Co-suppression by Nicardipine, a Calcium Antagonist, of Induction of Microsomal Lauric Acid Hydroxylation with Peroxisome Proliferation in Clofibrate-Treated Rat Liver

Takafumi Watanabe, Hiroki Itoga, Sumito Okawa, Hiroshi Tamura and Tetsuya Suga

Department of Clinical Biochemistry, Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan. Received October 23, 1990

The in vivo effect of nicardipine, a well-known calcium antagonist, on microsomal ω-oxidation of laurate in clofibrate-treated rat liver was studied. The 15.3-fold induction of the activity by 2 weeks administration of 0.25% clofibrate in the diet was markedly suppressed to about 6-fold by co-administration of nicardipine at 100 mg/kg body weight. Similarly, the induction of peroxisomal β-oxidation and carnitine acetyltransferase activities were also suppressed by this simultaneous administration by more than 50%. Although clofibrate also induced the activity of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome c reductase and increased the hepatic content of cytochrome P-450, no suppressive effect of nicardipine was observed. Contrarily, nicardipine induced the reductase activity and increased the hepatic content of cytochromes P-450 and b_5. These results provide the first demonstration of a calcium antagonist, e.g. nicardipine acting as inhibitor of the induction of microsomal ω-oxidation, in association with the inhibition of peroxisome proliferation in animals. The suppression of drug-induced peroxisome proliferation and microsomal ω-oxidation by the calcium antagonist may help in elucidating the causal relationship of the induction mechanisms between peroxisomal and microsomal enzymes.

Keywords: peroxisome proliferation; ω-oxidation; laurate hydroxylase; fatty acid oxidation; co-suppression; calcium antagonist

The effect of hypolipidemic drugs on rat liver has been extensively studied in terms of morphometric and biochemical responses in peroxisomes. 1-4 Certain hypolipidemic drugs such as clofibrate (CL) and its derivatives induce hepatomegaly and the proliferation of hepatic peroxisomes, in association with the induction of peroxisomal fatty acid oxidation-related enzymes. 5-7 These are classified as non-mutagenic hepatocarcinogens. 8-10 Thus, induction or inhibition of enzymes by drugs is important to evaluate their efficacy and toxicity. Many recent studies showed that peroxisome proliferators induced microsomal ω-oxidation activity of fatty acids through the induction of a cytochrome P-450 isozyme termed P-450 IVA1 (formerly termed cytochrome P-452), which has an unusually narrow substrate specificity for the terminal (or ω) hydroxylation of fatty acids including lauric acid. 11-13 From the finding of co-induction of peroxisomal fatty acid oxidation activity with microsomal lauric acid hydroxylation activity, it has been postulated that these responses are based on a mechanistic interrelationship. In a previous report, we first reported that CL-induced peroxisome proliferation was suppressed by calcium antagonists such as nicardipine and suggested that a calcium-related mechanism might participate in the process of peroxisome proliferation. 14,15 In this report, we examined whether the induction of microsomal lauric acid hydroxylation activity by CL was suppressed by nicardipine (NC) in rat liver, in an attempt to clarify the mechanistic interrelationship between such induction and the peroxisome proliferation.

Experimental
Male Wistar rats weighing about 120 g were divided into 4 groups (5 animals/group), fed standard laboratory diet (CE-2, Clea Japan) and served drinking water ad lib. Groups 2 and 3 were orally administered 0.25% clofibrate (CL) in the diet and nicardipine at 100 mg/kg body weight (b.w.) daily for 2 weeks suspended in 0.5% methylcellulose (1 ml/100 g b.w.), respectively. Group 4 was simultaneously administered the above quantities of clofibrate and nicardipine. The control group (group 1) received an equal volume of the same vehicles. After treatment the livers were removed and 10% (w/v) homogenates were prepared in 0.25 M sucrose-1 mM ethylenediamine tetraacetic acid (EDTA)-10 mM Tris-HCl (pH 7.4). The homogenate was centrifuged at 10000 × g for 20 min at 4 °C, then at 105000 × g for 20 min and the resulting pellet was suspended in 0.25 M sucrose at 0.2 g original tissue/ml. The suspension was used as a microsomal fraction for assay of the microsomal enzyme activities and the content of cytochromes P-450 and b_5 content. The activity of the cyanide-insensitive fatty acyl-CoA oxidizing system (FAOS) was determined using palmitoyl-CoA as substrate. 16 The activity of carnitine acetyltransferase (CAT) was determined using acetyl-CoA as substrate. 17 Unless otherwise stated, 1 unit of each enzyme activities was defined as the amount of the enzyme that produced 1 nmol of reaction product per min per g of liver. The activity of microsomal laurate hydroxylase (LH) was determined using (1-14C)lauric acid as substrate. 18 Aminoxypropine demethylase (AD) and aniline hydroxylase (AH) activities were determined by the method of Mazel. 19 Reduced nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome c reductase (Cyt. C-R) activity was determined by the method of Beaufoy et al. 20 Cytochromes P-450 and b_5 contents were determined by the method of Omura and Sato, 21 and protein content was determined by the method of Lowry et al. 22 using bovine serum albumin as a standard.

Results
The effects of in vivo administration of NC on body weight gain, liver weight and hepatic protein content of normal and CL treated rats are summarized in Table I. The body weight gain of the group administrated both NC and CL decreased slightly compared with the control, whereas no significant alteration was observed in the groups treated with each drug separately. Hepatomegaly was observed in all of the treated groups. There was no significant difference in the liver protein among the groups. Table II shows the effects of drugs on microsomal enzyme activities. Although the activity of AD was decreased to 70% of the control by CL, no significant change was observed in the NC- and NC plus CL-treated groups. No significant change in AH activity was observed in any groups compared with the control. Cytochrome c reductase activity was increased 1.5-fold and 1.9-fold in NC- and CL-treated groups, respectively, and 2.11-fold in the group treated with both drugs. The hepatic content of total cytochrome P-450 species was increased 2-fold in NC- and CL-treated groups, and
TABLE I. In Vivo Effect of Nicardipine (NC) on Body Weight Gain, Liver Weight and Hepatic Protein Content

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CL</th>
<th>NC</th>
<th>CL + NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g)</td>
<td>103 ± 8</td>
<td>97 ± 8</td>
<td>82 ± 22</td>
<td>83 ± 7</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>4.3 ± 0.1</td>
<td>5.6 ± 0.6*</td>
<td>5.2 ± 0.5*</td>
<td>5.7 ± 0.3*</td>
</tr>
<tr>
<td>(as % of body weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver protein (mg/g)</td>
<td>205 ± 15</td>
<td>203 ± 15</td>
<td>197 ± 23</td>
<td>223 ± 11</td>
</tr>
</tbody>
</table>

Experimental conditions are described in Experimental. Each value is the mean ± S.D. of 5 rats. Statistical evaluation was performed by Student’s t-test:
- a) p < 0.05 vs. control.

TABLE II. In Vivo Effect of Nicardipine (NC) on Some Peroxiosomal and Microsomal Enzymes of Normal and Clofibrate (CL)-Treated Rat Liver

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CL</th>
<th>NC</th>
<th>CL + NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxiosomal FAOS</td>
<td>728 ± 50</td>
<td>587 ± 74*</td>
<td>660 ± 80</td>
<td>2214 ± 384</td>
</tr>
<tr>
<td>CAT</td>
<td>299 ± 81</td>
<td>17882 ± 348*</td>
<td>672 ± 66*</td>
<td>7555 ± 1384*</td>
</tr>
<tr>
<td>Microsomal LH</td>
<td>11 ± 4</td>
<td>169 ± 39*</td>
<td>10 ± 1</td>
<td>65 ± 29*</td>
</tr>
<tr>
<td>AD</td>
<td>129 ± 17</td>
<td>91 ± 16</td>
<td>109 ± 26</td>
<td>127 ± 37</td>
</tr>
<tr>
<td>AH</td>
<td>47 ± 5</td>
<td>37 ± 11</td>
<td>42 ± 8</td>
<td>47 ± 13</td>
</tr>
<tr>
<td>NADPH-Cyt. C-R</td>
<td>3228 ± 255</td>
<td>3586 ± 302*</td>
<td>4426 ± 600*</td>
<td>5578 ± 766*</td>
</tr>
<tr>
<td>Cytochrome P-450</td>
<td>55 ± 16</td>
<td>110 ± 30*</td>
<td>109 ± 18*</td>
<td>158 ± 44*</td>
</tr>
<tr>
<td>Cytochrome b3</td>
<td>41 ± 4</td>
<td>36 ± 8</td>
<td>53 ± 8*</td>
<td>50 ± 7*</td>
</tr>
</tbody>
</table>

Experimental conditions are described in Experimental. Each value is the mean ± S.D. of 5 rats. Statistical evaluation was performed by Student’s t-test:
- a) p < 0.05 vs. control.
- b) p < 0.05 vs. clofibrate. The activities of enzyme activities are expressed as nmol/min/g liver; cytochromes P-450 and b3 contents, nmol/g liver.

the increase was enhanced 3-fold by the simultaneous administration of the two drugs. The oxidation activity of the control group was 11.0 ± 4.4 U/g liver. With CL administration the activity was increased by 15.3-fold, however, NC suppressed the increase to about 6-fold.

Discussion

The administration of peroxiosome proliferators induced the activities of microsomal LH,11-13 cytosolic epoxide-dehydrogenase23 and acyl-CoA hydrolase24 together with the peroxiosomal β-oxidation in liver of rats and mice. Although these results suggest that a similar biochemical mechanism might participate in the induction process of these enzymes housed in different subcellular compartments, the detailed mechanism has not yet been clarified. Studies of the mechanism of ω-oxidation induction by drugs, such as hypolipidemic peroxiosome proliferators reveal that agents with the ability to inhibit this induction may be a useful tool. Although some drugs are known to be inhibitors of peroxiosomal β-oxidation,25,26 to the author’s there has to date been no report concerning drugs which can inhibit microsomal ω-oxidation induction in vivo. Our present experiments demonstrate for the first time that the calcium antagonist, NC, when administered simultaneously with CL in vivo, suppresses the induction of ω-oxidation activity by the latter drug (Table II). In the present experiments we chose a dose of 100 mg/kg b.w. However, in our previous report the suppression of peroxiosomal enzyme induction by NC was dose dependent in the range of 20—100 mg/kg. A 2-week time course study of the induction of peroxiosomal enzymes when CL and NC were administered together showed the suppressive effect of NC on the CL-induced increase in an activity of an enzyme such as the cyanide-insensitive fatty acyl-CoA oxidizing system (FAOS) after 3—5 d treatment.15 Thus, in the early phase of the treatment with CL there was no significant difference in the peroxiosomal enzyme activities between the CL-group and the group treated with CL and NC, and the activity of both groups was increased by about 4-fold of the control. This finding clearly shows that NC does not effect the disposition, i.e., the pharmacokinetics of CL. Furthermore the suppressive effect of NC on CL-induced peroxiosome proliferation was also found when CL was administered intraperitoneally (data not shown). From these results we concluded that the drug’s suppressive effect on enzyme induction might be due to a modulation of the cellular response, i.e., the biosynthetic process of the enzymes. The finding that the suppression was present even after 2 weeks of treatment when the inductive potency of CL would be at its height supports this conclusion. The in vitro experiment also showed that the inhibitory effect of NC was not due to its direct action on the LH molecule itself (data not shown). Microsomal drug metabolizing enzymes such as AH and AD were not influenced by either CL or NC, and the hepatic content of cytochrome P-450 was slightly increased by both drugs. Compared with those enzymes, ω-oxidation is remarkably unique in character because its response to CL is quite similar to that to peroxiosomal enzymes such as FAOS and CAT. Also, the induction was markedly suppressed by NC in a manner similar to that by FAOS and CAT. Although the mechanism of suppression of peroxiosome proliferation by calcium antagonist has not been clarified, a similar mechanism might underlie the suppression of induction of ω-oxidation by NC. If this induction was mediated by a specific receptor (binding protein), the calcium antagonists could compete with the drugs, or affect the metabolism of the drugs, causing a decrease in their inducement activities. There might also be another possible explanation: that the induction process is mediated by a calcium-related mechanism. Thus, our present results suggest that a calcium-related mechanism including protein phosphorylation and/or a calmodulin-dependent process might participate during drug-induced ω-oxidation, as suggested in drug-induced peroxiosome proliferation. Although the enzyme participating in ω-oxidation is housed in the microsomes, the biosynthesis may be regulated by a mechanism common to that contributing to the regulation of biosynthesis of peroxiosomal enzymes.

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References