Detection of Peroxisome Proliferators Using a Reporter Construct Derived from the Rat Acyl-CoA Oxidase Promoter in the Rat Liver Cell Line H-4-II-E

Michael J. Lee, Pauline Gee, and Shannon E. Beard

ABSTRACT

Peroxisome proliferators are nongenotoxic carcinogens capable of causing rapid transcriptional activation of genes comprising the rodent β-oxidation pathway. Numerous compounds, such as hypolipidemic drugs, herbicides, plasticizers, and analgesics have been identified as peroxisome proliferators in rodents. We have developed a whole-cell in vitro assay to detect peroxisome proliferators in approximately 48 h. A promoter::chloramphenicol acetyltransferase (CAT) fusion construct for rat acyl-CoA oxidase (ACOX), the rate-limiting enzyme in the peroxisomal β-oxidation pathway, was stably transfected into the rat liver cell line H-4-II-E. Treatment of the recombinant cell line (ACOX::CAT) with peroxisome proliferators, WY 14,643, dolfibrate, diethylstilbestrol, and ethyl methanesulfonate, as negative controls and showed no significant induction of CAT protein 48 h after exposure. Nonsteroidal anti-inflammatory drugs including ibuprofen, fenbupe, naproxen, and acetaminophen also up-regulated ACOX::CAT. Phorbol 12-myristate 13-acetate, a nongenotoxic carcinogen that is not classified as a peroxisome proliferator, also resulted in a slight induction of ACOX::CAT, consistent with the role of cell proliferation in tumor progression. The carcinogenic compounds 4-nitroquinoline N-oxide, ethyl methanesulfonate, diethylstilbestrol, and 2-aminoanthracene did not induce ACOX::CAT. Although the significance of peroxisome proliferators and their impact on humans is still unknown, this ability to identify them of interest to the pharmaceutical and chemical industries. This assay was able to detect known peroxisome proliferators tested in approximately 48 h of exposure and to distinguish them from genotoxic carcinogens.

INTRODUCTION

Peroxisomes, cytoplasmic organelles found in virtually all eukaryotic cells, are present in very high numbers in hepatocytes and epithelial cells of the kidney (1). They are involved primarily in oxidative catabolism of long chain fatty acids via the peroxisomal β-oxidation pathway (2). Normally, peroxisomes comprise less than 2% of the total volume of hepatocytes. Exposure to a group of xenobiotics classified as peroxisome proliferators leads to a dramatic increase in the size, number, and activity of peroxisomes in hepatic and renal tissues of rodents, accounting in some cases for 15–25% of the total cellular volume (3). In addition, hepatic hyperplasia, hepatomegaly, and hepatocarcinogenesis are observed in rodents after chronic exposure to peroxisome proliferators (3).

Peroxisomal proliferators are structurally diverse compounds with widespread use as industrial plasticizers and solvents, herbicides, hypolipidemic drugs, and anti-inflammatory agents. These compounds have not been found to interact directly with DNA but are thought to exert their carcinogenic effect through disruptions of cellular processes (4, 5).

Interestingly, peroxisomal proliferation or the resulting hepatocarcinogenesis seen in rats has not been demonstrated to the same extent in other species, including humans. Possible explanations for this may be low levels of the receptor activated by peroxisome proliferators or differences in transcriptional activation (6, 7). Nonetheless, until a mechanism is found for rodent carcinogenesis, human safety with these compounds will continue to be questioned (8). Some peroxisome proliferators have shown genotoxic effects such as sister chromatid exchange and chromosomal aberrations, but experimental evidence suggests that genotoxic events are compound-specific. Therefore, indicators of genotoxic events are not good general markers for detecting peroxisome proliferators (9). It has been shown that a correlation does exist in rats between the induction of β-oxidation enzymes and the amplification of peroxisomes (9, 10). Also, there is an 80% correlation between the induction of peroxisome proliferation and hepatocarcinogenicity in rats (9, 11).

Most peroxisome proliferators activate a member of the hormone receptor superfamily found to be highly expressed in both the liver and kidney (12, 13). A nuclear receptor, the PPAR,2 heterodimerizes with the RXR, also abundant in the liver and kidney (14). Binding of the dimer to the PPRE, a short regulatory stretch of DNA found in the promoters of many genes including ACOX, has been demonstrated to activate transcription of the structural gene (12, 15, 16). ACOX produces H2O2 as a byproduct in the β-oxidation of long chain fatty acids (17). ACOX is the primary and rate-limiting enzyme in the peroxisomal β-oxidation pathway. Exposure to peroxisome proliferators can lead to a 20-fold increase in ACOX mRNA and a subsequent increase in the corresponding protein (1).

We have developed an in vitro assay that monitors induction of peroxisomal ACOX, the most widely used marker to measure peroxisome proliferator-induced responses (18). We stably transfected a plasmid containing the rat ACOX promoter spanning the region of the -1273/-471 nucleotides upstream of the transcription initiation site for the ACOX gene and the rabbit β-globin promoter fused to a CAT reporter gene (Fig. 1) into the rat hepatoma cell line H-4-II-E. ACOX, which contains PPRE motifs in this region of its promoter, has been shown to be transcriptionally activated directly by binding of ligand-bound PPAR (17). Upon exposure to a variety of compounds, the assay was able to detect all known peroxisome proliferators tested, as measured by a CAT ELISA. Ames-positive and -negative carcinogens were tested as negative controls and showed no significant induction of ACOX::CAT.

MATERIALS AND METHODS

Cell Lines. The transformed rat hepatoma cell line H-4-II-E was used for all experiments in this study (American Type Culture Collection, Rockville, MD). Both the recombinant H-4-II-E cells containing the rat ACOX promoter::CAT reporter fusion constructs and the parental cell line were maintained as described previously (19).

Plasmid/Reporter Constructs. The plasmid used to transfect the H-4-II-E cells, pACO(-1273/-471)G.CAT, was obtained from Dr. Jonathan D. Tugwood (Zeneca Central Toxicology Laboratory, Cheshire, United Kingdom). The plasmid contains the 5′ flanking sequence between -1273 and -471 bp

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2 The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; PPRE, peroxisome proliferator response element; ACOX, acyl-CoA oxidase; CAT, chloramphenicol acetyltransferase; PMA, phorbol 12-myristate 13-acetate; 4-NQO, 4-nitroquinoline N-oxide; DEHP, di(2-ethylhexyl) phthalate; EMS, ethyl methanesulfonate; DES, diethylstilbestrol; TPA, 12-O-tetradecanoylphorbol-13-acetate.
of the rat ACOX promoter. This region is fused to the rabbit β-globin promoter (−109 to +10 bp), which is in turn linked to the bacterial CAT reporter gene.

**Stable Transfections.** H-4-II-E cells were stably transfected as described previously (19). G418 (Life Technologies, Inc.; 150 μg/ml) was added 2 days after transfection. Media containing G418 were changed 2 and 10 days after the initial addition. Single-cell clones were identified, transferred, and grown to greater cell numbers before being transferred to 96-well microtiter plates for screening.

**Clone Screening.** Individual clones were screened by exposure to WY 14,643 (200 μM) in 1% DMSO for 48 h. Cells were then lysed with 1× lysis buffer (5 mM 4-morpholinepropanesulfonic acid, 2.5 mM NaCl, 38 μM MgCl₂, and 0.25% Triton X-100 [pH 6.5]). The lysate was transferred to CAT ELISA plates (Boehringer Mannheim, Mannheim, Germany), and the ELISA was performed as described previously (19). Positive clones were frozen in supplemented MEM containing 10% DMSO and stored in liquid nitrogen.

**Chemicals.** WY 14,643 (14-chloro-6-(2,3-xylidino)-2-pyrimidinyllithio)acetic acid) was a gift from Mark Shiganaga and was purchased from Chemsys Science Laboratories. 2-AA was obtained from Aldrich Chemical Co. (Milwaukee, WI). DMSO was obtained from Fisher Biotech (Pittsburgh, PA). (S)-6-methoxy-α-methyl-2-naphthaleenecarboxylic acid (naproxen), γ-oxo-1,1′-binaphthyl-4-butoanionic acid (fenbufen), acetylsalicylic acid (aspirin), ibuprofen, 2-(p-chlorophenoxy)-2-methylpropionic acid ethyl ester (clofibrate), salicylic acid, PMA, c-RA, t-RA, 4-NQO, 4-acetamidophenol (acetaminophen), DEHP, acetylsalicylic acid, ibuprofen, 2-(p-chlorophenoxy)-2-methylpropionic acid ethyl ester (clofibrate), salicylic acid, PMA, c-RA, t-RA, 4-NQO, 4-acetamidophenol (acetaminophen), DEHP, EMS, and DES were obtained from Sigma Chemical Co. (St. Louis, MO).

**Assay Conditions.** Frozen stocks were thawed and passaged twice a week for 2–3 weeks before use. Cells (55,000) were plated into each well of a 96-well microtiter plate. Cells were exposed to chemicals 24 h later. Chemicals were prepared as 10× stocks. For all nine replicates of each test sample, the final solvent concentration for DMSO-soluble samples was 0.5%, except for clofibrate, which was at 1%. Cells were exposed to the compound for 48 h before assaying for CAT induction as described previously (19). Fold induction values were calculated as the average of nine replicates tested over a 2-week period.

**RESULTS**

**Activity of c-RA and t-RA.** To test for activation of the PPAR in this system, we investigated the effects of c-RA and t-RA on ACOX::CAT induction. The RXR is the heterodimerization partner for the PPAR, and both receptors have been shown to be necessary for activation of ACOX (20). c-RA is a specific ligand for the RXR receptor, whereas its isomer, t-RA, is preferred by the RAR. The results for nine replicates for each dose of both c-RA and t-RA are summarized in Table 1. c-RA (5 μM) induced the ACOX::CAT fusion 9-fold over basal levels. Inductions decreased only slightly over the dose range, reaching 6.8-fold at 313 nM. In contrast, t-RA showed a 5-fold induction at 5 μM that dropped to below 2-fold at 625 nM.

**Activity of Known Peroxisome Proliferators.** The ability to induce the ACOX::CAT promoter fusion was tested with compounds known to cause peroxisome proliferation and hepatocarcinogenicity at various potencies. The cells were exposed to WY 14,643, clofibrate, DEHP, acetylsalicylic acid, and salicylic acid for 48 h. The results for nine replicates for each dose for all compounds tested are summarized in Table 2. WY 14,643, a very strong peroxisomal proliferator, exposure to which is correlated with a high incidence of tumor formation, showed a greater than 14-fold induction of the ACOX::CAT fusion over all 5 doses tested. The system was already approaching saturation at 6 μM, our lowest test concentration. Clofibrate, another potent peroxisome proliferator associated with high rodent hepatocarcinogenicity, elicited a maximal induction of 4.7-fold at 50 μM and slowly decreased to less than 2-fold at 6 μM. These data are consistent with in vivo results that show that clofibrate induces peroxisome proliferation at doses five times higher than WY 14,643 (9). DEHP is a weak peroxisome proliferator relative to WY 14,643 and clofibrate (9). It induced ACOX::CAT 5-fold at 600 μM, approximately 10 times greater than the dose needed to elicit a similar response to clofibrate. DEHP inductions also followed a dose-response curve, decreasing to less than 2-fold at 75 μM. These data indicate a potential correlation between ACOX::CAT induction and the ability for these compounds to mediate peroxisome proliferation.

Acetylsalicylic acid was chosen as a test chemical because it is classified as a very weak inducer of peroxisome proliferation and β-oxidation enzymes in comparison to WY 14,643, clofibrate, and DEHP (21–24). In this assay, acetylsalicylic acid induced the ACOX::CAT fusion only to 2-fold at the highest dose of 1.25 mM, and salicylic acid induced the ACOX::CAT fusion to 2.4-fold at a maximum dose of 1.25 mM. Higher doses were not used because 1.25 mM
DISCUSSION

Although there is little evidence to indicate that peroxisome proliferators pose a significant health risk to humans, the underlying cellular processes involved in the carcinogenic effect of these compounds have yet to be determined. The need to identify these compounds will remain of vital interest until the mechanisms by which they induce rodent hepatocarcinogenesis are determined (8). Over 70 compounds have been shown to proliferate peroxisomes (9). Identifying potential peroxisome proliferators is difficult due to the wide variety of compounds and the lack of any significantly useful predictive chemical structures that unify this group of carcinogens.

Table 2 Fold inductions of the ACOX:CAT fusion construct for known peroxisome proliferators

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Fold induction ± SD</th>
</tr>
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<tbody>
<tr>
<td>WY 14,643</td>
<td>6.25 μM</td>
<td>14.8 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>12.5 μM</td>
<td>13.8 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>25 μM</td>
<td>15.5 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>50 μM</td>
<td>15.5 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>100 μM</td>
<td>16.2 ± 3.2</td>
</tr>
<tr>
<td>Clofibrate</td>
<td>3.13 μM</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>6.25 μM</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>12.5 μM</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>25 μM</td>
<td>2.8 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>50 μM</td>
<td>4.7 ± 1.3</td>
</tr>
<tr>
<td>DEHP</td>
<td>37.5 μM</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>75 μM</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>150 μM</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>300 μM</td>
<td>3.5 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>600 μM</td>
<td>4.9 ± 1.0</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>78 μM</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>157 μM</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>313 μM</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>625 μM</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>1.25 mM</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>78 μM</td>
<td>1.0 ± 0.1</td>
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<td></td>
<td>157 μM</td>
<td>1.1 ± 0.2</td>
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<tr>
<td></td>
<td>313 μM</td>
<td>1.4 ± 0.2</td>
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<tr>
<td></td>
<td>625 μM</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>1.25 mM</td>
<td>2.4 ± 0.4</td>
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was the maximum attainable solubility for these compounds in 0.5% DMSO. These low level inductions indicate that some activation of the PPAR occurs, resulting in induction of the ACOX:CAT fusion, although only at relatively high doses.

Activity of NSAIDs. Ibuprofen, a NSAID, has been reported previously to be an inducer of peroxisome proliferation in vivo in male Wistar rats at 150 mg/kg, well above the standard administered therapeutic dose (25). In that study, ibuprofen and clofibrate were shown to have similar results, showing comparable abilities to interact with the PPAR. We tested ibuprofen and three other NSAID compounds, fenbufen, naproxen, and acetaminophen, to determine if they elicited a response in our test system. The results for nine replicates at each dose are shown in Table 3. Ibuprofen stimulated inductions of 4- and 7-fold at 30 μM and 60 μM, respectively (Table 3), which were comparable to the inductions seen with clofibrate (Table 2). Fenbufen, a NSAID structurally similar to ibuprofen, failed to induce peroxisome proliferation at 150 mg/kg in Wistar rats (25). In our study, fenbufen induced ACOX:CAT 3-fold at 0.5 mM and 5.5-fold at 1 mM, concentrations approximately 10 times higher than were required for ACOX:CAT induction with either clofibrate or ibuprofen. Naproxen induced strong responses of 13-fold at 400 μM and showed ACOX:CAT induction in a dose-responsive manner down to 50 μM. Acetaminophen was able to induce ACOX:CAT at 1.9-fold at 625 μM and 6-fold at 2.5 mM.

Activity of Ames-positive and -negative Carcinogens. Ames-positive and -negative carcinogens were tested to ensure that these compounds could be distinguished from peroxisome proliferators. 2-AA, EMS, and 4-NQO are all Ames-positive carcinogens, whereas DES and PMA are Ames-negative carcinogens. The results for nine replicates at each dose are shown in Table 4. DES, EMS, and 4-NQO were tested within the toxic range (data not shown) with no appreciable effect on ACOX:CAT induction. 2-AA was tested at 16 μM, its maximum solubility in 0.5% DMSO, and showed no inductions.

Interestingly, PMA was tested as Ames-negative control and elicited an approximately 3-fold induction at all concentrations tested. Because PMA is a tumor promoter and activates the protein kinase C signal transduction pathway (26), induction of ACOX:CAT may represent a downstream effector for protein kinase C-mediated cell proliferation.
**IN VITRO SYSTEM FOR DETECTION OF PEROXISOME PROLIFERATORS**

Peroxisome proliferators are typically nongenotoxic carcinogens and do not seem to initiate hepatocarcinogenesis by interacting directly with cellular DNA. The use of *in vitro* and *in vivo* genotoxicity assays is therefore unreliable for detecting these compounds. Some peroxisome proliferators, notably WY 14,643 and nafenopin, have tested positive in sister chromatid exchange and chromosomal aberration assays (27), but there is no consistent pattern of genotoxicity among those peroxisome proliferators for which data are available. Genotoxic effects may enhance the carcinogenicity of a peroxisomal proliferator, but they do not seem to be the predominant event related to their common carcinogenicity (9, 28).

The use of ACOX and transcriptionally regulated systems as an alternative method to screen compounds for peroxisome proliferation is based on several factors (9, 11). Peroxisomal proliferation has been correlated with the up-regulation of peroxisomal enzymes involved in the β-oxidation pathway. The key factor involved in ACOX regulation is the PPAR, the nuclear hormone receptor shown to bind to the PPRE found within the ACOX promoter (12, 29). PPAR mRNA induction localized specifically to the liver, which is the main target organ for peroxisome proliferators (30). Because there is increased β-oxidation associated with peroxisomal proliferation and hepatocarcinogenesis, ACOX is a good marker for measuring changes in gene expression that occur in the presence of peroxisome proliferators. It should be noted, however, that the magnitude of an increase in ACOX::CAT may not quantitatively predict the extent of peroxisome proliferation *in vivo* (31).

We tested t-RA, the ligand for the RAR, and c-RA, the natural ligand for the RXR (18), for their ability to induce ACOX::CAT. c-RA induced CAT protein at doses starting at 5 μM, and induction did not decrease significantly even at 313 nm. This is consistent with previous findings that c-RA, via the RXR, could induce ACOX through heterodimerization with the PPAR (20). RXR/PPAR heterodimer interaction with the PPRE is necessary for transcriptional activation of ACOX (18). Neither RXR nor PPAR alone has been shown to bind to the PPRE in the ACOX promoter (14). RAR and RXR have been shown to act together to stimulate transcription of ACOX (14). t-RA induced CAT protein at 5 μM but to lower levels than for c-RA, and inductions eventually decreased below 2-fold at the lower end of the dose range. This dose response is consistent with a previous demonstration of t-RA induction of ACOX in primary rat hepatocytes (32). Because RXR has been shown not to bind t-RA (33), this activity is probably due to either cell-mediated isomerization of t-RA to c-RA or binding of the RAR to the PPRE, which can be mediated through the RXR (13, 14, 20). These data indicate that the construct is working in a manner consistent with the previously published mechanism(s) of receptor binding to the PPRE.

Induction of ACOX has been demonstrated with a large number of peroxisome proliferators (17). We chose to test four known peroxisomal proliferators of varying abilities to induce peroxisome proliferation and hepatic tumors in rats *in vivo*. WY 14,643 was the most potent peroxisome proliferator tested. At 1,000 ppm, WY 14,643 induced the number of peroxisomes greater than 8-fold in F344 rats and had a strong hepatocarcinogenic effect (9). Concordant with this, WY 14,643 elicited very large fold inductions at every dose tested in our reporter system. Clofibrate, at five times the concentration of WY 14,643, elicited similar effects on peroxisomal number and hepatocarcinogenicity *in vivo* as WY 14,643 (9). Consistent with these data, clofibrate induced the ACOX::CAT fusion at levels several-fold lower than those observed for WY 14,643 at comparable doses. DEHP induced moderate increases in peroxisome number and has a weaker hepatocarcinogenic effect than either WY 14,643 or clofibrate (9). DEHP induced the ACOX::CAT fusion at doses approximately 10 times greater than those needed to elicit a response from clofibrate. These data suggest that the level of induction of the ACOX::CAT fusion may be predictive of the ability of a compound to induce peroxisome proliferation.

Acetylsalicylic acid, and its analogue, salicylic acid, were tested as weak inducers of peroxisome proliferation and the peroxisomal β-oxidation enzymes. Both compounds were reported to induce peroxisome proliferation and up-regulation of ACOX in male Wistar rats (21). The slight up-regulation of ACOX::CAT by these compounds in our study is consistent with the increased β-oxidation activity described *in vivo* (21). In addition, several reports state that acetylsalicylic acid is a much weaker inducer of peroxisome proliferation and β-oxidation enzymes than WY 14,643, clofibrate, or DEHP (34–36). Gray et al. (34) also reported inductions of ACOX activity for acetylsalicylic acid of approximately 4-fold at 2.5 μM in cultured primary hepatocytes from Sprague Dawley rats, which is consistent with the 2-fold increase in ACOX::CAT that we observed at 1.25 μM.

We tested several other NSAIDs. Ibuprofen has been reported previously to be an inducer of peroxisome proliferation *in vivo* in male Wistar rats and is comparable to clofibrate in its ability to interact with the PPAR (25). Both clofibrate and ibuprofen stimulated the production of ACOX::CAT in a similar fashion, suggestive of a common mechanism to promote peroxisome proliferation. This was not the case in the rat study because very high doses of ibuprofen were needed to elicit a response, although no direct comparison between the two compounds was made (25). Fenbufen, a NSAID found to be negative in the rat study, induced the ACOX::CAT fusion but required doses far above those needed with ibuprofen. It may be that peroxisome proliferation with fenbufen was not observed *in vivo* because the effective dose was not high enough to elicit a response.

The presence of a propionic acid moiety in ibuprofen, clofibrate acid, and benoxaprofen, another NSAID peroxisome proliferator, may enhance activation of the PPAR for this group of compounds (25). This moiety is absent in fenbufen, for which much higher doses were required to induce the ACOX::CAT fusion. To address this hypothesis, we tested two other anti-inflammatory drugs, acemetacin and naproxen. Naproxen, but not acemetacin, contains the aryl propionic acid moiety present in clofibrate and ibuprofen. Naproxen induced ACOX::CAT at doses slightly higher than those required for clofibrate and ibuprofen, consistent with the findings of Rekka et al. (25). Naproxen inhibits acyl-CoA ligase (37), an enzyme involved with the transport of fatty acids across the mitochondrial membrane, whereupon they undergo β-oxidation. Inhibition of fatty acid transport results in an accumulation of fatty acids within the cell. Excess fatty acid ligands have been shown to bind to the PPAR and activate transcription of peroxisomal enzymes (38, 39). Thus, one potential mechanism for up-regulation of ACOX by naproxen may involve indirect activation of the PPAR.

Acemetacinophen, in contrast, showed ACOX::CAT inductions at doses 10–15 times higher than those required for naproxen, clofibrate, and ibuprofen. Acemetacinophen, which lacks an aryl propionic acid moiety, induced the ACOX::CAT fusion at dose ranges similar to fenbufen. It seems, therefore, that the presence of the aryl propionic acid moiety is not required for activation of the PPAR; however, it may enhance activation.

A series of Ames-positive and -negative chemicals were run to serve as negative controls. EMS, 4-NQO, 2-AA, and DES were all negative in this study. These compounds were chosen as negatives because as a group, they represent compounds that either are directly mutagenic, initiate redox cycling, interfere with replication, or, in the case of DES, interact with another steroid hormone receptor, the estrogen receptor. These negative results suggest that induction of ACOX::CAT is specific to peroxisome proliferators. Interestingly, PMA was capable of inducing ACOX::CAT approximately 3-fold over the entire dose range tested. Another phorbol ester, TPA, was initially suggested to be a peroxisome proliferator.
based on up-regulation of certain peroxisomal enzymes in mouse C3H10T1/2 cells (40). In addition, Bronfman et al. (41) showed protein kinase C up-regulation in the presence of some hypolipidemic drugs, suggesting that up-regulation of protein kinase C may mediate the effects of some peroxisome proliferators through downstream effectors. In contrast, Mikelsen et al. (42) contend that TPA is not a peroxisome proliferator based on data obtained with embryonic hamster and rat cells. In that study, minimal and transient up-regulation of catalase and differential effects on peroxisomal enzyme patterns led the authors to conclude that TPA did not behave like a classical peroxisome proliferator in rat and hamster embryos. However, data showing up-regulation of ACOX was also presented and was consistent with the Bronfman et al. (41) data. The ability of some hypolipidemic peroxisome proliferators to activate protein kinase C-dependent pathways and induce ACOX suggests that up-regulation of the PPAR might involve this pathway as well.

In conclusion, this assay has shown the ability to reproducibly detect all known peroxisome proliferators tested in this study. The level of ACOX:CAT induction corresponds to the ability of the compound to activate the PPAR. Our results from this reporter system ranked the nine peroxisome proliferators tested in the following order: acetylsalicylic acid < salicylic acid < acetaminophen < fenbufen < DEHP < naproxen < ibuprofen < clofibrate < WY 14,643. There are clear consistencies between our data and previously published in vitro and in vivo data for the compounds tested in this study. However, retinoic acid analogues may elicit a positive response because of the heterodimerization of the RXR with the PPAR. Nonetheless, an understanding of compounds capable of this interaction may aid in the design of compounds to elicit this specific response.

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