REVIEW

Peroxisome proliferator-activated receptor alpha: role in rodent liver cancer and species differences

P R Holden and J D Tugwood

Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, SK10 4TJ, UK
1Safety of Medicines Department, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, SK10 4TG, UK

(Requests for offprints should be addressed to P R Holden)

ABSTRACT

Peroxisome proliferators (PPs) are chemicals of industrial and pharmaceutical importance that elicit liver carcinogenesis by a non-genotoxic mechanism. One of the intriguing properties of PPs is that the pleiotropic effects of these compounds (including increased DNA synthesis and peroxisome proliferation) are seen in rats and mice only, but not humans. It is important to determine the risks to humans of environmental and therapeutic exposure to these compounds by understanding the mechanisms of non-genotoxic hepatocarcinogenesis in rodents. To understand this apparent lack of human susceptibility, attention has focused on the peroxisome proliferator-activated receptor alpha (PPARα), which appears to mediate the effects of PPs in rodents. It is also known to mediate the hypolipidaemic effects that fibrate drugs exert on humans with elevated plasma cholesterol and triglyceride levels. Human PPARαs share many functional characteristics with the rodent receptors, in that they can be transcriptionally activated by PPs and regulate specific gene expression. However, one key difference is that PPARα is less abundant in human than in rodent liver, which has led to the suggestion that species differences result from quantitative differences in gene expression. In this review we describe the effects of PPs and what is known of the molecular mechanisms of action and species differences with respect to rodents and man. Attention will be given to differences in the amounts of PPARα between species as well as the 'qualitative' aspects of PPARα-mediated gene regulation which might also explain the activation of some genes and not of others in human liver by PPs.

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BACKGROUND

Peroxisome proliferators (PPs) are a class of non-genotoxic carcinogens that cause liver tumours in rodents (Moody et al. 1991, Ashby et al. 1994). Included in this class are hypolipidaemic drugs, industrial plasticisers, herbicides and some leukotriene antagonists (Reddy & Lalwani 1983, Marsman et al. 1988, Eacho et al. 1991). When administered to rats and mice, PPs cause a marked increase in the size and number of hepatocyte peroxisomes. Peroxisomes are subcellular organelles which perform a number of functions that include the β-oxidation of fatty acids and cholesterol metabolism. PPs also cause liver hypertrophy and hyperplasia in rodents, leading to the growth of hepatocellular carcinomas (Reddy et al. 1980, Kraupp-Grassl et al. 1991, Moody et al. 1991). There have been a number of explanations for the observed hepatocarcinogenicity in rodents. One suggestion was that PPs caused an increase in hydrogen peroxide by altering the activity of the peroxisomal hydrogen peroxide-producing enzymes leading to oxidative stress and DNA damage (Reddy & Rao 1986, Rao & Reddy 1991, Clayson et al. 1994). Subsequent data have suggested that PPs can perturb the mechanisms controlling hepatocyte growth and apoptosis. It has long been accepted that PP-induced DNA synthesis is likely to play a major role in their hepatocarcinogenicity (Ashby et al. 1994). In addition, PPs can suppress hepatocyte apoptosis, possibly preventing the

A number of studies have shown that humans do not display the same range of PP-induced responses seen in rats and mice (Frick et al. 1987, Blaauboer et al. 1990). The guinea pig and non-human primates also appear unaffected by PPs (Foxworthy et al. 1990, Makowska et al. 1992, Chinje et al. 1994, Pacot et al. 1996). Establishing an explanation for species differences and an understanding of the risks to man from exposure to these chemicals has been a major focus for research. Published data over recent years have provided compelling evidence for a ligand-activated transcription factor, peroxisome proliferator-activated receptor alpha (PPARα), as a major mediator of PP action. This receptor has also provided some clues on why differences in response exist between species.

**PPARα MEDIATES RESPONSES TO PPS**

It was suggested in 1988 that PPs might act like steroid and retinoid hormones, through a nuclear receptor (Evans 1988). Thus, PPAR was isolated following a screen for nuclear receptors of a mouse liver cDNA library (Issemann & Green 1990). It was shown to be highly expressed in rat and mouse and activated by a number of PPs (Issemann & Green 1990, Kliever et al. 1994). The receptor was later designated PPARα, as it turned out to be the ‘prototype’ of a family of PPARs that now includes three other isoforms, β, γ and δ (Dreyer et al. 1992, Schmidt et al. 1992, Kliever et al. 1994) which have been cloned from a number of species (Gottlicher et al. 1992, Aperlo et al. 1995, Tugwood et al. 1998). PPARα is expressed predominantly in rodent liver and kidney, which are known target tissues for PPs (Issemann & Green 1990, Braissant et al. 1996, Lambe & Tugwood 1996). Although receptor activation is a post-translational event, little is known about the activating ligand for PPARα. It has been shown to be activated by leukotriene B4 and the eicosanoid 8(S)-hydroxyeicosatetraenoic acid (Yu et al. 1995, Devchand et al. 1996) as well as a number of fibrate drugs and fatty acids (Forman et al. 1997, Kliever et al. 1997).

In rodents, PPARα regulates transcription of a number of genes involved in both peroxisome proliferation and the β-oxidation of fatty acids by binding to a specific DNA regulatory element located in the upstream promoter region of these genes (Fig. 1). First it forms a heterodimer with another nuclear receptor, the retinoid X receptor (RXR) and then the heterodimer binds to the DNA element termed the peroxisome proliferator response element (PPRE). The PPRE consists of a direct repeat of two copies of an TGACCT-like sequence separated by a single base (a DR-1 repeat) and has been identified in the promoters of rat genes such as acyl CoA oxidase (ACO), bifunctional

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**Figure 1.** Proposed model of PP action. PPs enter the cell and cause heterodimerisation of PPARα with RXR, which binds to the PPRE DNA element. The PPRE consists of a direct repeat of two copies of a TGACCT-like sequence separated by a single base (a DR-1 repeat) and has been identified in the promoters of genes such as ACO and LPL. Binding modulates transcription of target genes resulting in changes in peroxisome proliferation, lipid metabolism, growth and apoptosis.
dehydrogenase/hydration (BFE) and microsomal cytochrome P450IVA1 (Tugwood et al. 1992, Zhang et al. 1992, Bardot et al. 1993, Aldridge et al. 1995) (Fig. 1). PPARα is also involved in modulating serum cholesterol levels, particularly high density lipoprotein cholesterol, in humans as well as rodents, through the regulation of target genes such as apolipoprotein (Apo) A-I, A-II and C-III (Schoonjans et al. 1996, Latruffe & Vamecq 1997, Peters et al. 1997).

Perhaps the most compelling data implicating PPARα in peroxisome proliferation and hepatocarcinogenicity come from studies of PPARα-null mice. Mice homozygous for a disrupted PPARα gene showed no apparent gross phenotypic abnormalities either externally or internally (Lee et al. 1995). In addition these mice showed no hepatic enzyme induction, liver growth or peroxisome proliferation in response to the PPs WY-14,643 and clofibrate (Lee et al. 1995, Gonzalez 1997). Further studies have shown that basal levels of serum cholesterol, high density lipoprotein and hepatic Apo A-I increase, supporting a role for PPARα in cholesterol regulation (Peters et al. 1997).

SPECIES DIFFERENCES IN RESPONSE TO PPS

PPs show a high degree of species specificity in their toxicity and carcinogenicity (Stringer 1992). The increase in growth and peroxisome proliferation seen in rat and mouse liver is not seen in guinea pigs, dogs, non-human primates and, more importantly, humans. In humans these findings have come from epidemiological studies with hypolipidaemic drugs (Hanefeld et al. 1983, Frick et al. 1987) and from in vitro experiments with human hepatocytes (Elcombe & Styles 1989, Parzefall et al. 1991). However, these drugs do lower elevated plasma cholesterol levels by acting through genes such as Apo A-I, -II, C-III and also lipoprotein lipase (LPL). This would suggest that humans are responsive, at least in part, to the effects of PPs. These reports are supported by the finding that active PPARα has been found in human and guinea pig liver (Sher et al. 1993, Mukherjee et al. 1994, Tugwood et al. 1994) and that genes such as Apo A-II and LPL contain a responsive PPRE (Vu-Dac et al. 1995, Schoonjans et al. 1996). Since humans clearly respond to PPs, but differently from rodents, it is difficult to determine the risk to humans from PPs based on rodent data. There are several explanations for these observed differences in response between rodents and humans. There may be a difference in the relative amounts of active PPARα receptor mRNA required to modulate growth, peroxisome proliferation and cholesterol metabolism, suggesting a quantitative difference between species. Alternatively, there may be a qualitative difference between species, in that human hepatocytes may lack an activator or have an active repressor of PPARα function, or that target genes (for DNA synthesis, peroxisome proliferation) contain altered or unresponsive PPRE sequences.

QUANTITATIVE DIFFERENCES BETWEEN SPECIES

It has been suggested previously that the relative amounts of PPARα mRNA differ between responsive and non-responsive species since lower transcript levels are detected in human and guinea pig liver compared with rat and mouse (Tugwood et al. 1996, 1998, Auboeuf et al. 1997, Palmer et al. 1998). On average it is estimated that human hepatocytes express PPARα at 5–10% of the levels found in rodent hepatocytes. However, despite the low levels of mRNA, humans are still able to respond to PPs by changes in serum lipid levels. Therefore there may be sufficient amounts of PPARα mRNA in human and guinea pig liver to maintain lipid homeostasis but not growth and peroxisome proliferation. A model for this is illustrated in Fig. 2, showing the different activation thresholds required to drive the peroxisomal (Tp) and lipid homeostasis (Tl) genes. It would be useful to test this hypothesis by increasing experimentally the levels of guinea pig or human PPARα mRNA in respective tissues. It would follow from this model that those genes that require a lower threshold of PPARα mRNA would also respond to lower concentrations of the ligand. However, evidence shows that genes associated with the peroxisome proliferation response in rodents are very sensitive to low levels of PPs (Wada et al. 1992, Berthou et al. 1995).

QUALITATIVE DIFFERENCES BETWEEN SPECIES

There may be a qualitative difference between species in response to PPs with respect to growth and peroxisome proliferation. For example, it has been suggested that the human hepatocyte may lack a transcriptional activator or conversely may contain a transcriptional inhibitor. A number of co-activators have been described that interact with PPAR/RXR heterodimers to facilitate transcriptional activation. These include the steroid receptor...
co-activator 1 (SRC-1) (Kamei et al. 1996) and the integrator protein p300 (Dowell et al. 1997). Whether these proteins are involved in species differences is unknown. It has also been shown that the PPARα/RXR heterodimer can behave as either a positive or negative regulatory factor in the same cell. The receptor can activate LPL by binding to its PPRE (Schoonjans et al. 1996) and inhibit Apo C-III by displacing the positive regulator HNF-4 (Hertz et al. 1995). Therefore, in the same cell, PPARα/RXR could behave as either a positive or negative regulatory factor, depending on the structure of the gene promoters and the other proteins that interact with them. Also PPARα/RXR has been shown to interact with the constitutive factors AP-1 (Sakai et al. 1995) and SP-1 (Krey et al. 1995). Some of the factors that potentially regulate the function of PPARα/RXR, either by protein–protein interactions or by competition for DNA binding, are depicted in Fig. 3. In rodents, other transcription factors can modulate the effects of PPARα/RXR depending on the promoter context. The hepatic factors COUP-TF1 and HNF-4 recognise direct repeat elements very similar to PPREs and both can influence the regulation of ACO and BFE (Chu et al. 1995). More recently, the thyroid hormone receptor TRα (Miyamoto et al. 1997) and the nuclear orphan receptor TAK1 (Yan et al. 1998) have been shown to repress the activity of PPAR/RXR by competitive binding to a PPRE-like element.

Qualitative differences might exist within the PPARα gene itself. There are two published full length sequences of the human PPARα gene that differ at two amino acid positions (Sher et al. 1993, Mukherjee et al. 1994). Both cognates are able to bind DNA and are responsive to PPs. Following a

**FIGURE 2.** Schematic representation of relative PPARα abundance in rodent and human livers. In rat and mouse, PPARα is present at sufficiently high levels to exceed theoretical thresholds both for 'lipid homeostasis' gene activation (T_p1), and for 'peroxisome proliferation' gene activation (T_p2). Human livers contain lower amounts of PPARα, and so can regulate only the lipid homeostasis pathway. However, since there is considerable inter-individual variation in PPARα expression in humans, a small proportion of individuals may lie below T_1, and be unresponsive to fibrate therapy for hyperlipidaemia (depicted by the error bar intersecting with T_1). Conversely, if the 'peroxisome proliferation' activation threshold is lower (T_p2), some individuals may show rodent-like toxic responses to PPs (depicted by the error bar intersecting with T_p2).

**FIGURE 3.** Regulation of PPARα function by other nuclear factors. The PPARα/RXR heterodimer is shown binding to a PPRE direct repeat sequence, and potential interactions with other homo- or heterodimeric nuclear proteins are depicted by arrows (see text for details). The constitutive factors AP-1 and SP-1 are also shown, together with the co-factors N-CoR and SRC-1 that interact with nuclear receptors.

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screen of human liver PPARα cDNAs, another human PPARα has been isolated that has four amino acid differences from the wild-type sequence. Although this receptor was able to bind to a PPRE it could not be activated by a number of PPs (Myers et al. 1997) and could also act as a dominant-negative repressor of PPARα-mediated gene expression (Roberts et al. 1998). Recently another human mutant PPARα has been cloned that lacks exon 6 as a result of alternate RNA splicing (Palmer et al. 1998). How and if these mutants act as dominant-negative repressors of peroxisomal and/or lipid homeostasis gene expression is unknown.

Finally, evidence suggests that PPREs may be altered or even absent in certain target genes. The PPRE for the human ACO, the first and rate-limiting enzyme of the peroxisomal β-oxidation system, has been cloned and sequenced (Varanasi et al. 1996). Located at −1918 to −1906 bp upstream of the transcription initiation site, this human sequence has several differences from the rat ACO PPRE. However, it was found to bind directly to PPAR/RXR and was responsive to a number of PPs in a reporter gene assay suggesting that this ‘qualitative’ sequence change is not a complete explanation for species differences. However, polymorphisms may exist in PPRE sequences among the human population.

CONCLUSIONS

The extensive information that has accumulated on the mechanism of PP action in rodents, and the responses of humans to these compounds, has yet to provide a definitive explanation for species differences in the effects of PPs. However, since its discovery, PPARα has been shown to be an essential mediator of PP-induced responses in rodents and humans. The receptor is abundant in tissues that show the physiological changes associated with PPs and can be activated by a large number of PPs in vivo assays. Perhaps the most convincing demonstration of PPARα involvement in peroxisome proliferation, growth and lipid homeostasis comes from the PPARα-null mouse. The possibility that PPARα holds the key to species differences is still a plausible premise irrespective of whether the quantity or the quality of the receptor is more relevant. In fact, the most likely explanation is that both qualitative and quantitative factors are important to human and rodent responses to PPs. While it may be tempting to conclude that a lack of induction of certain classes of genes in humans is due solely to reduced PPARα levels, there is a considerable weight of evidence to suggest this is an over-simplification. Clearly, there are a number of proteins, including other nuclear receptors, that are potent regulators of PPARα function in the liver. The relative abundance of hepatic factors including PPARα, and the structures of gene promoters with which these factors interact, are critical to the quality and quantity of PP-mediated gene regulation.

Future work may reveal whether there are positive- or negative-acting nuclear factors that may interact with PPARα. In addition, there may be polymorphisms in humans in both the PPRE and PPARα sequence. This may relate to the apparent differential responses of some human individuals to hypolipidaemic drugs. Studies are in progress to introduce the human PPARα into PPARα-null mice by gene replacement experiments, generating transgenic animals with different levels of the receptor (Gonzalez 1997, and F Gonzalez, personal communication). Finally, it is unlikely that a single receptor alone will elicit such a complex pleiotropic response but likely, rather, that other mediators are required for the changes in growth, lipid perturbation and peroxisome proliferation. Genes associated with cell survival and proliferation, such as tumour necrosis factor alpha, are under investigation as potential candidates. This cytokine has received a great deal of interest of late as it can both suppress apoptosis and induce DNA synthesis in a manner similar to PPs (Bojes et al. 1997, Rolfe et al. 1997, P.R. Holden, N.H. James, R. Brindle & R.A. Roberts, unpublished observations).

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