Effect of Clofibrate on Intravascular Coagulation in Hyperlipoproteinemia

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SUMMARY Intravascular coagulation (IVC) was evaluated in 19 patients with type II and 11 with type IV hyperlipoproteinemia before and after clofibrate therapy by measurement of soluble fibrin complexes (SFC) in plasma; fibrinolysis was estimated by quantitation of fibrinogen degradation products in serum. Untreated type II and type IV patients had increased SFC (P < 0.01). The former also had activation of the intrinsic coagulation pathway as evidenced by decreased plasma prekallikrein (P < 0.001), kallikrein inhibitors (P < 0.001), and factor XII (P < 0.02).

SERIOUS ILLNESS AND DEATH in patients with the common hyperlipoproteinemias — type II and type IV — are usually caused by thrombotic complications of atherosclerosis. We have previously demonstrated that both type II and type IV patients have intravascular coagulation characterized by the presence in plasma of elevated concentrations of heavy molecular weight fibrin(ogen) derivatives.1 Activation of the coagulation system appeared to have been initiated in the type II but not the type IV patients by hyperreactive platelets2 with subsequent activation of the intrinsic pathway. The platelet abnormalities in type II patients can be partially reversed by clofibrate.3 We report here studies which show that clofibrate therapy reverses Hageman factor activation and decreases the level of plasma soluble fibrin complexes of type II patients toward normal. Clofibrate had no effect in these studies on the increased level of soluble fibrin complexes found in the plasma of type IV patients, and the coagulation changes induced by clofibrate did not correlate with the lipid-lowering ability of this drug.

Materials and Methods

Patient Selection. Nineteen type II patients were selected on the basis of elevated serum and low density lipoprotein (LDL) cholesterol and at least two characteristic lipoprotein electrophoretic patterns.4 All patients had at least one first degree relative with either documented hypercholesterolemia or xanthomatosis. Ten of the 19 untreated type II patients had clinical evidence of atherosclerosis (angina, previous myocardial infarction, or transient cerebral ischemic attack). Each patient was studied before and during clofibrate therapy (1.5–2g/day, depending on body weight) which had been given for a minimum of six weeks and a maximum of six months. Eleven type IV patients were also diagnosed by plasma lipid and lipoprotein analysis. Six of the type IV patients had clinical evidence of atherosclerosis. All but two patients were on low cholesterol diets. Nineteen normal individuals were selected from healthy laboratory personnel. All were on regular diets. As a crude parameter of the relationship between laboratory findings and thrombotic sequelae, the incidence of clinical thromboembolic events in the two patient groups was compared retrospectively, by dividing the number of events for each patient by the number of months of patient follow-up in our clinic, to obtain the number of events per month. Cerebral thrombosis or embolus, acute myocardial infarction, peripheral arterial thrombosis or embolus, and thrombophlebitis were considered as thromboembolic events.

Blood Sampling. Venous blood was collected with a siliconized needle and plastic syringes. Nine volumes of blood were added to one volume of 3.8% sodium citrate (final concentration 0.013M) in plastic tubes. For determination of factor XII, and prekallikrein and kallikrein inhibitors, the citrated blood was chilled in ice as soon as drawn and centrifuged immediately at 4°C for 15 min at 2500g. The separated plasma was frozen in aliquots at −60°C. For determination of soluble fibrinogen complexes (SFC) the whole blood was collected in epsilon-amino caproic acid (EACA), final concentration 10−3M, in addition to sodium citrate. All samples were centrifuged at 2500g for 15 min and at 25°C to avoid possible precipitation in the cold of cryofibrinogen-like material. These samples were also kept frozen at −60°C and thawed only once. For the determination of the fibrinogen degradation products (FDP) in serum, whole blood was collected in glass tubes containing EACA (10−8M) and thrombin (20 NIH units/ml), incubated at 37°C for two hours, and centrifuged at 4°C for 15 min at 2500g. The assay of FDP was done immediately or on occasion frozen at −60°C for no more than three days.

Hageman Factor (Factor XII). Determination was made by one stage assay, a modification of the method of Proctor and Rappaport5 for partial thromboplastin time, using plasma from a patient with a congenital deficiency of the Hageman factor. The results were expressed as percent of normal plasma concentration.

Prekallikrein and Kallikrein Inhibitors. The esterase
assay method of Colman et al. was used. This assay is based on the observation that citrated human plasma collected without glass contact contains very low levels of arginine esterase activity. The addition of kaolin transforms inactive factor XII (Hageman factor) to activated Hageman factor and its fragments which in turn convert prekallikrein to kallikrein. Although other enzymes may be activated by contact, it appears that most of the enzymatic activity at 1 min in the absence of calcium (necessary for thrombin activation), using L-tosyl arginine methyl ester (TAME) as a substrate, results from the formation of kallikrein. Evidence that kaolin-activated arginine esterase activity primarily reflects kallikrein is provided by identical substrate specificity to purified kallikrein, similar inhibition profiles, release of bradykinin in parallel with arginine esterase activity, and exclusion of other enzymes such as plasmin and thrombin.

In addition, immunochemical measurements of prekallikrein in a normal individual and a patient with cirrhosis and depressed prekallikrein closely correlate with the arginine esterase assay. Although several arginine esterase inhibitors exist in plasma, 85% to 100% of the decrease in arginine esterase activity from 1 to 5 min is due to the stoichiometric combination of kallikrein with the C1 esterase inhibitor. This conclusion is supported by similar kinetics of inhibition by plasma with purified enzyme and by the small loss of esterase activity (<15%) in patients with hereditary angioedema who lack the C1 esterase inhibitor.

Further, purified kallikrein reacts with plasma to form a complex detected immunochemically with C1 esterase inhibitor but not α1-antitrypsin. Although α1-macroglobulin can also inhibit the proteolytic activity of plasma kallikrein, it has little effect on the esterolytic activity. One inhibitor unit is defined as that amount which produces 50% inhibition of the arginine esterase activity formed at 1 min. To measure the hydrolysis of TAME, the methanol formed is oxidized to formaldehyde and coupled to chromotropic acid to give a purple color. Duplicate assays agreed within ±5%.

Gel filtration. Fibrinogen derivatives were determined by a modification of the method of Fletcher et al. Two ml of citrated plasma collected in EACA and sodium citrate were filtered through a 1.5 × 90 cm column containing Sepharose 4B, previously calibrated with standard proteins of known molecular weight. The fibrinogen reactive material was measured by the staphylococcal clumping test (SCT). The results are expressed in µg/ml in reference to a purified human fibrinogen standard. The percentage of total fibrinogen reactive material was estimated by integration of the area under each peak. The SFC are defined as fibrinogen reactive material eluting prior to the fibrinogen peak. These fibrinogen derivatives all contained clottable protein.

Assay of FDP. The SCT was used to measure the FDP in the sera of patients and normal subjects and expressed in µg/ml of fibrinogen equivalents. The staph-reagent (staph aureus, Newman strain D) was a gift of Dr. Kent Miller, University of Miami Medical School.

Antithrombin III. This was measured by radial immuno-diffusion. Antithrombin III (ATIII) serum from Behring-werke, A.G., Germany, gave a single line by immunoelectrophoresis against normal plasma, which was identical to the one obtained by highly purified ATIII (kindly supplied by Dr. Robert Rosenberg). ATIII results were expressed as a percentage of normal plasma activity determined with a pool of 12 normal individuals. The values were also checked with individual normal plasma samples and related to the mean value of 15 normal plasmas.

Complement. CIINH and C3 were also determined by radial immuno-diffusion using antiserum from Behring-werke. Results were expressed as a percentage of normal plasma activity determined with a pool of 15 normal individuals. A group of 24 normal persons were also checked individually.

Statistics. All values are expressed as mean ± SEM and differences evaluated by Student's t-test.

**Results**

**Plasma Lipids**

The plasma lipid concentrations of the patients studied are shown in table 1. The mean concentration of total and LDL cholesterol in the plasma of type II subjects was elevated and did not change after clofibrate. Untreated type IV patients had significantly elevated plasma triglycerides which returned to normal with clofibrate treatment.

**Prekallikrein-Kallikrein Inhibitors**

Activation of the intrinsic coagulation pathway was evaluated by depletion of prekallikrein and kallikrein inhibitors, a method that has been shown to be more sensitive than depletion of factor XII itself. Untreated type II patients had significantly low levels of prekallikrein (61.2 ± 6) and kallikrein inhibitors (0.50 ± 0.18) compared with normal (P < 0.001) as shown in figure 1. Clofibrate treatment of type II patients caused an increase of prekallikrein (P < 0.001) and kallikrein inhibitor (P < 0.02) concentrations to normal values. The prekallikrein levels of untreated type IV patients were 124 ± 7 and kallikrein inhibitors 0.97 ± 0.09, values which were not significantly different from normal. After treatment, no change in the kallikrein system was observed in type IV patients. The

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**Table 1. Characteristics of the Patients Studied**

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Age (yrs)</th>
<th>Total Cholesterol (mg/100ml)</th>
<th>LDL* (mg/100ml)</th>
<th>Triglycerides (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type II</td>
<td>19</td>
<td>38 ± 31</td>
<td>353 ± 23</td>
<td>298 ± 25</td>
<td>114 ± 10</td>
</tr>
<tr>
<td>Type II on Clofibrate</td>
<td>19</td>
<td>38 ± 4</td>
<td>340 ± 29</td>
<td>272 ± 29</td>
<td>119 ± 14</td>
</tr>
<tr>
<td>Type IV</td>
<td>11</td>
<td>52 ± 2</td>
<td>213 ± 12</td>
<td>126 ± 13</td>
<td>194 ± 31</td>
</tr>
<tr>
<td>Type IV on Clofibrate</td>
<td>8</td>
<td>50 ± 3</td>
<td>222 ± 5</td>
<td>134 ± 9</td>
<td>121 ± 16</td>
</tr>
<tr>
<td>Normal</td>
<td>17</td>
<td>30 ± 2</td>
<td>160 ± 9</td>
<td>119 ± 8</td>
<td>66 ± 7</td>
</tr>
</tbody>
</table>

*Low density lipoprotein.
†Mean ± SEM.
of factor XII (84 ± 5.5) compared with normal. However, after clofibrate therapy (108 ± 8.2) there was a significant rise in factor XII ($P < 0.02$). No change in factor XII levels was noted with treatment in type IV patients. The normal group had levels of factor XII of 99 ± 6.

**Hageman Factor (Factor XII)**

Untreated type II patients had slightly but not significantly depressed levels of factor XII (84 ± 5.5) compared with normal. However, after clofibrate therapy (108 ± 8.2) there was a significant rise in factor XII ($P < 0.02$). No change in factor XII levels was noted with treatment in type IV patients. The normal group had levels of factor XII of 99 ± 6.

**Gel Filtration of Fibrinogen Derivatives**

The size and distribution of circulating fibrin derivatives were determined by gel filtration on Sepharose 4B columns. The calibration of the columns has been described previously.$^1$ A representative normal individual (fig. 2, top) shows a single symmetric peak of fibrinogen-like material at the same volume as purified fibrinogen.$^1$ In contrast, plasma from an untreated type II shows two heavy derivatives which elute prior to fibrinogen (fig. 2, middle). The first peak is identical to the fibrinogen polymer formed by action of thrombin on purified fibrinogen.$^1$ The second peak has the $K_d$ of a fibrin dimer.$^{16}$ By integrating the areas under these two peaks, it was found that 51% of the total fibrinogen reactive material was present in the form of soluble fibrinogen complexes. After two months on clofibrate therapy, these high molecular weight fibrinogen derivatives in the same patient decreased to 10% (fig. 2, bottom).

Figure 3 represents the concentration of soluble fibrin complexes (SFC) in type II, type IV, and normal individuals. The untreated type II patients had markedly elevated concentrations of SFC (31.6% ± 3.3) compared with the normal ($P < 0.0001$). Clofibrate therapy resulted in a decrease to 11 ± 3% which differed from the untreated patients ($P < 0.001$). Untreated type IV patients had increased SFC (19.8% ± 3) levels but they were not as striking as in untreated type II subjects. No changes occurred after treatment with clofibrate.

The normal levels of SFC were 3.2% ± 1.2 (mean ± SEM).

**Serum Fibrinogen Derivatives**

The untreated type II patients had low levels of FDP in serum (1.93% ± 9.3μg/ml), in the low range of normal. After clofibrate treatment a significant rise ($P < 0.0001$) in FDP was noted (31.4% ± 10.8μg/ml) (fig. 3). Untreated type II patients had low levels of FDP in serum (1.93% ± 9.3μg/ml), in the low range of normal. After clofibrate treatment a significant rise ($P < 0.0001$) in FDP was noted (31.4% ± 10.8μg/ml) (fig. 3). Untreated type II patients had low levels of FDP in serum (1.93% ± 9.3μg/ml), in the low range of normal. After clofibrate treatment a significant rise ($P < 0.0001$) in FDP was noted (31.4% ± 10.8μg/ml) (fig. 3). Untreated type II patients had low levels of FDP in serum (1.93% ± 9.3μg/ml), in the low range of normal. After clofibrate treatment a significant rise ($P < 0.0001$) in FDP was noted (31.4% ± 10.8μg/ml) (fig. 3). Untreated type II patients had low levels of FDP in serum (1.93% ± 9.3μg/ml), in the low range of normal. After clofibrate treatment a significant rise ($P < 0.0001$) in FDP was noted (31.4% ± 10.8μg/ml) (fig. 3). Untreated type II patients had low levels of FDP in serum (1.93% ± 9.3μg/ml), in the low range of normal. After clofibrate treatment a significant rise ($P < 0.0001$) in FDP was noted (31.4% ± 10.8μg/ml) (fig. 3). Untreated type II patients had low levels of FDP in serum (1.93% ± 9.3μg/ml), in the low range of normal. After clofibrate treatment a significant rise ($P < 0.0001$) in FDP was noted (31.4% ± 10.8μg/ml) (fig. 3). Untreated type II patients had low levels of FDP in serum (1.93% ± 9.3μg/ml), in the low range of normal. After clofibrate treatment a significant rise ($P < 0.0001$) in FDP was noted (31.4% ± 10.8μg/ml) (fig. 3).
IV patients had normal levels of FDP before and after treatment.

**Antithrombin III**

Untreated type II patients' levels were 118 ± 4.6% and after treatment 114 ± 6.4%. Untreated type IV subjects had 114 ± 6.0% and after treatment these levels were unchanged. Our control group had an ATIII value of 106 ± 8% (mean ± SEM).

**C1NH and C3 Levels**

These parameters were normal in both groups of patients before and after treatment.

**Clinical Events**

The incidence of clinical thromboembolic events over the two to five year period of follow-up was 0.013 per month for the type II patients and 0.005 per month for the type IV patients (P < 0.02).

**Discussion**

The data show that clofibrate reversed the increased soluble fibrin complexes and the activated intrinsic coagulation pathway in type II but did not change the elevated SFC levels in type IV hyperlipoproteinemia. These changes were independent of the drug's effects on plasma lipids, since clofibrate therapy lowered plasma triglycerides in our type IV patients but did not significantly change the plasma lipids of the type II patients. The marked differences in coagulation abnormalities between type II and type IV patients cannot be explained by differences in age alone, since type II patients are younger (mean age 39) and are more severely affected with atherosclerosis than are type IV patients (mean age 51). The normal coagulation results obtained in a group of healthy elderly patients (mean age 65) undergoing hip surgery supports this hypothesis.

Type II patients have abnormal platelet responsiveness to aggregating agents and increased nucleotide release; these abnormalities also move toward normal on clofibrate therapy. In addition, untreated type II patients have both low prekallikrein and kallikrein inhibitors and depressed factor XII levels. This finding is indicative of prekallikrein activation since the active enzyme kallikrein is known to form a complex with Cl inhibitor, deleting the activity of both enzyme and inhibitor. In contrast, since the reaction produces an inactive enzyme-inhibitor complex but one which remains immunologically detectable, the Cl esterase inhibitor antigen remains normal. This activation may result in thrombin generation and thus cause intravascular coagulation. Factor XII and its derivatives appear to be essential for the conversion of prekallikrein to kallikrein. The findings of low preK and K1 have been used as an indicator of factor XII activation in patients with disseminated intravascular coagulation due to gram negative sepsis, myocardial ischemia, dumping syndrome, and polycythemia vera. However, activation of the kallikrein system does not occur in consumption coagulopathy associated with neoplasia or liver disease. Our data show that clofibrate inhibits the activation of factor XII and the kallikrein system in type II patients in vivo. In contrast to type II patients, untreated type IV patients have normal or increased prekallikrein and K1 levels.

Activation of factor XII and the kallikrein system in type II patients may be mediated by the release of intracellular enzymes from these patients' hypersensitive platelets. The combination of hypersensitive platelets and activation of factor XII and the kallikrein system is unique for type II hyperlipoproteinemia. The improvement toward normal in platelet aggregation observed in clofibrate-treated type II patients, in addition to the reversal of factor XII activation, suggests a platelet-mediated mechanism for the latter. In contrast, patients with type IV disease have normal platelet sensitivity and the kallikrein system and factor XII level were not different from normal (fig. 1).

To determine whether intravascular coagulation was occurring in vivo in hyperlipidemia we evaluated the products of reaction of thrombin on fibrinogen and searched for secondary fibrinolysis.

Substantial evidence has accumulated that SFC may serve as markers for intravascular fibrin deposition. In particular, Fletcher, Alkaersig, and colleagues have demonstrated that the determination of SFC in plasma through gel exclusion chromatography represents a sensitive and specific assay for detecting ongoing intravascular coagulation. Ly and Jakobson found that SFC contain partially crosslinked fibrin as a result of the action of factor XII, but that most of the polymer fibrin complexes are held together by noncovalent bonds. We have also demonstrated that the SFC in type II patients contain partially crosslinked fibrin. Soluble fibrin complexes determined by plasma gel filtration have been found to be elevated in postoperative thrombophlebitis, acute myocardial infarction, acute cerebral vascular occlusion, in women taking oral contracept...
tives,29 31 in disseminated intravascular coagulation and other fibrinolytic states,19 in polycthemia vera,14 and in patients with hyperlipidemias. The data presented here show that clofibrate treatment decreased the elevated SFC concentration threefold in type II patients but did not alter the increased levels in type IV patients. The SFC concentration in treated type II patients was only half that of treated type IV patients.

Follow-up of both groups of patients over a two to five year period showed a threefold incidence of thromboembolic events in type II patients compared with type IV patients. A correlation, therefore, appeared to exist between the laboratory evidence of intravascular coagulation in type II patients and their increased incidence of thromboembolic complications.

Our finding of activation of the intrinsic clotting pathway in type II patients and elevation of soluble fibrin complexes in both type II and IV led us to search for changes in fibrinolysis. There were low normal serum fibrinogen degradation products (FDP) in both untreated groups of hyperlipidemic patients. The low FDP levels in the presence of intravascular coagulation may reflect an inadequate fibrinolytic response for the degree of intravascular coagulation or inhibition of the fibrinolytic system by the plasma lipoproteins. Another possibility is that plasma FDP can form complexes in vivo with both fibrinogen and fibrin monomer, contributing to the increased formation of SFC as they do in vitro.32 Fibrin degradation products complexed in this manner would not be available for measurement by the SCT assay which is performed in serum because SFC are clottable and hence would be excluded from serum. Clofibrate stimulates the production of FDP in type II hyperlipoproteinemia more profoundly than in type IV patients (P < 0.01).

Cotton and Wade28 have show that clofibrate increases fibrinolytic activity in patients post coronary thrombosis and that the changes persisted over 9 months of observation. These authors also noted that clofibrate had no effect on the fibrinolytic system if the level of fibrinogen was normal before treatment. The high fibrinogen level of the patients studied by Cotton and Wade28 may represent an increase in plasma SFC since these complexes are clottable and are included in the plasma fibrinogen. In contrast, Chakrabarti and Fearnley reported an inconstant antifibrinolytic effect of clofibrate in eight patients with high plasma cholesterol.30 Since their patients’ cholesterol decreased by a mean of 30% on clofibrate and plasma triglycerides were not measured, they were most likely studying type IV patients and their data are therefore probably consistent with ours. Epstein et al.31 reported that type IV patients have an impairment of the normal fibrinolytic response to exercise.

Taken together, these observations emphasize that the effects of clofibrate on coagulation depend upon the patient’s plasma lipoprotein pattern. The equivocal results seen with clofibrate in studies such as the Coronary Drug Project29 may reflect the nature of the patients studied, rather than the potential usefulness of the drug in a discrete patient group such as those with type II hyperlipoproteinemia. Our data suggest strongly that in the latter group clofibrate decreases intravascular coagulation and may help to prevent its thrombotic complications.
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