimulation on standing. Therefore, it is interesting to investigate the effects of the drugs on vasoconstrictor response to sympathetic or adrenoceptor stimulations. In the present study, we examined whether the drugs have such effects or not in normal subjects using 2 noninvasive methods. This is a new approach to evaluating the drugs used for orthostatic hypotension.

First, the finger-tip vasoconstrictor response to cold stimulation was examined using a laser Doppler flowmeter. Laser Doppler analysis allows real-time and noninvasive measurement of skin blood circulation. We used an integrating probe employing a bundle of 7 efferent fibers, which increases the total measuring volume in order to allow a more precise detection of the blood flow. Although measurements obtained with a laser Doppler flowmeter are of a relative nature, the method is particularly appropriate for the study of local skin vasomotor responses in human subjects. Cold stimulation rapidly increases an endogenous noradrenaline concentration, which, in turn, causes adrenoceptor-mediated vasoconstriction and a reduction in blood flow. This method is used for the evaluation of autonomic failure, including Shy-Drager syndrome, Parkinson’s disease, amyloidosis and diabetic neuropathy. We demonstrated that α-adrenoceptor antagonists, which could induce orthostatic hypotension, suppress the finger skin vasoconstrictor response and the degree of the suppression is correlated with the drug plasma concentration.

Second, the vasoconstrictor response to noradrenaline was examined using LVDT. The dorsal hand vein technique provides a sensitive, easy and reproducible means of assessing the effect of drugs on peripheral veins. The effects of oral drugs on vasoconstrictor response caused by the infusion of a vasoconstricting agent can be detected without affecting systemic hemodynamics, because the measurement is made within the vicinity of the infusion site, allowing the amount of the agent to be extremely small. The vasoconstrictor response of dorsal hand veins to phenylephrine or noradrenaline is reported to reflect the responsiveness of the foot vein and the overall vascular system. Several investigators have applied this method to the evaluation of the pathophysiological conditions of patients with orthostatic hypotension or diabetic autonomic neuropathy.

Reproducibility of the finger-tip vasoconstrictor response to cold stimulation and the vasoconstrictor response to noradrenaline were reported to be excellent. In the present study, we examined the vascular responses under standardized conditions including room temperature, time of the measurements and effect of food, to ensure the reliability of the experiments.

The 10 mg of amezinium and 2 mg of midodrine used in this study were the recommended starting oral doses in Japan for the treatment of orthostatic hypotension. The difference in pharmacokinetic properties of the drugs prompted us to select different time points for this single
dose comparative study. As the t\textsubscript{max} of amezinium\textsuperscript{13} and that of dimethoxyphenyl-aminoethanol (DMAE), an active metabolite of midodrine, are around 2.5 h and 1.5 h, respectively, the measurements were scheduled at 2.5 h, 1.5 h and 2 h after amezinium, midodrine, and placebo administration, respectively.

In the present study, the increase in blood pressure by amezinium or midodrine was not statistically significant. Other studies in healthy volunteers\textsuperscript{13,14} also showed that neither amezinium nor midodrine at the doses used in this study caused significant change in blood pressure or pulse rate. However, pretreatment with amezinium at the dose used in this study increased the finger-tip vasoconstrictor response to cold stimulation and venoconstrictor response to noradrenaline. These findings indicate that amezinium enhances vasoconstriction induced by sympathetic stimulation and venoconstriction induced by noradrenaline, and this mechanism by amezinium might contribute to the improvement of the symptoms of orthostatic hypotension. On the other hand, midodrine did not exert such an effect. The mechanism of these findings might be explained by the pharmacological properties obtained in in-vitro studies. Amezinium is shown to inhibit MAO activity inside noradrenergic neurons and the uptake of noradrenaline in the peripheral monoamine nervous system.\textsuperscript{2} Therefore, it can be speculated that the activity of noradrenaline released by cold stimulation and exogenously infused noradrenaline, which is inactivated by MAO, is enhanced by amezinium pretreatment. On the other hand, this study showed that midodrine did not affect the responsiveness of vasoconstriction to the stimulation, and this might be because it directly stimulates \(\alpha\)-adrenoceptors on blood vessels.\textsuperscript{10} It would enhance vascular tone irrespective of the stimulation, although this phenomenon is hard to detect by the methods used in the present study. According to studies using the doral hand vein method\textsuperscript{31,32} some patients with orthostatic hypotension showed increased vasoconstriction to exogenous noradrenaline due to denervation. Mido- drine might be preferable in such patients, and this assumption is supported by a study in patients with diabetic orthostatic hypotension.\textsuperscript{25}

**Study Limitations**

This study was performed in 8 healthy young subjects, using a single oral dose to characterize the properties of the drugs. Further studies are needed to elucidate whether the findings are similar after long treatment with the drugs and to evaluate the effects of the drugs on the vasoconstrictor responses as well as on blood pressure or symptoms due to hypotension in older subjects or in patients with various types of orthostatic hypotension.

From the findings observed in this study, amezinium might be effective for the treatment of patients with an impaired vasoconstrictor response. Applying the methods used in the present study in further investigations of the pathophysiology of orthostatic hypotension, in order to characterize the effects of the drugs in such patients, might give suggestions for the selection of the most appropriate drug in clinical practice.

**Conclusion**

In the present study, we showed, using a new approach, that a single oral dose of amezinium increases the vasoconstrictor response to sympathetic stimulation and exoge-