Suppression of Tumor-associated Hyperfibrinogenemia and Free Fatty Acidemia with p-Phenoxybenzalbutyrate (Clofibrate)¹

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ABSTRACT

Previous work in our laboratory has indicated that free fatty acids stimulate synthesis of fibrinogen by the liver. The effect of the hypolipidemic agent clofibrate on hyperfibrinogenemia associated with tumors was evaluated by monitoring clofibrate-induced changes in plasma fibrinogen concentration and biosynthesis of the protein in Buffalo rats implanted with a localized, nonmetastasizing neoplasm derived from a tumorigenic hepatoma cell line (HTC₄). In tumor-bearing animals not treated with clofibrate, cancer growth was associated with elevated rates of fibrinogen synthesis and a doubling of plasma fibrinogen concentrations. Plasma free fatty acid concentrations and serum free fatty acid/albumin molar ratios were also increased in tumor-bearing rats. Treatment with clofibrate in doses which normalized the plasma free fatty acid/albumin ratio also prevented the tumor-associated rise in plasma fibrinogen. Rates of fibrinogen synthesis were lowered significantly in clofibrate-treated animals. Tumor growth was not affected by clofibrate. These results indicate that hyperfibrinogenemia associated with nonmetastasizing tumors may reflect changes in lipid metabolism which are neutralized by clofibrate.

Thus, treatment with clofibrate or other hypolipidemic agents should be evaluated in cancer patients with elevated plasma fibrinogen levels and their attendant complications.

INTRODUCTION

Patients with neoplastic disease frequently develop hyperfibrinogenemia (11, 17, 33, 36) due to enhancement of fibrinogen synthesis with rapid tumor growth (22, 33, 36, 42). Tumor-induced hyperfibrinogenemia is associated with a poor prognosis (42) which may be improved by treatment directed toward reduction of plasma fibrinogen levels (2). The exact role of plasma fibrinogen in mortality and morbidity of cancer is unclear. Thromboembolic complications due to hypercoagulability and accelerated extension of metastatic neoplasms may be among the deleterious effects of hyperfibrinogenemia in cancer patients (2, 16, 21, 22, 24, 31, 40). Reduction of plasma fibrinogen concentrations with defibrinating enzymes has been repeatedly reported to inhibit or to delay the metastatic spread of clinical and experimental tumors (21, 23, 24, 27, 30, 31, 45). This form of therapy has, however, produced relatively transient improvement, due largely to development of antibodies against defibrinogenating enzymes (23).

We have previously demonstrated that elevated intrahepatic FFA³ concentrations may be a key factor in development of experimental hyperfibrinogenemia (38). Should a similar mechanism operate in tumor-associated hyperfibrinogenemia, treatments which reduce plasma FFA levels could prevent the elevation of plasma fibrinogen observed with neoplastic tumors. The present study examines the effect of the hypolipidemic agent clofibrate (p-phenoxybenzalbutyrate) on fibrinogen synthesis and plasma concentrations in rats bearing a rapidly expanding neoplasm derived from a tumorigenic hepatoma cell line.

MATERIALS AND METHODS

Induction of Tumors. The cultured rat hepatoma (HTC₄) is an established tumorigenic cell line chemically induced in Buffalo rats and propagated in spinner culture (39). Injection of 10⁶ HTC₄ cells into the thigh muscles of Buffalo rats produces a nonmetastasizing tumor which proves fatal in approximately 5 weeks.

Male Buffalo rats (80 g) were injected with tumor cells and maintained on a diet of normal rat chow supplemented with clofibrate. The drug was administered by impregnating chow pellets with a solution of clofibrate in hexane and allowing the solvent to evaporate at room temperature. The weight of chow consumed daily was carefully monitored, and the clofibrate concentration in the pellets was adjusted to ensure that each rat received a measured amount of the drug (0, 5, 20, 50, or 100 mg/kg/day). Control animals, which included rats with and without tumors, received chow treated with hexane only.

Determination of Fibrinogen and Albumin Concentrations and Specific Activities in Plasma. Plasma for isolation and measurement of fibrinogen and albumin was obtained after rats were injected with 1000 units of heparin, followed after 30 min by heart puncture, using 0.1 volume of 1% EDTA in phosphate-buffered saline, pH 7.4 (Grand Island Biological Co., Grand Island, N.Y.), as anticoagulant. Fibrinogen was measured as fibrin produced by addition of thrombin to plasma to induce clotting, and albumin was determined by paper electrophoresis as fully described elsewhere (38). Proteins were radiolabeled in vivo with L-[4,5-³H]leucine (55 μCi/mmol; Schwarz/Mann, Orangeburg, N. Y.) injected i.p. (20 μCi) on the morning 24 hr prior to sacrifice. Protein specific activity was calculated after measurement of albumin and fibrin isolated from heparinized plasma and counting of incorporated radioactivity as described previously.

Determination of Free Fatty Concentrations in Plasma. FFA's were measured in serum obtained each morning from a group of rats which received no heparin. Details of the procedure were previously published (38).

Experiments with Animals. The effects of various dosages of clofibrate (0, 5, 20, 50, or 100 mg/kg/day) on tumor-induced hyperfibrinogenemia were determined in preliminary experiments conducted after tumor growth could be detected.

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³ The abbreviation used is: FFA, free fatty acid.

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by visual inspection (2 weeks). Plasma albumin, fibrinogen, FFA concentrations, and rates of incorporation of \(^{3}H\)leucine into the 2 proteins were measured in subsequent experiments at the drug dosage found to be optimal (50 mg/kg/day). Finally, tumors in treated and control animals were excised and weighed at the termination of each experiment.

**Statistical Analysis.** Results were statistically compared after an analysis of variance among groups, and values were calculated from the \(t\)-distribution (10).

**RESULTS**

**Effects of Clofibrate on Plasma Fibrinogen and Albumin.** Plasma fibrinogen concentrations gradually increased in all tumor-bearing animals. Maximal plasma fibrinogen concentrations were reached within 14 days after implantation of hepatoma cells and remained stable thereafter (Chart 1). On the 14th day after implantation, fibrinogen plasma concentrations had reached nearly twice control values (Chart 1; Table 1). Clofibrate administered at doses varying from 20 to 100 mg/kg/day reduced tumor-induced fibrinogen concentrations in a dose-dependent fashion. Maximal reductions in plasma fibrinogen were achieved at a dose of 50 mg/kg/day, reaching values only marginally above control values (Chart 2). In contrast, plasma fibrinogen levels in normal controls were not significantly affected by clofibrate (Table 1).

At the 14-day stage of tumor growth, the synthetic rate of plasma fibrinogen was increased 2.5-fold in tumor-bearing rats compared with controls (Table 1). Clofibrate prevented this increase.

Plasma albumin concentrations were reduced, and incorporation of \(^{3}H\)leucine into albumin was decreased in tumor-bearing animals compared with controls (Table 1). Overall synthetic rates could not be calculated for albumin as for fibrinogen due to distribution of albumin both within and outside of the vascular compartment (60% of rat albumin is extravascular). However, if uniform labeling of albumin is assumed, the synthesis of albumin appeared to be reduced in tumor-bearing animals. Treatment with clofibrate had no significant effect on plasma albumin concentrations in normal rats, while plasma albumin concentrations in tumor-bearing rats were significantly \((p < 0.05)\) higher in those treated with clofibrate (Table 1).

**Effects of Tumors and Clofibrate on Plasma FFA.** Plasma FFA was consistently elevated after 14 days of tumor growth (Table 1). Treatment with clofibrate maintained plasma FFA at control levels. The FFA/albumin molar ratio, an indicator of FFA transport from plasma to tissues, was markedly increased in untreated tumor-bearing rats. In contrast, the FFA/albumin ratio remained at nearly normal values in comparable rats treated with clofibrate (Table 1).

**Effect of Fibrinogen Reduction on Tumor Growth.** The implanted hepatoma cells produced a slowly growing tumor which averaged 110 g in weight 5 weeks after implantation in clofibrate-treated rats. The average tumor weight in control rats was 29 ± 2 (S.D.) g at 14 days, 45 ± 3 g at 21 days, and 105 ± 8 g at 28 days (Chart 3).

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**DISCUSSION**

These experiments establish that p.o. treatment with the hypolipidemic drug clofibrate prevents the increase in plasma fibrinogen concentrations and synthetic rates associated with the HCTC tumor in rats. In addition, the results suggest that synthesis of albumin was reduced in tumor-bearing rats and was returned toward normal by clofibrate. While a direct effect of clofibrate on protein synthesis was not ruled out, the correlation between the effects of clofibrate on fibrinogen synthesis and FFA metabolism in tumor-bearing animals compared with controls, taken together with previous observations (38), support the interpretation that the fibrinogen-lowering effects of clofibrate reflect its hypolipidemic properties. Clofibrate has been shown to cause concurrent reductions in resting FFA and fibrinogen concentrations in patients with angina pectoris (44). While these effects of clofibrate may be partially due to displacement of thyroxine from albumin and consequent alterations in hepatic energy metabolism, treatment of human subjects with an analog of clofibrate which has no effect on thyroxine metabolism (ICI 55,897) also produced reductions in both plasma fibrinogen and FFA (44).

Lipidemia and hyperfibrinogenemia often coexist in patients with rapidly growing or metastasizing tumors (23, 24). The present observations raise the possibility that these metabolic abnormalities may be causally related. Our previous studies in mice and in incubated mouse liver slices have implicated plasma FFA’s as active in the production of hyperfibrinogenemia, since changes in the FFA/albumin ratio in vivo and in vitro were reflected in corresponding alterations in hepatic synthesis of fibrinogen (38). In addition, dietary saturated fats and fat-mobilizing hormones have been shown to induce marked elevations in plasma fibrinogen and fibrinogen synthesis (14, 38, 41).

These observations suggest a mechanism by which tumor growth may induce secondary hyperfibrinogenemia. As outlined in Chart 4, many tumors secrete thromboplastin-like substances which are capable of generating thrombin and initiating clot formation (17). In addition to its coagulative actions, thrombin is a potent mobilizer of FFA’s (32, 38). We have recently demonstrated that thrombin also produces hyperfibrinogene-

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\(^4\) The synthetic rate of fibrinogen can be approximated by the product of the specific activity of fibrin and the plasma fibrinogen concentration (measured as fibrin) (37), since approximately 85% of total rat fibrinogen is in the vascular pool and radiolabeled amino acids administered i.p. as a pulse have been shown to be incorporated into fibrinogen within 1 hr (5).

Suppression of Hyperfibrinogenemia with Clofibrate

Table 1

Effects of tumor and clofibrate treatment on fibrinogen and albumin metabolism and serum FFA’s

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Plasma fibrinogen</th>
<th>Synthetic rate of fibrinogen (cpm in fibrin/ml plasma)</th>
<th>Plasma albumin</th>
<th>Serum FFA (µg/ml)</th>
<th>Molar ratio FFA/albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/ml</td>
<td>cpm/mg</td>
<td>mg/ml</td>
<td>cpm/mg</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>3.08 ± 0.15 b c</td>
<td>483 ± 52</td>
<td>30.1 ± 1.8 c</td>
<td>640 ± 72</td>
<td>0.42 ± 0.03 e</td>
</tr>
<tr>
<td>Controls + clofibrate (50/ mg/kg/day)</td>
<td>2.78 ± 0.24 a</td>
<td>465 ± 67</td>
<td>30.4 ± 0.6</td>
<td>621 ± 40 a</td>
<td>0.37 ± 0.04 a</td>
</tr>
<tr>
<td>Tumor bearing</td>
<td>5.66 ± 0.55 g</td>
<td>637 ± 100</td>
<td>24.8 ± 3.6 i</td>
<td>478 ± 55 j</td>
<td>0.74 ± 0.10 k</td>
</tr>
<tr>
<td>Tumor bearing + clofibrate (50/mg/kg)</td>
<td>3.21 ± 0.32 m</td>
<td>561 ± 73</td>
<td>27.0 ± 1.7 o</td>
<td>553 ± 64 p</td>
<td>0.43 ± 0.06 q</td>
</tr>
</tbody>
</table>

* Album molecular weight assumed to be 64,000.
* Mean ± S.D.
* p values: a versus g, <0.001; g versus m, <0.001; b versus h, <0.001; h versus n, <0.001; b versus n, <0.025; c versus i, <0.02; c versus o, <0.01; i versus o, <0.05; d versus j, <0.005; d versus p, <0.025; j versus p, <0.05; e versus k, <0.001; k versus q, <0.001; f versus i, <0.001; l versus r, <0.001. Other comparisons were not significant at p < 0.05.

Chart 2. Effect of clofibrate dosage on tumor-associated hyperfibrinogenemia. Increasing amounts of clofibrate reduced elevated tumor-associated fibrinogen levels in a dose-dependent manner up to a dose of 50 mg body weight per kg per day. Clofibrate at this dose had a minimal effect on fibrinogen levels in tumor-free rats (O). BW, body weight.

Analogous to its effects on tumor-induced hyperfibrinogenemia reported in this paper, the amount of thrombin generated by a low-grade coagulative process at the tumor periphery may be adequate to induce and maintain a hyperfibrinogenemic condition. One ml of plasma contains about 330 units of thrombin (20). Infusion of 15 units of highly purified thrombin into rabbits is followed by a detectable increase in hepatic fibrinogen synthesis (26), and 300 units will triple fibrinogen production (3). Previous enzymatic defibrinogenation does not diminish this effect of thrombin (4). Thus, tumor-induced generation of thrombin may trigger mobilization of FFA, in turn stimulating hepatic fibrinogen synthesis and development of hyperfibrinogenemia (Chart 4).

Tumor-associated hyperfibrinogenemia may contribute to circulatory complications encountered in neoplastic disease (21, 23, 24, 31). Elevated fibrinogen levels induced rouleaux formation and RBC sludging and may precipitate thromboembolic episodes (2, 16). In addition, the greater resistance to endogenous fibrinolytic dissolution of fibrin formed from plasma at elevated, compared with normal, fibrinogen concentrations (7, 18) may play a role in the accumulation of fibrin deposits at the periphery of tumors, thereby facilitating their extension or invasiveness. A similar mechanism for accelerated spread of metastases in cancer patients with hyperfibrinogenemia has been postulated by others (21, 24, 31).

Various clinical and experimental treatments of hyperfibrinogenemia have been attempted which use enzymatic fibrinogen-lowering agents (21, 23, 24, 28, 30, 36, 45). These procedures utilize proteins which either activate the fibrinolytic system (e.g., streptokinase from streptococci, human uroki-
nase, porcine or bovine plasmin) or which directly lyse the fibrinogen molecule (e.g., "Arvin" and "Defibrase" from viper venom). However, the usefulness of exogenously derived enzymes which reduce fibrinogen concentrations is limited in practice by their rapid clearance from the circulation and by the development of host antibodies against these foreign proteins within 1 to 2 months (23).

In contrast to enzymatic treatments, hypolipidemic agents are well tolerated and may be useful in prolonged suppression of tumor-associated hyperfibrinogenemia. In addition to clofibrate and its analogs, other p.o. administered drugs which have been shown to reduce both circulatory lipids and fibrinogen in humans include allyl propyl disulfide (6, 9), aspirin (35), β-benzalbutyrate (34, 37), dextran sulfate (1), phenformin (12, 29), tetracnicotinyl fructose (8), and testosterone analogs such as ethylestrenol (12, 19), furabazol (19, 28), and stanazol (15, 19). Combinations of these drugs, e.g., clofibrate plus nicotinic acid, (43) or phenformin plus ethylestrenol, furabazol, or stanazol (12, 15, 25) may be even more effective in control of hyperfibrinogenemia. Turnover studies in vivo have demonstrated that reductions in plasma fibrinogen during treatment with combinations of aspirin, ethylestrenol, and phenformin are due to decreased fibrinogen synthesis (13, 22). Thus, a wide assortment of drugs potentially effective in control of tumor-associated hyperfibrinogenemia is currently available. Long-term clinical and animal studies conducted with these agents may establish their therapeutic effectiveness and may clarify the role of hyperfibrinogenemia in the extension and complications of cancer.

REFERENCES


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