Platelet-Activating Factor Acetylhydrolase in Plasma Lipoproteins From Patients With Ischemic Stroke

Kei Satoh, MD; Hidemi Yoshida, PhD; Tada-atsu Imaizumi, MD; Shigeru Takamatsu, MD; and Seitoku Mizuno, MD

Background and Purpose: Platelet-activating factor is a potent bioactive phospholipid and may play an important role in occlusive vascular diseases. To assess the inactivation of this autacoid in plasma, we measured platelet-activating factor acetylhydrolase activity in plasma low density and high density lipoproteins from patients with ischemic stroke.

Methods: Low density and high density lipoproteins were separated by ultracentrifugation from plasma of 33 patients with cerebral thrombosis and 31 age-matched healthy control subjects, and platelet-activating factor acetylhydrolase activity in each fraction was assayed.

Results: The average values of platelet-activating factor acetylhydrolase activity in low density lipoprotein from patients and control subjects were $41 \pm 18$ and $29 \pm 17$ nmol/ml per minute, respectively, and the difference was statistically significant ($p<0.01$, U test). There was no difference in activity in high density lipoprotein between the two groups ($16\pm 11$ versus $14\pm 9$ nmol/ml per minute, respectively).

Conclusions: The increased plasma platelet-activating factor acetylhydrolase activity in stroke patients is primarily attributable to the increased binding to low density lipoprotein, and this increase may be an adaptation to the augmented generation of platelet-activating factor in ischemic stroke. (Stroke 1992;23:1090-1092)

KEY WORDS • cerebral ischemia • lipoproteins • platelet-activating factor

Platelet-activating factor (PAF) is a bioactive phospholipid identified as 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine. It is produced by various types of cells including neuronal cells1-3 and possesses neurorregulatory functions.4-6 Platelet-activating factor is inactivated by an enzyme called PAF acetylhydrolase, which removes the sn-2 acetyl moiety.7 There are intracellular and extracellular (plasma) forms of PAF acetylhydrolase,8 and in plasma this enzyme is associated with low density and high density lipoproteins (LDL and HDL).9 In our previous study we demonstrated age- and sex-related changes in the distribution of PAF acetylhydrolase activity among LDL and HDL in healthy subjects.10 The distribution of this enzyme among plasma lipoproteins may be of critical importance because the inactivation of PAF is achieved more effectively in LDL than in HDL.9 Although we previously observed an increased total plasma activity of this enzyme in stroke patients,11 the actual inactivation of PAF in plasma may also be affected by the relative distribution among LDL and HDL. Since PAF is known to play an important role in ischemic brain injury,12,13 inactivation of PAF may be one of the determinants of a pathophrophysiological state after an ischemic event. Therefore, in this study we measured PAF acetylhydrolase activity in plasma LDL and HDL in patients with ischemic stroke.

Subjects and Methods

Subjects

The subjects studied included 33 consecutive patients (17 men and 16 women) with ischemic stroke, with an average age of $65\pm 9.9$ years (mean±SD). These patients were studied after their clinical condition had been stabilized (i.e., >2 months after the stroke). They did not receive any agents known to affect lipid or lipoprotein metabolism and platelet function. A computed tomographic scan was performed in all patients. Those who were diagnosed as having embolic stroke or lacunar infarcts were excluded from this study. The control group consisted of 31 healthy subjects (16 men and 15 women) who had participated in the annual health checkup program from April to October 1991. Their average age was $64\pm 8.7$ years (mean±SD). They had not received any medication during at least the preceding 4 weeks and did not show any abnormality on physical examination, urinalysis, chest x-ray film, electrocardiogram, and blood chemical and hematological screenings. Fasting venous blood was obtained in the morning using ethylenediaminnetetraacetate as an anticoagulant.
Acetylhydrolase Activity
Tokyo, Japan) and HDL-cholesterol by the dextran-
Plasma Lipids and Apolipoproteins
previously. Briefly, the fraction containing LDL and very
low density lipoprotein; HDL, high density lipoprotein.
*p<0.01, tp<0.05 significantly higher than control value (t test).

Plasma Lipoproteins and PAF Acetylhydrolase Activity
The separation of plasma lipoproteins and PAF acetylhydrolase assay were performed as described previously.10 Briefly, the fraction containing LDL and very low density lipoprotein was separated from the rest (containing HDL and other plasma proteins) by single ultracentrifugation at the density of 1.063 g/ml. The activity of PAF acetylhydrolase in each fraction was assayed according to Stafforini et al.14 The details of this method have been described elsewhere.10

Plasma Lipids and Apolipoproteins
Plasma cholesterol was determined by an enzymatic method using a Hitachi 726 autoanalyzer (Hitachi, Tokyo, Japan) and HDL-cholesterol by the dextran-Mn2+ precipitation method using an Abbott ABA 200 autoanalyzer (Abbott, North Chicago, Ill.). Low density lipoprotein–cholesterol concentration was calculated according to Friedewald et al.15 Plasma apolipoproteins (apo) A-I and B were determined by single radial immunodiffusion using antisera obtained from Daiichi Pure Chemical (Tokyo, Japan) and Hoechst Japan (Tokyo, Japan), respectively.

Statistical Analysis
Statistical significance was tested by the Mann-Whitney U test or by Student’s t test.

Results
The average values of PAF acetylhydrolase activity in LDL, HDL, and plasma (LDL+HDL) are summarized in Table 1. The LDL fraction from stroke patients contained higher PAF acetylhydrolase activity compared with control subjects (p<0.01, U test). The activity associated with HDL was almost equal in the two groups, thus most of the difference in total plasma activity was accounted for by the difference in activity associated with LDL. There was no difference by sex in either patients or control subjects. High density lipoprotein contained roughly one fourth of the total plasma activity, and no significant difference was observed between the two groups in the percent distribution of the activity between LDL and HDL.

Plasma levels of lipids and apoproteins in patients and control subjects are summarized in Table 2. Plasma levels of HDL-cholesterol and apo A-I were significantly higher in control subjects than in patients (p<0.01, t test).

To assess the PAF acetylhydrolase content in lipoprotein particles, the ratio of PAF acetylhydrolase activity to cholesterol in each lipoprotein was calculated. As shown in Table 3, the LDL fraction from stroke patients retained more PAF acetylhydrolase per cholesterol than the LDL from control subjects (p<0.01, U test). A similar difference was observed for the HDL fraction; however, it was not statistically significant.

Discussion
In the present study we demonstrated that the plasma LDL fraction from stroke patients contained higher PAF acetylhydrolase activity than that from healthy subjects. Since there was no virtual difference in the activity associated with HDL, the difference in LDL-associated activity accounted for the difference in total plasma activity between stroke patients and control subjects. Although plasma PAF acetylhydrolase activity is altered in dyslipoproteinemia,16,17 the difference observed in the present study was not related to LDL levels because the PAF acetylhydrolase/cholesterol ratio in LDL was larger in patients than in control subjects. In addition, there was no significant difference in the levels of LDL-cholesterol, apo B, and total cholesterol between the two groups.

The mechanism that regulates the distribution of PAF acetylhydrolase among lipoproteins is not clear. We have previously observed in normal subjects an increase in LDL-associated PAF acetylhydrolase along with an age-dependent increase in total plasma activity.18 Therefore, the capacity of the HDL fraction to bind PAF acetylhydrolase may be relatively limited and fixed, and the increase in total plasma activity may be attributable primarily to the increase in the binding of

**Table 1. Platelet-Activating Factor Acetylhydrolase Activity in Plasma Lipoproteins of Stroke Patients and Control Subjects**

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>41±18*</td>
<td>29±17</td>
</tr>
<tr>
<td></td>
<td>(72±11)</td>
<td>(67±11)</td>
</tr>
<tr>
<td>HDL</td>
<td>16±11</td>
<td>14±9</td>
</tr>
<tr>
<td></td>
<td>(28±11)</td>
<td>(33±12)</td>
</tr>
<tr>
<td>Total (LDL+HDL)</td>
<td>56±24†</td>
<td>43±22</td>
</tr>
</tbody>
</table>

Values are mean±SD of platelet-activating factor (PAF) acetylhydrolase activity in 33 patients and 31 control subjects. Numbers in parentheses denote percentage of total activity. LDL, low density lipoprotein; HDL, high density lipoprotein.

**Table 2. Plasma Levels of Lipids and Apolipoproteins**

<table>
<thead>
<tr>
<th>Lipids or apolipoproteins</th>
<th>Patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>192±42</td>
<td>195±42</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>125±37</td>
<td>123±39</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>41±12*</td>
<td>50±13</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>126±62</td>
<td>123±39</td>
</tr>
<tr>
<td>Apo A-I (mg/dl)</td>
<td>108±18*</td>
<td>136±24</td>
</tr>
<tr>
<td>Apo B (mg/dl)</td>
<td>117±27</td>
<td>128±31</td>
</tr>
</tbody>
</table>

Values are mean±SD of plasma lipids or apolipoproteins (Apo) in 33 patients and 31 control subjects. LDL, low density lipoprotein; HDL, high density lipoprotein.

*p<0.01 significantly lower than control value (t test)."
the enzyme to LDL. Platelet-activating factor acetylhydrolase may be secreted by the liver as a complex with HDL, and in plasma it may be transferred to LDL. Higher activity in LDL may be advantageous for the inactivation of PAF in plasma because the inactivation proceeds more effectively in LDL than in HDL. Also, in our previous study we observed sex-related changes in LDL-associated PAF acetylhydrolase activity in healthy subjects. However, we did not find such differences in the present study, and this result is explained by the fact that enzyme activity in women had almost reached that in men by the sixth decade. Recent studies have shown that PAF acetylhydrolase also hydrolyzes oxidized derivatives of phosphatidylcholine, which have a short chain acyl residue at the sn-2 position of the molecule. Such derivatives also exhibit PAF-like activity through binding to the PAF receptor. Although the occurrence in vivo of such phospholipid derivatives is not demonstrated, our previous study suggested the existence of bioactive phospholipids other than PAF in plasma from patients with ischemic stroke. It is also known that lipid oxidation is enhanced in stroke patients. Higher levels of PAF or PAF-like lipids may serve as a stimulus to increase the production of PAF acetylhydrolase by cells such as hepatocytes and macrophages, and the higher LDL-associated activity in stroke patients may be an adaptation to the increased generation of PAF or PAF-like lipids.

Lindsberg et al detected a substantial level of PAF bioactivity in the rabbit spinal cord subjected to ischemic injury. Platelet-activating factor induces differentiation, enhances the expression of c-fos and c-jun, and elevates intracellular Ca in neuronal cells. It may also act as a general membrane perturbant. All of these factors may influence brain injury in ischemic stroke. Therefore the rate of PAF inactivation in vivo may be of critical importance in determining the outcome of an acute ischemic event.

In conclusion, the activity of PAF acetylhydrolase in LDL is higher in stroke patients than in healthy control subjects, and this increase may not be due to the difference in the levels of plasma lipids or lipoproteins. In addition to the higher total plasma PAF acetylhydrolase, the increase in LDL-associated activity may favor the inactivation of PAF in plasma. Also, these results may be regarded as evidence of another aspect of LDL function.

Acknowledgments
We thank the staff of the Aomori Physical Checkup Center for their help in collecting samples. We also thank Miss Kumiko Satoh for her help in preparing the manuscript.

References
Platelet-activating factor acetylhydrolase in plasma lipoproteins from patients with ischemic stroke.

K Satoh, H Yoshida, T Imaizumi, S Takamatsu and S Mizuno

Stroke. 1992;23:1090-1092
doi: 10.1161/01.STR.23.8.1090

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/23/8/1090

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/