Pharmacologic Analysis of 7-O-Ethyl-fangchinoline-Induced Vasodilation Properties in Isolated Perfused Common Carotid Arteries of Wistar Kyoto Rats and Spontaneously Hypertensive Rats

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Using the cannula insertion method, we investigated vascular effects of 7-O-ethyl-fangchinoline (TJN-220) derived from tetrandrine in isolated and perfused common carotid arteries of Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). A single dose of TJN-220 caused a vasodilation in a dose-related manner in arteries preconstricted by phenylephrine. The vasodilation was not inhibited by propranolol, a potent beta-adrenergic antagonist, although the methyl group of TJN-220 slightly but significantly attenuated the KCl-induced one in large doses. The vasodilation of TJN-220 was not abolished after removing the endothelium by an intraluminal administration of saponin

Keywords 7-O-ethyl-fangchinoline; tetrandrine; spontaneously hypertensive rat; cannula insertion method; calcium antagonist

Tetrandrine is one of alkaloids isolated from Chinese herb Radix Stephaniae Tetrandrae. Tetrandrine has been used clinically in the management of hypertension, especially hypertensive crisis and coronary heart diseases in China. Experimental studies of tetrandrine on smooth muscle, cardiac papillary muscle, left atrial tissue and GH₃ anterior pituitary cells were reported. These suggested that these effects of tetrandrine might be related to an inhibition of Ca²⁺ entry. Recently, several tetrandrine derivatives were studied to obtain an even more effective compound. 7-O-Ethyl-fangchinoline (TJN-220) is a compound which is substituted at the 7-position to the ethyl group from the methyl group of tetrandrine. Kawashima et al. demonstrated that TJN-220 had more hypotensive potency than tetrandrine in unanesthetized and freely moving stroke-prone spontaneously hypertensive rats (SHRSP) however, they did not make a pharmacologic analysis. Thus, in the present study we tried pharmacologically to analyze vascular responses to TJN-220 in isolated common carotid arteries of Wister Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) using the cannula insertion method.

Methods
Male WKY and SHR rats (weighing 150—250 g: aged 7—13 weeks) were used in the present study. After treatment with sodium heparin (200 units/kg, i.v.), rats were killed by rapid exsanguination, and common carotid arteries (0.8 to 1.3 mm in length and 0.8 to 1.2 mm in outer diameter) were carefully isolated. A stainless steel cannula with small holes at 2 mm distance from the distal sealed end (23 or 25 gauge; 0.5 to 0.6 mm in outer diameter and 3 cm in length) was inserted into each vessel segment to avoid injury of the intraluminal surface of the isolated vessel. Segments were set up in the bath as described by Tsuji and Chiba. The perfusion solution contained (mmol/l): NaCl 118, KCl 4.7, CaCl₂ 2.5, NaHCO₃ 25, MgSO₄ 1.2, KH₂PO₄ 1.2, and glucose 10: and bubbled with 95% O₂ and 5% CO₂ to maintain the pH of the solution at 7.2—7.4. The bath and perfusion circuit were warmed at 37°C with a thermopump (Model FE2, Haake, Karlsruhe, FRG). Speed of the perfusion pump was determined at a resting perfusion pressure of 40—60 mmHg. The vasodilation and vasoconstriction were presented as a decrease and an increase in perfusion pressure, respectively, which was continuously measured with an electronic manometer. TJN-220 used in the present study was obtained from the Research Institute for Biology and Chemistry, Tsumura Co., Ltd., and the procedure for its preparation has been published elsewhere. Other drugs used were isoproterenol hydrochloride (Nikken, Tokyo), propranolol hydrochloride (Sumitomo, Tokyo), acetylcholine chloride (Daiichi, Tokyo), atropine sulfate, potassium chloride, diltiazem hydrochloride (Tanabe, Tokyo), norepinephrine hydrochloride (Sankyo, Tokyo), phenylephrine hydrochloride (Kowa, Tokyo), and bunazosin hydrochloride (Eisai, Tokyo). Drug solution was injected intraluminally into the isolated vessel from the rubber tubing close to the cannula in a volume of 0.01—0.03 ml for a period of 4 s.

Results
Vasodilator Responses to TJN-220 and Isoproterenol in Isolated Common Carotid Arteries of WKY and SHR The vascular effect of TJN-220 in non-treated WKY and SHR common carotid arteries was tested but no vascular responses were observed. Thus, a preconstricted condition was induced by treatment with phenylephrine (1 μg/ml). The increase in perfusion pressure was approximately 30 mmHg, and each experiment was performed in a stable state. When

![Fig. 1. Vascular Responses of Isolated and Perfused Common Carotid Arteries to Isoproterenol and TJN-220](image-url)

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Table I. Vasodilation Effects of Isoproterenol, TJN-220 and Papaverine on Isolated Common Carotid Arteries of WKY and SHR

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (µg)</th>
<th>Maximum decrease in P.P. (%)</th>
<th>Time to maximum response (min)</th>
<th>50% recovery time (min)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>0.01</td>
<td>36.7 ± 4.1</td>
<td>0.5 ± 0.03</td>
<td>1.2 ± 0.1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>50.8 ± 3.5</td>
<td>0.5 ± 0.05</td>
<td>1.8 ± 0.2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>67.8 ± 4.3</td>
<td>1.0 ± 0.1</td>
<td>3.9 ± 0.5</td>
<td>8—10</td>
</tr>
<tr>
<td>TJN-220</td>
<td>30</td>
<td>25.6 ± 2.9</td>
<td>1.4 ± 0.2</td>
<td>3.8 ± 0.6</td>
<td>7—9</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>51.3 ± 5.0</td>
<td>2.2 ± 0.3</td>
<td>8.8 ± 1.0</td>
<td>9—10</td>
</tr>
<tr>
<td>Papaverine</td>
<td>100</td>
<td>100</td>
<td>4.6 ± 0.6</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>0.01</td>
<td>39.6 ± 2.1</td>
<td>0.5 ± 0.02</td>
<td>1.0 ± 0.1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>53.8 ± 2.4</td>
<td>0.5 ± 0.02</td>
<td>1.4 ± 0.2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>68.4 ± 3.3</td>
<td>0.8 ± 0.05</td>
<td>3.5 ± 0.4</td>
<td>8—10</td>
</tr>
<tr>
<td>TJN-220</td>
<td>30</td>
<td>19.5 ± 2.5</td>
<td>1.0 ± 0.2</td>
<td>4.5 ± 0.7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>37.2 ± 4.6</td>
<td>1.8 ± 0.4</td>
<td>7.8 ± 1.2</td>
<td>8—10</td>
</tr>
<tr>
<td>Papaverine</td>
<td>100</td>
<td>100</td>
<td>4.7 ± 0.5</td>
<td>—</td>
<td>6</td>
</tr>
</tbody>
</table>

a) P.P., perfusion pressure.

Fig. 2. Vasodilation to Isoproterenol and TJN-220 in Isolated Common Carotid Arteries of WKY and SHR Preconstricted by Phenylephrine

- ○, isoproterenol-induced vasodilation in WKY; △, in SHR; ○, TJN-220-induced vasodilation in WKY; ▲, in SHR. Each point and vertical bar represent the mean ± S.E. (n = 6—10).

A single dose of TJN-220 or a beta-adrenoceptor agonist, isoproterenol was intraluminally administered in vessels of both WKY and SHR, vasodilation was readily observed as a decrease in perfusion pressure (Fig. 1). Slight fluctuation just after drug administration was an artifact caused by injection of the drug solution. In each preparation, the maximum decrease in perfusion pressure by 100 µg papaverine was defined as a 100% decrease. In both WKY and SHR, the threshold doses for inducing a vasodilation to TJN-220 and isoproterenol were 10 µg and 0.0003 µg, respectively. These responses were induced in a dose-related manner (Fig. 2). The maximum decrease induced by TJN-220 at a dose of 300 µg was 67 ± 5.5% in of WKY and 53 ± 3.9% in SHR. The maximum decrease induced by 0.3 µg isoproterenol was 80% in WKY and 78% in SHR, showing no difference between the two preparations. The response to TJN-220 was markedly longer than that to isoproterenol (Table I). The vasodilation of 100 µg TJN-220 lasted 30—40 min, with 50% recovery time lasting 7—9 min in both preparations.

Effects of Propranolol on Vasodilations Induced by TJN-220 and Isoproterenol

After administration of 1 µg propranolol, the vasodilation of isoproterenol was markedly inhibited in both WKY and SHR in the preconstricted state. In contrast, the vasodilation of TJN-220 was not clearly modified by propranolol, although it was attenuated slightly by propranolol in WKY. When a relatively large dose of TJN-220 (100—300 µg) was used,
propranolol was injected just before each TJN-220 administration. Summarized data are shown in Fig. 3.

**Effects of Atropine on Acetylcholine- and TJN-220-Induced Vasodilations** Acetylcholine usually caused a vasodilation in a dose-related manner in vessels with the presence of the endothelium preconstricted by phenylephrine. After an administration of 10 μg atropine, the vasodilation induced by acetylcholine was significantly inhibited. However, the vasodilation induced by TJN-220 was not significantly influenced by atropine treatment (Fig. 4).

**Effects of Endothelium Removal on Acetylcholine- and TJN-220-Induced Vasodilations** In a preconstricted state, intraluminal saponin treatment (1—3 mg) readily removed the endothelium, because acetylcholine-induced dilation disappeared thereafter. Since saponin caused a long-lasting increase in perfusion pressure (approximately 20 mmHg), acetylcholine or TJN-220 was administered under a stable condition of perfusion pressure higher than control. In both WKY and SHR vessels, TJN-220-induced dilations were not modified after saponin treatment. Summarized data are shown in Fig. 5.

**Effects of TJN-220 and Diltiazem on KCl-Induced Vasocostrications** When KCl (1, 3 mg) was injected intraluminally, a vasoconstrictor response was clearly produced in a dose-related manner in both WKY and SHR common carotid arteries. The vasoconstriction to KCl was inhibited by pretreatment with diltiazem, a Ca^{2+} entry antagonist, or with TJN-220. In both WKY and SHR arteries, the vasoconstrictor responses to 1 mg KCl were decreased to
approximately 50% of control by 1 µg diltiazem, and were markedly inhibited by 10 µg diltiazem \( (p<0.005) \) (Fig. 6A). However, the effects of TJN-220 for the KCl-induced vasconstriction were much less than those of diltiazem. After the 100 µg TJN-220 was administered, the vasoconstrictions induced by 1 mg and 3 mg KCl were reduced to approximately 40–60% and 70–80% of the controls, respectively (Fig. 6B).

**Effects of TJN-220 and Bunazosin on Norepinephrine-Induced Vasconstrictions** When norepinephrine was administered into common carotid arteries, an increase in perfusion pressure was usually observed in a dose-related manner. After bunazosin (0.1–10 µg), an alpha-adrenoceptor antagonist, was injected, the vasconstriction induced by norepinephrine was completely inhibited in both WKY and SHR carotid arteries. In both strains, TJN-220 did not suppress norepinephrine-induced constrictions, indicating that TJN-220 has no alpha-adrenoceptor blocking activity. In most preparations, TJN-220 slightly enhanced norepinephrine-induced vasconstrictions but not significantly. Summarized data are shown in Fig. 7.

**Discussion**

When the cannula insertion method was used in isolated and perfused rat common carotid arteries, preconstriction was required to investigate the vascular effects of vasoconstrictors. A potent beta-adrenoceptor agonist, isoproterenol induced a vasodilation in a dose-related manner. In contrast, TJN-220 caused less potent vasodilator responses, although its action was long-lasting.

Tetrandrine and several of its derivatives have a hypotensive effect of gradual onset and long duration of action after oral administration in SHRSP. Among the derivatives, 7-O-methyl, 7-O-ethyl (TJN-220) and 7-O-isopropyl derivatives showed the most marked and almost equivalent hypotensive activity. In conscious normotensive Wistar rats, SHR, renal hypertensive and deoxycorticosterone (DOCA)-salt rats, i.v. administration of tetrandrine caused an abrupt and long-lasting decrease in mean arterial pressure, which proved dose-dependent. After the injection of tetrandrine, the decrease of mean arterial pressure in conscious normotensive rats was slightly greater than that in SHR.

In our experimental data of TJN-220, we were unable to detect a clear difference between WKY and SHR vessels. The vasodilation to isoproterenol was readily inhibited by propranolol, while the vasodilation to TJN-220 was not, indicating that TJN-220 has no beta-adrenoceptor agonistic activity in vessels. In the isolated rabbit atrium, propranolol exhibited competitive antagonism with isoproterenol while tetrandrine did not, indicating that tetrandrine has no beta-adrenoceptor blocking activity in cardiac tissue.

Acetylcholine produces vasodilator responses in preconstricted vessels existing in the intact endothelium. In this study, it induced a vasodilation in a dose-related manner in both WKY and SHR vessels. This vasodilation was markedly inhibited by atropine. The vasodilation of TJN-220, however, was not modified by atropine in either WKY or SHR, indicating that TJN-220 has no muscarinic properties. Acetylcholine dilation was readily abolished by removing the endothelium, while that of TJN-220 was not modified by such removal, indicating that TJN-220 induced no release of endothelium derived relaxing factor (EDRF).

Diltiazem significantly inhibited vasconstriction induced by KCl in both WKY and SHR. TJN-220 also showed inhibitory effect on the KCl-induced constrictions, but a large dose was required and its blocking effects were not strong. Thus, the vasodilating effects of TJN-220 might, in part, be due to blocking of Ca\(^{2+}\) entry to vascular smooth muscle.

Norepinephrine produced a vasoconstriction in both WKY and SHR common carotid arteries in a dose-related manner. The pressor responses to exogenous norepinephrine were greater in SHR than in WKY perfused mesenteric arterial beds. However, in the present study, the increase in perfusion pressure in SHR was slightly but not significantly greater than that in WKY. In both WKY and SHR isolated arteries, vasoconstrictions of norepinephrine were readily abolished by a potent alpha-adrenoceptor...
antagonist, bunazosin, while TJN-220 did not influence the norepinephrine-induced vasoconstriction even in large doses. The vasodilation of TJN-220 was thus not explained by blocking action of alpha-adrenoceptors in isolated perfused common carotid arteries.

It was indicated that TJN-220 caused a long-lasting vasodilation in isolated WKY and SHR common carotid arteries, in part by a partial Ca\(^{2+}\) entry blocking action in addition to unknown vasodilatory mechanisms.

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References