Original Article

Cilnidipine More Highly Attenuates Cold Pressor Stress-Induced Platelet Activation in Hypertension than Does Amlodipine

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The clinical significance of N-type calcium channel blockade has not been fully examined. We here compared the effects of the N-type calcium channel blockers cilnidipine and amlodipine on the sympathetic nervous system and platelet function in hypertension under resting and stressed conditions. Thirty-two patients with hypertension (58±9 years) received cilnidipine or amlodipine for 4 weeks in this crossover study. On day 28 of each treatment, plasma levels of epinephrine (EP), norepinephrine (NEP), and β-thromboglobulin (BTG), and ECA of ADP-induced platelet aggregation (ADP-Ca) were determined at rest and after a cold pressor test. On day 29, the group receiving cilnidipine was switched to amlodipine treatment, and vice versa. At rest, the blood pressure, heart rates, EP, NEP, ADP-Ca, and BTG, were similar in both treatments. After the cold pressor test, increases in EP (35±17 to 44±25 pg/ml; p<0.05) and BTG (40±13 to 49±22 ng/ml; p<0.01) and a decrease in ADP-Ca (32±26 to 27±24 μmol; p<0.05) were observed in the amlodipine treatment, but not in the cilnidipine treatment. In addition, the increase in NEP was significantly greater (p<0.05) in the amlodipine (276±78 to 318±87 pg/ml; p<0.01) than in the cilnidipine treatment (273±88 to 291±100 pg/ml; p<0.05). Cilnidipine more highly attenuates the activation of the sympathetic nervous system via N-type calcium channel blockade may contribute to this phenomenon. (Hypertens Res 2001; 24: 679-884)

Key Words: calcium channel blocker, N-type calcium channel, sympathetic nervous system, platelet function

Introduction

Accumulated evidences suggest that platelet activation may play important roles in atherosclerosis and thrombotic complications (1). Stress is known to be one of the risk factors of cardiovascular diseases (2), and enhancement of platelet activity by stress is thought to be an underlying mechanism contributing to this risk (3). Sympathetic activation may contribute to enhanced platelet activity (4). While calcium channel blockers that induce vasodilation mediated by L-type channel blockade are widely used as a first-line treatment for hypertension, adverse effects of these compounds have also been suggested to exert adverse effects that are mediated by activation of sympathetic activity induced by abrupt vasodilatation (5). Accordingly, long lasting L-type calcium channel blockers that exert less influence on the sympathetic nervous system are now recommended for treatment of hyper-
Table 1. Clinical Data Obtained in Routine Examinations before Entry.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58±9</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>16/16</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24±3</td>
</tr>
<tr>
<td>Smokers (numbers)</td>
<td>6</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>99±9</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>203±27</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>47±14</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>137±27</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
<td>106±14</td>
</tr>
</tbody>
</table>

Medication

- Amlodipine (5–10 mg/day) n=16
  - cilnidipine 10 mg/day → amlodipine 5 mg/day, n=6
  - cilnidipine 20 mg/day → amlodipine 10 mg/day, n=10

- Cilnidipine (10–20 mg/day) n=12
  - amlodipine 5 mg/day → cilnidipine 10 mg/day, n=5
  - amlodipine 10 mg/day → cilnidipine 20 mg/day, n=7

- Benidipine (4 mg/day) n=3
  - cilnidipine 10 mg/day → amlodipine 5 mg/day, n=2
  - amlodipine 5 mg/day → cilnidipine 10 mg/day, n=1

- Nifedipine retard (40 mg/day) n=1
  - amlodipine 5 mg/day → cilnidipine 10 mg/day, n=1

LVMI, left ventricular mass index; Medication, calcium channel blockers prescribed to subjects before entering the protocol; →, medication in two sequences in protocol.

Subjects

Thirty-two subjects with mild essential hypertension [16 men and 16 women (11 women were in menopausal state); mean age, 58±9 years] who had been followed for at least 6 months at Teikyo University Ichihara Hospital while receiving monotherapy with calcium channel blockers were enrolled in this study. Table 1 gives the details of the calcium channel blockers administered to subjects prior to this study. All subjects had undergone routine blood, urine, electrocardiogram, chest X-ray, and echocardiogram examinations within 3 months before entering the protocol. Left ventricular mass index (LVMI) was obtained from the echocardiogram (10). Subjects with other serious medical problems requiring specific treatments were excluded.

Study Protocol

Informed consent to participate in the study was obtained from all subjects. The protocol was approved by the Ethics Committee of Teikyo University School of Medicine, Ichihara Hospital. The doses of amlodipine and cilnidipine were considered equivalent (i.e., 5 mg amlodipine is considered equivalent to 10 mg cilnidipine, 10 mg amlodipine to 20 mg cilnidipine, and 4 mg benidipine or 40 mg nifedipine retard to 5 mg amlodipine) (11, 12). The patients were assigned to one of two sequences: amlodipine-cilnidipine or cilnidipine-amlodipine. Patients who were previously prescribed amlodipine were switched to cilnidipine and vice versa, and continued until day 28. Patients who were previously prescribed benidipine or nifedipine retard were randomly allocated to receive amlodipine (5 mg) or cilnidipine (10 mg) until day 28 (Table 1). On day 28, the cold pressor test was performed.

On day 29, the patients were switched to the other calcium channel antagonist and continued until day 56. On day 56, the cold pressor test was again performed.

Cold Pressor Test

On the night before the cold pressor test, patients were asked to maintain overnight fasting, with the exception of taking a calcium channel blocker at 6:30 AM. At 8:00 AM, an 18 G plastic needle was inserted into the antecubital vein of the right arm for blood collection. The needle was connected to an infusion pump and 0.9% saline was infused at a speed of 10 ml/h to prevent blood coagulation in the needle. A cold pressor test was performed after 30 min bed rest. At 8:30 AM, the left hand was immersed up to the wrist in ice-cold water for 1 min. Blood pressure was measured at the left arm using an automatic arterial sound recording device (ST12B; Nihon Colin Co., Ltd., Komaki, Japan) and cardiac output.
was measured by the pulsed Doppler technique using an echocardiograph equipped with a 2.5 MHz transducer (SSH-140; Toshiba Co., Ltd., Tokyo, Japan) before and immediately after the cold pressor test (13). Heart rate was monitored using a system attached to the echocardiographic apparatus.

Before and after the cold pressor test, a blood sample was collected to measure plasma levels of β-thromboglobulin (BTG), epinephrine (EP), norepinephrine (NEP), fibrinogen, tissue plasminogen activator (tPA) antigen, and plasminogen activator inhibitor-1 (PAI-1) antigen and plasma renin activity, and also to determine cyclic AMP and cyclic GMP levels in platelets and platelet function. First, 3 ml of blood was dripped into a precooled tube containing 0.3 ml of a platelet-stabilizing solution (10 mM theophylline, 0.33 µg/ml prostaglandin E1, and 3.3% ethylenediaminetetraacetic acid) to determine the plasma BTG level without stasis. Then, blood was drawn with stasis to determine other variables.

Platelet Function

Platelet function was assessed using a Born’s aggregometer (HEMA tracer IV, NBS). Nine ml of blood was collected in a syringe containing 1 ml of 3.8% sodium citrate. Whole blood was centrifuged at 600 rpm for 10 min and platelet-rich plasma was collected. The remaining blood was then centrifuged at 3,000 rpm for 10 min and platelet-poor plasma was obtained. Both plasma samples were transferred to polystyrene tubes. The extent of aggregation was expressed as a percent of the baseline. The light transmission in platelet-poor plasma was set at 100% and untreated platelet-rich plasma at 0%. ADP (MCM Medica, Tokyo, Japan) was used as an aggregating reagent and the ECso value (i.e., the concentration of agonist required to induce half-maximal aggregation) was determined (14). The ECso for ADP was determined by a dose-response procedure in which the extent of aggregation was measured. Seven different concentrations of ADP (100, 40, 30, 20, 15, 10 and 5 µmol/l) were investigated.

Determinations of Cyclic AMP and Cyclic GMP Levels in Platelets

In 26 patients, platelet levels of cyclic AMP and cyclic GMP were obtained on day 28 of each treatment protocol. Platelet-rich plasma was prepared. Platelet-rich plasma and test compounds were incubated at 37°C for 2 min. The reaction was stopped by addition of 10% perchloric acid, the reaction mixture was centrifuged, and the resulting supernatant was assayed for cyclic AMP and cyclic GMP levels using radioimmunoassay kits (Yamasa, Chiba, Japan).

Other Determinations

Plasma norepinephrine and epinephrine concentrations were determined by high-performance liquid chromatography with electromechanical detection. Plasma renin activity was measured by radioimmunoassay using a PRA kit (SRL Co., Tokyo, Japan). Plasma tPA and PAI-1 antigen levels were measured by enzyme-linked immunosorbent assays (Imulise tPA kit, Biopool AB; PAI-1 ELISA kit, Yuka Medias Co., Tokyo, Japan). Plasma fibrinogen levels were determined with a one-stage clotting assay kit (Fibrinogen A; Roche Co., Barzel, Switzerland). Plasma BTG was determined by radioimmunoassay (Diagnostica Stago Co., Kirr, Germany).

Statistics

Data are expressed as the means ± SD. Statistical analysis was performed using software from SPSS Inc. (Chicago, USA). For each treatment period, the paired t test was used to assess changes in each parameter before and after the cold pressor test. The effects of amiodipine and cildipidine on each variable and on stress-induced changes (delta changes before and after the cold pressor test) were evaluated in terms of intrapatient differences and sums [general two-paired crossover study test (15)] to avoid the time effect of the crossover design. Values of p < 0.05 were considered to indicate statistical significance.

Results

Table 1 shows the data obtained in routine examinations before entry. Table 2 depicts the clinical and biomedical characteristics before the cold pressor test after 28 days of treatment with amiodipine or cilnidipine. There was no significant difference in effect between the two treatments. Cyclic AMP and cyclic GMP levels in platelets were also similar in both treatments. Figure 1 shows the changes in hemodynamic variables during the cold pressor test. In both treatments, the cold pressor stress significantly elevated systolic and diastolic blood pressure, but did not affect heart rate or cardiac output. The total peripheral resistance increased from 1,920 ± 473 to 2,104 ± 523 dyns/cm² by amiodipine treatment (p < 0.01) and from 1,834 ± 559 to 2,164 ± 706 dyns/cm² by cilnidipine treatment (p < 0.01). The changes in blood pressures and total peripheral resistance before and after the cold pressor test were similar by both treatments. Figure 2 shows changes in the plasma levels of catecholamines and BTG and the ECso value of ADP-induced platelet aggregation during the cold pressor test. Cold pressor stress significantly increased the plasma levels of EP (35 ± 17 to 44 ± 25 pg/ml; p < 0.05) and BTG (40 ± 13 to 49 ± 22 ng/ml; p < 0.01) and decreased the ECso of ADP-induced platelet aggregation (ADPECo) (32 ± 26 to 27 ± 24 µmol; p < 0.05) in the amiodipine treatment, but these parameters did not change in the cilnidipine treatment. However, the plasma level of NEP was increased after the cold pressor test in both treatments. The general two-paired crossover study test was used to evaluate the difference between the effects of amiodipine and cilnidipine. The increases of plasma levels of NEP and BTG,
Table 2. Characteristics of Subjects before the Cold Pressor Test during the Amlodipine and Cilnidipine Treatment Periods

<table>
<thead>
<tr>
<th></th>
<th>Amlodipine</th>
<th>Cilnidipine</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic pressure</td>
<td>128±11</td>
<td>126±8</td>
<td>ns</td>
</tr>
<tr>
<td>(mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic pressure</td>
<td>78±7</td>
<td>79±7</td>
<td>ns</td>
</tr>
<tr>
<td>(mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate</td>
<td>65±9</td>
<td>67±10</td>
<td>ns</td>
</tr>
<tr>
<td>(beats/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac output</td>
<td>4.2±1.2</td>
<td>4.4±1.2</td>
<td>ns</td>
</tr>
<tr>
<td>(L/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPR (dyn/s/cm²)</td>
<td>1,920±472</td>
<td>1,834±472</td>
<td>ns</td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
<td>10.3±2.6</td>
<td>10.2±3.5</td>
<td>ns</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>36.1±20</td>
<td>35.3±19</td>
<td>ns</td>
</tr>
<tr>
<td>Fibrinogen (ng/ml)</td>
<td>245±40</td>
<td>253±15</td>
<td>ns</td>
</tr>
<tr>
<td>BTG (ng/ml)</td>
<td>41±13</td>
<td>43±17</td>
<td>ns</td>
</tr>
<tr>
<td>EP (pg/ml)</td>
<td>35±17</td>
<td>31±13</td>
<td>ns</td>
</tr>
<tr>
<td>NEP (pg/ml)</td>
<td>276±78</td>
<td>270±88</td>
<td>ns</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/h)</td>
<td>1.8±0.7</td>
<td>1.8±0.7</td>
<td>ns</td>
</tr>
<tr>
<td>Cyclic AMP (pmol/10⁶ platelet)</td>
<td>0.41±0.12</td>
<td>0.36±0.18</td>
<td>ns</td>
</tr>
<tr>
<td>Cyclic GMP (pmol/10⁶ platelet)</td>
<td>1.05±0.40</td>
<td>0.92±0.36</td>
<td>ns</td>
</tr>
</tbody>
</table>

TPR, total peripheral resistance; tPA, plasma level of tissue plasminogen activator antigen; PAI-1, plasma level of plasminogen activator inhibitor-1 antigen; cyclic AMP, cyclic AMP level in platelets; cyclic GMP, cyclic GMP level in platelets. BTG, β-thromboglobulin; EP, epinephrine; NEP, norepinephrine.

**Fig. 1.** Changes in hemodynamic variables during the cold pressor test. Before, before cold pressor test; after, after cold pressor test. Open circles represent treatment with amlodipine and closed circles represent treatment with cilnidipine. **p<0.01** vs. before cold pressor test as assessed by paired t-test.

but not EP and EC₅₀ in ADP, after the cold pressor test were significantly greater by amlodipine treatment (NEP: 276±78 to 318±87 pg/ml; BTG: 40±13 to 49±22 ng/ml) than by cilnidipine treatment (NEP: 273±88 to 291±100 pg/ml; BTG: 41±14 to 44±20 ng/ml) (p<0.05).

**Fig. 2.** Changes in plasma levels of catecholamines and β-thromboglobulin and EC₅₀ of ADP-induced platelet aggregation during the cold pressor test. Before, before cold pressor test; after, after cold pressor test; EC₅₀ in ADP, EC₅₀ of ADP-induced platelet aggregation. Open circles represent treatment with amlodipine and closed circles represent treatment with cilnidipine. *p<0.05 and **p<0.01 vs. before cold pressor test as assessed by paired t-test; p<0.05: between amlodipine and cilnidipine as assessed by the general two-paired crossover study test.

**Discussion**

Experimental studies have demonstrated that amlodipine and cilnidipine inhibit the peripheral neural N-type calcium channel (6, 16). This inhibition may attenuate sympathetic nervous activity. However, no study has yet confirmed the clinical significance of N-type channel blockade in the treatment of hypertension. However, it has been reported that amlodipine increases the plasma level of norepinephrine in hypertensive patients (17). On the other hand, cilnidipine
and amlodipine have been shown to have less influence on the sympathetic nervous system compared with other long lasting L-type calcium channel blockers, even in clinical studies (9, 18). At rest (before the cold pressor test), the plasma catecholamine levels in the present study were similar in both the amlodipine and cilnidipine treatments, which was consistent with Sakata’s report (9). To our knowledge, this study is the first to compare the difference in the potency of N-type channel blockade by amlodipine and cilnidipine under a stressed condition. The increase of plasma NEP level after the cold pressor test was greater by amlodipine than by cilnidipine treatment. Thus, at clinical dosages, while the influences of amlodipine and cilnidipine on basal NEP release from neural terminals were similar, cilnidipine seemed to more highly attenuate the stress-induced sympathetic activation via N-type channel blockade than did amlodipine.

The sympathetic nervous system affects hemostasis and fibrinolysis (4, 19). It is possible that inhibition of the sympathetic activity via N-type calcium channel blockade is beneficial for hemostasis and fibrinolysis. In the present study, plasma levels of catecholamines as well as hemostatic and fibrinolytic parameters at rest (before the cold pressor test) were similar in both treatments. It is well noted that stress induces platelet activation (3), although the precise mechanism has not been fully elucidated. A change in regional blood flow that affects shear stress, neurohormonal factors, and vasoactive substances is thought to contribute to platelet activation (20, 21). While some clinical studies have demonstrated an anti-platelet effect of L-type calcium channel blockers (22), the beneficial effect of these agents under condition of stress-induced platelet activation has not been fully examined. In the present study, cilnidipine attenuated platelet activation in the cold pressor test more pronouncedly than amlodipine. In both treatments, changes in hemodynamic variables (blood pressure and cardiac output) were similar, so that changes in regional blood flow might not account for the difference in degree of platelet activation between the two treatments. Catecholamines induce platelet activation via α1-adrenoreceptors on the platelet membrane (23). The suppressed elevation of plasma NEP level by cilnidipine treatment may have contributed to the attenuation of platelet activation in the cold pressor test.

Cold pressor stress increases blood pressure and peripheral resistance, mainly through sympathetic activation (24). In the present study, while the increase of plasma NEP level after the cold pressor test was greater by amlodipine than by cilnidipine treatment, blood pressure was elevated similarly by both treatments. The present data could not explain this apparently paradoxical phenomenon. We propose two possible explanations.

1. Other vasoactive substances such as angiotensin II (25) may contribute to the increase in blood pressure.
2. The method used for blood pressure measurement—automatic arterial sound recording once before and once after the cold pressor test—may not have been sensitive enough to detect slight differences in blood pressure.

**Clinical Implications and Limitations**

Hemostatic and fibrinolytic abnormalities contribute to the progression of cardiovascular diseases (26), and may also trigger stress-induced onset of cardiovascular accidents (1). Our finding that cilnidipine attenuated stress-induced platelet activation suggests the possibility that cilnidipine plays roles in the prevention of cardiovascular accidents via N-type channel blockade. However, the greater attenuation by cilnidipine was apparent only under an “unnatural” stress condition. Further studies are required to evaluate whether the potency of N-type channel blockade by cilnidipine is clinically beneficial in the management of hypertension. On the other hand, it must also be examined whether this effect augments a prohemorrhagic state, which would clearly constitute a disadvantage (27).

L-type calcium channel blockers that do not induce N-type channel blockade have been shown to have an antiplatelet effect (22). On the other hand, other studies have shown that such channel blockers do not exert an antiplatelet effect (28, 29). Since platelet membrane lacks a voltage-dependent calcium channel, the precise mechanism of the antiplatelet effect remains unclear. Change in shear stress because of vasodilatation, neurohormonal factors, and vasoactive substances are thought to contribute to this effect (22). Some studies have suggested that L-type calcium channel blockers inhibit phosphodiesterase activities (30, 31). Such an effect may increase the cyclic AMP level in platelets, thereby contributing an antiplatelet effect. In addition, it has been suggested that the nitric oxide-cyclic GMP process in platelets contributes to the antiplatelet effect of amlodipine (32). In the present study, platelet levels of cyclic AMP and cyclic GMP were similar in the two treatment groups, and it was shown that neither phosphodiesterase activity nor the nitric oxide-cyclic GMP process contributed to the difference in antiplatelet effects between the two drugs. Thus, while the present study suggests that suppressed sympathetic activation during the cold pressor test may contribute to the attenuation of platelet activation during cilnidipine treatment, the precise mechanism of this attenuation remains to be clarified.

In conclusion, when administered at a clinical dosage, cilnidipine induced greater attenuation of the activation of platelet function in response to cold pressor stress than did amlodipine. Attenuation of the activation of the sympathetic nervous system via N-type calcium channel blockade may have been one of the mechanisms involved.

**References**


