Captopril Administration Reduces Thrombus Formation and Surface Expression of Platelet Glycoprotein IIb/IIIa in Early Postmyocardial Infarction Stage

María J. Zurbano, Ignasi Anguera, Magda Heras, Eulàlia Roig, Miguel Lozano, Ginés Sanz, Ginés Escolar

Abstract—Long-term administration of the angiotensin-converting enzyme inhibitor captopril in survivors of myocardial infarction (MI) reduces the risk of cardiovascular death, recurrence of MI, and unstable angina, suggesting that captopril may possess antithrombotic properties that have not been clearly elucidated. We assessed the short-term antithrombotic effects of captopril on platelet aggregation, platelet-subendothelium interaction, and the expression of major glycoproteins on platelet surface. A double-blind study was carried out in 25 patients with MI. Blood samples were taken before (baseline) and 12 days after treatment in both the control and captopril groups. Platelet aggregation was tested by conventional aggregometry using common activating agents. Platelet interaction with deeply damaged subendothelial surface was evaluated in a perfusion model, with blood maintained under flow conditions. Deposition of platelets was quantified by using computer-assisted morphometric techniques on histological sections, and it was expressed as a percentage of total vessel surface covered by platelets (CS) and as a ratio between large aggregates (T) and surface covered by platelets (100 × T/CS). Glycoprotein expression was measured using flow cytometric techniques. Aggregometric responses showed no significant variations; however, in the captopril group, 100 × T/CS decreased after 12 days of treatment (100 × T/CS: 36 ± 12.1% captopril versus 64 ± 8.0% baseline; \( P < 0.05 \)). This parameter was also significantly decreased from that found in control group patients (100 × T/CS: 67 ± 4.5%; \( P < 0.008 \)). Flow cytometry showed a 30% reduction in glycoprotein IIb/IIIa expression (\( P < 0.02 \)). Captopril reduced the formation of large aggregates in a perfusion system, which might be related to a down-regulation of glycoprotein IIb/IIIa complex on the platelet surface. These results suggest that captopril exerts an antiplatelet effect that may contribute to its beneficial action in MI. (Arterioscler Thromb Vasc Biol. 1999;19:1791-1795.)

Key Words: captopril • platelets • vessel wall • flow cytometry

Angiotensin-converting enzyme inhibitors (ACEIs) reportedly reduce the rate of recurrent myocardial infarction (MI), sudden death, development of unstable angina, and the need for revascularization in cardiovascular disease.\(^1,2\)

These drugs can change the hemodynamic parameters in patients surviving MI by both reducing blood pressure and remodeling the left ventricle, which will lead to a reduction in ventricular wall stress.\(^3\) ACEIs may decrease coronary vasoconstrictor influences by inhibiting the production of angiotensin II and suppressing the degradation of the vasodilator bradykinin.\(^4\) These inhibitors might also increase the production of nitric oxide, prostacyclin, and tissue plasminogen activator and reduce plasminogen activator inhibitor-I activity.\(^5-11\)

Recent observations raise the possibility that angiotensin II may be involved in the thrombotic process. Reportedly, the infusion of large amounts of angiotensin II leads to MI.\(^12\) Angiotensin II can adversely affect the balance between increased cardiac oxygen demand and supply by either a direct coronary vasoconstrictor effect or increased inotropy by using its ability to raise cytosolic Ca\(^{2+}\) concentrations in the myocardium. Inhibition of the angiotensin system might have a beneficial effect in acute coronary syndromes.

In the present study, we investigated the possibility that the antithrombotic effects of captopril could be mediated through an inhibitory action on platelet function. Thus, we analyzed modifications in platelet aggregometry and platelet-subendothelium interactions in a group of patients subjected to treatment with captopril (37.5 to 75 mg/d). Modifications in major glycoproteins were monitored by flow cytometry in a subgroup of the study population.

Methods

Patients
A total of 25 patients experiencing a Q-wave MI were evaluated. They ranged in age from 45 to 84 years (mean, 60 ± 7 years) and
TABLE 1. Baseline Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control (n=14)</th>
<th>Captopril (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, yr</td>
<td>58</td>
<td>61</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>78</td>
<td>100</td>
</tr>
<tr>
<td>Clinical history, % of patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>57</td>
<td>63</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Hypertension</td>
<td>64</td>
<td>45</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>21</td>
<td>36</td>
</tr>
<tr>
<td>Stroke</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Previous MI</td>
<td>38</td>
<td>35</td>
</tr>
<tr>
<td>Infarct location, % of patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>Inferior</td>
<td>78</td>
<td>81</td>
</tr>
<tr>
<td>Thrombolysis, % of patients</td>
<td>65</td>
<td>90</td>
</tr>
<tr>
<td>Concomitant use of (\beta)-blockers, % of patients</td>
<td>42</td>
<td>63</td>
</tr>
<tr>
<td>Creatine kinase, U/L</td>
<td>2037±440</td>
<td>2239±425</td>
</tr>
<tr>
<td>Creatine kinase-MB fraction, U/L</td>
<td>171±32</td>
<td>185±27</td>
</tr>
</tbody>
</table>

were admitted to the coronary care unit of the Hospital Clinic of Barcelona with acute MI. A diagnosis of MI was established if no abnormal Q waves (30 ms) appeared in at least 2 adjacent leads and if 1 of the following criteria was also present: chest pain lasting 30 minutes or an increase in the serum creatine kinase MB fraction. Acetylsalicylic acid (125 mg/d) was administered to all patients. Exclusion criteria were (1) secondary coagulation abnormalities associated with the condition and/or known altered platelet function diseases (liver, kidney, or hematologic diseases) and (2) treatment required with antplatelet agents other than aspirin, with other drugs that might have a negative effect on platelet function (such as nitroglycerin, heparin, and ticlopidine), or with angiotensin-converting enzyme inhibitors, because of the presence of congestive heart failure. Patients with intraventricular thrombus, reinfarction, postinfarction angina, or those undergoing coronary bypass surgery were also excluded.

Study Design

Patients were admitted to the coronary care unit and received treatment with aspirin, thrombolytic agents, vasodilators, or \(\beta\)-blockers, according to standard guidelines of management of acute MI. The baseline demographic characteristics of the patients are summarized in Table 1.

Perfusion and aggregating studies were performed before and after the treatment was initiated. Platelet glycoprotein (GP) studies were also performed in some patient blood samples. Patients were randomized to either the captopril (37.5 to 75 mg/d) or control group. The dose of captopril was the highest that the patient could tolerate. Patients were supine for 60 minutes before venipuncture. Blood was taken from the antecubital vein and collected in acid-citrate-dextrose (final concentration, 19 mmol/L).

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Institutional review board approval was obtained, and all patients gave written informed consent. All the procedures were performed in accordance with our institutional guidelines.

Aggregation Studies

An aliquot of the sample (10 mL) was used to prepare platelet-rich plasma (PRP). The capability of PRP to aggregate was tested by conventional turbidimetric procedures. 13 The following agonists were used in all the experiments in this study. Aortic segments were mounted on plastic rods and rinsed with PBS for 10 minutes. After 10 minutes of perfusion with the blood samples, segments were rinsed with the same phosphate buffer and fixed with 3% glutaraldehyde. After 1 hour, segments were separated from the rod, washed with PBS, dehydrated through a graded series of ethanol, embedded in JB-4 embedding material (Polysciences), thin-sectioned for light microscopy (3 \(\mu\)m), and stained with methylene blue.

Morphometric Evaluation

Platelet interactions with subendothelium were morphometrically evaluated using a computerized image-analysis system. 16 The morphometric parameters were determined using the criteria previously established by Baumgartner. 14 Aggregated platelets were classified as 1 of the following: contact (percentage of platelets attached to but not spread on the subendothelium); adhesion (percentage of platelets spread on the subendothelial surface, including small aggregates \(<5 \mu\)m in height); or thrombi (T; percentage of platelet aggregates \(\geq 5 \mu\)m in height). All these parameters were expressed as a percentage of the total surface examined. Another parameter related to those previously defined is the total covered surface (CS), which was determined by adding the previous parameters (contact, adhesion, and thrombi). The rate of thrombus formation (100 \(
\times \)CS/TS) was calculated from the former parameters and used as an estimate of platelet-platelet interaction.

Platelet Glycoprotein Studies

Platelet immunolabeling with monoclonal antibodies conjugated with either phcoeritrin (PE) or fluorescein (FITC) was performed in whole blood using dual-color analysis. 17,18 Briefly, 5 mL of whole blood collected in citrate-phosphate-dextrose with paraformaldehyde (final concentration, 0.3%) was added to polypolyene tubes preloaded with 50 mL PBS. The following monoclonal antibodies were used for labeling (all from Immunotech): anti-CD41-PE clone P2 (GP Ib/IIa), anti-CD42b-FITC (GP Ibα, clone SE2), and anti-CD36-FITC (GP IV, clone FA6.152). Samples for dual-color analysis were first incubated with saturating concentrations of anti–CD41a-PE in the dark, without stirring, for 15 minutes at room temperature; this was followed by the addition of FITC-conjugated monoclonal antibodies and further incubation for 15 minutes in the dark. Samples were then diluted with 1 mL of PBS for analysis.

Blood samples were evaluated with a FACScan flow cytometer (Becton-Dickinson) at an excitation wavelength of 488 nm. Fluorescence and scatter signals were calibrated with 2 Tm Calibrite beads (Becton-Dickinson) at an excitation wavelength of 488 nm. Fluorescence data obtained in the logarithmic mode from 5000 events were analyzed. Blood fluorescence intensities corresponding to antibody binding (GP Ib, GP Ib/IIa, and GP IIb/IIIa) and thromboxane-endoperoxide analogue (Upjohn), 5 \(\mu\)g/mL collagen, 10 \(\mu\)mol/L epinephrine, and 1 mg/mL ristocetin (all from Menarini).

Perfusion Studies

A second aliquot of the sample (25 mL) was used to perform perfusion studies. Perfusions were performed at 37°C in perfusion chambers, as developed by Baumgartner, 14 with an effective annular width of 2.2 mm and a rod length of 72 mm. Flow was obtained by pumping blood through a peristaltic pump, and perfusion lasted for 10 minutes at 140 mL/min (the wall shear rate was 800 s \(^{-1}\), which is similar to that found in normal coronary arteries). 15 a-Chymotrypsin–denuded rabbit aortas were used in all the experiments in this study. Aortic segments were mounted on plastic rods and rinsed with PBS for 10 minutes. After 10 minutes of perfusion with the blood samples, segments were rinsed with the same phosphate buffer and fixed with 3% glutaraldehyde. After 1 hour, segments were separated from the rod, washed with PBS, dehydrated through a graded series of ethanol, embedded in JB-4 embedding material (Polysciences), thin-sectioned for light microscopy (3 \(\mu\)m), and stained with methylene blue.

Statistical Analysis

An unpaired Student’s \(t\) test was used for statistical comparisons. The results are expressed as mean±SEM. \(P \leq 0.05\) was considered statistically significant.
TABLE 2. Maximal Aggregation Values

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Captopril</th>
<th>Control</th>
<th>Captopril</th>
<th>Baseline</th>
<th>ASA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachidonic acid (1.4 mmol/L)</td>
<td>15±1</td>
<td>12±7</td>
<td>17±7</td>
<td>24±3</td>
<td>95±2</td>
<td>10±2</td>
</tr>
<tr>
<td>Collagen (5 μg/mL)</td>
<td>47±2</td>
<td>47±2</td>
<td>44±2</td>
<td>40±2</td>
<td>90±1</td>
<td>50±1</td>
</tr>
<tr>
<td>ADP (4 μmol/L)</td>
<td>83±1</td>
<td>75±1</td>
<td>82±9</td>
<td>78±5</td>
<td>90±1</td>
<td>85±1</td>
</tr>
<tr>
<td>U46619 (1 μmol/L)</td>
<td>90±1</td>
<td>86±1</td>
<td>84±1</td>
<td>85±8</td>
<td>95±2</td>
<td>95±2</td>
</tr>
<tr>
<td>Ristocetin (1 mg/mL)</td>
<td>68±2</td>
<td>67±2</td>
<td>75±2</td>
<td>69±3</td>
<td>61±1</td>
<td>61±1</td>
</tr>
<tr>
<td>Epinephrine (10 μmol/L)</td>
<td>70±5</td>
<td>68±3</td>
<td>71±1</td>
<td>69±4</td>
<td>75±2</td>
<td>75±2</td>
</tr>
</tbody>
</table>

Values are mean±SEM and expressed as percentages. Data obtained from control and captopril-treated patients. Normal values were obtained from a normal healthy population. ASA indicates acetyl salicylic acid; and U46619, synthetic thromboxane-endoperoxide analogue.

Results

No significant differences existed in the clinical characteristics of patients treated with captopril and those in the control group.

Platelet Aggregation Studies

In baseline tests, the sensitivity of platelets to some of the routine aggregating agents used was diminished when compared with normal values (Table 2). The magnitude of this decreased platelet response was similar to that commonly observed in blood samples obtained from patients treated with aspirin.19

Aggregometric tracings remained unmodified after 12 days in both the control and captopril groups (Table 2). Moreover, neither among the baseline and follow-up studies for each group nor among groups in the follow-up study were statistically significant differences found.

Interaction of Platelets With the Subendothelium

Captopril reduced the formation of large aggregates after 12 days of treatment. In the captopril group, a 56% reduction of thrombi formation existed when values obtained after 12 days were compared with those obtained at baseline conditions (64±8.0% before treatment versus 36±12.1% after treatment; P=0.05). Conversely, the control group did not show any significant change after 12 days (56±5.8% before treatment versus 67±4.5% after 12 days) (Figures 1 and 2).

Furthermore, after 12 days of treatment with captopril, a 53% reduction in thrombi-covered surfaces existed when results were compared with those obtained in the control group using the same follow-up tests (67±4.5% control versus 36±12.1% captopril; P=0.008) (Figures 1 and 2).

Total surface covered by platelets was similar in both groups at baseline conditions (18.8±2.2% control versus 22.7±3.4% captopril). After 12 days, no significant changes existed in this morphometric parameter between the control and captopril groups (22±1.8% control versus 19.8±1.8% captopril) (Figures 1 and 2).

Surface Expression of Platelet Glycoproteins

No relevant changes were noted in the expression of major glycoproteins in platelets from patients in the control group. In the group treated with captopril, no significant variations existed in mean fluorescence intensities for CD42b (GP Ibα) or CD36 (GP IV), before (50±7.6% and 99.9±11.2%, respectively) or after treatment (51.2±3.5% and 98.3±19.8%, respectively). However, a statistically significant decrease existed in the binding of monoclonal antibodies directed against CD41 (GP IIb/IIIa) after 12 days of treatment with captopril (119±28.27 before versus 77±19.4 after treatment; P=0.02). Figure 3 shows the percentage of reduction in GP IIb/IIIa expression in the captopril group.

Discussion

We investigated the possible antiplatelet effects of the ACEI captopril in patients with MI. Results of this study indicate that captopril reduces the formation of large aggregates when blood is exposed to a thrombogenic vascular surface under flow conditions. Flow cytometry studies revealed a concomitant decrease in the expression of platelet GP IIb/IIIa in these patients. These results suggest that administering captopril to patients with MI may cause additional antiplatelet effects, which could explain its beneficial effects in cardiovascular events.

Figure 1. Bar diagram expressing percentages of vessel surface covered by platelets in perfusion experiments performed with blood obtained from patients in control or captopril groups at baseline conditions and after 12 days of treatment. The open portion of bar corresponds to surface covered by adhesive platelets (adhesion), and dashed segments indicate surface covered by groups of platelets forming aggregates >5 μm in height (thrombi). Figures given inside bars correspond to rates of thrombus formation, which are estimates of platelet-platelet interactions occurring on initially spread platelets. Rates of thrombus formation decreased significantly in group treated with captopril. Observed differences reached levels of statistical significance with respect to control group (P=0.05, n=14) and with respect to same captopril group at baseline conditions (P=0.008, n=11). Values are expressed as mean±SEM.
Captopril is used in the treatment of hypertension and congestive heart failure. Several clinical trials have shown an improvement in exercise capability and life quality in patients taking it. Moreover, the administration of ACEIs in patients who have suffered MI reduces cardiovascular mortality and reinfarction.

The present study investigated the antiplatelet effects of captopril after its oral administration in patients who had suffered a first MI. Our routine aggregometric studies were unable to demonstrate important modifications in the aggregometric patterns other than those that could be ascribed to the standard treatment with acetylsalicylic acid (ASA), which is recommended for these patients.

Data from perfusion studies performed with blood from the same patients showed a statistically significant reduction (56%) in the morphometric parameters that quantify the building of large aggregates onto previously spread platelets (100×T/CS) without affecting the overall ability of platelets to interact with the subendothelium (CS). These results are consistent with the idea that captopril treatment could interfere with mechanisms mediating platelet-platelet interactions.

Discrepancies between the effects of captopril in aggregation versus perfusion studies could be explained by the different conditions of the tests. The shear rate is high in perfusion studies and low in standard aggregometric procedures. Moreover, the rheological effects of red blood cells are present in the perfusion system and absent in standard aggregometric procedures, which were performed in PRP. Red blood cells have a dramatic impact on platelet diffusivity, which is the rapidity with which platelets move perpendicular to flow streamlines. The rate of platelet deposition is markedly influenced by the presence of red blood cells. Baumgartner and Muggli reported a 50-fold increase in platelet deposition when red blood cells were present. Moreover, the administration of ACEIs in patients with acute MI reduces levels of free circulating tissue factor. Experimental studies by the same group suggest that there would be a reduction in the expression of tissue factor on monocytes and smooth muscle cells in human coronary artery injuries. Exposure of tissue factor at sites of vascular damage increases local thrombin generation. Thrombin is a powerful aggregating agent for platelets, and even at very low concentrations. Moreover, several studies have shown that treatment with captopril can reduce catecholamine production. Increased levels of catecholamines potentiate platelet aggregation induced by several agents and can reverse the antiplatelet effects of ASA. Platelet exposition to excessive circulating levels of catecholamines or to trace amounts of thrombin might increase the expression of GP IIb/IIIa. We think that the reduction of GP IIb/IIIa observed with captopril might be related to a down-regulation of the proaggregatory mechanisms (thrombin and catecholamines) and due to desensitization of the prothrombotic mechanisms subjacent in these patients. The antithrombotic effects of captopril were observed in vivo. The sole incubation of isolated platelets from normal individuals or from patients with captopril had no effects on the expression of major glycoproteins (data not shown). These observations would agree with the concept that the antiplatelet action of captopril is only noticed in vivo and that it is probably related to a general effect on vasculature.
In conclusion, the results from the present study indicate that the oral administration of captopril to patients suffering from MI reduces excessive platelet aggregate formation in an in vitro perfusion model. Such an antithrombotic effect may explain, at least in part, the beneficial action of captopril in survivors of acute cardiovascular events. The antiplatelet role observed with captopril might be common with other ACEIs. However, specific studies are required to test this hypothesis.

**Study Limitations**

In the authors’ experience, the platelet-covered surfaces measured in these perfusion studies were relatively low. In fact, in studies by our group under similar flow conditions in normal donors, covered surfaces ranged from 30% to 45%.

In addition, rates of thrombus formation (100 × T/CS) were also higher than those measured in normal donors treated with ASA. We believe that these discrepancies may be related to the following 2 facts: (1) the study was performed in patients (not in normal donors) with an undergoing thrombotic state that had probably caused the coronary event; and (2) current therapeutic protocols may interfere with our tests.

**Acknowledgments**

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**References**


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