Clofibrate Reversal of Platelet Hypersensitivity in Hyperbetalipoproteinemia

By Angelina C. A. Carvalho, M.D., Robert W. Colman, M.D., and Robert S. Lees, M.D.

SUMMARY
Platelet function was evaluated in 29 patients with familial hyperbetalipoproteinemia; 17 were untreated and 12 were receiving clofibrate (Atromid-S). In comparison with 26 normal subjects, the untreated patients aggregated in response to \( \frac{1}{3} \) the concentration of adenosine diphosphate (ADP), \( \frac{1}{4} \) the concentration of epinephrine, and \( \frac{1}{5} \) the concentration of collagen. Treatment with clofibrate, 2 g daily, returned the ADP sensitivity of the platelets to normal and returned epinephrine and collagen sensitivity towards normal, even though it did not alter total cholesterol, low-density-lipoprotein (LDL) cholesterol, or triglyceride concentrations significantly.

Platelet nucleotide release was also 3-to-5 fold increased in untreated type II patients. Clofibrate treatment failed to diminish the elevated platelet nucleotide release.

Incubation of clofibrate at therapeutic concentrations (100–200 \( \mu \)g/ml) with normal platelets in vitro decreased their sensitivity to ADP and epinephrine and reduced \( ^{14} \)C-serotonin release by these agents. The effect on the release was probably mediated through decreased platelet sensitivity since higher doses of epinephrine could reverse the inhibitory effect of clofibrate.

Clofibrate may decrease the incidence of thrombotic complications of atherosclerosis by altering platelet sensitivity to aggregation.

Additional Indexing Words:
Type II hyperbetalipoproteinemia
Hypercholesterolemia
Atherosclerosis
Thrombosis

FAMILIAL HYPERBETALIPOPROTEINEMIA (type II hyperbetalipoproteinemia), one of the most common hyperlipidemic syndromes, is characterized by the early occurrence of atherosclerosis and its thrombotic complications. We have shown that type II patients have increased sensitivity to aggregating agents and increased nucleotide release, suggesting that abnormal platelet function may contribute to the thrombotic complications of the type II syndrome. Since clofibrate has been reported to reduce the incidence of thrombotic sequelae of atherosclerosis, we investigated the effect of this drug on platelet function in type II patients. In addition, platelet function was evaluated after incubating normal platelets and type II platelets with clofibrate in vitro.

Methods

Patient Selection
Twenty-nine patients were studied. All had positive family histories and more than one lipoprotein electrophoretic pattern characteristic of type II hyperbetalipoproteinemia. They were all on low cholesterol diets; 17 patients (9 women and 8 men) were untreated and 12 were receiving treatment with 2 g clofibrate/day. Twenty-six normal individuals (12 women and 14 men) were selected from healthy laboratory personnel. They had had no medications for more than 15 days, and had normal plasma concentrations of total and low density lipoprotein cholesterol and of triglycerides.

Platelet Function

Preparation of platelet-rich and platelet-poor plasma. Venous blood was collected through siliconized needles into plastic syringes. Nine volumes of blood were added to one volume of 3.8% aqueous sodium citrate in plastic tubes. Samples were centrifuged at 230 for 10 min at 100g; the resultant platelet-rich plasma (PRP) had a platelet count by Coulter counter of 180,000 to 330,000/\( \mu l \). The remaining blood was centrifuged at 3,000g for 15 min at 4\( ^{\circ} \). The platelet count of this platelet-poor plasma (PPP) was below 20,000/\( \mu l \).

Platelet aggregation was studied at 37°C according to a modification of the method of Born. The aggregometer

From the Arteriosclerosis Center, Massachusetts Institute of Technology, Cambridge, and the Hematology and Cardiac Units, Massachusetts General Hospital, Boston, Massachusetts.

Supported by Grant HL-14209 from the National Heart and Lung Institute for a Specialized Center of Research in Arteriosclerosis. Dr. Colman is the recipient of NIH Career Development Award HL-48075.

Dr. Colman’s present address is Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104.

Address for reprints: Robert S. Lees, M.D., Massachusetts General Hospital, Boston, Massachusetts 02114.

Received April 8, 1974; revision accepted for publication May 10, 1974.
CLOFIBRATE IN HYPERBETA LIPOPROTEINEMIA

was set up so that the percent transmittance of platelet-rich plasma was recorded as 0 and that of platelet-poor plasma as 100. The difference between 0 and the maximal transmittance produced by a stimulus was defined as the amplitude of response to that stimulus. Each aggregating agent was tested to determine the lowest concentration capable of inducing the same maximum degree of aggregation characteristic of that substance; the latter was defined as the full response to that agent within the range of platelet counts used; aggregation was independent of platelet concentration.

**Aggregating agents.** ADP* was stored frozen at a concentration of 5 X 10−6 M in 0.1 M sodium phosphate buffer, pH 6.8, containing 0.15 M NaCl. A fresh solution of l-epinephrine (Sigma) (2.5 X 10−5 M) in distilled water was prepared daily. Acid-soluble calf skin collagen, at a concentration of 5.5 mg/ml (as determined by measuring the hydroxyproline content and multiplying by 7.1) was donated by Dr. Joseph Teller (Worthington Biochemicals).

**Platelet nucleotide release** was studied by the method of Wolfe and Shulman substituting gel-filtered for washed platelets. The nucleotide release of the supernate from perchlorate-precipitated platelets was measured spectrophotometrically at 260 mM.

**Platelet 14C-serotonin release** was measured according to the method of Jerusalmy and Zucker. Clofibrate incubation with platelet-rich plasma (PRP). One mg of sodium ethyl-p-chlorophenoxyisobutyrate (clofibrate) was dissolved in one ml of distilled water. The effect of clofibrate concentrations of 100 µg/ml and 200 µg/ml of PRP was evaluated after incubation at 37°C for 10, 20, 30, 60, 90, and 120 min by assays of platelet aggregation and 14C-serotonin platelet release. Addition of equivalent volumes of distilled water alone did not affect platelet function. Both normal and type II platelets were studied.

**Gel-filtered platelets.** Platelets were separated from plasma proteins by gel filtration using a modification of the method of Tangen et al. Tyrode’s buffer, without albumin, was used to suspend the platelets. After gel filtration, platelets were incubated with sodium clofibrate at 37°C for 30 min. Human purified fibrinogen (Kabi, Stockholm), 2 mg/ml platelets, was added prior to the aggregating agents. The following concentrations of aggregating agents were used: 8 µM ADP, 25 µM epinephrine, 110 µg/ml collagen, per ml platelets. Gel-filtered platelets were also labeled with 14C-serotonin and the effect of clofibrate on the release reaction was studied.

**Statistical methods.** Student’s t-test, with the Bessel cor-

---

**Table 1**

<table>
<thead>
<tr>
<th>Characteristics of the Patients Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Type II</td>
</tr>
<tr>
<td>Type II on clofibrate</td>
</tr>
<tr>
<td>Normal</td>
</tr>
</tbody>
</table>

*Low density lipoprotein.
†Mean ± SEM.
Figure 1
Platelet aggregation studies. The minimum concentration of each aggregating agent (ADP, l-epinephrine, collagen) to give a full response is plotted for each patient. Note the logarithmic scale. The triangles represent type II hyperlipoproteinemic patients; open circles, clofibrate-treated type II patients; filled circles, normal subjects.

type II patients treated with clofibrate showed no change in nucleotide release in comparison with untreated type II patients.

Effect of Incubation with Clofibrate on Platelet Aggregation
The mean amplitude of aggregation of normal platelets with each aggregating agent was significantly depressed after treatment with clofibrate for 1 hr at 25°C followed by aggregation at 37°C (fig. 3). When platelets from treated type II patients were used under the same conditions, the results were similar to those in normal subjects (table 2). When platelets from an untreated type II patient (fig. 3) were aggregated with 2.5 μM of epinephrine, a full response was obtained (curve A). By contrast, after incubation with clofibrate (100 μg/ml) a marked decrease of aggregation using the same concentration of epinephrine was observed (curve B). However, the inhibitory effect of clofibrate could be overcome (curve C) by a higher concentration of epinephrine (6.2 μM).

Effect of Clofibrate on Platelet Release Reaction
Clofibrate (200 μg/ml) also inhibited the release of 14C-serotonin from normal platelets (table 3) when ADP and epinephrine were used as aggregating agents.

Figure 2
Platelet nucleotide release. The concentration of total nucleotides released is plotted on a logarithmic scale.
agents. After incubation of PRP with 200 μg/ml of clofibrate for 1 hr at 37°, the mean release with ADP dropped from 51 to 13 (% P < 0.01), and with epinephrine from 65 to 25 (% P < 0.05). With collagen no significant differences were obtained (P > 0.1). The release of 14C-serotonin from type II platelets was unchanged after incubation with clofibrate. This finding is in agreement with the results obtained with platelets from clofibrate-treated type II patients.

Platelets separated from the bulk of plasma proteins by gel filtration responded to clofibrate as did unwashed platelets.

Discussion

Platelets from patients with hyperbetalipoproteinemia are hypersensitive in vitro to aggregating agents, and exhibit increased nucleotide release. The data presented above suggest that clofibrate returns the platelet sensitivity of type II patients towards normal even though the drug has little effect on plasma lipid and lipoprotein concentrations in these patients. We had previously noted a dissociation between the extent of platelet hypersensitivity and the LDL cholesterol concentration in individual patients. Both observations suggest that clofibrate has a direct effect on platelets. Previous studies have shown a decrease in platelet aggregation on latex particles, and a decrease in platelet adhesiveness after clofibrate therapy, although the latter finding has been disputed. In our studies, clofibrate in vitro as well as in vivo was able to decrease aggregation of normal or type II platelets.

This inhibition was reversible in vitro when higher concentrations of aggregating agents were used.

Nucleotide release by platelets from patients treated with clofibrate was unchanged despite their decreased sensitivity to aggregation. Thus, the action of clofibrate is different from that of nonsteroidal anti-inflammatory drugs such as aspirin, which primarily affect the release reaction. Perhaps clofibrate alters the platelet membrane to decrease the sensitivity of receptor sites for aggregating agents. The drug itself in its active form is a fatty acid and may be taken up by the platelets.

Although in normal platelets clofibrate inhibits serotonin release, no decrease in serotonin release was noted after incubation of type II platelets with clofibrate. This difference may occur because a given concentration of aggregating agent represents a much greater stimulus to the sensitive type II platelets than to normal platelets.

These observations may have important implications for the treatment of type II hyperlipoproteinemia. Since the platelet abnormality appears to be independent of the plasma cholesterol and LDL concentration and the effect of clofibrate in platelets independent of its effect on lipids, it may be worthwhile to treat all type II patients with clofibrate. It is noteworthy that the drug appears in epidemiologic studies to have a beneficial effect which is independent of any effect on plasma lipids. It appears as well to have a distinct effect on easily noted sequelae of atherosclerosis, such as transient cerebral ischemia.

Table 2

<table>
<thead>
<tr>
<th>Incubation</th>
<th>ADP (2.0 μM)</th>
<th>Epinephrine (3.5 μM)</th>
<th>Collagen (94 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>76 ± 8</td>
<td>90 ± 7</td>
<td>92 ± 10</td>
</tr>
<tr>
<td>Clofibrate</td>
<td>33 ± 10</td>
<td>36 ± 7</td>
<td>53 ± 15</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Incubation</th>
<th>ADP (4 μM)</th>
<th>Epinephrine (12.5 μM)</th>
<th>Collagen (119 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>51 ± 7*</td>
<td>65 ± 4</td>
<td>56 ± 8</td>
</tr>
<tr>
<td>Clofibrate</td>
<td>13 ± 8</td>
<td>25 ± 12</td>
<td>23 ± 8</td>
</tr>
</tbody>
</table>

(200 μg/ml)
Since clofibrate appears to alter platelet sensitivity to aggregation and aspirin abolishes the release reaction, the combination of the two drugs might be expected to have a greater beneficial effect on intravascular thrombosis than either drug alone. In our limited clinical experience, this appears to be the case; a larger clinical trial of such therapy is now being conducted.

Acknowledgments

We are very grateful for the excellent technical assistance of Rae B. Cabral and Raymond Vaillancourt in performing the platelet studies and of Gene DeBenedetto and Josefina Sabio for the determination of plasma lipids.

References
9. Wolfe MS, Schulman NR: Inhibition of platelet energy production and release reaction by PGE1, theophylline and cAMP. Biochem Biophys Res Commun 41: 128, 1970

Circulation. Volume 50, September 1974
Clofibrate Reversal of Platelet Hypersensitivity in Hyperbetalipoproteinemia
ANGELINA C. A. CARVALHO, ROBERT W. COLMAN and ROBERT S. LEES

Circulation. 1974;50:570-574
doi: 10.1161/01.CIR.50.3.570
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1974 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/50/3/570

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 Circulation is online at:
http://circ.ahajournals.org//subscriptions/