Combination of Fosinopril and Pravastatin Decreases Platelet Response to Thrombin Receptor Agonist in Monkeys

L. Paulette Hale, Karen T. Craver, Alan M. Berrier, Matthew V. Sheffield, L. Douglas Case, John Owen

Abstract—Both angiotensin-converting enzyme (ACE) inhibitors and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors have been shown to decrease cardiovascular morbidity and mortality. Results from clinical trials have suggested that HMG-CoA reductase inhibition might exert a beneficial effect independent of its lipid-lowering effect, and ACE inhibition may exert a benefit independent of blood-pressure lowering. To test the hypothesis that such an effect might be mediated by alteration in platelet reactivity, we studied 55 monkeys receiving both, 1, or neither of the ACE inhibitor fosinopril and the HMG-CoA reductase inhibitor pravastatin. Platelet responsiveness to collagen and to the thrombin receptor agonist (TRA) SFLRRN-NH₂ was determined by aggregometry. For each agonist, the maximum rate and extent of aggregation were measured for each dose, and the concentration required for half-maximal response (C₅₀) was determined. Each drug, when given alone, slightly decreased the dose of agonist required to produce 50% response in the rate and extent of platelet aggregation relative to control. The combination of the 2 drugs, however, produced a significant increase in the dose of TRA required to produce 50% response in the rate and extent of aggregation relative to either drug alone or the control group. This was not true for collagen. The magnitude of the change relative to the control group, 47% for rate and 30% for extent of aggregation, could confer considerable protection by changing the threshold for thrombin-induced platelet aggregation and, thus, decrease thrombosis. (Arterioscler Thromb Vasc Biol. 1998;18:1643-1646.)

Key Words: angiotensin-converting enzyme inhibitors ▪ platelet aggregation ▪ 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors

Cardiovascular disease is the leading cause of death in the United States.¹ Most of the morbidity and mortality of this disease results from the rupture of atherosclerotic plaques in the vessel wall. This event leads to exposure of the thrombogenic inner components of the plaque to circulating blood, resulting in platelet activation and aggregation and activation of the coagulation system. Subsequently, a thrombus forms.² The use of angiotensin-converting enzyme (ACE) inhibitors and lipid-lowering drugs has decreased cardiovascular morbidity and mortality below that expected from the resulting mild reduction in blood pressure and minor reduction in severity of coronary stenosis.²,³,⁴ Therefore, in addition to their well-known actions, these drugs probably have other protective effects on the processes that lead to cardiovascular disease and thrombosis. It is possible that these drugs may affect platelet function.

In this randomized trial, we studied platelet responsiveness to collagen and a thrombin receptor agonist (TRA) in monkeys that were treated with an ACE inhibitor, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, both drugs, or neither drug.

Materials
The highly purified peptide TRA SFLRRN-NH₂ (PMIS387) was purchased from BACHEM California; type I collagen was purchased from Chronolog.

Animals
This research was a randomized, therapeutic study of syngeneic, atherogenesis-prone monkeys (Macaca fascicularis) that initially included 63 individuals; however, 8 were not evaluable because they expired from unrelated causes. The monkeys were treated for 2 years and fed a nutritionally complete, low-cholesterol diet. The monkeys were sedated with ketamine before venipuncture. Blood was drawn by venipuncture from the left femoral vein into 3.8% sodium citrate. This institution’s animal use and care committee reviewed and approved the study.

Platelet Preparation
Platelet-rich plasma was prepared by centrifugation of the blood at 900 rpm for 15 minutes at 20°C. Platelet counts were adjusted with platelet-poor plasma from the same monkey to 200 000 to 300 000/μL.
Platelet Aggregation

Platelet aggregation studies were performed by using standard techniques without knowledge of the specific monkey group. Each platelet-rich plasma was incubated at 37°C for 2 minutes and stirred for 1 minute at 1100 rpm in an aggregometer (PAP 4-C, Biodata). Platelets were then stimulated with a range of concentrations of the TRA (5 to 1000 μmol/L) and collagen (0.4 to 1000 μg/mL), and the optical density was recorded for 4 minutes. Serial dilutions of the agonists were tested to span the range of full aggregation to zero aggregation. For each dose of agonist used, the maximum rate and extent of aggregation were measured.

Interpretation

A logistic dose-response curve was fitted to the response data for each agonist with each monkey and was used to determine the relationships between aggregation response and agonist dose. An example of a typical dose-response curve is shown in the Figure. The fitted curves allowed the determination of the agonist concentration required to produce half-maximal response (C_{50}).

Statistical Methods

Four outcome measures are considered in this report, namely, the concentration of agonist required to produce half-maximal (ie, C_{50}) rate and extent of platelet aggregation after stimulation with either the TRA or collagen. Means and SDs for these measures were calculated separately for each treatment arm. Each outcome measure was analyzed by ANOVA. Rank transformations were used owing to some outliers and heteroscedasticity. Initial models included terms for each treatment and their interaction. When interactions were nonsignificant, only main effects were fitted in the models. When interactions were significant, pairwise contrasts were tested to assess treatment differences. For those monkeys not receiving pravastatin, the addition of fosinopril slightly, but nonsignificantly, decreased the C_{50} for both the extent (1.01 versus 0.79, P=0.3290) and rate (0.84 versus 0.71, P=0.5081) of aggregation. However, for those monkeys receiving pravastatin, the addition of fosinopril significantly increased the C_{50} for both the extent (0.92 versus 1.31, P=0.0210) and the rate (0.82 versus 1.23, P=0.0162) of aggregation.

Similarly, for those monkeys not receiving fosinopril, the addition of pravastatin had little effect on the C_{50} for either the extent (1.01 versus 0.92, P=0.7386) or rate (0.84 versus 0.82, P=0.9409) of aggregation. However, for those monkeys not receiving pravastatin, the addition of fosinopril significantly increased the C_{50} for both the extent (0.79 versus 1.31, P=0.0043) and rate (0.71 versus 1.23, P=0.0026) of aggregation.

Collagen Stimulation

In this setting, the interaction between fosinopril and pravastatin was nonsignificant for the C_{50}s for extent (P=0.6997) and rate (P=0.9024) of aggregation. In addition, neither fosinopril nor pravastatin significantly affected the C_{50} for

<table>
<thead>
<tr>
<th>Group</th>
<th>No Drug</th>
<th>Pravastatin Alone</th>
<th>Fosinopril Alone</th>
<th>Both Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>T, extent*</td>
<td>1.007 (0.523)</td>
<td>0.917 (0.389)</td>
<td>0.791 (0.254)</td>
<td>1.306 (0.543)</td>
</tr>
<tr>
<td>T, rate†</td>
<td>0.841 (0.454)</td>
<td>0.815 (0.342)</td>
<td>0.705 (0.237)</td>
<td>1.233 (0.597)</td>
</tr>
<tr>
<td>C, extent‡</td>
<td>0.216 (0.268)</td>
<td>0.199 (0.189)</td>
<td>0.146 (0.059)</td>
<td>0.151 (0.096)</td>
</tr>
<tr>
<td>C, rate‡</td>
<td>0.249 (0.309)</td>
<td>0.190 (0.176)</td>
<td>0.152 (0.062)</td>
<td>0.164 (0.089)</td>
</tr>
</tbody>
</table>

Values are means (with SDs in parentheses). T indicates TRA in μmol/L; C, collagen in μg/mL.

*Pravastatin alone vs pravastatin and fosinopril (P=0.0210), fosinopril alone vs pravastatin and fosinopril (P=0.0043), other contrasts not statistically significant.

†Pravastatin alone vs pravastatin and fosinopril (P=0.0162), fosinopril alone vs pravastatin and fosinopril (P=0.0026), other contrasts not statistically significant.

‡No significant differences between groups.
either extent or rate of aggregation. The mean C50 for extent was 0.172 and 0.188 for monkeys that did and did not receive pravastatin, respectively (P=0.7968). The mean C50 for rate was 0.175 and 0.210, respectively (P=0.7632). The mean C50 for extent was 0.149 and 0.209 for monkeys that did and did not receive fosinopril, respectively (P=0.7468). The mean respective C50 for rate was 0.159 and 0.224 (P=0.7800).

Neither fosinopril nor pravastatin, alone or in combination, had a significant effect on systolic blood pressure or total cholesterol. Interestingly, however, the monkeys that received both drugs had a significantly lower diastolic blood pressure than did those that received neither drug or either drug alone. The adjusted mean diastolic blood pressure for the group receiving both drugs was 40.4, compared with 52.3 for the group receiving neither drug (P=0.0014), 50.9 for the group receiving fosinopril alone (P=0.0087), and 53.2 for the group receiving pravastatin alone (P=0.0012). In addition, there was no significant difference in the intimal area of the coronary arteries among monkey groups, which suggests that there was no significant alteration in atherosclerosis.

**Discussion**

Conflicting results have been published regarding the effect of ACE inhibitors on platelet function. Patients on captopril therapy had decreased platelet aggregation in response to ADP, epinephrine, collagen, and arachidonic acid; however, platelet aggregation induced by epinephrine, ADP, and collagen in patients on quinapril therapy and by ADP and arachidonic acid in patients on lisinopril therapy was unchanged. Recently, the effect of fosinopril on platelet aggregation was evaluated by using collagen and ADP in hypertensive patients. ADP-induced platelet aggregation decreased by 31% after treatment with fosinopril, whereas collagen response was unchanged.

Platelet aggregability has been shown to decrease with decreasing levels of LDL in patients with familial hyperlipidemia and in normolipidemic males. HMG-CoA reductase inhibitors, such as lovastatin and pravastatin, lower LDL cholesterol, which should result in decreased platelet sensitivity to aggregation. However, the actual effect of HMG-CoA reductase inhibitors on platelets is unclear. One study showed an increase in platelet sensitivity to ADP in patients treated with lovastatin for 26 weeks, despite a significant decrease in their LDL cholesterol levels. In contrast, another study evaluated platelet aggregation in patients treated with lovastatin for 1 year and found a significant decrease in ADP-induced platelet aggregation.

We studied platelet function by using collagen and TRA in all 4 monkey groups. Because it was not possible to obtain large quantities of platelet-rich plasma, we chose to study only these 2 agonists but in detail. By using a wide range of concentrations of each agonist, we were able to determine the dose-response curves and C50 for each agonist, thus producing quantitative results of platelet function. In our study, a significant difference in platelet responsiveness was not seen with either drug alone; however, the combination of the 2 drugs resulted in a significant increase in the dose of TRA required to produce a 50% response in both the rate and extent of aggregation compared with either drug alone or with controls. No significant change was observed in response to collagen, possibly indicating a selective effect. It is not possible to know whether these effects are drug- or class-specific; however, this is a very interesting question that needs to be studied. It is quite intriguing that the difference in aggregation was present even though there was no significant change in systolic blood pressure, total plasma cholesterol, or intimal area of the coronary arteries and that the significant change in diastolic blood pressure occurred only in the group receiving both drugs. These observations suggest that the platelet effect may be independent of the well-known effects of these 2 drugs.

This study was a randomized trial in animals, and the results may not extrapolate to humans. However, a trial such as this provides a good starting point for preliminary data. Animal studies, when compared with human studies, have very definite advantages that stem from a tightly controlled study environment: the animals are syngeneic, are fed the same diet, and are not on other medications that might interfere with the aggregation results or study drugs; the dropout rate is low; and noncompliance is not an issue.

Previous trials evaluating platelet function in patients on ACE inhibitors and HMG-CoA reductase inhibitors have yielded conflicting results. In our study, although platelet function was not significantly affected by either drug alone, the combination resulted in a surprising decrease in platelet sensitivity to the TRA. Response to collagen was not significantly affected. One must consider that the combination of these drugs is somehow affecting the thrombin receptor, because the response to collagen was unaffected. Several possible explanations for these results come to mind: drug alteration of the thrombin receptor leading to decreased sensitivity of the receptor; direct interference with the receptor by the drugs; a decrease in the number of receptors; and a decrease in the recycling of the receptors. These results lead one to ponder whether affects on platelets may also contribute to the decreased cardiovascular morbidity and mortality that is associated with treatment with ACE inhibitors and HMG-CoA reductase inhibitors.

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