Peroxisome Proliferation, Pparalpha Agonists and Hepatocarcinogenesis (13-Nov-2004)

R. C. Cattley
Department of Pathology, Amgen Inc., Thousand Oaks, CA, USA.

Introduction
Peroxisome proliferation is an adaptive response caused by several chemical entities. Peroxisome proliferation is typically observed in hepatocytes, where it is characterized by changes in cellular morphology, metabolic capacity, proliferation, and survival (Cattley et al, 1998; Cattley, 2003). The morphological changes include an increase in the volume density of peroxisomes, with or without fluctuations in the endoplasmic reticulum and mitochondria. Susceptibility to peroxisomal proliferation is dramatically influenced by species and to a much lesser degree by sex and strain of animal (Lake et al, 2000; Reddy et al, 1974; Tanaka et al, 1992; Butler et al, 1988; Choudury et al, 2000).

Peroxisomal proliferation was first identified as an ultrastructural change accompanying increased liver weights in rodents following oral administration of the hypolipidemic drug, clofibrate (Hess et al, 1965). Later studies identified several enzymes (notably, those of the peroxisomal fatty acid beta oxidation pathway and the microsomal CYP4A family of enzymes) that were increased in expression and activity in livers undergoing peroxisomal proliferation (Lazarow and deDuve, 1976; Gibson et al, 1982). About the same time, several hypolidemic drugs were observed to increase the incidence of hepatocellular neoplasia following long-term oral administration to rats or mice (Reddy et al, 1980). This association of peroxisomal proliferation and risk of liver tumors was extended to other orally administered chemical entities including phthalate ester plasticizers such as di-(2-ethylhexyl)phthalate (DEHP) and diphenyl ether herbicides such as lactofen (Kluwe et al, 1982; Butler et al, 1988).

The mechanism of peroxisomal proliferation was clarified by the identification of a group of nuclear receptors known as "Peroxisome Proliferator-Activated Receptors" or PPARs (see review by Escher and Wahl, 2000). PPARs are members of the steroid-thyroid hormone receptor superfamily, and act as heterodimeric partners with retinoid X receptor at peroxisome proliferator response elements in specific genes by mediating transcription. PPARα was cloned and identified as a transcription factor that mediated the expression of genes of the peroxisomal fatty acid beta oxidation pathway and that was inducible by peroxisome proliferators in cells (Issemann and Green, 1990; Issemann et al, 1993).

Additional PPAR subtypes (γ and δ/β) were subsequently identified, and their expression patterns and activators suggested that they mediate responses other than peroxisomal proliferation. The critical and specific role of PPARα in peroxisomal proliferation was defined by the study of PPARα knockout mice, in which a germline mutation in the PPARα gene prevented expression of the receptor (Lee et al, 1995). Mice that are homozygotic for this mutation are viable, but lack the features of peroxisomal proliferation (hepatomegaly, increased peroxisomal volume density, and enzyme induction) caused by several peroxisome proliferators (clofibrate, WY-14,643, and DEHP) in wild type mice that express functional receptor (Lee et al, 1995; Ward et al, 1998). The cell proliferation induced by peroxisome proliferators in livers of wild type mice also was absent in studies of PPARα knockout mice. A single exception to these observations has been observed in that treatment of PPARα knockout mice with a very potent entity with combined PPARγ agonist and PPARδ agonist activities (DeLuca et al, 2000) resulted in a minimal increase in liver weights and peroxisomal enzyme induction. Despite this exception, the available evidence shows that PPARα is critical to induction of peroxisomal proliferation by most chemical entities.
Mechanism of Hepatic Carcinogenesis

Peroxisome proliferators in general are not genotoxic (see review by Galloway et al, 2000). Therefore, activation of PPARα maybe the key event in the mechanism of hepatic carcinogenesis. The availability of PPARα knockout mice has so far enabled one chronic study of a peroxisome proliferator to determine the role of this receptor in hepatic carcinogenesis. This study used the experimental hypolipidemic drug WY-14,643, fed in the diet for 37 weeks (Peters et al, 1997). In contrast to the robust induction of hepatocellular neoplasia in 100% of wild type mice fed diet-containing WY-14,643, PPARα knockout mice had no gross or microscopic evidence of hepatocellular neoplasia. These results clearly demonstrated the central role of PPARα in the carcinogenic activity of peroxisome proliferators.

With evidence for the central role of PPARα in the carcinogenic activity of peroxisome proliferators, 2 additional questions deserve consideration. First, which of the events mediated by the activation of PPARα is responsible for the inductions of neoplasia? Second, is activation of PPARα only necessary to mediate the induction of hepatic neoplasia, or is it sufficient to mediate the induction of hepatic neoplasia?

PPARα Mediation of Procarcinogenic Effects

Several effects of peroxisome proliferators in rodent liver have been hypothesized to mediate the development of neoplasia observed after chronic treatment. These effects include both the induction of peroxisomal fatty acid beta oxidation and the modulation of hepatocellular replication and death, either generally or in preneoplastic lesions specifically.

The induction of peroxisomal fatty acid beta oxidation was the earliest proposed response to be implicated in the mechanism of carcinogenesis by peroxisome proliferators (Yeldandi et al, 2000). The first step in peroxisonal fatty acid beta oxidation pathway is mediated by acyl CoA oxidase. Peroxisomal acyl CoA oxidase produces a molecule of H2O2 for each 2-carbon chain shortening of the fatty acyl CoA. It is the reactivity of H2O2 with cellular macromolecules, particularly genomic DNA, that was hypothesized to account for the carcinogenic activity of peroxisome proliferators. There is evidence that elevated production of H2O2 in peroxisomes can result in this escape, under certain conditions, from the detoxication by peroxisomal catalase (Conway et al, 1988; Conway et al, 1989). However, it is uncertain whether DNA damage occurs or contributes to the formation of genetic alterations in hepatocytes that are causally related to the development of tumors.

The increased cell replication in hepatocytes resulting from treatment of mice and rats with peroxisome proliferators is a complex response. Typically there is an increase of hepatocytes in S-phase reflecting the DNA replication that occurs upon initiation of treatment with peroxisome proliferators and is associated with hepatomegaly (Cattley, 2003). The development of hepatomegaly due to hyperplasia of hepatocytes occurs soon after initiation of treatment, and the liver stops increasing in size. As treatment continues, the rate of increase in liver weights decreases, as does the replication of hepatocytes. At some doses of certain peroxisome proliferators, the replication of hepatocytes continues at a rate slightly higher than in controls (Marsman et al, 1988; Marsman et al, 1992). However, in other studies, the level of replication of hepatocytes may return to that of controls or decrease to a level below that of controls (Tanaka et al, 1992). Therefore, sustained cell replication may adjust the rate of tumor induction, but other responses are likely to be central to the mechanism of hepatic carcinogenesis.

While sustained cell proliferation in the livers of mice and rats has been variably observed under continuous treatment conditions, studies that examine this response have been frequently concentrated on non-neoplastic hepatocytes. In a few studies, however, cell proliferation and cell death have been characterized in preneoplastic and neoplastic hepatocytes. Such studies demonstrate that peroxisome proliferators directly increase hepatocyte replication within preneoplastic foci, and thereby cause the growth of foci and increase the number of preneoplastic hepatocytes at risk for neoplastic development (Marsman et al, 1991). Results of further studies indicate that even upon reaching the stage of benign, grossly visible neoplasia, there was still a dependence of hepatocytes within the lesions upon peroxisome proliferator for stimulation of cell replication and survival (Miller et al, 2000).

Sufficiency of PPARα for Hepatic Carcinogenicity of Peroxisome Proliferators

The possibility that some response mechanism distinct from PPARα might cooperate with PPARα activation and downstream events cannot be excluded based on available data. While acknowledging this possibility, it may also be concluded that there is no proposed cooperative mechanism in the carcinogenicity of peroxisome proliferators that can be supported by available data.
A proposed mechanism for the increased hepatocyte replication observed upon treatment of rodents with peroxisome proliferators has invoked the activation of Kupffer cells and cytokine signaling from Kupffer cells to hepatocytes. There is conflicting evidence that Kupffer cell activation occurs and may be involved in the hepatocyte replication that follows treatment with peroxisome proliferators. Some studies have shown that Kupffer cell activation, as evidenced by increased phagocytosis (Bojes and Thurman, 1996) and nuclear translocation of NF-κB (Rusyn et al, 1998), may occur soon after treatment of rats with peroxisomal proliferators. Attempts to prevent Kupffer cell activation in mice (rose et al, 1997) rats (Rusyn et al, 2000) resulted in inhibition of replicative DNA synthesis in response to the peroxisome proliferator WY-14,643. Treatment of rats with anti-TNFα antibodies was reported to prevent the hepatocyte replication caused upon initiation of treatment with WY-14,643 (Bojes et al, 1997). These findings suggested that the hepatocyte replication induced by peroxisome proliferators could depend on Kupffer cell cytokine signaling, in cooperation with PPARα.

The evidence for the role of Kupffer cell activation in the hepatocyte replication that follows treatment with peroxisome proliferators was countered by studies of IL-6, TNFα, TNFR-1 and 2 knockout mice. Taken together the results suggest that the hyperplasia induced by peroxisome proliferators is distinct from regenerative cell replication, and that Kupffer cell-derived cytokines TNFα and IL-6 are not part of the mechanism of action of peroxisome proliferators in rodent liver. (Lawrence et al, 2001; Anderson et al 2001; Wallenius et al, 2000; Ledda-Columbano et al, 1998).

The significance of findings regarding the role of Kupffer cell activation and cytokine release in mediating responses to peroxisome is uncertain. To date, no putative activity of Kupffer cells has been experimentally linked to the induction of liver tumors by peroxisome proliferators.

**Species Differences in Peroxisomal Proliferation**

Recent findings suggest that liver of non-human primates is considerably less responsive or non-responsive to peroxisome proliferators as compared to rodent liver (Pugh et al, 2000; Kurata et all, 1998; Hoivik et al, 2004). These findings raise the possibility that humans treated with these intitites may not experience peroxisome proliferation. Some characterization of human response to peroxisome proliferators has been enabled by the clinical use of fibrate drugs that are peroxisome proliferators in rodent liver. Various older studies using liver biopsies from patients placed on fibrate drugs have indicated a lack of increase in peroxisomal volume density by subjective (de la Iglesia, 1992; Blumcke, 1993) or objective (Gariot, 1987; Hanefeld, 1983) assessment. In a more recent study, the lack of changes in peroxisomal acyl CoAoxidase mRNA levels in human patients given oral fibrate therapy for 8 weeks was observed (Roglans et al, 2002). Taken together, these findings suggest that human liver is non-responsive to peroxisomal proliferation by drugs that are clearly defined as peroxisome proliferators in rodent liver.

Other than studies in mice and rats, only one study provides an objective assessment of the carcinogenic activity of peroxisomal proliferators (Lake et al, 1993). In this study, groups of Syrian hamsters were fed diets containing nafenopin or WY-14,643 for 60 weeks. While these diets were carcinogenic in rat liver, no tumors were detected in livers of Syrian hamsters.

**Conclusions**

Peroxisome proliferators are an important group of hepatic carcinogens in rodents. The primary role of PPARα in mediating this response had led to the further characterization of potential events downstream that likely enable the carcinogenic response, including increased peroxisomal fatty acid beta oxidation and the modulation of hepatocellular replication and death. Kupffer cell activation has been proposed to function in the modulation of hepatocellular proliferation in rodent liver by peroxisome proliferators, but data that confirm or refute this proposal are mixed. There are marked species differences in susceptibility to peroxisomal proliferation and associated hepatic carcinogenesis.

**References**


All rights reserved. This document is available on-line at www.ivis.org. Document No. P1243.1104. This manuscript is reproduced in the IVIS website with the permission of the ACVP & ASVCP www.acvp.org