Environmental Mutageus & Carcinogens 17-2 : 78-91 (1997)

- REVIEW -

Peroxisome Proliferators and Hepatocarcinogenesis

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(Received July 3, 1997)

ABSTRACT : **Peroxisome is a single membrane-bounded organelle found in hepatic parenchymal cells and kidney tubular epithelial cells. A number of enzymes exist in peroxisome contributing to anabolic and catabolic peroxisomal functions. Extramitochontrial β-oxidation of fatty acid is a major function of peroxisome. Peroxisomes can be proliferated by many structually unrelated compounds such as hypolipidemic drugs, plasticizers, pesticides, some pharmaceutical agents and high fat diet. These chemicals, called peroxisome proliferators, act via the peroxisome proliferator activated receptor, to induce peroxisome proliferation, hepatomegaly and hepatocellular carcinoma in rodent. The clear mechanisms of peroxisome proliferator-induced hepatocarcinogenesis have been not demonstrated. Since they are not genotoxic, biochemical changes or changes in gene expressions may be involved. A free radical theory has been suggested based on the finding of oxidative damages of macromolecules by hydrogen peroxide released in the peroxisomal β-oxidation of fatty acid. Increased cell proliferation by a peroxisome proliferator has been also thought to be an important factor in the hepatocarcinogenesis as suggested in other cases of nongenotoxic carcinogenesis. The alternation of eicosanoid concentrations by peroxisome proliferators may be important in the peroxisome proliferator-induced hepatocarcinogenesis since peroxisome proliferators decrease the concentration of eicosanoids, and the peroxisome proliferator ciprofibrate-eicosanoid combination is comitogenic and costimulates some mitogenic signals in hepatocytes. All of proposed mechanisms should be considered in the peroxisome prolifrator-induced hepatocarcinogenesis.**

Key words : Peroxisome, Peroxisome proliferator, Hepatocarcinogenesis

1. Peroxisomes

The peroxisomes were first found in mouse kidney tubular epithelial cells by A.G. Rhodin of the Karolinska Institution (Stockholm, Sweden) in the mid-1950s (Reddy and Lalwani, 1983). Since then, these single membrane-bounded organelles have been found in various mammalian and plant tissues as well as eukaryotic microorganisms such as yeast, protozoa, and fungi (Master and Holmes, 1977; Reddy *et al*, 1988).

Peroxisomes were also found in hepatic parenchymal cells by Rouiller and Bernhard (1956). In hepatic parenchymal cells, peroxisomes are usually spherical or oval in shape, approximately 0.3 to 1.0 μm in diameter and contain a finely granular electron-dense matrix with a characteristic electron-dense crystalloid or nucleoid core signifying the presence of urate oxidase in many species. However, in humans, peroxisomes lack urate oxidase and the nucleoid core (Reddy and Lalwani, 1983; Lock *et al.*, 1989).

Peroxisomes can be readily differentiated from mitochondria by their single limiting membrane, electron-dense matrix and absence of cristae. Peroxisomes appear to be clustered in cells and are often interconnected at the times of peroxisome proliferation and also are often found physically associated with the endoplasmic reticulum (Lazarow, 1981; Lazarow and Fujiki, 1985), which may facilitate biochemical cooperation between the organelles. The lipid composition of peroxisomal membrane is different from the endoplasmic reticulum, however (Stott, 1988). Peroxisomes are highly permeable to small molecules such as sucrose, H_2O and inorganic ions (Masters and Holmes, 1977).

Peroxisomes can be identified at the ultrastructural level by immunocytochemical procedures demonstrating the presence of catalase, which is the only common enzyme found in peroxisomes (Stott, 1988). There are approximately 1000 peroxisomes per parenchymal cell, and they constitute 1.5-2% of total cell volume in the rat, but primate hepatocytes contain a

lower number (De Duve and Baudhuin, 1966). In the rodent, half-lives of hepatic peroxisomes are estimated to be about 1 to 3.5 days (Chance, 1979; Miyazawa *et al.*, 1980).

2. Enzymes in peroxisome

A number of enzymes exist in peroxisomes such as oxidases, dehydrogenases, acyltransferases, aminotransferases, reductases, hydrolases, catalase, and a number of the enzymes of fatty acid oxidation pathways and glycolate cycle. These enzymes contribute to anabolic and catabolic peroxisomal functions, including plasmalogen, cholesterol, and bile acid synthesis, gluconeogenesis, hydrogen peroxide-based respiration, polyamine and purine catabolism, ethanol oxidation, and lipid metabolism (Lazarow and Fujiki, 1985; Watkins *et al.*, 1989; Tolbert, 1981).

The synthesis of plasminogens, ether-linked glycerolipids, starts with acylation of dihyroxyacetone in peroxisomes, unlike in the endoplasmic reticulum where the synthesis of plasminogens occurs mainly with glycerol 3-phosphate. The further reactions of plasmalogen synthesis are carried out in the endoplasmic reticulum. The function of plasmalogens is not known, but it has been found that plasmalogens constitute a part of the phospholipids of heart and brain membrane which may protect the cell by scavenging oxygen radicals. Peroxisomes also contain enzymes of cholesterol synthesis, for example, 3-hydroxy, 3-methylglutaryl-CoA reductase, which is responsible for catalyzing the terminal reaction of cholesterol synthesis. Peroxisomes may be also involved in gluconeogenesis to form α-keto acids (De Duve and Baudhin, 1966).

Respiratory pathways mediated by peroxisomal oxidases account for 20% of the oxygen consumption of the liver (De Duve and Baudhin, 1966). The substrates for peroxisome oxidases include D-amino acids, L-pipecolate, uric acid, lactic acid, polyamines, fatty acyl CoAs and gylcolate. Unlike mitochondrial oxidation, peroxisomal oxidation is characterized by the lack of energy conservation. Instead, peroxisome oxidation results in the production of hydrogen peroxide, which is degraded by catalase to water. It was suggested that the heat dissipated from peroxisomal respiration may be involved in thermogenesis in brown fat of cold-adapted rats (Lazarow, 1981). Peroxisomes also play an important role in purine catabolism. In many species, except humans, birds and some reptiles, uric acid, the end-product of purine catabolism, is further degraded by hepatic peroxisomal enzymes such as xanthine dehydrogenase, urate oxidase, and allantoicase. These enzymes in the human have been lost in the course of evolution. This loss may be an advantage since oxygen free radicals can be scavenged by uric acid. With high cellular concentrations of ethanol, peroxisomes also play a role in ethanol metabolism. With the dysfunction or lack of alcohol dehydrogenase, peroxisomal catalase becomes a major metabolic pathway of ethanol oxidation to form acetaldehyde (Lazarow and Fujiki, 1985).

Peroxisomes also can degrade a variety of lipids, including fatty acid derivatives such as decarboxylic acids, prostaglandins, leukotrienes, some xenobiotics and the side chain of cholesterol. In contrast to mitochondria, the peroxisomal membrane possesses a long-chain acyl-CoA synthetase for the activation of very long chain fatty acids. In peroxisomes, acyl-CoA ester can cross the peroxisomal membrane by an unknown mechanism. In addition, peroxisomal NADPH can serve as a reductant for oxidized substrates. Unlike mitochonrial β-oxidation, peroxisomal β-oxidation has a relatively high pH optimum, and is not dependent upon an active electron transport system coupled to a phosphorylation system, and does not completely degrade fatty acids. Indeed, peroxisome β-oxidation functions as a chain-shortening system. Peroxisomal β-oxidation is not sensitive to the classic cytochrome inhibitors such as cyanide.

Three proteins are involved in peroxisomal β-oxidation instead of the four required in mitochondria. In peroxisomes, the first step is catalyzed by a fatty acyl CoA oxidase which reduces the acyl-CoA substrate with molecular oxygen to form trans-2-enoyl-CoA and hydrogen peroxide. The second reaction is catalyzed by the trifunctional protein; its activities include enoyl-CoA hydratase, β-hydroxy-acyl-CoA dehydrogenase and Δ3-Δ2 enoyl-CoA isomerase. Enoyl-CoA hydratase hydrates trans-2-enoyl-CoA to L-(+)-β-hydroxyacyl-CoA thioester, which is further catalyzed by hydroxy-acyl-CoA dehydrogenase to form 3-ketoacyl-CoA through the reduction of NAD. Δ3-Δ2 enoyl-CoA isomerase is required for the metabolism of unsaturated acyl-CoA containing double bonds at odd-numbered positions from the carboxyl end (Hiltunen *et al.*, 1993; Milila *et al.*, 1993). The last cleavage step is catalyzed by the peroxisomal β-ketothiolase, which cleaves a 3-ketoacyl-CoA group, yielding acetyl-CoA and a new acyl-CoA, depending on chain length. The newly formed acyl-CoA can either reenter the peroxisomal pathway or be transported out of the peroxisome by carnitine acyl-CoA transferases (Vamecq and Draye, 1989). Hydrogen peroxide is generated by fatty acyl-CoA, the first and rate limiting enzyme of the pathway. Catalase degrades hydrogen peroxide to water.

3. Peroxisome proliferators

Since peroxisome proliferation in the liver of rats fed a diet

containing clofibrate was first demonstrated in the early 1960's, several structurally related hypolipidemic drugs, such as ciprofibrate, gemfibrozil, and bezafibrate have been shown to induce peroxisome proliferation in many species including primates (Reddy and Lalwani, 1983; Moody and Reddy, 1978a, 1978b).

Compounds which are not structually related to clofibrate have also been shown to induce peroxisome proliferation. These compounds include [4-chloro-6-(2,3-xylidino)2-pyriminidinylthioacetic acid (Wy-14,643), 4-chloro-6-(2,3-xylidino)2-pyriminidinylthio(N-b-hydroxy-ethyl)actamide (BR-931) and tibric acid (Moody and Reddy,1978; Lake *et al.*, 1984). Plasticizers such as di-(2-ethylhexyl)phthalate (DEHP) and di-(2-ethylhexyl)adipate are also peroxisome proliferators (Marsman and Popp, 1994; Lake *et al.*, 1993; Rao and Reddy, 1987; Elcombe, 1985). DEHP is one of the widely used plasticizing agents employed in the manufacture of polyvinyl chloride plastics, including cellophane, polyvinylchloride pipe, kidney dialysis tubing and blood transfusion bags (Graham, 1973). Other environmental chemicals such as perfluoro-n-decanoic acid, trichloroacetic acid and the herbicides 2,4-D and 2,4,5-T were also found to induce peroxisome proliferation (Borges *et al.*, 1991; Glauert *et al.*, 1992; Borges *et al.*, 1993; Kawashima *et al.*, 1984; Sundberg *et al.*, 1994; Lundgren *et al.*, 1987; DeAngelo *et al.*, 1993). Some pharmaceutical agents such as acetylsalicylic acid, the leukotriene antagonist, LY171883, and the antidiabetic agent, dehydroepiandrosterone, have been identified as peroxisome proliferators (Eacho *et al.*, 1991; Rao *et al.*, 1992; Foxworthy *et al.*, 1993; Yamada *et al.*, 1992; Sakuma *et al.*, 1992). Natural factors such as high fat diets, vitamin E deficiency, retinoic acid, and hormonal imbalances like hyperthyroidism have been also found to induce peroxisome proliferation (Ishii *et al.*, 1980; Reddy *et al.*, 1981; Sugiyama *et al.*, 1994). The wide range of compounds which induce peroxisome proliferation are listed in the Table 1.

Table 1. Peroxisome Proliferators

Hypolipidemic Agents
Structurally Related to Clofibrate
Nafenopin
Gemfibrozil
Ciprofibrate
Benzafibrate
Structurally Unrelated to Clofibrate
Tibric acid
Wy-14643
BR-931
Phthalates and Related Compounds
Di(2-ethylhexyl) phthalate
Di(2-ethylhexyl) adipate
2-Propylhexanol
Environmental Compounds
2,4-dichlorophenoxy acetic acid
Perflouro-n-decanoic acid
Perflourosulfonic acids
Trichloroacetic acid
Pharmaceutical Agents
Salicylic acid
Valproic acid
LY-17883
Dehydroepiandrosterone
Dietary Manipulations
High fat diets
Prologed vitamin E deficiency

From: Moody and Reddy, 1978; Lock *et al.*, 1989; Reddy *et al.*, 1984; Graham *et al.*, 1994; Reddy, 1978; Cattley, 1987; Lake *et al.*, 1984; Borges *et al.*, 1991; Rao and Reddy, 1987; Elcombe, 1985; Graham, 1973; Kawashima *et al.*, 1984; Eacho *et al.*, 1991; Foxworthy *et al.*, 1993; Yamada *et al.*, 1992; Sakuma *et al.*, 1992; Ishii *et al.*, 1980; Moody *et al.*, 1991; Yamada *et al.*, 1993; Sugiyama *et al.*, 1994.

4. Peroxisome proliferation

Peroxisomes normally comprise about 2% of volume of hepatocyte, but the administration of peroxisome proliferators increases the number of peroxisomes as well as their size, and the enzymes in peroxisomal β-oxidation pathway. The magnitude of the induction of peroxisome proliferation is not equally increased by all peroxisome proliferators. For example, ciprofibrate is more efficacious than acetylsalicylic acid and DEHP in the induction of peroxisomal β-oxidation enzymes (Reddy *et al.*, 1986; Marsman *et al.*, 1988). Peroxisome proliferators were found to increase the mRNAs encoding the peroxisomal fatty acyl-CoA oxidase (FAO) and bifunctional protein about 20 fold (Rao and Reddy, 1987; Reddy *et al.*, 1986; Caira *et al.*, 1995; Gibson, 1992). Induction of peroxisome proliferation in the liver is associated with proliferation of smooth endoplasmic reticulum with an increase in the microsomal cytochrome P-450 4A subfamily (Lock *et al.*, 1989; Sharma *et al.*, 1988; Gibson, 1989; Grasl-Kraupp *et al.*, 1993a, 1993b; Pacot *et al.*, 1993; Makowska *et al.*, 1990; Makowska *et al.*, 1992).

1) Peroxisome proliferator activated receptor

The receptor-mediated mechanism was suggested to explain the mechanism of peroxisome proliferation (Lock *et al.*, 1989; Rao and Reddy 1987). Peroxisome proliferators and/or their metabolites interact with a receptor in the hepatic cytoplasm to form a peroxisome proliferator-receptor complex, and the complex activates the genes coding for the enzymes in the perox-

isomal β-oxidation pathway at the transcriptional level. Peroxisome proliferator activated receptor (PPAR), a member of the nuclear hormone receptor superfamily, has been isolated (Issemann and Green, 1990), and has been shown to confer peroxisome proliferator responsiveness in some cells using transactivation assay (Issemann and Green, 1990; Green, 1992). There are good correlations between the activation of PPARα and peroxisome proliferation potency (Issemann and Green, 1990; Isseman *et al.*, 1993). In a chimeric receptor assay, for example, Wy-14,643 is more potent than mono (2-ethylhexyl) phthalate. In addition, the tissue specific expression of PPARα correlates well with the induction of FAO enzyme. It is also noteworthy that PPARα is directly responsible for the transcriptional activation of peroxisome responsive genes which have peroxisome proliferator response elements (PPRE). Furthermore, mice with targeted disruption of the PPARα gene are resistant to peroxisome proliferation and induction of the genes of enzymes in the peroxisomal β-oxidation pathway and the cytochrome P-450 4A family, demonstrating the importance of PPARa in peroxisome proliferation (Lee *et al.*, 1995). Since neither peroxisome proliferators nor fatty acids are ligand (s) of PPAR, peroxisome proliferators may perturb intracellular lipid metabolism in such a way causing accumulation of lipid metabolites which would increase the induction of PPAR activity that regulates gene coding peroxisomal enzymes.

The inducers of peroxisome proliferation have been found to activate PPAR (Issemann and Green, 1990; Dreyer *et al.*, 1992). A broad range of fatty acids, except very long unsaturated and short chain fatty acids, have been found to activate PPAR in a number of studies (Schmidt, 1992; Zhu *et al.*, 1993; Keller, *et al.*, 1993; Isseman *et al.*, 1993). Some eicosanoids such as PGD_1 and D_2 activated PPAR α, δ, and γ in an in vitro assay (Yu *et al.*, 1995). Thiazolidinedione was found to bind selectively PPARγ, whereas arachidonic acid metabolite, prostaglandin J_2 is the natural activator and its ligand, suggesting that fatty acid derivate may be the endogenous ligands of some subtype of PPAR (Kliewer *et al.*, 1995; Yu *et al.*, 1995). Yu *et al.*(1995) have also demonstrated that activator-dependent activation of PPAR isoform. WY 14,643 activates PPARα and δ much more than γ, whereas clofibrate and 5,8,11,14-eicosatetraynoic acid (ETYA) activate only PPARa. However, the true ligand(s) of PPARs is(are) still unclear.

PPARs activate expression of target genes by recognizing PPRE composed of TGACCT-related direct repeats that are spaced by one nucleotide (DR1), which is the so-called space element (Tugwood *et al.*, 1992; Yu *et al.*, 1991; Schoonjans *et al.*, 1996). PPARα does not bind to PPRE directly. The retinoid X receptor (RXR) acts as the preferential partner in a functional PPAR complex (Green, 1995; Zhang *et al.*, 1992; Yu *et al.*, 1991; Leid *et al.*, 1992), suggesting physiological cross-talk between PPAR and RXR signaling pathways. RXR is activated by its ligand 9-cis retinoic acid; some eicosanoids were also found to activate RXR (Eager *et al.*, 1992). The reciprocal modulation of thyroid hormone and peroxisome proliferator-responsive genes through cross-talk between their receptors suggests diverse functional interaction between PPARs and other nuclear receptor (Bogazzi *et al.*, 1994). Negative regulation of PPAR activation was also found. Chicken ovalbumin upstream promoter transcription factor (COUP-TF) blocks peroxisome proliferator responsiveness of genes such as P-450 4A6 and multienzyme via competition of COUP-TF homodimer with PPAR/RXR heterodimer complex for occupancy of DNA biding site of the genes (Miyata *et al.*, 1993). The competition between hepatic nuclear factor-4 and PPAR/RXR in the response element of apolipoprotein C-III results in decreased gene transcription (Hertz *et al.*, 1995). Recently, it was also demonstrated that the human orphan nuclear hormone receptor, liver X receptor α (LXRα), interacted with PPAR and inhibited peroxisome proliferator signaling (Miyata *et al.*, 1996). PPRE containing genes have been found in enzymes such as FAO, bifunctinal enzyme (Zhang *et al.*, 1992; Zhang *et al.*, 1993; Dreyer *et al.*, 1992; Tugwood *et al.*, 1992), P-450 4A1 and 6 (Muerhoff *et al.*, 1992), fatty acid binding protein (Besnard *et al.*, 1993), malic enzyme (Castelein *et al.*, 1994), phosphoenolpyruvate carboxykinase (Tontonoz *et al.*, 1995) and apolipoprotein A-II gene (Vu-Dac *et al.*, 1995).

2) Species differences

Species differences in peroxisome proliferation and hepatocarcinogenesis have been studied. Generally, rats and mice are sensitive, and the hamster is only slightly responsive in terms of enzyme activity and peroxisome proliferation. Guinea pigs, dogs, marmosets, monkeys and humans are nonresponsive (Lake *et al.*, 1989; Bentley *et al.*, 1993; Price *et al.*, 1992; Pacot *et al.*, 1996).

Since PPARs have been demonstrated to mediate the effect of peroxisome proliferators, alterations in these receptors may explain the species differences. As suggested by Ashby (1994), PPAR may not function because of its inability to heterodimerize with RXR or because it lacks other factors necessary to mediate PPAR activity in nonresponsive species. Because of studies showing that human liver has a functional PPAR, and a functional active PPRE in the FAO promoter, the nonresponsiveness of humans may be from other factors, such

as inability of ligand binding or interference of PPAR binding to PPRE by other factors (Varanasi *et al.*, 1996). COUP-TF1 and HNF-4 were found to antagonize PPAR signaling via competition in occupying the PPRE (Miyata *et al.*, 1993; Hertz *et al.*, 1995). The competition in dimerization of PPAR with RXR by other unknown factors may possibly inhibit PPAR activation in nonresponsive species, as seen in a recent study demonstrating that the inhibitory effect of LXRα in peroxisome proliferator signaling (Miyata *et al.*, 1996).

5. Mechanisms of peroxisome prolifrator-induced hepatocarcinogenesis

It was first reported that the peroxisome proliferator napenofin induced hepatocellular carcinoma in mice (Reddy *et al.*, 1976). Since then, several studies have shown that many peroxisome proliferators induce hepatocellular carcinoma in rodents after long term exposure (Svoboda and Azarnoff, 1979; Rao *et al.*, 1984; Abdellatif *et al.*, 1990; Rao *et al.*, 1992) (Table 2). The mechanism by which peroxisome proliferators induce hepatocellular carcinoma is not clear yet. Although some studies showed that the peroxisome proliferators WY-14,643, napenopin and ciprofibrate increased sister chromatid exchanges, chromosomal aberrations and micronuclei in cultured hepatocytes (Hwang *et al.*, 1993; Reisenbichler and Eckl, 1993), most studies have not shown them to be directly genotoxic, using number of methods including bacterial mutagenesis, unscheduled DNA synthesis, DNA modification, lac I and Z mutation in transgenic animal models (Warren *et al.*, 1980; Glauert *et al.*, 1984; Gupta *et al.*, 1985; Elliott and Elcombe, 1987; Bentley *et al.*, 1993; Gunz *et al.*, 1993; Lefevre *et al.*, 1994; Ashby *et al.*, 1994). Peroxisome proliferators thus are considered to be nongenotoxic agents (Table 3).

Table 2. Hepatocarcinogenesis by peroxisome proliferators

Peroxisome proliferator	Rat			Mouse		
	strain	M	F	strain	M	F
Ciprofibrate	F344	+		C57BI	+	
Clofibrate	F344	+		C57BI	-	
	SD	+	+	Swiss	-	
DEHP	Wister	-				
Gemfibrozil	F344	+	+	B6C3F1	+	
LY171883	SD	+				
Nafenopin				B6C3F1	+	
Perchloroethylene	F344	+				
Percloroacetic acid	F344	+		B6C3F1	+	+
Tribric acid	F344	+		B6C3F1	+	
Wy 14,643	F344	+	+			

DEHP; Di-(2-ethylhexyl) phthalate, M; male, F; female.
+; Induction of hepatocellular carcinoma
-; No induction of hepatocellular carcinoma
SD; Sprague Dawley

Table 3. Genotoxicity of peroxisome proliferators

Coumpounds	Assay[a]	Results[b]
Clofibrate	16 (7)	13/16
Nafenopin	6 (6)	4/7
Wy-14,643	9 (6)	6/9
Trichloroacetic acid	8 (6)	6/8
Perchloroethylene	20 (7)	16/20
Benzyl butyl phthalate	14(10)	14/14
Di-(2-ethylhexyl) phthalate	41(18)	32/41
Trichloroethylene	21(12)	10/21

[a]Total number of reports, ( ); Number of assay tested
[b]Negative results/total number of reports

1) Role of oxidative damages of maromolecules

Several possible hypotheses for the carcinogenesis of peroxisome proliferators have been suggested. One of them is the free radical theory which was suggested by Reddy *et al.* (1986) based on the correlation between peroxisomal proliferation and incidence of hepatic tumor. It was proposed on the basis of the finding that there is a 20-30 fold increase of fatty acyl CoA oxidase, which produces hydrogen peroxide as a by-product, whereas catalase is only increased about 2 fold, which may not be sufficient to detoxify the overproduction of hydrogen peroxide. The hydrogen peroxide may diffuse to the cytosol and to the nucleus, and lead to oxidative damages, such as lipid peroxidation and DNA damages (Reddy and Rao, 1986; Rao and Reddy, 1991; Tamura,1990). Several studies have been shown peroxisome proliferators elevate 8-oxo-deoxyguanosine and 5-hydroxymethyl-2'-deoxyuridine (HMdU), DNA damaged forms (Kasai *et al.*, 1989; Takagi, 1990; Srinivansan and Glauert, 1990). However, with purified nuclei or mitochondria, peroxisome proliferators did not increase 8-oxo-deoxyguanosine formation (Cattley and Grover, 1993; Sausen *et al.*, 1995). It maybe possible that the increased 8-oxo-deoxyguanosine in unfractioned liver may result from either damage of constitutive DNA during homogenization or contamination of mitochondrial DNA, which has higher levels of 8-oxo-deoxyguanosine than nuclear DNA. In addition, PFDA, which inhibits peroxisomal β-oxidation and does not have tumor promoting activity, increases 8-oxo-deoxyguanosine in the nuclear fraction (Huang *et al.*, 1994). It was also found that expression of catalse, which is the key enzyme in detoxifying of hydrogen peroxide, did not block peroxisome proliferator-induced ox-

idative damages such as formation of 8-oxodeoxyguanosine and lipid peroxidation in transgenic mice liver (personal communication). It was reported that overexpression of FAO in the African green monkey kidney cell line CV-1 cells resulted in increased hydrogen peroxide production, and transformed foci that grew in soft agar and formed tumors when transplanted into nude mice (Chu *et al.*, 1995). These data suggest the association between hydrogen peroxide production and cellular transformation. However, whether this transformation was due to the DNA damage or other mechanisms and whether the CV-1 cell system is relevant to liver are not clear. Furthermore, other evidences have shown a lack of correlation between hydrogen peroxide-induced oxidative damage and hepatocarcinogegesis by peroxisome proliferators (Elliott and Elcombe, 1987; Hegi *et al.*, 1990; Glauert *et al.*, 1992; Cattley and Glover, 1993; Marsman *et al.*, 1992).

2) Role of cell proliferation

Another possible mechanism in peroxisome proliferation-induced hepatocarcinogenesis is increased cell proliferation. Chemically induced cell proliferation is an important factor in the development of tumors (Butterworth, 1989) since cell proliferation can increase the mutation frequency. Increased cell replication can also play a role in the carcinogenic process by an increased error in DNA repair, by activation of protooncogenes, or by the promotion of initiated cells (Butterworth, 1989; Popp and Goldsworthy, 1989; Schulte-Hermann *et al.*, 1989; Goldsworthy,1994; Ashby, 1994).

Two distinct phases of cell proliferation have been studied in the liver. Cell proliferation is increased early after the administration of peroxisome proliferators (Reddy and Lalwani, 1983). At later times, DNA synthesis remains significantly elevated above control levels in rats receiving the peroxisome proliferator Wy-14,643, but not for peroxisome proliferators such as nafenopin, clofibrate, and ciprofibrate (Wada *et al.*, 1992; Marsman *et al.*, 1988; Eacho *et al.*, 1991; Yeldandi *et al.*, 1989; Chen *et al.*, 1994). It has been suggested that a long-term low-level increase in cell proliferation may be important in hepatocarcinogenesis since sustained cell proliferation can amplify the initiated cell by promotion or/and progression process (Wada *et al.*, 1992; Marsman *et al.*, 1988). It may be that both phases of cell proliferation are important because, as suggested by Ames and Gold (1991), early proliferation events lead to an initiated cells, whereas latter events may give a preferential selective growth advantage to initiated cells over normal hepatocytes. Quantitatively, there are good correlations between increased cell proliferation (both short and long term) and hepatocarcinogenesis, suggesting that increased cell proliferation is important in the process (Ashby, 1994). However, this theory has been challenged by other studies showing that no relation exists between hepatocellular carcinoma and cell replication in the chronic phase (Eacho *et al.*, 1991; Chen *et al.*, 1994). Peroxisome proliferators have also been found to increase cell proliferation in putative preneoplastic lesions of the liver (Schulte-Hurmann *et al.*, 1983; Grasl-Kraupp *et al.*, 1993a, 1993b), enhance the tumor incidence in the old animals more than in young animals (Cattley *et al.*, 1991) and promote clonal expansion of initiated rat hepatocytes with EGF synergistically (James and Roberts, 1994).

The target genes that alter the pathways allowing for selective growth of cells in hepatocarcinogenesis have not been identified. However, the peroxisome proliferators have been found to induce several oncogenes, such as c-jun, c-fos, jun-B, jun-D, myc, and Ha-ras, in liver cell lines and *in vivo* (Ledwith *et al.*, 1993; Goldsworthy *et al.*, 1994; Hsieh *et al.*, 1991; Cherkaoui Malki *et al.*, 1990). Futhermore, expression of these genes was modulated by signal transduction inhibitors (Ledwith *et al.*, 1996). The signal transduction system plays a central role in cell growth, and alteration of this system would have significant effects on cellular proliferation and thereby on hepatocarcinogenesis by peroxisome proliferators.

Peroxisome proliferators act via the PPAR. However, either direct activation of membrane-triggered signaling or PPAR-mediated signaling in cell proliferation by peroxisome proliferators has not been demonstrated. Peroxisome proliferators, however, have been found to increase protein kinase C (PKC) activity and free cytosolic calcium. The peroxisome proliferators Wy-14,643 and perfluorooctanoate significantly increased PKC in rat liver microsomes, whereas other peroxisome proliferators, including ciprofibrate, slightly increased it (Bojes *et al.*, 1992; Bojes and Thurman, 1994). In other studies, acyl CoA or acyl-CoA thioesters of peroxisome proliferators, instead of peroxisome proliferators themselves, activated PKC in rat liver and brain (Bronfman *et al.*, 1988; Bronfman *et al.*, 1989; Orellana *et al.*, 1990). The activation of PKC may be involved in the phosphorylation of the epidermal growth factor (EGF) receptor by peroxisome proliferators (Orellana *et al.*, 1993). As discussed by Nishizuka (1988), the activation may be invoved in cellular oncoprotein activation and tumor promotion in hepatocarcinogenesis by peroxisome proliferators (Ledwith *et al.*, 1996). Furthermore, the induction of cell proliferation by EGF and the peroxisome proliferator nafenopin was abolished in the presence of a PKC inhibitor, suggeting that PKC activation may play a role in the mitogenic response

to peroxisome proliferators (Bieri *et al.*, 1993). The increase of calcium mobilization by peroxisome proliferators has also been studied in hepatocytes (Bennett and Williams, 1992; Ochsner *et al.*, 1990; Shackleton *et al.*, 1995). The peroxisome proliferator nafenopin was found to inhibit the suppressive effect of a PKC inhibitor on phenylephrine-induced calcium mobilization in hepatocytes, suggesting involvement of the PKC pathway in this signaling (Liebold *et al.*, 1996). A direct relationship between early increased intracellular calcium concentrations and later hepatocyte proliferation was reported (Bennett and Williams, 1993). The inositol phosphate pathway has also been studied. Inositol triphosphate production is related to mobilization of calcium and activation of PKC. However, nafenopin did not induce the production of inositol triphosphates while increasing the intracellular calcium concentration, but it impaired vasopressin-induced inositol triphosphate production (Ochsner *et al.*, 1990), suggesting that inositol triphosphate therefore may not be directly involved in cell proliferation by peroxisome proliferators.

3) Role of eicosanoids

The role of eicosanoids in carcinogenesis has also been studied. Increased eicosanoid concentrations have been found during the tumor promotion in many tissues, such as colon and skin (Marnett, 1992; McCormick *et al.*, 1985). The promotion of hepatocarcinogenesis has also generally been associated with increased prostaglandin (PG) levels (Denda *et al.*, 1989; Hendrich *et al.*, 1991; Tang *et al.*, 1993). The inhibitors of arachidonic acid metabolism inhibit the development of γ-GT-positive foci in rats fed phenobarbital (Denda *et al.*, 1989). The mechanism by which eicosanoids enhance carcinogenesis may brought about in several ways, but the effect on cell proliferation by eicosanoids in normal or/and tumor cells may be important. In fact, PGE_2 was found to increase cell proliferation in hepatoma cells (Trevisani *et al.*, 1980). Leukotriene C4 (LTC_4) and D_4 have been found to increase the proliferation of AGS cells, a gastric cancer cell line, and of U937 cells, a transformed monocyte/macrophage cell line (Shimakura & Boland, 1992; Ondrey *et al.*, 1989).

Production of eicosanoids, particularly PGs, has also been suggested to be related to liver regeneration (MacManus and Braceland, 1976; Miura and Fukui, 1979; Tsujii *et al.*, 1993). In cultured hepatocytes, eicosanoids have also been found to induce DNA synthesis (Andreis *et al.*, 1981; Armato *et al.*, 1984; Skouteris etal., 1988; Refsnes *et al.*, 1994; Adachi *et al.*, 1995; Marchesini, 1986). Inhibitors of PG synthesis suppressed cell proliferation in hepatocytes (MacManus and Braceland, 1976; Mirura and Fukui, 1979). LTC_4 has also been found to be important in cell proliferation in hepatocytes (Romano *et al.*, 1986).

In mitogenic signaling of hepatocytes, prostaglandins, including PGE_2 and $PGF_{2\alpha}$, increase cellular concentrations of cAMP, inositol triphosphate, and free calcium (Andreis *et al.*, 1981; Melien *et al.*, 1988; Athari and Jungerman, 1989), and increase the expression of c-myc induced by epidermal growth factor (EGF) and TGF-α (Skouteris and Kaser, 1992; Skouteris and Mcmenamin, 1992). The production of PGE_1, PGE_2 and $PGF_{2\alpha}$ was found to play a pivotal role in MAP kinase activity in hepatocytes (Adachi *et al.*, 1996). This fundamental property of eicosanoids may contribute to the sustained modulation of signal transduction pathways in biological responses such as cell proliferation and differentiation, either directly or by interaction with other mitogenic signals (Marzo, 1995).

In cultured hepatocytes, peroxisome proliferators have been found to increase DNA synthesis less than or about two-fold, which is much lower compared to that seen in the early phase in vivo, and they are not comitogenic with epidermal growth factor (EGF) (Marsman *et al.*, 1993; Bennett and William, 1993; Bieri *et al.*, 1984; Mukkassah-Kelly, 1988). Cell proliferation in cultured hepatocytes from rats fed the peroxisome proliferator ciprofibrate was lower than from rats fed a normal diet (Lindroos and Michalopoulos, 1993). These studies suggest that additional cellular responses or endogeneous factors influencing cell proliferation may be essential factors in the cell proliferation process in both normal and initiated cells. These include oxidative stress, expression of oncogenes and tumor suppressor genes, modulation of growth factors and their receptors, and metabolic changes such as alterations in fatty acids, eicosanoids, and other cytokines.

We also found that the peroxisome proliferators decreased eicosanoid concentration in the rat liver and cultured hepatocytes (Hong and Glauert, 1995; Glauert *et al.*, 1996). Furthermore, it was also found that the peroxisome proliferator ciprofibrate and eicosanoids such as PGE_2, $PGF_{2\alpha}$ and LTC_4 are comitogenic (Hong and Glauert, 1996), and this combination treatment was also found to costimulate PKC activity, mitogen activated pwtein (MAP) kinase activity, AP-1 DNA binding activity, and increase intracellular calcium concentration more than additively (unpublished data). It is noteworthy that peroxisome proliferators activate kupffer cells which release mitogenic factors such as eicosanoids and other cytokines, and could be involved in cell proliferation of nearby hepatocytes (Borgies and Thurman, 1996). These results demonstrate that modulation of eicosanoid concentrations may play an important

roles in peroxisome proliferator-induced mitogenic signals.

None of these proposed mechanisms alone account for the mechanism by which peroxisome proliferators induce hepatocarcinogenesis. More than one mechanism is likely involved in the process. However, cell proliferation may be more involved not only in the promotion/progression but in the initiation of carcinogenesis by peroxisome proliferators.

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Peroxisome proliferators and Hepatocarcinogenesis

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적 요

Peroxisome은 간 및 신장에 존재하는 **organelle**로써 여러가지 효소들이 존재하고 있는데 그중에서 **peroxisomal β-oxidation**에 관여하는 효소들이 있어 **extramitochondria lipid metabolism**에 중요한 역활을 하는것으로 알려지고 있다. **Peroxisome proliperator**는 **peroxisome**의 크기와 수를 증가 시키는**chemicals** 또는 **physiological factors**들을 말하며 이에는 **clofibrate**와 같은 **hypolipidermic drug** 뿐만 아니라 **pesticides,** 그리고 **di-(2-etheylhexyl)phthalate**와 같은 **plasticizer** 또는 **dehydroepiandrosterone** 같은 **hormone**과 **high fat diet**같은 **physiological factor**도 이에 속한다. **Peroxisome proliferator**는 **peroxisome proliferator activated receptor**을 통하여 **biological**한 **action**을 하는데 실험동물에서 **peroxisome proliferation, liver hyperplasia** 및 **hepatocellular carcinoma**를 일으킨다. 이들의 발암과정에 대한 구체적인 메카니즘은 밝혀지지 않았으나 **peroxisomal β-oxidation**의 부산물로 생성되는 H_2O_2에 의한 생체의 **macromolecules (DNA or lipid)**의 산화적손상이 가설로 제기되고 있으며, **cell proliperation** 증가에 의한 **promotion activity** 증가가 다른 한 가설로 제기 되고있다. 한편 **peroxisomal β-oxidation**에 의한 **biological active lipid metabolite** (특히 **eicosanoids**)의 농도변화가 **peroxisome proliferator**에 의한 **mitogenic signaling pathway**에 변화를 주어 **multihepatocarcinogenesis**에 영향을 줄 것이란 의견도 고려되고 있다.