Effect of Manidipine on Balloon Catheter-Induced Arterial Smooth Muscle Cell Proliferation in Spontaneously Diabetic GK Rats

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ABSTRACT—The inhibitory effect of manidipine, a long acting calcium channel blocker, on vascular smooth muscle cell proliferation was investigated in spontaneously diabetic GK rats with balloon catheter-induced denudation of the carotid artery. Treatment with manidipine at doses of 4.6 and 15.1 mg/kg/day inhibited thickening of the neo-intima in the balloon catheter-injured artery without any effect on blood pressure and lowered the ratio of intima to wall areas and wall to total vascular areas in a dose-dependent fashion. These results suggest that manidipine inhibits an abnormal proliferation of the intima in the carotid artery of spontaneously diabetic rats.

Keywords: Manidipine, Neo-intima, Antimyoproliferative action

It has been reported that calcium channel blockers suppress atherogenesis in cholesterol-fed animals (1-3) and the development of new occlusive lesions in patients with coronary atherosclerosis (4, 5). The possible mechanisms of this antiatherogenic effect are probably related to lowering of arterial pressure, prevention of dyslipidemic endothelial injury and inhibition of arterial smooth muscle cell (SMC) proliferation (6). In this paper, we examined the effects of manidipine, a long acting calcium channel blocker (7), on SMC proliferation after vascular injury induced by a balloon catheter in spontaneously diabetic Goto-Kakizaki (GK) rats (8), in which severe thickening of intima in the injured artery is observed.

Male spontaneously diabetic GK rats were bred in our Laboratory Animal Unit. They were housed in individual wire cages and fed a commercial stock diet (CE-2, Clea Japan Inc., Tokyo) in a room with controlled temperature (23 ± 1°C), humidity (55 ± 5%) and lighting (08:00-20:00).

Thirteen-week-old, male GK rats were given a powdered CE-2 diet with or without manidipine at a concentration (w/w) of 0.01 or 0.03% for 20 days. The concentrations were selected as those producing slight and mild antihypertensive effects in spontaneously hypertensive rats (9). The drug intake was calculated from the food intake and expressed as mg/kg/day. On day 6, these rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and subjected to balloon catheter-induced denudation of the left carotid artery according to the method described by Baumgartner (10). In brief, a 2F balloon catheter (Edwards Laboratories, Santa Ana, CA) was introduced into the left carotid artery via the left femoral artery. The balloon was then inflated with saline and drawn back and forth 3 times over a distance of 1 to 1.5 cm to remove the endothelium of the carotid artery. On day 20, the systolic blood pressure was measured by a tail pulse pick-up method in unanesthetized rats. Thereafter, a catheter was inserted into the ascending aorta via the left ventricle under pentobarbital anesthesia. Phosphate-buffered saline with 4% formaldehyde (pH 7.4) was perfused under a pressure of 90 to 100 mmHg for 10 min to fix the carotid artery. Five frozen cross-sections (12 µm in thickness) from each injured artery were stained with oil red O and hematoxylin for light microscopic observation.

The images of sections were projected on the tracing easel of an IBAS-2000 (Carl Zeiss, FRG) image analyzer. Cross-sectional areas of the intima, media, wall (intima and media) and the total (wall and lumen) were obtained by tracing the outline of the respective images. The largest three lesion areas were selected to
estimate the lesion size, and the extent of the lesion is shown as an average of these three sections.

Blood samples were collected in the heparinized tubes from the orbital plexus and centrifuged immediately. Plasma cholesterol and triglyceride were measured enzymatically using an Encore™ (Baker Instruments Co., Allentown, PA).

The data are expressed as the mean ± S.D. and were statistically analyzed using Duncan’s multiple range test.

The dietary administration of manidipine at the average dose of 4.6 or 15.1 mg/kg/day did not alter body weight, food intake, and plasma lipid levels (data not shown). The 20 day-treatment with 15.1 mg/kg/day also did not change the systolic blood pressure (144 ± 3 or 141 ± 6 mmHg for the control or manidipine group, respectively).

Thickening of intima in the denuded part of the injured artery was prominent, but that of the media was very slight (Fig. 1a). The neo-intima was filled with actin-positive cells, indicating proliferation of vascular SMC (data not shown). Treatment with manidipine at a dose of 15.1 mg/kg/day suppressed thickening of the intima in the denuded part (Fig. 1b). The morphometrical analysis also revealed that the inhibitory action of manidipine on thickening of the intima in the injured artery was roughly dose-dependent (Table 1).

**Fig. 1.** Cross-sections of the balloon catheter-injured carotid artery from GK rats with (b) or without (a) manidipine (15.1 mg/kg/day). NI: Neo-intima. M: Media.

**Table 1.** Effect of manidipine on cross-sectional area of the balloon injured carotid artery of GK rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Manidipine (mg/kg/day)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>4.6</td>
<td>15.1</td>
</tr>
<tr>
<td>Number of animals</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Intima ( × 10⁻³ mm²)</td>
<td>160 ± 3²a</td>
<td>127 ± 24²ab</td>
</tr>
<tr>
<td></td>
<td>141 ± 12²a</td>
<td>144 ± 14²a</td>
</tr>
<tr>
<td>Intima/Wall (%)</td>
<td>52.8 ± 6.0³a</td>
<td>46.6 ± 4.6³b</td>
</tr>
<tr>
<td></td>
<td>38.4 ± 8.3³a</td>
<td>30.8 ± 3.8³b</td>
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</tbody>
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Three cross-sections of the balloon catheter-injured carotid artery in each rat were made, and the area of the intima and media were measured by an image analyzer. Mean ± S.D. without a common superscript letter differs significantly (P < 0.05) by Duncan’s multiple range test.
Manidipine at doses of 4.6 and 15.1 mg/kg/day caused 20.6 and 27.5% reduction of the cross-sectional area of the neo-intima, respectively. As a result, manidipine lowered the ratio of intima to wall areas and wall to total vascular areas in a dose-dependent fashion. The drug showed no effect on the area of media of the aorta.

Vascular SMC proliferation in intima of the artery plays the most important role in the development of atherosclerosis (11). The present study shows that manidipine, a dihydropyridine-type of calcium antagonist, has antiproliferative action on vascular SMC in the in vivo experiments.

We previously observed that SMC of spontaneously diabetic GK rats showed high proliferative responses to the balloon catheter injury of the artery when compared with those of Wistar rats (unpublished data). Therefore, we used GK rats to examine the inhibitory activity of manidipine on the myoproliferative response to the balloon catheter injury. Manidipine suppressed thickening of the intima in the denuded part of the injured carotid artery without any effects on body weight, food intake, plasma lipids and blood pressure. As a result, manidipine lowered atherogenic indices, such as the ratio of intima to wall areas and wall to total vascular areas in a dose-dependent fashion.

It has been reported that several calcium antagonists, such as nifedipine, verapamil, diltiazem or lanthanum, also inhibited arterial DNA synthesis and thickening of the intima in the denuded part of the artery in rats and rabbits, respectively (12). However, the exact mechanism for how these calcium antagonists including manidipine prevent the development of the neo-intima in the denuded part of the artery is not fully understood.

More recently, Ferns et al. reported that the development of the neo-intima induced by the balloon catheter injury was inhibited by a polyclonal antibody of PDGF, suggesting that endogenous PDGF is involved in the proliferation of SMC in the neo-intima (13). Block and Emmons have introduced a new perspective on the antiatherosclerotic activity of calcium antagonists including manidipine, a dihydropyridine-type of calcium antagonist, in three rat models of hypertension. The present study shows that manidipine, a dihydropyridine-type of calcium antagonist, has antiproliferative action on vascular SMC in the in vivo experiments.

In conclusion, manidipine inhibited a proliferative response of the intima to the balloon catheter-induced injury in the carotid artery of spontaneously diabetic rats. It is noted that the inhibitory action was observed at the dose in which the blood pressure was not decreased.

REFERENCES