

Suppression of clofibrate-induced peroxisome proliferation in rat liver by nicardipine, a calcium antagonist

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In vivo administration of nicardipine, nifedipine and diltiazem, known as calcium antagonists, suppressed the clofibrate-evoked induction of activities of peroxisomal enzymes, such as the peroxisomal fatty acyl-CoA oxidizing system and carnitine acetyltransferase. The inhibition activity of nicardipine with respect to clofibrate induction of the two enzyme systems was 62 and 33%, respectively. Induction of the peroxisomal bifunctional protein, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, by clofibrate was suppressed about 60% by nicardipine on analysis of the hepatic protein composition by SDS-polyacrylamide gel electrophoresis. Other drugs also exhibited similar inhibitory activity. These results provide the first demonstration of calcium antagonists, e.g. nicardipine, nifedipine and diltiazem, acting as inhibitors of peroxisome proliferation in animals. Such drugs might become useful as tools for elucidating the mechanism of peroxisome proliferation and for determination of the pathological conditions under which peroxisomal function is impaired.

Nicardipine; Nifedipine; Diltiazem; Enzyme inhibition; Peroxisome proliferation

1. INTRODUCTION

Peroxisomes are single membrane-bound cytoplasmic organelles which are present in a wide variety of both animal and plant cells [1,2]. It is well known that certain hypolipidemic drugs, such as clofibrate, induce marked proliferation of hepatic peroxisomes and increase the activity of peroxisomal β -oxidation in association with hepatomegaly [3,4]. The development of hepatocellular carcinomas in rodents fed on a diet containing a peroxisome proliferator was first reported in 1976 [5]. Since then, several hypolipidemic compounds have been shown to in-

duce liver tumors in rats and mice [6–8]. Furthermore, peroxisome diseases, such as Zellweger syndrome, have become the subjects of increasing interest since the discovery that peroxisomes in hepatocytes and renal tubular cells are absent in patients with this syndrome [9,10]. However, the mechanism by which peroxisome proliferators exert their pleiotropic responses still remains unelucidated. To gain more insights into the mechanism of peroxisome proliferation, specific inhibitors of peroxisome proliferation or peroxisomal enzymes would be of great value, especially if they can be used in vivo. Although attention has been paid to peroxisome proliferators, there is no description of inhibitors of peroxisome proliferation, except the studies of Leighton et al. [11] and Van den Branden and Roels [12] who reported that phenothiazine drugs could inhibit peroxisomal β -oxidation in vivo and in vitro. We describe here the influence of the calcium antagonists, nicardipine, nifedipine and diltiazem, on the induction of enzyme activity by clofibrate in hepatic peroxisomes and peroxisome-associated enzymes from the rat.

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Abbreviations: DAAO, D-amino acid oxidase; FAOS, cyanide-insensitive fatty acyl-CoA oxidizing system; FADH, fatty acyl-CoA dehydrogenase; CAT, carnitine acetyltransferase; CPT, carnitine palmitoyltransferase

Table 1

In vivo effect of nicardipine on some biochemical parameters of normal and clofibrate-treated rats

	Control	Nicardipine		Clofibrate	Cl + NC	
		50 mg/kg	100 mg/kg		50 mg/kg	100 mg/kg
Body weight gain (+ g)	40 ± 15	31 ± 9	31 ± 8	29 ± 6	29 ± 11	22 ± 17
Liver weight (% of body wt)	4.0 ± 0.2	4.4 ± 0.1 ^a	4.7 ± 0.2 ^a	4.9 ± 0.2 ^a	4.9 ± 0.1 ^a	5.5 ± 0.5 ^a
Liver protein (mg/g)	188 ± 23	206 ± 5	201 ± 8	232 ± 18	223 ± 18	225 ± 5 ^a
Cholesterol						
Serum (mg/dl)	76 ± 6	68 ± 4 ^a	73 ± 10	52 ± 8 ^a	55 ± 5 ^a	49 ± 8 ^a
Liver (mg/g)	3.6 ± 0.5	4.3 ± 0.2 ^a	4.5 ± 0.2	4.2 ± 0.2	4.3 ± 0.6	3.9 ± 0.6
Triglyceride						
Serum (mg/dl)	110 ± 20	112 ± 27	57 ± 8 ^a	57 ± 7 ^a	44 ± 6 ^{a,b}	27 ± 10 ^{a,b}
Liver (mg/g)	7.6 ± 1.1	12.2 ± 1.4 ^a	10.2 ± 1.5 ^a	13.6 ± 1.4 ^a	14.6 ± 2.5 ^a	14.0 ± 2.6 ^a

Experimental conditions are described in section 2. Each value is the mean ± SD of 5 rats. Statistical evaluations were performed by Student's *t*-test: ^a *p* < 0.05 vs control, ^b *p* < 0.05 vs clofibrate; Cl, clofibrate; NC, nicardipine

2. MATERIALS AND METHODS

Male Wistar rats of about 150 g were used. For the first experiment, 30 animals were divided into 6 groups. Groups 1–3 were fed on standard diet and groups 4–6 on a diet containing 0.25% (w/w) clofibrate for 2 weeks. Animals of groups 2, 5 and 3, 6 were orally administered with nicardipine suspended in 0.5% (w/v) methylcellulose-saline at dose levels of 50 and 100 mg/kg body wt daily, respectively, for 2 weeks via stomach tubes; control animals received an equivalent volume of the same medium. In the second experiment, concerning the effects of nifedipine and diltiazem, animals (5 per group) fed on the diet containing 0.25% (w/w) clofibrate were orally administered with nifedipine and diltiazem at a dose level of 50 mg/kg body wt daily for 2 weeks. After killing, the livers

were removed and 10% (w/v) homogenates were prepared in 0.25 M sucrose. Sera obtained from the animals were used for determination of the lipid level.

Catalase activity was determined as in [13]. One unit of activity was defined as the amount of enzyme resulting in a value of *K* = 1 where *K* is the rate constant. The activities of DAAO and urate oxidase were determined according to [14]. FAOS activity was assayed as described in [15] using palmitoyl-CoA as substrate. CAT and CPT activities were determined as in [16] using acetyl-CoA and palmitoyl-CoA as substrate, respectively. FADH activity was assessed as described [17] using palmitoyl-CoA as substrate. Unless otherwise stated, 1 unit of all enzyme activities was defined as the amount of enzyme that produced 1 nmol reaction product/min. Protein content was determined by the method of Lowry et al. [18] using bovine serum albumin as a standard.

Table 2

In vivo effect of nicardipine on some peroxisomal and mitochondrial enzymes of normal and clofibrate-treated rats

	Control	Nicardipine		Clofibrate	Cl + NC	
		50 mg/kg	100 mg/kg		50 mg/kg	100 mg/kg
Catalase	43.8 ± 5.5	44.5 ± 2.5	46.8 ± 4.4	100.7 ± 19.4 ^a	82.8 ± 13.0 ^a	76.0 ± 7.0 ^{a,b}
DAAO	1.05 ± 0.2	0.86 ± 0.2	0.76 ± 0.2 ^a	0.80 ± 0.2	0.63 ± 0.2 ^a	0.71 ± 0.2 ^a
Urate oxidase	2.57 ± 0.22	2.60 ± 0.22	2.57 ± 0.19	2.86 ± 0.21	2.22 ± 0.33 ^b	2.60 ± 0.33
FAOS	814 ± 51	740 ± 47	661 ± 59	4506 ± 435 ^a	2838 ± 231 ^{a,b}	2200 ± 231 ^{a,b}
FADH	1251 ± 171	1241 ± 27	1147 ± 121	2754 ± 336 ^a	2387 ± 191 ^a	2220 ± 293 ^{a,b}
CAT	424 ± 101	844 ± 125 ^a	788 ± 40 ^a	18560 ± 2290 ^a	10329 ± 1644 ^{a,b}	10400 ± 1280 ^{a,b}
CPT	1853 ± 308	1829 ± 272	1520 ± 433	5388 ± 618 ^a	4358 ± 890 ^a	4235 ± 726 ^{a,b}

Experimental conditions are described in section 2. Enzyme activities are expressed as U/g liver. Each value is the mean ± SD of 5 rats. Statistical evaluations were performed by Student's *t*-test: ^a *p* < 0.05 vs control, ^b *p* < 0.05 vs clofibrate; Cl, clofibrate; NC, nicardipine

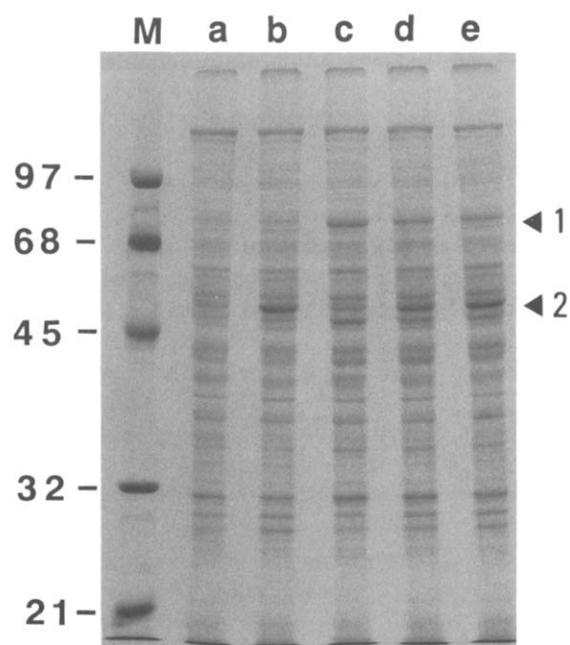


Fig.1. SDS-polyacrylamide gel electrophoresis of the homogenate. 30 μ g protein was applied on a 11% polyacrylamide gel, which was stained with Coomassie brilliant blue R. (a) Control, (b) nicardipine (100 mg/kg), (c) clofibrate, (d) clofibrate + nicardipine (50 mg/kg), (e) clofibrate + nicardipine (100 mg/kg); M, molecular mass markers (in kDa).

Liver and serum triglyceride and cholesterol levels were assayed according to [19,20], respectively. SDS-PAGE was performed using 11% polyacrylamide gels as described in [21].

3. RESULTS

The effects of nicardipine administration on some biochemical parameters for normal and

clofibrate-treated rats are summarized in table 1. Nicardipine induced hepatomegaly in normal rats in a dose-dependent manner and also had a tendency to enhance hepatomegaly induced by clofibrate. Although in clofibrate-treated animals a slight increase in hepatic protein content was observed that was not statistically significant, nicardipine had no apparent effect on the corresponding values for both groups. Nicardipine showed no effect on the hypocholesterolemic action of clofibrate. Serum triglyceride level in normal rats was markedly decreased by nicardipine at 100 mg/kg body wt and also in clofibrate-treated groups the decreased serum triglyceride level was further reduced to up to 25% of the control value by nicardipine in a dose-dependent manner. Nicardipine induced triglyceride accumulation in livers of normal rats.

The effects of nicardipine on a number of peroxisomal and mitochondrial enzymes are summarized in table 2. The activities of peroxisomal catalase, FAOS, CAT which was also distributed in mitochondria, and mitochondrial FADH and CPT were markedly increased by clofibrate administration. However, simultaneous administration of nicardipine suppressed the clofibrate-induced increase in activity of catalase, FAOS, FADH, CAT and CPT, the levels of inhibition being 44, 62, 47, 45 and 33%, respectively. In fig.1, the change in hepatic protein composition after administration of the drugs is depicted. Although one can observe a marked increase in the amount of a polypeptide of 76 kDa (band 1), which is known as a peroxisomal proliferation-associated polypeptide (e.g. enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase in the

Table 3

In vivo effect of nifedipine and diltiazem on some peroxisomal and mitochondrial enzymes of clofibrate-treated rat liver

	Control		Clofibrate		Cl + NF		Cl + DZ	
Catalase	48.2	$\pm$ 7.6	63.3	$\pm$ 11 ^a	68.8	$\pm$ 8 ^a	70.4	$\pm$ 8.4 ^a
DAAO	1.21	$\pm$ 0.14	1.00	$\pm$ 0.13 ^a	1.00	$\pm$ 0.11 ^a	0.95	$\pm$ 0.17 ^a
FAOS	617	$\pm$ 146	4823	$\pm$ 719 ^a	2926	$\pm$ 330 ^{a,b}	3108	$\pm$ 484 ^{a,b}
FADH	1785	$\pm$ 287	2545	$\pm$ 247 ^a	2240	$\pm$ 145 ^{a,b}	2766	$\pm$ 653 ^a
CAT	621	$\pm$ 120	21412	$\pm$ 2804 ^a	14588	$\pm$ 1655 ^{a,b}	19569	$\pm$ 3289 ^a
CPT	1987	$\pm$ 132	7003	$\pm$ 1181 ^a	5338	$\pm$ 1668 ^a	5784	$\pm$ 1360 ^a

Experimental conditions are described in section 2. Each value is the mean $\pm$ SD of five rats. Statistical evaluations were performed by Student's *t*-test: ^a *p* < 0.05 vs control, ^b *p* < 0.05 vs clofibrate; Cl, clofibrate; NF, nifedipine; DZ, diltiazem

peroxisomal β -oxidation system), in clofibrate-treated rats, the simultaneous administration of nicardipine significantly suppressed the increase in the amount of the polypeptide. After nicardipine treatment, a significant increase in the amount of a 49 kDa polypeptide (band 2), and slight changes in that portion corresponding to a molecular mass of less than 32 kDa were also observed. The modification by other calcium antagonists of the effects of clofibrate on rat liver peroxisomal enzymes is listed in table 3. On simultaneous administration of nifedipine at 50 mg/kg with the clofibrate diet, induction of the activities of peroxisomal FAOS, FADH and CAT by clofibrate was suppressed significantly and diltiazem also suppressed the induction of FAOS to a statistically significant extent. The effects of these two calcium antagonists were supported by examination of the protein compositions in the livers of treated rats with the use of SDS-polyacrylamide gel electrophoresis (not shown).

4. DISCUSSION

The mechanism by which peroxisome proliferators exert a pleiotropic response has not yet been clearly established. Two questions still remain to be resolved: (i) how do these peroxisome proliferators induce such an increase in the peroxisome population and selected peroxisomal enzymes in liver cells? (ii) How is xenobiotic-induced peroxisome proliferation related to the development of hepatic carcinogenesis? Two possibilities have been considered: these compounds (i) induce peroxisome proliferation by increasing lipid influx into liver cells [22,23], or serve as substrates for the peroxisomal β -oxidation system; (ii) exert their effect through a ligand-receptor-mediated mechanism [24]. On studying the mechanism of peroxisome proliferation induced by these compounds, agents with the ability to inhibit peroxisome proliferation must be a useful tool. Until now, although some drugs are known to be inhibitors of peroxisomal β -oxidation [11,12], there has been no report concerning drugs which can inhibit peroxisome proliferation *in vivo*.

Our present experiments demonstrate that the calcium antagonists nicardipine, nifedipine and diltiazem suppress the induction of peroxisomal enzyme activities by clofibrate *in vivo*. As shown in

table 1, administration of nicaldipine at 50 or 100 mg/kg body wt to rat simultaneously with a clofibrate diet suppressed induction of peroxisomal catalase, FAOS and CAT activities by clofibrate. Furthermore, even in control animals one may observe a tendency of nicardipine to suppress FAOS activity. In analysis of the hepatic protein composition by means of SDS-polyacrylamide gel electrophoresis, the increased quantity of peroxisome proliferation-associated polypeptide as a result of clofibrate treatment was lowered by nicardipine. This polypeptide is an enzyme present in the peroxisomal β -oxidation system and the change in its amount provides a biochemical measure of peroxisome proliferation. Thus, the results obtained from analysis of the protein composition of the liver show that nicardipine inhibits the enhancement in biosynthesis of certain peroxisomal enzyme proteins. Similar effects have been observed in the case of the other calcium antagonists, nifedipine and diltiazem, although a slight difference in potency was noted. Although the mechanism of suppression of peroxisome proliferation by these calcium antagonists remains unknown, a number of possibilities can be considered. If xenobiotic-induced peroxisome proliferation were mediated by a specific receptor (binding protein), the calcium antagonists could compete with a peroxisome proliferator on the receptor, or affect the metabolism of the xenobiotics, causing a decrease in the activity of the peroxisome proliferator. Another explanation would be provided by the existence of a calcium-related mechanism during the induction of peroxisome proliferation. Increasing interest has been focussed on Ca^{2+} with respect to the regulation at the molecular level of biological systems through calcium signaling. Nicardipine, nifedipine and diltiazem have been widely used as typical drugs. It has been shown that Ca^{2+} performs important functions in (i) regulation of contractile proteins, (2) the process of secretion of a secretory protein, and (3) enhancement of cell growth through protein phosphorylation, etc. Therefore, our present results, that all of the calcium antagonists used here showed similar suppressing activities on clofibrate-induced peroxisome proliferation, suggest the possibility that during the process of xenobiotic-induced peroxisome proliferation a calcium-related mechanism including protein

phosphorylation and/or calmodulin-dependent process might participate. This is an interesting problem awaiting clarification in the future.

As shown in table 1, nicardipine markedly decreased serum triglyceride levels in both control and clofibrate-treated groups, whereas the activities of peroxisomal enzymes related to lipid metabolism were decreased by nicardipine, suggesting dissociation of the hypotriglyceridemic effect from the capacity to carry out peroxisomal fatty acid metabolism. Hence, it was also concluded that triglyceride accumulation in the liver could not be a trigger for the induction of peroxisomal enzymes and/or peroxisome proliferation.

Furthermore, on SDS-PAGE analysis of the hepatic protein composition of nicardipine-treated rats a marked increase in amount of a 49 kDa polypeptide was observed. This increase was dose-dependent and was unaffected by simultaneous administration of clofibrate. Although identification of this polypeptide remains to be made, it would be most interesting to ascertain whether the increase in this polypeptide is associated with the pharmacological activity of nicardipine as a calcium antagonist.

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