Effect of Oral Nimodipine on Platelet Function

William M. Feinberg, MD, and Denise C. Bruck, AAS

Background and Purpose: Nimodipine, a calcium antagonist, has been reported to have beneficial effects in acute ischemic infarction. Some calcium channel antagonists have antiplatelet effects. We investigated the effect of oral nimodipine on platelet function in healthy volunteers.

Methods: Twelve healthy volunteers (6 men and 6 women, mean age 32.9±5.6 years) took 30 mg nimodipine every 6 hours for 24 hours, followed by a week with no medication, followed by 60 mg every 6 hours for 24 hours. Ex vivo platelet function was measured at baseline, 1 hour after the first dose at each dosage strength, and 1 hour after the last dose at each dosage. Platelet studies included aggregation and adenosine triphosphate release in response to collagen, epinephrine, and adenosine diphosphate; maximal rate of primary aggregation; threshold adenosine diphosphate concentration for second-phase aggregation; and thromboxane B2 release at threshold aggregation. The bleeding time was measured at baseline and after the last 60-mg dose of nimodipine.

Results: No change in any platelet function study was seen with 30 mg nimodipine every 6 hours. Platelet function studies were also unchanged after 60 mg every 6 hours, except for a slight decrease in aggregation and adenosine triphosphate release in response to suprathreshold (10 μM) adenosine diphosphate (p=0.001, Student's paired t test). There was no significant change in bleeding times.

Conclusions: Oral nimodipine has minimal antiplatelet activity in young, healthy subjects. (Stroke 1993;24:10–13)

Key Words • calcium channel blockers • nimodipine • platelet aggregation

In recent years calcium antagonists have been proposed as therapy for cerebrovascular disease.1–8 The properties of calcium antagonists that may be beneficial in cerebrovascular disease include vasodilation of cerebral vessels,8 and reduction of Ca2+ entry into neurons.1,3,8 Nimodipine, a dihydropyridine derivative, has been approved in the United States for treatment of ischemia after subarachnoid hemorrhage.8,9 A beneficial effect of nimodipine has been suggested in acute ischemic stroke, although clinical trials have produced conflicting results.1,2,4,5,7,8 A recent meta-analysis of the acute infarction trials suggested a beneficial effect only if treatment with nimodipine was begun within 12 hours of onset of ischemia.6 Calcium antagonists have also been noted to have antiplatelet effects,10–15 a property that might be important in the treatment of cerebral ischemia. The antiplatelet properties appear to vary among differing calcium antagonists.12,14,15 Calcium antagonists may inhibit ex vivo platelet aggregation in response to collagen, epinephrine, and serotonin and cause mild prolongation of the bleeding time.10–13,16 Because nimodipine's antiplatelet activity has not been extensively studied, we examined the effect of nimodipine on platelet function in a group of normal subjects.

Subiects and Methods

Twelve healthy volunteer subjects, 6 men and 6 women, participated in this study. The mean±SD age was 32.9±5.6 (range, 24–44) years. The subjects were instructed not to take any other medication for 2 weeks before the start of the study or during the 9 days of the study. They were specifically questioned about aspirin-containing compounds and nonsteroidal anti-inflammatory drugs. Informed consent was obtained from all subjects.

Baseline laboratory tests (chemistry panel, complete blood count, platelet count, and pregnancy test) were used to rule out underlying disease or pregnancy. A bleeding time test was performed using a Simplate II device (Organon Teknika, Durham, N.C.) before ingestion of any drug and 1 hour after the ingestion of the final dose.

During the initial study period, subjects took 30 mg nimodipine (Miles Inc., West Haven, Conn.) every 6 hours for a 24-hour period (120 mg/day). Blood for platelet aggregation and adenosine triphosphate release was drawn in the morning before the first dose and after a low-fat breakfast (first baseline sample). Blood was obtained 1 hour after the first dose, and again the next morning 1 hour after ingestion of the last dose of nimodipine.

The subjects then took no medications for 1 week, followed by 60 mg nimodipine taken every 6 hours for a 24-hour period (240 mg/day). Blood for platelet aggregation studies and ATP release was again obtained before the first 60-mg dose of nimodipine (second baseline sample), 1 hour after the first dose, and 1 hour after the final dose. A second bleeding time test was performed 1 hour after the last 60-mg dose of nimodipine.
TABLE 1. Percent Platelet Aggregation (Primary and Secondary Phases) in Response to Selected Agonists at Baseline and After Oral Administration of Nimodipine

<table>
<thead>
<tr>
<th>Agonist</th>
<th>First baseline</th>
<th>30 mg every 6 hours</th>
<th>60 mg every 6 hours</th>
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<tbody>
<tr>
<td></td>
<td>1 hour*</td>
<td>24 hours*</td>
<td>1 hour*</td>
</tr>
<tr>
<td>Collagen</td>
<td>91±5</td>
<td>89±5</td>
<td>92±3</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>79±13</td>
<td>73±12</td>
<td>77±11</td>
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<tr>
<td>ADP</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1.25 μM</td>
<td>57±11</td>
<td>58±13</td>
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<td>2.5 μM</td>
<td>72±8</td>
<td>74±10</td>
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<td>5.0 μM</td>
<td>83±7</td>
<td>81±6</td>
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</tr>
<tr>
<td>10 μM</td>
<td>87±5</td>
<td>83±5</td>
<td>90±3</td>
</tr>
</tbody>
</table>

Values (%) are mean±SE.

*Blood for platelet studies was obtained 1 hour after nimodipine dose.

†p<0.05 different from second baseline by paired t test.

Using a 21G butterfly needle, 22.5 ml blood was drawn into a syringe containing 2.5 ml 3.8% sodium citrate anticoagulant. Platelet-rich plasma was prepared and adjusted with platelet poor plasma to 2x10^9/mm^3 platelets. A model 660 Dual-Chamber platelet ionized calcium aggregometer was used. Agonists used were thrombin, collagen, adenosine diphosphate (ADP), and epinephrine. Chrono-Lume reagent was used to determine the ATP release. All reagents and the platelet aggregometer were products of Chrono-Log (Havertown, Pa.).

For platelet aggregation studies, 450-μl aliquots of platelet-rich plasma were pipetted into siliconized glass cuvettes. Individual cuvettes were allowed to warm to 37°C for at least 2 minutes but not more than 5 minutes. Fifty microliters of Chrono-Lume was added to the cuvette in the mixing chamber and allowed to equilibrate for 1 minute before the addition of the agonist. Final cuvette concentrations were as follows: thrombin 1 IU/ml, collagen 2 μg/ml, epinephrine 10 μM, and ADP 1.25, 2.5, 5, and (if time and sample permitted) 10 μM. Percent aggregation and ATP release were measured at each agonist concentration. All testing was completed within 2 hours. The maximal rate of primary aggregation in response to 2.5 and 5 μM ADP was calculated in terms of light transmittance units per minute at the steepest part of the primary aggregation curve, as described by Thompson and Vickers.17 Threshold for second-phase aggregation was defined as the ADP concentration required to produce irreversible aggregation accompanied by ATP release. ATP release of the samples was compared with the 2-nmol standard provided with the Chrono-Lume reagent.

Thromboxane B2 (TXB2) release was determined at the threshold ADP concentration. After addition of the ADP, the platelets were allowed to react for 5 minutes. A control sample, to which no ADP was added, was incubated under the same conditions in the other chamber. Both supernatants were quickly obtained by spinning in a microcentrifuge, then freezing at -20°C. TXB2 levels were measured using an enzyme-linked immunosorbent assay from Cayman Chemical (Ann Arbor, Mich.), according to manufacturer’s instructions. Briefly, plastic microtiter plates were coated with mouse monoclonal anti-rabbit immunoglobulin G. Dilutions of the standard provided in the kit and dilutions of samples were added to the plates. Acetycholinesterase tracer and thromboxane antiserum were added to the wells and allowed to incubate overnight at 4°C. After the excess sample and reagents were washed out, Ellman’s reagent was added to each well and incubated to allow color development. The color was read on a microtiter plate reader (Titertek, McLean, Va.). Standard readings were plotted against their concentrations, and the resultant curve was used to calculate sample concentrations. Units were picograms per milliliter and were converted to nanograms per 10^9 platelets.

Platelet aggregation studies were performed in control subjects (n=3) before and after aspirin ingestion. Aspirin abolished platelet aggregation in response to collagen and epinephrine and second-phase aggregation in response to ADP in all controls.

Platelet aggregation and ATP release (in response to the above agonists) at each dosage strength of nimodipine were compared with baseline values using Student’s two-tailed paired t test. Bleeding times at baseline and after 60 mg nimodipine were also compared using Student’s two-tailed paired t test. Primary aggregation rates in response to ADP were also compared with baseline using Student’s two-tailed paired t test. Threshold ADP concentration at each dosage strength was compared with baseline using the Wilcoxon signed rank test. Significance was sought at the α=0.05 level.

Results

Baseline blood tests revealed no underlying disease or pregnancy. The mean platelet count was 2.56±0.57x10^11/mm^3 platelets (range, 1.51-3.72x10^11/mm^3; laboratory normal range, 1.5-3.5x10^11/mm^3 platelets).

Percent aggregation in response to collagen, epinephrine, and ADP at each time period and nimodipine dosage is shown in Table 1. There were no significant differences noted at any time at 120 mg/day. At 240 mg/day, there was a minor decrease in aggregation in response to 10 μM ADP after the first 60-mg dose (p=0.038) but not after 24 hours. There was no significant change in the maximum primary aggregation rate in response to 2.5 or 5 μM ADP at either nimodipine dosage.

ATP release at each time period and dosage strength is shown in Table 2. No significant differences were noted at a dose of 120 mg/day. ATP release in response to 10 μM ADP at 1 hour after the first 60-mg dose of nimodipine showed a significant decline from baseline (0.50±0.08 nmol versus 0.28±0.05 nmol; p=0.001). At 24 hours, after the fourth 60-mg dose of nimodipine, this appeared to recover slightly but was still signifi-
TABLE 2. ATP Release in Response to Selected Agonists After Oral Administration of Nimodipine

<table>
<thead>
<tr>
<th>Agonist</th>
<th>First baseline</th>
<th>30 mg every 6 hours</th>
<th>60 mg every 6 hours</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1 hour*</td>
<td>24 hours*</td>
</tr>
<tr>
<td>Collagen</td>
<td>0.95±0.10</td>
<td>0.97±0.12</td>
<td>0.90±0.10</td>
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<tr>
<td>Epinephrine</td>
<td>0.55±0.13</td>
<td>0.53±0.12</td>
<td>0.49±0.15</td>
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<tr>
<td>ADP 1.25 µM</td>
<td>0.11±0.03</td>
<td>0.16±0.09</td>
<td>0.13±0.05</td>
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<tr>
<td>ADP 2.5 µM</td>
<td>0.26±0.08</td>
<td>0.18±0.05</td>
<td>0.29±0.11</td>
</tr>
<tr>
<td>ADP 5.0 µM</td>
<td>0.32±0.07</td>
<td>0.37±0.08</td>
<td>0.37±0.07</td>
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<tr>
<td>ADP 10 µM</td>
<td>0.24±0.08</td>
<td>0.30±0.08</td>
<td>0.27±0.08</td>
</tr>
</tbody>
</table>

Values for ATP release (nmol) are mean±SE.
*Blood for platelet studies was obtained 1 hour after nimodipine dose.
†p<0.02 different from first baseline by paired t test.
‡p<0.05 different from second baseline by paired t test.

significantly different from baseline (0.50±0.08 nmol versus 0.29±0.06 nmol; p=0.008) (Figure 1).

Threshold ADP concentration for second-phase platelet aggregation is shown in Figure 2. After 24 hours of 60-mg doses of nimodipine, threshold was increased in three subjects, decreased in one, and unchanged in six. These differences were not statistically significant. Threshold dose was not significantly different from baseline at any time period or nimodipine dosage.

Levels of TXB₂ are shown in Table 3. A slight increase in TXB₂ release was noted in the second versus the first baseline samples (9.5±2.4 versus 7.5±1.9 ng/10⁷ platelets; p=0.028). No other significant differences at any time period or dosage were seen.

Bleeding time after 24 hours of 60-mg doses of nimodipine was actually slightly shorter than at baseline (3.92±1.06 min versus 4.33±1.26 min). This difference was not statistically significant.

Discussion

These results demonstrate no significant effect of oral nimodipine at a dose of 120 mg/day on the ex vivo tests of platelet function or on bleeding time. At a dose of 240 mg/day, no significant effect was seen on collagen- or epinephrine-induced platelet aggregation, threshold for second-phase aggregation, or bleeding time. These data are consistent with animal studies showing no significant effect on ADP-induced aggregation by nimodipine in cat platelets.¹⁸

Only minor effects on platelet function were seen at a dose of 240 mg/day. There was a slight decrease in ADP-induced ATP release seen only with a supra-threshold (10 µM) concentration of ADP after the first 60-mg nimodipine dose when compared with the second baseline value. Of note, the second baseline value for ADP release showed a slight increase from the first baseline value; if ADP release in response to 10 µM is compared with the original baseline, there is no significant decrease (Table 2).

Because of the small number of patients in this study, we may have missed a small but statistically significant effect on platelet function. However, we do not feel that we have missed a clinically significant effect. Clinical trials have reported a beneficial effect of nimodipine at a dose of 120 mg/day,²⁶,⁷ and we saw no evidence of any antiplatelet effect at this dose.

Several caveats apply to this study. The effect of nimodipine ex vivo may be different from that in vivo.

FIGURE 1. Adenosine triphosphate (ATP) release (filled circles) and percent aggregation (filled squares) in response to 10 µM adenosine diphosphate (ADP) at baseline and after administration of 240 mg/day nimodipine. Blood for platelet studies was obtained 1 hour after first and fourth doses.

FIGURE 2. Threshold adenosine diphosphate (ADP) concentration for second-phase aggregation at baseline and after oral administration of 240 mg/day nimodipine. Blood for platelet studies was obtained 1 hour after first and fourth doses.
Other calcium channel blockers have been shown to have in vivo antiplatelet effects. Verapamil and nifedipine inhibit deposition of autologous platelets onto implanted Gore-Tex grafts in dogs. Verapamil infusion has also been reported to decrease circulating platelet aggregates in patients with coronary artery disease. However, in a cat global ischemia model, nifedipine had no effect on plasma TXB2 concentrations; this finding also supports nifedipine’s lack of in vivo effect in ischemia.

The effect of nifedipine on platelets in normal, healthy, young control subjects may be different from that in older patients with active vascular disease. For instance, platelets in patients with myocardial infarction are larger and show reduced sensitivity to aspirin. Thus, although we found no significant antiplatelet activity in young control subjects, it is possible that an antiplatelet effect might exist in older patients or those with acute cerebral ischemia. Despite the negative results of our study, it would be valuable to examine the antiplatelet effect of nifedipine in stroke patients.

Some studies have demonstrated a synergistic effect between calcium antagonists and aspirin. We chose to examine the effects of nifedipine alone because several of the major acute stroke treatment trials have specifically prohibited the use of aspirin or other antiplatelet agents. It is possible that nifedipine may have an additive antiplatelet effect if used with aspirin.

We studied platelet activity after 24 hours of drug administration. Although a number of studies have demonstrated antiplatelet activity after a single dose of calcium antagonist, Jones et al found no antiplatelet activity 2 hours after a single dose of verapamil but found inhibition of ex vivo epinephrine-induced aggregation after 4 days of therapy. Juvela et al found no antiplatelet effect during the first 5 days of intravenous nifedipine treatment in patients with subarachnoid hemorrhage but found inhibition of platelet thromboxane release thereafter. Future studies might look at a more prolonged administration of nifedipine. The slight increase in TXB2 and ATP release in response to 10 μM ADP that we found on the second baseline might suggest a rebound effect. Future studies might also look more closely at the recovery period after discontinuance of nifedipine.

In summary, our results show minimal antiplatelet effect of oral nifedipine in young, healthy subjects. No antiplatelet effect was seen at a dose of 120 mg/day, the dose reported to have a beneficial effect in acute stroke. These results do not support the hypothesis that nifedipine exerts an effect on cerebral ischemia through an inhibitory effect on platelet activity. These studies should be confirmed in older persons and in patients with acute cerebral ischemia.

Table 3. Thromboxane B2 Release at Baseline and After Oral Administration of Nimodipine

<table>
<thead>
<tr>
<th></th>
<th>30 mg every 6 hours</th>
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<th>60 mg every 6 hours</th>
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<tbody>
<tr>
<td>First baseline</td>
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<td>Second baseline</td>
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<tr>
<td></td>
<td>6.7±2.0</td>
<td>7.4±2.3</td>
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</table>

Values (ng/10⁶ platelets) are mean±SE. Thromboxane B2 release was measured in response to the threshold adenosine diphosphate concentration for second-phase aggregation.

*Blood for platelet studies was obtained 1 hour after nimodipine dose.

References

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