MICROBODY PROLIFERATION IN LIVER INDUCED BY NAFENOPIN, A NEW HYPOLIPIDEMIC DRUG: COMPARISON WITH CPIB

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SUMMARY. Nafenopin (2-methyl-2[P-(1,2,3,4-tetrahydro-1 naphthyl) phenoxy]- propionic acid, a phenolic ether with hypolipidemic properties, when administered by gavage at 100 mg/kg b wt daily for 1 to 2 weeks, caused a significant increase in the number of microbody profiles and simultaneous increase in catalase activity in livers of male rats. The concentration of catalase protein and the rate of incorporation of H^3-δ-aminolevulinic acid into catalase fraction, as determined by immunochemical methods were approximately twice that of controls. The microbody proliferation resulting from nafenopin treatment was comparable to that induced by CPIB.

Microbodies (peroxisomes) are cytoplasmic organelles described originally in kidney and liver and identified recently in several other mammalian cell types (1-4). Microbodies in rat liver possess catalase and several oxidative enzymes including uricase, d-amino acid oxidase, α-hydroxy acid oxidase and isocitrate dehydrogenase (5). Alterations in the number or morphology of microbody profiles are rarely encountered and it appears that these organelles are relatively unaffected by most experimental manipulations. Studies with ethyl-α-p-chlorophenoxyisobutyrate (CPIB), a hypolipidemic drug, demonstrated a significant increase in number of microbody profiles in the livers of male rats and in both sexes of acatalasemic mice (6-9). The CPIB-induced microbody proliferation in rat liver was associated with marked elevation in catalase activity resulting from enhanced rate of synthesis of this enzyme (10). The relationship between microbody proliferation and hypolipidemia resulting from CPIB treatment however, is not understood, since other hypolipidemic agents examined to date failed to induce microbody proliferation (11). We now report that a new hypolipidemic drug, nafenopin (Su-13437; 2-methyl-2-[P-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy]-propionic

acid) (12,13) induces a significant increase in the number of microbody profiles in male rat liver. A simultaneous increase in liver catalase activity and of catalase protein was also observed. Nafenopin, like CPIB, is a phenolic ether and these two compounds are structurally related (Fig. 1). Parallel studies with CPIB are included for comparison.

MATERIALS AND METHODS

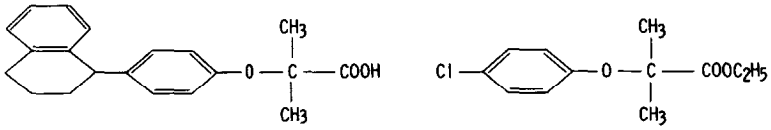
Treatment of animals: Inbred male F-344 rats (Simonson Laboratories Inc., Gilroy, Calif.) weighing between 140-175 grams were used in these experiments. Nafenopin was administered daily by stomach tube in a dose of 100 mg/kg b. wt., as 1% finely homogenized aqueous solution. CPIB was given daily by stomach tube as a 5% solution in olive oil in a dose of 250 mg/kg b. wt. Two to four animals were killed after 1,3,7,10 and 14 days on nafenopin or CPIB.

Ultrastructural studies: For electron microscopic study small segments of rat liver at selected intervals during nafenopin or CPIB treatment were fixed for 1-2 hours in 2% osmium tetroxide buffered to pH 7.4 with s-collidine. After fixation the tissues were dehydrated in graded series of alcohols and embedded in epoxy resin. Thin sections were cut with an ultramicrotome, stained with lead hydroxide and examined in an electron microscope.

Assay of catalase activity: Catalase activity was assayed spectrophotometrically at 25°C as described by Lück (14) on deoxycholate extracts of liver homogenates prepared according to the method of Ganschow and Schimke (15). Protein estimation was done by the method of Lowry et al (16).

Quantitation of catalase protein: Rat liver catalase was purified according to the procedure outlined by Price et al (17) and anticatalase serum was prepared by injecting the purified catalase into foot pads of rabbits (10). Quantitation of catalase protein in livers of normal rats and of rats treated with nafenopin or CPIB for 14 days was performed by immunotitration method (15).

Incorporation of H^3-δ-aminolevulinic acid into catalase: H^3-δ-aminolevulinic acid (specific activity 300 mc/m mole) was obtained from Amersham/Searle, Arlington Heights, Illinois. Control rats and rats treated with either



NAFENOPIN (Su-13437) CPIB

Figure 1. Structural formulas of Nafenopin and CPIB.

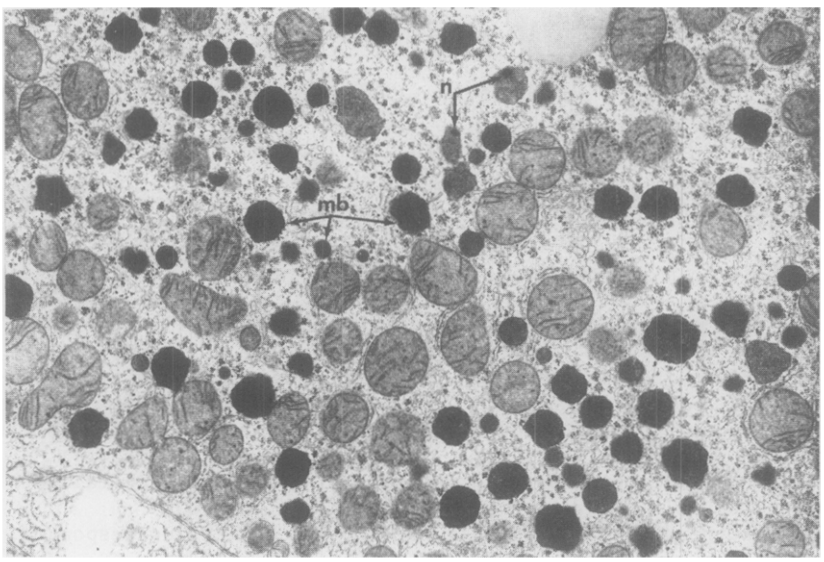


Figure 2. Portion of a liver cell from a male rat treated with Nafenopin (100 mg/kg b wt; daily by gavage) for 10 days. Marked increase in number with considerable variation in size of microbody profiles (mb) is evident. Nucleoid or crystalline core (n). X 14,500.

nafenopin or CPIB for 14 days were injected intraperitoneally with the isotope in a dose of 75 μc/100 gm body weight and killed 2 hours later. Deoxycholate extract of liver homogenate and immunoprecipitate of catalase with anticatalase serum were obtained and the radioactivity of catalase fraction determined as described previously (10).

RESULTS

Increase in the number of microbody profiles and proliferation of smooth endoplasmic reticulum were evident in the liver cells within 1-3 days after the initiation of nafenopin treatment. During the second week on nafenopin, the microbody profiles in liver cells were numerous and revealed considerable

Table 1. Liver weight and catalase activity in male rats treated with Nafenopin and CPIB.

Group[a]	No. of rats	Liver weight (gm/100 gm body wt) Mean ± standard error	Catalase activity (units/mg protein)
Untreated control	4	3.8 ± 0.31	39 ± 3.6
Nafenopin (14 days)	3	7.8 ± 0.38	81 ± 6.4
CPIB (14 days)	3	6.2 ± 0.35	85 ± 9.6

[a]Nafenopin (100 mg/kg b wt) and CPIB (250 mg/kg b wt) were administered daily by stomach tube.

variation in size and shape (Fig. 2). The microbody proliferation in rats treated with nafenopin for two weeks was roughly comparable to that induced by CPIB. The data on the effects of nafenopin and CPIB on liver weight and catalase activity are presented in Table 1. Both compounds produced significant increases in liver weight and in catalase activity. The increase in liver weight was more pronounced with nafenopin. The liver catalase activity of rats treated with nafenopin or CPIB for 2 weeks corresponded closely to the increase in the number of microbody profiles.

The results of immunoprecipitation of liver catalase in male rats treated with nafenopin and CPIB for 2 weeks are shown in Figure 3. The anticatalase serum required to precipitate completely the catalase activity from 1 ml of 5% liver extract from nafenopin and CPIB treated rats was 0.22 ml and 0.23 ml respectively. The amount of antiserum needed to precipitate catalase from 1 ml of 5% liver extract from normal rat liver was found to be 0.115 ml. These results suggest that the amount of catalase protein in liver extracts of rats given nafenopin or CPIB was approximately twice that of controls. In order to determine and compare the rates of synthesis of catalase in nafenopin and CPIB treated rats, the incorporation of H^3-δ-aminolevulinic acid into catalase fraction per gm of liver was studied at 14 days, with the assumption that the amount of labeled precursor incorporated into each newly synthesized catalase

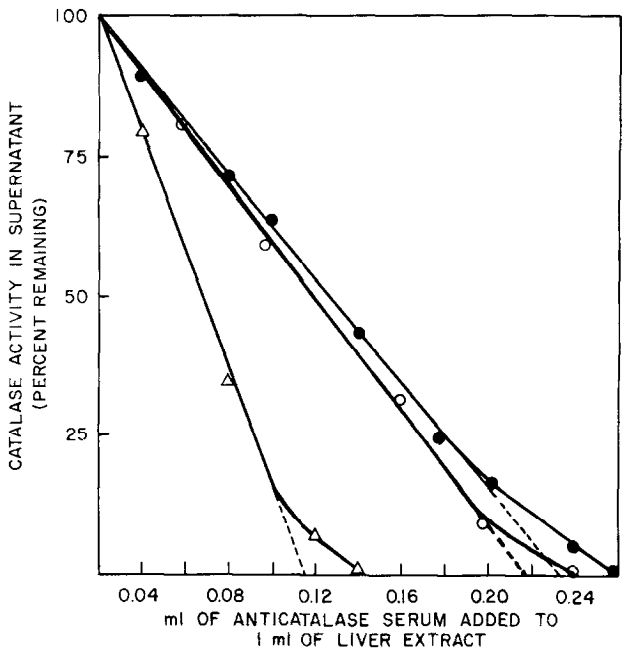


Figure 3. Immunoprecipitation of liver catalase in male rats treated with Nafenopin and CPIB for 2 weeks. Increasing amounts of anti-catalase serum were added to constant quantities of deoxycholate-treated liver extracts from normal (Δ-Δ), Nafenopin (o-o) and CPIB (•-•) treated rats. The resulting immunoprecipitates were sedimented and the catalase activity in the supernatant was determined by the spectrophotometric method. Dashed lines are extrapolations of the linear portions of the titration curves to zero catalase activity. Nafenopin (100 mg/kg b wt) and CPIB (250 mg/kg b wt) were administered daily by gavage.

molecule is the same in normal, nafenopin and CPIB-treated rat livers. Table 2 shows the results of this experiment and it is evident that there is a marked increase in radioactivity of catalase fraction of rats treated with nafenopin and CPIB when compared to controls.

DISCUSSION

Unlike other cytoplasmic constituents, microbodies (peroxisomes) in liver cells are infrequently altered in number or morphology by experimental manipulations (1,19). CPIB and acetylsalicylic acid are the only two compounds known to induce significant increase in the number of microbody profiles in liver cells (6-9,18,19). The CPIB-induced microbody proliferative response is more pronounced when compared to that resulting from the administra-

Table 2. Comparison of radioactivity of catalase fraction per gm of liver in male rats treated with nafenopin and CPIB[a].

Group[b]	dpm	Ratio
Control	6,552	1
Nafenopin	13,942	2.1
CPIB	12,528	1.9

[a] H^3-δ-aminolevulinic acid 75 μc per 100 gm of body weight was given intraperitoneally 2 hours before sacrifice. Catalase was precipitated from the liver extract by the addition of anticatalase serum and the radioactivity of catalase per gm of liver was calculated.

[b] Nafenopin (100 mg/kg b wt) and CPIB (250 mg/kg b wt) were given daily by gavage for 14 days.

tion of acetylsalicylic acid. The biological significance of microbody proliferation and stimulation of catalase synthesis in liver induced by CPIB and the regulatory mechanism(s) involved in the initiation of these events remain to be elucidated. Although a possible relationship between microbody proliferation and cholesterol metabolism was invoked because of the hypolipidemic properties of CPIB, additional studies suggested that the microbody proliferation resulting from CPIB treatment may be independent of its hypolipidemic effect (7,11).

The present investigation identifies another compound capable of inducing significant increase in the number of microbody profiles. Nafenopin, a hypolipidemic phenolic ether which is structurally similar to CPIB, produced rapid proliferation of microbody profiles and concomitant elevation in catalase activity in the livers of male rats. Nearly two-fold increase in the quantity of catalase protein and the rate of synthesis of catalase were also observed in the livers of rats treated with nafenopin for 14 days. It is evident from these studies that nafenopin is as effective as CPIB in inducing hepatic microbody proliferation in male rats.

Nafenopin causes marked enlargement of the liver presumably resulting

from hypertrophy and hyperplasia of liver cells (20). The nafenopin induced hepatomegaly may, in part, be attributed to the marked proliferation of microbody profiles and smooth endoplasmic reticulum in liver cells observed in the present study.

Identification of chemicals and altered metabolic states which are capable of producing microbody proliferation is essential in understanding the role of microbodies in cellular metabolism. Additional studies are necessary to delineate the relationship, if any, between microbody proliferation and hypolipidemic changes resulting from nafenopin and CPIB treatment. Further experiments are in progress in our laboratory in an attempt to determine which portion of the chemical configuration of the respective molecules of these two hypolipidemic agents is necessary to induce hepatic microbody proliferation.

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