Peroxisome proliferators are a diverse group of chemicals that include several therapeutically used drugs (e.g., hypolipidemic agents), plasticizers and organic solvents used in the chemical industry, herbicides, and naturally occurring hormones. As the name implies, peroxisome proliferators cause an increase in the number and size of peroxisomes in the liver, kidney, and heart tissue of susceptible species, such as rats and mice. Long-term administration of peroxisome proliferators can cause liver cancer in these animals, a response that has been the central issue of research on peroxisome proliferators for many years. Peroxisome proliferators are representative of the class of nongenotoxic carcinogens that cause cancer through mechanisms that do not involve direct DNA damage. The fact that humans are frequently exposed to these agents makes them of particular concern to government regulatory agencies responsible for assuring human safety. Whether frequent exposure to peroxisome proliferators represents a hazard to humans is unknown; however, increased cancer risk has not been shown to be associated with long-term therapeutic administration of the hypolipidemic drugs gemfibrozil, fenofibrate, and clofibrate. To make sound judgments regarding the safety of peroxisome proliferators, the validity of extrapolating results from rodent bioassays to humans must be based on the agents’ mechanism of action and species differences in biologic activity and carcinogenicity. The peroxisome proliferator-activated receptor α (PPARα), a member of the nuclear receptor superfamily, has been found to mediate the activity of peroxisome proliferators in mice. Gene-knockout mice lacking PPARα are refractory to peroxisome proliferation and peroxisome proliferator-induced changes in gene expression. Furthermore, PPARα-null mice are resistant to hepatocarcinogenesis when fed a diet containing a potent nongenotoxic carcinogen WY-14,643. Recent studies have revealed that humans have considerably lower levels of PPARα in liver than rodents, and this difference may, in part, explain the species differences in the carcinogenic response to peroxisome proliferators. [J Natl Cancer Inst 1998; 90:1702–9]

Many human cancers can result from exposure to chemical carcinogens derived from tobacco, diet, and the environment. Two classes of carcinogens can be distinguished based on their mechanisms of action: genotoxic and nongenotoxic. Genotoxic carcinogens are inert chemicals that must be metabolically activated by cellular enzymes to electrophilic derivatives that can bind to DNA and other macromolecules. Mutation of oncogenes and tumor suppresser genes derived from carcinogen–DNA adduction are the lesions that cause cell transformation. Aflatoxin B1 and 4-aminobiphenyl are examples of genotoxic carcinogens that are known to cause cancer in humans (1). Genotoxic carcinogens are easy to detect using assays such as the Ames test that measure carcinogen activation and binding to DNA and the resultant gene mutations. In contrast, nongenotoxic carcinogens cause cell transformation indirectly, since they are not activated to electrophilic derivatives. Chloroform, furan, and 2,3,7,8-tetrachlorodibeno-p-dioxin are all examples of nongenotoxic carcinogens (2). Among the most widespread nongenotoxic carcinogens are a group of compounds collectively referred to as peroxisome proliferators. Peroxisome proliferators are a diverse class of chemicals (3), including the lipid and cholesterol lowering fibrate drugs (clofibrate, fenofibrate, and gemfibrozil), leukotriene antagonists (e.g., LY-171883), herbicides (e.g., lactofen), plasticizers (phthalate esters), solvents (e.g., trichloroethylene), and naturally occurring chemicals (e.g., phenyl acetate) or hormones (e.g., dehydroepiandrosterone sulfate).

Administration of nongenotoxic peroxisome proliferators results in a marked increase in the number and size of peroxisomes and an increase in liver size. In addition, long-term administration of peroxisome proliferators results in hepatocellular carcinomas in rats and mice (4). The carcinogenicity of these chemicals in rodents, coupled with their widespread exposure, has generated concern that peroxisome proliferators may contribute to human cancer risk. Thus, they are subject to regulatory control. The regulatory status of several peroxisome proliferators is shown in Table 1. While these chemicals are of major concern for human risk assessment, their carcinogenic risk for humans lacks confirmation. Long-term pharmacologic exposure of patients to the hypolipidemic drugs gemfibrozil (5) and clofibrate, both potent rodent peroxisome proliferators (6), has not revealed
any increased risk of liver cancer or significant elevation of other cancers (7). This species difference does not appear to result from differences in dose because serum levels in rodents receiving carcinogenic levels of drug in the diet are similar to those in humans receiving drug therapy (7). In addition, peroxisome proliferation has not been demonstrated in human liver biopsy samples following oral clofibrate or gemfibrozil therapy (7) or in human hepatocytes treated in vitro with a variety of peroxisome proliferators (8).

Whether long-term exposure to peroxisome proliferators that are potent rodent hepatocarcinogens represents a hazard to humans is unknown (9). On the basis of this uncertainty, the regulatory agencies are especially cautious in licensing chemicals that exhibit peroxisome proliferation and carcinogenesis. Since peroxisome proliferation and enzyme induction can be demonstrated after short-term administration of test compounds to rodents, avoiding development of peroxisome proliferators has become standard practice in the pharmaceutical and chemical industries, especially if long-term human exposures are anticipated. To determine with certainty whether peroxisome proliferators can be carcinogenic in humans, however, the mechanisms of carcinogenicity and species differences in response need to be determined. Studies of peroxisome proliferator-activated receptor α (PPARα) have brought us closer to this goal.

**PPARα and Peroxisome Proliferation**

Peroxisomes are membrane-bound organelles that contain enzymes responsible for β-oxidation of fatty acids, the biosynthesis of cholesterol, and other biochemical pathways (10). Peroxisome proliferation is coincident with increases in many peroxisomal and mitochondrial enzymes involved in fatty acid β-oxidation and microsomal cytochrome P450 fatty acid ω-hydroxylases (11). PPARα mediates the activation of genes encoding these enzymes through dimerization with the retinoid X receptor α and binding to cis-acting regulatory elements upstream of the promoter regions in target genes (12,13). In rats and mice, PPARα is expressed at high levels in the liver and kidney, the primary sites of peroxisome proliferation. Furthermore, PPARα-null mice lack the morphologic and biochemical responses to peroxisome proliferators commonly observed in wild-type mice (14).

In addition to PPARα, two other nuclear receptors share significant sequence similarity to PPARα. PPARγ is highly expressed in white and brown adipose tissues and macrophages; it is not found at significant levels in the liver (15). PPARγ is required for the differentiation of adipocytes and the tissue-specific regulation of genes in white and brown fat cells (16). It is the target for the thiazolidinedione drugs used to control adult-onset diabetes (17). In contrast to PPARα and PPARγ, PPARβ is more ubiquitously expressed and is most abundant in the central nervous system (15). The target genes and physiologic function of PPARβ are currently unknown.

**Physiologic Role of PPARα**

On the basis of target gene expression patterns, PPARα appears to have critical roles in regulation of fatty acid metabolism, including fatty acid β-oxidation, apolipoproteins, and fatty acid

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**Table 1. Peroxisome proliferators: examples of regulatory interests**

<table>
<thead>
<tr>
<th>Classes of chemicals and representative examples</th>
<th>U.S. Government constituency</th>
<th>EPA cancer potency estimate and classification</th>
<th>Potential avenues for human exposure</th>
<th>Potential impacts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasticizers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEHP</td>
<td>EPA, FDA, CPSC, OSHA</td>
<td>0.014 [mg/kg per day]−¹ (B2)</td>
<td>Water, food, air, industrial, consumer products, medical devices</td>
<td>Waste management workplace regulation, reporting. (Hazardous Substance Priority 1)</td>
</tr>
<tr>
<td>DBP</td>
<td>EPA, FDA, OSHA</td>
<td>(D) [Currently under study by NTP]</td>
<td>Water, air, industrial</td>
<td>Waste management workplace regulation, reporting</td>
</tr>
<tr>
<td>DEHA</td>
<td>EPA, FDA</td>
<td>0.0012 [mg/kg per d]−¹ (C)</td>
<td>Water, food, air, industrial</td>
<td>‘Unregulated’ contaminant... EPA may regulate these contaminants in the future</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>EPA, DOD</td>
<td>0.011 [mg/kg per day]−¹ (B2)</td>
<td>Water, air, industrial, consumer products</td>
<td>Waste management reporting (Hazardous Substance Priority 1)</td>
</tr>
<tr>
<td>Herbicides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactofen</td>
<td>EPA</td>
<td>(Tolerance = 1.13% ADI, based on l.o.d.)</td>
<td>Agricultural workers</td>
<td>Registration approval and labeling</td>
</tr>
<tr>
<td>Fomasafen</td>
<td>EPA</td>
<td>0.19 [mg/kg/d]−¹ (C)</td>
<td>Not approved in U.S.</td>
<td></td>
</tr>
<tr>
<td>Haloxytop</td>
<td>EPA</td>
<td>NA</td>
<td>Patient therapy</td>
<td>Approval, labeling, monitoring</td>
</tr>
<tr>
<td>Drugs</td>
<td>FDA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHEA</td>
<td>Not regulated</td>
<td></td>
<td></td>
<td>Health supplement</td>
</tr>
</tbody>
</table>

*U.S. Government entities: EPA = Environmental Protection Agency; FDA = Food and Drug Administration; CPSC = Consumer Product Safety Commission; OSHA = Occupational Safety and Health Administration; DOD = Department of Defense; NTP = National Toxicology Program. Carcinogen classification: B2 = probable human carcinogen (based on inadequate evidence or no data from epidemiologic studies and sufficient evidence from animal studies); C = possible human carcinogen (based on limited evidence from animal studies and no human data); D = not classifiable as to human carcinogenicity (based on inadequate human and animal data or no data). Plasticizers: DEHP = di(2-ethylhexyl)phthalate; DBP = di(n-butyl)phthalate; DEHA = di(2-ethylhexyl) adipate. ADI = acceptable daily intake; l.o.d. = level of detection; DHEA = dehydroepiandrosterone (the precursor for the peroxisome proliferator DHEA-3β-sulfate).
transport proteins \((14, 18–22)\). Identification of endogenous ligands has provided evidence of physiologic roles for PPARα. For example, leukotriene \(B_4\) \((\text{LTB} _4)\) was found to be a ligand for PPARα \((23)\). Binding of \(\text{LTB} _4\) to the receptor stimulates the activation of genes encoding fatty acid-catalyzing enzymes that degrade \(\text{LTB} _4\) and abrogate the inflammatory response. PPARα was also shown to bind to eicosanoids such as \(8(\text{S})\)-HETE, suggesting that it may be involved in control of other pathways of immunomodulation \((24)\). PPARα regulates serum cholesterol, in particular, high-density lipoprotein cholesterol, and triglyceride levels, as indicated by the results obtained in mice lacking PPARα. Constitutive levels of serum cholesterol are markedly higher in PPARα-null mice than in wild-type controls, and fibrate-induced reductions of serum triglycerides do not occur in these mice as well. Both of these effects are due in part to PPARα-dependent modulation of apolipoprotein gene expression \((20)\). PPARα also mediates expression of genes encoding both peroxisomal and mitochondrial fatty acid-metabolizing enzymes via regulatory events at both the constitutive and inducible level. For example, PPARα affects constitutive expression of mitochondrial enzymes involved in fatty acid catabolism as well as induction of mitochondrial and peroxisomal fatty acid \(\beta\)-oxidation pathways by peroxisome proliferators and perhaps other fatty acid metabolites \((19)\). Finally, peroxisome proliferators activate fatty acid transport proteins \((22)\), and this effect is mediated by PPARα \((25)\). Thus, genes encoding peroxisomal and mitochondrial fatty acid-metabolizing enzymes, apolipoproteins, and fatty acid transport proteins are all regulated by PPARα, indicating that this receptor has important physiological roles in control of fatty acid metabolism. In fact, PPARα also regulates gene expression in response to dietary polyunsaturated fatty acids \((21)\). Delineating the role of PPARα in lipid metabolism is an active area of investigation. PPARα also may regulate other genes not involved in lipid metabolism such as those controlling the cell cycle.

**ROLE OF PPARα IN HEPATOCARCINOGENESIS BY PEROXISOME PROLIFERATORS**

Long-term administration of peroxisome proliferators to rodents is associated with increased risk of hepatocellular neoplasia \((4, 26, 27)\). Chemicals that induce significant peroxisome proliferation \((i.e.,\) greater than fourfold increase in peroxisomal acyl-CoA oxidase) almost always cause liver cancer in mice and rats in long-term studies \((3)\). The carcinogenic potency of peroxisome proliferators spans a broad range. The potent compound WY-14,643 caused a 100% incidence of hepatocellular tumors after 1 year when rats are fed at a level of 0.1% or less in the diet \((28)\). In contrast, the weaker chemical di(2-ethylhexyl)phthalate caused a more modest increase in incidence of hepatocellular tumors when administered to rats at a 1.2% level in the diet \((29)\). In mice, trichloroethylene caused an increased incidence of hepatocellular tumors when administered by gavage at the high level of 1000 mg/kg per day 5 days/week for 103 weeks \((30)\).

Since the receptor PPARα was shown to be essential to mediating the hepatocellular responses to peroxisome proliferators, a long-term study was initiated to determine if the carcinogenicity of a potent peroxisome proliferator, WY-14,643, was dependent on PPARα \((31)\). After feeding WY-14,643 \((0.1%\) of diet) for nearly 1 year, all PPARα wild-type mice had multiple hepatocellular adenomas and in some cases carcinomas. In contrast, PPARα-null mice that were treated in the same way had no hepatocellular adenomas or carcinomas; in fact, microscopic evaluation of the livers from null mice failed to identify any preneoplastic foci. This striking difference in response between wild-type and null mice demonstrated the essential nature of PPARα in mediating the carcinogenicity of one of the most potent peroxisome proliferators known and indicates that all peroxisome proliferators are likely to cause liver tumors through activation of PPARα. Furthermore, the results suggested that PPARγ, PPARβ, or other nuclear receptors are not likely to substitute for PPARα in mediating the carcinogenic response to peroxisome proliferators.

**ROLE OF OXIDATIVE STRESS IN HEPATOCARCINOGENESIS BY PEROXISOME PROLIFERATORS**

The mechanism by which peroxisome proliferators cause liver cancer is not currently understood. One hypothesis is that peroxisome proliferation causes gene mutations indirectly by increasing intracellular \(H_2O_2\) \((32)\). In rodent liver, peroxisome proliferators markedly increase peroxisomal fatty acid \(\beta\)-oxidation and the \(H_2O_2\)-generating enzyme acyl-CoA oxidase but only modestly elevate catalase. In addition, activity of glutathione peroxidase, a cytoplasmic enzyme capable of degrading \(H_2O_2\), is often depressed following long-term administration of peroxisome proliferators \((33)\). Thus, excess \(H_2O_2\) could potentially escape the peroxisomes and react with cellular macromolecules. Indeed, peroxide-modified lipids have been found in hepatocytes of peroxisome proliferator-treated rats \((33, 34)\). However, other more sensitive indicators of oxidative damage have not consistently been affected, even by the most potent peroxisome proliferators. For example, ethane exhalation was not altered by administration of WY-14,643 in rats \((35)\). In addition, measurements of esterified F2-isoprostanes, a sensitive intracellular indicator for oxidative damage, revealed that the extent of oxidative damage is minimal in mice chronically administered WY-14,643 \((36)\).

Oxidatively damaged DNA in the form of 8-hydroxydeoxyguanosine has been detected in livers of rats chronically exposed to different peroxisome proliferators \((37, 38)\). These adducts could lead to gene mutations during DNA cell replication. However, a comparative study \((39)\) in rats failed to link the magnitude 8-hydroxydeoxyguanosine levels under bioassay conditions to tumor multiplicity. Another study \((40)\) in rodent models reported no increases in 8-hydroxydeoxyguanosine modification in liver DNA of treated rats compared to controls. In experiments where DNA damage was detected, total liver DNA was analyzed; in studies \((39, 40)\) where no increase in DNA adducts were found, DNA was derived from purified nuclei. More recent evidence supporting a role for \(H_2O_2\)-mediated carcinogenesis was provided by showing that stable overexpression of peroxisomal acyl-CoA oxidase in NIH3T3 cells produced cell transformation, as assessed by cell growth in soft agar and nude mice \((41)\). Although this is among the most direct and compelling evidence that oxidative damage can lead to cell
transformation, possibly via oxidative DNA damage, it does not establish that H2O2 is responsible for carcinogenesis in an intact animal model.

While acyl-CoA oxidase activity and its byproduct H2O2 may contribute to the underlying mechanism of peroxisome proliferator-induced carcinogenicity, it was recently suggested to also have a more direct role in cell proliferation (42). Spontaneous peroxisome proliferation, induction of many of the target genes associated with this process, and hepatocellular carcinomas were found in acyl-CoA-oxidase-null mice after 10–15 months of age. Since the phenotype of these mice is similar to the effects observed in animals fed peroxisome proliferators, it was suggested that in the absence of peroxisomal β-oxidation of very long chain and long-chain fatty acids, that a fatty acid metabolite metabolic intermediate, that shares properties with peroxisome proliferators, accumulates and may stimulate the PPARα chain and long-chain fatty acids, that a fatty acid metabolite that in the absence of peroxisomal β-oxidation of very long chain and long-chain fatty acids, that a fatty acid metabolite metabolic intermediate, that shares properties with peroxisome proliferators, accumulates and may stimulate the PPARα chain and long-chain fatty acids. The role of peroxisome proliferators, the mitogenic response is not found (31) nor is there evidence for elevation of cell cycle control proteins (46). This establishes with certainty that PPARα mediates cell cycle regulation that is required for the carcinogenic response to peroxisome proliferators.

A role for cell division in the carcinogenic effect of peroxisome proliferators and perhaps other nongenotoxic carcinogens is also indicated by the effects of these agents on apoptosis or programmed cell death (47,48). Incubation of rat hepatocytes with the potent peroxisome proliferator nafenopin results in maintenance of viability for at least 4 weeks in contrast to 8 days with control cultures (49). This increased viability was associated with a decrease in cells exhibiting signs of apoptosis, such as condensed and fragmented nuclei. Apoptosis in control primary cultures could be increased by transforming growth factor-β1, and this increase is reduced by nafenopin (50). A similar effect was seen in the Reuben hepatoma cell line. Nafenopin was also found to synergize with epidermal growth factor to promote clonal outgrowth of primary rat hepatocytes due to an alteration of balance between mitosis and apoptosis (51). Inhibition of apoptosis by peroxisome proliferators is due to PPARα; the inhibitory effect can be eliminated by introduction of a dominant negative effector regulator of PPARα (52). The receptor appears to alter production of a soluble effector of apoptosis. Inhibition of apoptosis could be responsible for the carcinogenic effects of peroxisome proliferators if cells destined for programmed cell death that have genetic damage continue to proliferate as a result of mitogenic stimulus (48).

If peroxisome proliferation and acyl-CoA oxidase induction, which generates H2O2, are required for carcinogenesis, there should be a direct relationship between potency of the chemical for peroxisome proliferation and potency for hepatocarcinogenesis. However, this was not found when a weak peroxisome proliferator (DEHP) was compared with the potent WY-14,643 (33). An initial burst of DNA replication was found for both compounds within a few days after initiation of feeding either compound. After 18 days, livers from WY-14,643-treated animals showed a persistent increase in DNA labeling over DEHP-fed rats that ranged from 0.6-fold to 15-fold throughout the treatment period of 369 days. The amount of peroxisome proliferation and increase in peroxisomal enzyme induction were only 10%–20% higher for the WY-14,643. This shows that peroxisome proliferation alone is not associated with carcinogenicity but that persistent increased cell replication is an important factor (33,44,53). Other studies (54,55), however, have suggested an association between peroxisome proliferation and hepatocarcinogenesis. Additional evidence for a dissociation between peroxisome proliferation and cell proliferation was provided by showing that after treatment with a peroxisome proliferator the localization of acyl-CoA oxidase expression and replicative DNA synthesis in the liver were different, and the extent of DNA synthesis and enzyme induction were uncoupled (56). Studies (57,58) on cytokine-stimulated hepatocyte proliferation mediated by Kupffer cells also suggest that peroxisome proliferation may be uncoupled from mitogenesis. Interestingly, peroxisomal proliferators can cause cell transformation in cultured cells in the absence of apparent peroxisome proliferation (59,60). This and other studies (see below) showing that the processes of peroxisome proliferation and mitogenesis can be functionally distinguished lend support to the hypothesis that excess H2O2 is not absolutely required for causing genetic damage leading to carcinogenesis.

The mechanism of action of nongenotoxic carcinogens is thought to be due to a mitogenic effect accompanied by genetic alterations that arise spontaneously. Genetic damage could be the results of errors during DNA replication or direct damage caused by electrophiles generated by cellular metabolism. In the case of peroxisome proliferators, excess H2O2 or other metabolic effects could produce DNA damage and mutations that are not reflected by the amount of 8-hydroxydeoxyguanosine or other surrogate markers for oxidative damage. Extensive genetic damage in cells is thought to be dealt with by apoptosis, if this process is inhibited by peroxisome proliferators, DNA damage could be fixed as mutations. However, mutations in oncogenes and tumor suppressor genes have not been demonstrated in carcinomas and adenomas produced by peroxisome proliferators, suggesting that epigenetic changes immortalizing cells may also

ROLE OF MITOGENESIS AND CELL CYCLE CONTROL

Cell replication appears to be required for transformation stimulated by both genotoxic and nongenotoxic carcinogens (43). Genotoxic tumor initiators may induce mutations by producing both genotoxicity and cell division or may require a separate stimulus of cell division. Tumor promoters stimulate cell division, which functions in the fixation of mutations and in clonal expansion of initiated cells. Peroxisome proliferators cause hepatomegaly and hyperplasia that are due to an immediate mitogenic effect. Throughout the course of feeding peroxisome proliferators, stimulation of replicative DNA synthesis in the liver may persist or wane (44). Any DNA replication may serve to promote hepatocytes that have been initiated through either oxidative stress or some other unidentified mechanism. Administration of peroxisome proliferators to rats was found to increase expression of several proteins commonly associated with cell division (45). In PPARα-null mice fed peroxisome proliferators, the mitogenic response is not found (31) nor is there evidence for elevation of cell cycle control proteins (46). This establishes with certainty that PPARα mediates cell cycle regulation that is required for the carcinogenic response to peroxisome proliferators.

A role for cell division in the carcinogenic effect of peroxisome proliferators and perhaps other nongenotoxic carcinogens is also indicated by the effects of these agents on apoptosis or programmed cell death (47,48). Incubation of rat hepatocytes with the potent peroxisome proliferator nafenopin results in maintenance of viability for at least 4 weeks in contrast to 8 days with control cultures (49). This increased viability was associated with a decrease in cells exhibiting signs of apoptosis, such as condensed and fragmented nuclei. Apoptosis in control primary cultures could be increased by transforming growth factor-β1, and this increase is reduced by nafenopin (50). A similar effect was seen in the Reuben hepatoma cell line. Nafenopin was also found to synergize with epidermal growth factor to
have a role in tumorigenesis. Increases in expression of the oncogenes c-met and c-myc have been found on treatment with WY-14,643, but whether these increases are due to genetic changes is not known (61). While the mechanism of and occurrence of genetic changes are uncertain, cell division is clearly required for hepatocarcinogenesis. In fact, when WY-14,643 is co-administered with rotenone, a natural insecticide, to B6C3F1 mice, it still induces peroxisome proliferation and fatty acid β-oxidation, but hepatocyte proliferation is inhibited (62). Rotenone is known to reduce the spontaneous incidence of hepatocellular carcinoma in this mouse strain (63), thus providing additional support for the view that peroxisome proliferation (and generation of H2O2) is uncoupled to mitogenesis in the mechanism of action of peroxisome proliferators. One mechanism for mitogenesis, involving increased expression of proteins involved in cell cycle control, such as cyclin-dependent kinases and cyclins, has been found on peroxisome proliferator treatment (45,64,65), and these effects are PPARα dependent (46).

PPARα is required for transcriptional control of genes encoding enzymes involved in fatty acid metabolism, and this has been confirmed by transactivation assays and receptor binding to peroxisome proliferator response elements (PPREs). How PPARα controls cell division and apoptosis and the hepatocarcinogenic response, however, is not certain. PPARα has been shown to be required for peroxisome proliferator-induced hepatocarcinogenesis (31) and the inhibitory action of peroxisome proliferators on apoptosis (52). The receptor probably also alters the production of cytokines that stimulate hepatocyte proliferation. Kupffer cells, the macrophage-like cells that populate the liver, are known to secrete tumor necrosis factor-α (TNF-α) and hepatocyte growth factor/scatter factor (HGF), known mitogens for hepatocytes. A role of Kupffer cells and TNF-α in WY-14,643-induced mitogenic response has been established. In these studies (57,58), the proliferative response could be functionally distinguished from peroxisome proliferation. In contrast to TNF-α, whose production was stimulated, hepatic HGF expression was significantly lower in rats fed WY-14,643, suggesting that dysregulation of this growth factor may also contribute to peroxisome proliferator-induced mitogenesis (66). Further work is needed to delineate how TNF-α and/or HGF contribute to the underlying mechanisms of peroxisome proliferator-induced hepatocarcinogenicity.

Much of the emphasis on the contribution of mitogenicity to the liver carcinogenicity of peroxisome proliferators has been derived from studies of the entire liver or primary hepatocyte cultures. However, the mitogenic response in hepatocellular foci, precursors of the rodent liver tumors induced by peroxisome proliferators, also deserves attention. The proliferative activity of the hepatocytes in these lesions greatly exceeds that of the surrounding liver under conditions of continuous peroxisome proliferator administration and this property may be critical to the tumor-promoting activity of these compounds (67). This proliferation appears to be dependent on the continuous administration of the peroxisome proliferator, since cessation of treatment leads to regression of lesions (68,69). Since hepatocellular tumors caused by peroxisome proliferators continue to express PPARα (61), the mitogenicity in preneoplastic lesions is also likely to be mediated by this receptor.

### Table 2. Differences between mice and humans in response to peroxisome proliferators

<table>
<thead>
<tr>
<th>Response to peroxisome proliferators</th>
<th>Mice and rats</th>
<th>PPARα-null mice</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARα expression</td>
<td>+</td>
<td>−</td>
<td>+/10</td>
</tr>
<tr>
<td>Increase in peroxisomes</td>
<td>+</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Enzyme induction</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Cell proliferation</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Apoptosis inhibition</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Hypolipidemic effects</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Anti-inflammatory effects</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Increased risk of cancer</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*PPARα = peroxisome proliferator-activated receptor α; +/10 represents the fact that humans have less than one tenth the level of hepatic PPARα than mice and rats.
mutations that are generated by endogenous metabolites such as \( \text{H}_2 \text{O}_2 \). The question mark leads to a stimulation of mitogenesis and an inhibition of apoptosis that serves to fix gene as clofibrate and gemfibrozil. Higher expression of PPAR may result in activation of genes encoding lipid-metabolizing enzymes, apolipoproteins, fatty acid transport proteins, and genes responsible for mitogenesis. Genes encoding proteins involved in cell proliferation may not respond when receptor levels are low, such as in human liver. Alternatively, if human PPAR\(\alpha\) was expressed only in hepatocytes and not in Kupffer cells, the mechanism for stimulation of mitogenesis would be incomplete. Additional studies are required to distinguish among these possibilities.

**CONCLUSION**

The physiologic roles of PPAR\(\alpha\) and its role in hepatocarcinogenesis is summarized in Fig. 1. The PPAR\(\alpha\)-null mice have been essential to clarify these pathways. Any responses to hypolipidemic agents and other peroxisome proliferators can be clearly dissected by studying the biologic effects in this animal model as compared with wild-type mice. These studies have demonstrated that PPAR\(\alpha\) was responsible for peroxisome proliferation, activation of target gene encoding fatty acid-metabolizing enzyme, mitogenesis, and, ultimately, hepatocarcinogenesis. Other in vitro studies using trans-activation transfection studies have established that PPAR\(\alpha\) directly controls lipid metabolism through its activation of genes encoding enzymes for fatty acid \( \beta \)-oxidation and transport proteins. While PPAR\(\alpha\) clearly controls cell proliferation, it is not known whether this is a direct result of gene activation or an indirect response that is the result of perturbations in levels of cellular metabolites that cause the mitogenic responses through stimulation of other receptors. A role for Kupffer cells and cytokines also seems likely to contribute to hepatocyte proliferation. The PPAR\(\alpha\)-null mice have established that PPAR\(\alpha\) is required for hepatocarcinogenesis in rodents. The recent studies showing that humans have lower hepatic levels of PPAR\(\alpha\) suggest that this may be a major clue to understanding species-specific responses to peroxisome proliferators. Perhaps at the levels of expression of PPAR\(\alpha\) in humans, the only responses are through activation of fatty acid metabolism; the mitogenic responses may require much higher hepatic levels of receptor as found in rats and mice. Further studies of PPAR\(\alpha\) should clarify the processes involved in tumorigenesis caused by nongenotoxic agents, and this information will aid in the assessment of safety and risk for humans.

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NOTES

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