PPARs and Liver Disease
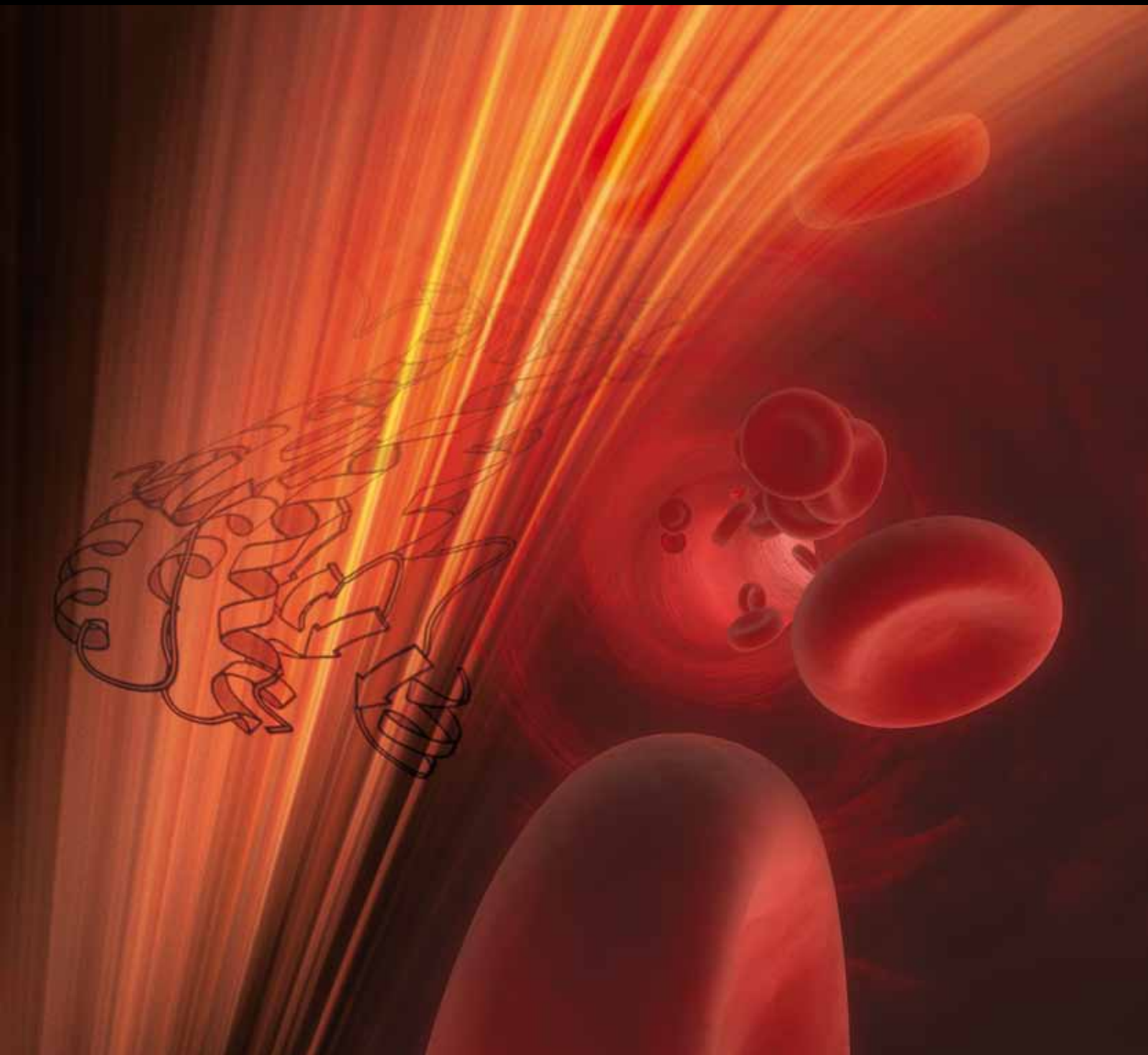
Guest Editors: Yasuteru Kondo, Kenji Uno, Keigo Machida, and Masanori Terajima

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Editorial Board

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Editorial
PPARs and Liver Disease

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This special issue of PPAR Research contains four interesting reviews and a research article examining the relevance of PPARs to liver diseases. Peroxisome proliferation-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily and have been implicated in a variety of pathologic processes. PPARs require heterodimerization with retinoid X receptors (RXRs) to function. PPARs $\alpha/\beta/\delta/\gamma$, with RXR, are important nuclear receptors expressed in the liver and contribute to the control of glucose and lipid metabolism, cell proliferation and inflammation, and so forth. PPARs were considered target molecules of human metabolic disease such as nonalcoholic fatty liver diseases (NAFLDs) including nonalcoholic steatohepatitis (NASH), a condition that might progress to cirrhosis. In this special issue, two review articles mention the relationship between PPARs and NAFLD. In regard to inflammation, a review article summarizes the antioxidant stress and anti-inflammation of PPARα. On the other hand, a research article mentions that PPARγ exacerbated concanavalin A (Con A)-induced liver injury. In our review article, we summarize the relevance of PPARs and hepatocellular carcinoma (HCC) including cancer stem cells. These five articles have interesting and valuable points of views regarding PPARs and liver diseases.

In the review article "*Antioxidant stress and anti-inflammation of PPARα on warm hepatic ischemic-reperfusion injury*" by Z. Gao and Y. H. Li, the authors focus on hepatic ischemic-reperfusion injury, since PPARα could have a role in organ protection in addition to regulating lipid and lipoprotein metabolism. They concluded that oxidant stress and inflammation are the most critical mechanisms in organ pathophysiology after warm hepatic ischemia reperfusion. The most significant mechanisms of PPARα hepatoprotective abilities have been demonstrated through antioxidant stress and anti-inflammation functions. Moreover, they mention that PPARα agonists such as N-3 polyunsaturated fatty acids, eicosapentaenoic acid, and docosahexaenoic acid could decrease the expression of proinflammatory genes by preventing IκB phosphorylation and NF-κB translocation into the nucleus. On the other hand, Y. Ogawa et al. published the research article "*Peroxisome proliferation-activated receptor gamma exacerbates concanavalin A-induced liver injury via suppressing the translocation of NF-κB into the nucleus.*" Using a mice model, this article surprisingly shows that the administration of PPARγ ligands exacerbated Con A-induced liver injury. They concluded that PPARγ suppressed the translocation of NF-κB into the nucleus, thereby inhibiting the suppression of liver cell apoptosis. In clinical settings, liver damage would occur with inflammation and apoptosis. Therefore, we need to consider the opposite effects of PPARs on liver injury.

NAFLD, a major cause of progressive liver disease, is increasing worldwide at an alarming rate. Defined by an increased hepatic lipid content, NAFLD varies widely from simple steatosis to NASH and has a strong genetic component. PPARs, including PPARα, PPARγ, and PPARδ, play an important role in hepatic lipid metabolism and also have

several genetic variants (polymorphisms). In the review article "*Peroxisome proliferator-activated receptor genetic polymorphisms and nonalcoholic fatty liver disease: any role in disease susceptibility?*" by P. Dongiovanni et al., the authors conducted a meta-analysis of previously reported evidence based upon which they describe the possible association between PPARs genetic polymorphisms and the susceptibility to NAFLD and NASH in specific subgroups. This review may contribute to new insight into the management of a therapeutic strategy for NAFLD, targeting PPARs. In the review article "*Misregulation of PPAR functioning and Its pathogenic consequences associated with nonalcoholic fatty liver disease in human obesity*" by L. A. Videla and P. Pettinelli, the authors mention that NASH is involved in the misregulation of PPARs signaling, accompanied by PPAR-γ and SREBP-1c-mediated metabolic disturbances (obesity-induced oxidative stress and related long-chain polyunsaturated fatty acid n-3 (LCPUFA n-3) depletion, insulin resistance, hypoadiponectinemia, and ER stress, due to lipogenesis and fatty acid oxidation. Targeting PPAR-α is problematic since fibrates have poor effectiveness, thiazolidinediones have weight gain limitations, and dual PPAR-α/γ agonists have safety concerns. The authors describe that supplementation of LCPUFA n-3 is a novel therapeutic modality since it reduces liver steatosis scores and inflammatory response, since the LCPUFA n-3 depletion reduces PPAR-α, leading to enhanced DNA binding of proinflammatory factors (NF-κB and AP-1) and the progression of steatosis to steatohepatitis.

In our review article "*PPAR could contribute to the pathogenesis of hepatocellular carcinoma*," we summarize the relevance of PPARs to the pathogenesis of HCC and cancer stem cells and possible therapeutic options through modifying PPAR signaling, since PPARs could contribute to the mechanisms of cell cycling, anti-inflammatory responses, and apoptosis. Abnormal stimulation of PPARα generates HCC through fatty liver. In HCCs, it is not clear whether PPARγ promotes cancer or can control it. PPARs might be useful target cancer stem cells in inducing the differentiation of HCC, because the expression of PPARs has been implicated in the regulation of the cell cycling of hepatocytes.

In conclusion, we hope that you will find these recent advances in elucidating the roles of PPARs in the various kinds of liver diseases. We expect that the reviews presented in this special issue, on the interplay between PPARs and liver disease, will be highly useful for those with interest in this field.

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Review Article

Peroxisome Proliferator-Activated Receptor Genetic Polymorphisms and Nonalcoholic Fatty Liver Disease: Any Role in Disease Susceptibility?

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Nonalcoholic fatty liver disease (NAFLD) defines a wide spectrum of liver diseases that extend from simple steatosis, that is, increased hepatic lipid content, to nonalcoholic steatohepatitis (NASH), a condition that may progress to cirrhosis with its associated complications. Nuclear hormone receptors act as intracellular lipid sensors that coordinate genetic networks regulating lipid metabolism and energy utilization. This family of transcription factors, in particular peroxisome proliferator-activated receptors (PPARs), represents attractive drug targets for the management of NAFLD and NASH, as well as related conditions such as type 2 diabetes and the metabolic syndrome. The impact on the regulation of lipid metabolism observed for PPARs has led to the hypothesis that genetic variants within the human PPARs genes may be associated with human disease such as NAFLD, the metabolic syndrome, and/or coronary heart disease. Here we review the available evidence on the association between PPARs genetic polymorphism and the susceptibility to NAFLD and NASH, and we provide a meta-analysis of the available evidence. The impact of PPAR variants on the susceptibility to NASH in specific subgroup of patients, and in particular on the response to therapies, especially those targeting PPARs, represents promising new areas of investigation.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), a major cause of progressive liver disease, is defined by an increase in hepatic fat content not related to toxics and has a strong genetic component. As the peroxisome proliferator-activated receptors (PPARs) represent major regulators of lipid metabolism in the liver, a few studies have tested the hypothesis that genetic variants in these hormone receptors may influence the susceptibility to NAFLD, but with controversial results. In this paper, we provide an overview of the published evidence in the field, and a meta-analysis of the available results on the role of the Pro12Ala PPARγ single nucleotide polymorphism (SNP), the most studied genetic variant to date. As PPARs are also the target of several drugs under evaluation for the treatment of NAFLD, this evidence may lay the basis to design pharmacogenetic studies to assess the role of PPARs SNPs

in predicting the response to drugs targeting these nuclear receptors.

2. Nonalcoholic Fatty Liver Disease (NAFLD)

Liver fat deposition related to systemic insulin resistance (IR) defines NAFLD [1]. The acronym NAFLD defines a wide spectrum of liver disease ranging from simple uncomplicated hepatic fat accumulation in the form of triglycerides exceeding 5% of liver mass in the absence of significant alcohol consumption to severe steatohepatitis characterized by severe steatosis, lobular inflammation, and hepatocellular damage and apoptosis with the activation of fibrogenesis [2], which can progress to cirrhosis and hepatocellular carcinoma [3]. Due to the epidemic of obesity and the metabolic syndrome, NAFLD is now the most frequent liver disease (prevalence

20–34%) and the leading cause of altered liver enzymes in Western countries [4, 5]. Hepatic fat accumulation results from an unbalance between triglycerides acquisition and removal [6] and initially represents a protective mechanism to shield hepatocytes from the toxicity resulting from an increased flux of free fatty acids (FFAs) to the liver [7]. Several lines of evidence support the hypothesis that most of the FFAs accumulated as triglycerides during steatosis derive from increased peripheral lipolysis [8] related to adipose tissue IR [9], followed by increased lipogenesis induced by hyperinsulinemia and diet. Indeed, the major risk factor for NAFLD is represented by systemic IR related to central obesity and the metabolic syndrome [1, 10]. Steatosis *per se* may then precipitate hepatic IR contributing to metabolic disturbances and cardiovascular damage [11, 12]. Impaired ability to secrete lipoproteins [13] and decreased β-oxidation due to mitochondrial damage (in particular in the presence of NASH) may also play a role in hepatic fat accumulation.

Epidemiological, familial, and twin studies have recently provided clear evidence for an element of heritability of NAFLD [14–16]. During the last years, genetic modifiers of disease severity and progression have been identified through genome-wide association studies [17, 18]. These include the Patatin-like phospholipase domain-containing 3 (PNPLA3) I148M gene variant, which has been demonstrated to represent a major determinant of interindividual and ethnicity-related differences in hepatic fat content independent of IR and serum lipid concentration, and a determinant of the progression towards NASH and fibrosis [19, 20]. Furthermore, a few large multicenter case-control studies demonstrated a role of SNPs implicated in insulin signalling [21], oxidative stress [22], and fibrogenesis [23] in the progression of NAFLD towards NASH confirming that hepatocellular fat accumulation and IR are key operative mechanisms in the pathophysiology of NAFLD and are closely involved in the progression of liver damage. New genetic risk factors could prove useful for the clinical management of patients with NAFLD and for the identification of novel therapeutic targets for NASH, for which specific treatments are still lacking.

3. Peroxisome Proliferator-Activated Receptors: The PPARs

The PPARs represent novel targets for the development of therapeutic agents for the treatment of metabolic syndrome, obesity, dyslipidemia and type 2 diabetes. Nuclear receptors are transcription factors that serve as intracellular receptors for endocrine hormones and dietary lipids. Differently from extracellular receptors which bind to peptide ligands and activate cytoplasmic kinase cascades, nuclear receptors interact directly with lipophilic ligands and regulate the expression of target genes [26]. These receptors can be considered the body's lipid sensor that can monitor the concentration of bioactive lipids and coordinate the enzymatic cascades that regulate lipid synthesis and utilization. There are three members of the PPAR family each encoded by a different gene: PPARα (NR1C1), PPARγ (NR1C3), and PPARδ (NP1C2).

All three PPARs bind to DNA as heterodimers with the retinoid X receptor (RXR).

4. PPARα

PPARα directly regulates a network of genes encoding protein involved in fatty acids uptake, enzymes required for the oxidation of fatty acids (β-oxidation), and enzymes required for ketogenesis by binding to control regions in the promoter of these genes and by promoting fat utilization [27]. The net effect is increased fatty acids oxidation, decreased serum triglycerides, and an increase in cholesterol efflux. PPARα is predominantly expressed in tissues capable of oxidizing fatty acids such as liver, heart, muscle, brown adipose tissue, and the kidney. PPARα can be activated by natural lipophilic ligands such as fatty acids and by drugs approved for the treatment of hypertriglyceridemia, such as fibrates [28]. The role of PPARα in the pathogenesis of fatty liver became evident in PPARα KO mice. These mice are unable to upregulate fatty acid catabolism and develop steatosis, myocardial lipid accumulation, and hypoglycaemia during short-term starvation or after high-fat diet [29, 30]. Taken together, mouse models suggest that PPARα functions to increase fatty acid use in the fasting state, and that in the context of a high-fat diet PPARα, inducing fatty acid catabolism, might prevent hepatocellular fat accumulation and hypertriglyceridemia. PPARα downregulation is involved in NASH pathogenesis by reducing FFA catabolism [31].

5. PPARα Polymorphisms and NAFLD

The role of PPARα gene polymorphisms in NAFLD and the regulation of lipid metabolism has been investigated in a few studies. Chen et al. hypothesized that the coding Val227Ala SNP in the PPARα gene may be implicated in the pathogenesis of NAFLD. In 79 NAFLD patients and 63 healthy controls, it was found that the PPARα Val227Ala genotype frequency was significantly different between NAFLD and control subjects and that the fat-related index such as weight, body mass index (BMI), hip circumference, waist circumference, waist-to-hip ratio, and the percentage of body fat of the carriers of the Ala227 allele was lower than that in noncarriers [24]. Yamakawa-Kobayashi et al. evaluated the Val227Ala SNP in 401 healthy Japanese subjects. Total cholesterol was lower in Ala227 carriers than in noncarriers, and the lipid profiles of Ala227 carriers appeared favourable compared with those of non-carriers. Since the Val227Ala variant is located in the region between the DNA-binding and ligand-binding domains, which is also thought to contain the dimerisation domain of the protein, it has been hypothesized that the substitution of Valine to Alanine at codon 227 causes a functional change in PPARα, and that the Ala227 isoform has higher activity than the Val227 isoform [32], thus leading to enhanced ability to burn fatty acids, and potentially explaining the association with lower lipid levels and the protection from steatosis development. However, Sparsø et al. genotyped the Leu162Val SNP in the PPARα gene in 5799 middle-aged white people, but they did not find any association

TABLE 1: Characteristics of the studies on the association between the PPARα polymorphisms and nonalcoholic fatty liver disease.

First author, year	Ref.	PPARα variant	Population ethnicity, country	Sample size (N)	Patients characteristics	Liver biopsy (N)	Female sex, N (%)	Conclusions
Chen, 2008	[24]	Val227Ala	China	N = 79	Unspecified NAFLD	N = not specified	40 (51)	Association with NAFLD
Dongiovanni, 2010	[25]	Leu162Val	Caucasian, Italy	N = 202	Histological NAFLD	N = 202	41 (20)	No association with NAFLD

Ref: reference number; N: number; NAFLD: nonalcoholic fatty liver disease.

with obesity or type 2 diabetes. Though, another PPARα coding polymorphism, the Leu162Val variant, was possibly associated with increased fasting cholesterol and triglyceride concentrations [33]. The relationship between the Leu162Val SNP and NAFLD was further evaluated in 202 Italian subjects compared to 346 healthy controls. The frequency of this SNP did not differ between patients and controls, but the presence of the PPARα 162Val allele was associated with higher IR, but not histologically assessed disease severity [25], suggesting that the risk related to increased IR may be balanced by the protective effect of decreased oxidative stress, the other key player in the progression of liver disease in patients with NASH. Results of the published association studies between PPARα polymorphisms and NAFLD are summarized in Table 1.

6. PPARγ

PPARγ is the master regulator of adipogenesis and plays an important role in the process of lipid storage [34]. PPARα and PPARγ have therefore opposing functions in the regulation of fat metabolism; PPARα promotes fat utilization while PPARγ promotes fat storage. PPARγ is expressed in adipocytes, macrophages, and muscle, where it regulates development, lipid homeostasis, and glucose metabolism. Mice lacking PPARγ are embryonically lethal, but the development of conditional PPARγ knockouts has confirmed the essential role of PPARγ in adipocytes differentiation and survival [35]. Moreover, specific deletion of PPARγ in fat and muscle causes IR underlying its importance in peripheral insulin sensitivity [36]. Possible mechanisms underlying the insulin sensitizing activity of PPARγ include increased lipid uptake and storage leading to decreased free fatty acids and serum triglycerides, induction of the expression of adiponectin, a molecule with anti-inflammatory and insulin-sensitizing effect by adipocytes [37], and the suppression of hepatic gluconeogenesis and increased glucose uptake by adipose tissue through GLUT4 upregulation [38]. Fatty acids and prostanoids act as PPARγ agonists. However, also the thiazolidinediones (TZDs), a class of insulin sensitizers that are approved for the treatment of type 2 diabetes and have been shown to decrease steatosis in patients with NASH [39, 40], function as high affinity PPARγ agonists. The activation of PPARγ by TZDs induces the expression of a set of genes involved in adipocytes differentiation and lipogenesis and induces adiponectin, thus explaining the insulin-sensitizing action of these drugs [41, 42].

7. Role of PPARγ Polymorphisms: Rationale and Available Studies

Human genetics has provided evidence of a role of PPARγ in the metabolic syndrome [43]. Dominant negative mutations in PPARγ are the cause of a monogenic disease characterized by severe insulin resistance, type 2 diabetes, and hypertension [44]. Importantly, a frequent coding SNP in the PPARγ gene, the Pro12Ala variant, has consistently been associated in metabolic studies with BMI, insulin sensitivity, the metabolic syndrome [45]. The N-terminal proline to alanine exchange (Pro12Ala) occurs in the extra domain of the PPARγ2 transcript: this PPARγ splice isoform includes 30 additional amino acids [46], which are responsible for an increase of PPARγ transcriptional activity in the adipose tissue. The Pro12Ala exchange results from a cytosine to guanine substitution in the PPARγ gene, encoding the Ala allele form with a strongly reduced function [47]. The association between the positivity for the 12Ala variant and IR, type 2 diabetes, higher BMI, and obesity has already been well described and confirmed in several studies [48–50]. This association may be explained by the lower activity of the 12Ala variant in the adipose tissue, favouring IR and potentially the flux of FFAs to the liver and NAFLD. However, the role of this SNP in the pathogenesis and progression of fatty liver disease is still debated.

Rey et al. analyzed the presence of the Pro12Ala polymorphism in 622 German Caucasian subjects suffering from fatty liver (263 NAFLD patients and 100 with alcoholic fatty liver disease (AFLD) subjects) or being healthy blood donors (n = 259). In fatty liver disease patients the Ala allele was more represented than in controls. In NAFLD patients the higher prevalence of the 12Ala allele was not associated with the progression of liver disease, whereas AFLD patients carrying the 12Ala allele had a higher risk of severe steatohepatitis and fibrosis [53]. Similarly, the 12Ala allele was not associated with NAFLD susceptibility, liver damage, or IR in 212 Italian patients with NAFLD [25]. Gupta et al. analyzed the genotype frequencies of the Pro12Ala variant in 98 NAFLD patients and 280 matched controls and found a higher prevalence of heterozygosity for the Ala variant in patients. Moreover, in NAFLD patients the 12Ala variant was also associated with overweight (BMI > 25 Kg/m^2), suggesting an important role of Pro12Ala variant in the obesity-related NAFLD disease pathogenesis [52]. Gawrieh et al. investigated the association between two PPARγ variants (the Pro12Ala and a second common SNP, the C1431T) with NAFLD and its histological features. They considered 212 patients with NAFLD and 63

TABLE 2: Characteristics of the studies on the association between the Pro12Ala variant of PPARγ and nonalcoholic fatty liver disease.

First author, year	Ref.	Ethnicity, country	Study design, sample size (N)	Patients characteristics	Liver biopsy (N)	Female sex, N (%)
Dongiovanni, 2010	[25]	Caucasian, Italy	Case-control $N = 202$	Histologically proven NAFLD	$N = 202$	41 (20)
Gawrieh, 2011	[51]	Caucasian, USA	Case-control $N = 212$	Histologically proven NAFLD	$N = 212$	145 (68)
Gupta, 2011	[52]	Asian, India	Case-control $N = 98$	Diagnosis based on ultrasound	$N = 71$	32 (33)
Rey, 2010	[53]	Caucasian, Germany	Case-control $N = 263$	Histologically proven NAFLD	$N = 263$	Not specified
Yang, 2012	[54]	Asian, China	Case-control $N = 436$	Diagnosis based on ultrasound	—	280 (64)

Ref: reference number; N: number; NAFLD: nonalcoholic fatty liver disease.

controls and found that individual SNPs did not show significant association with NAFLD. The haplotype defined by the presence of both minor alleles (GT) was less enriched, whereas an haplotype, comprised of the two major alleles (CC), was more enriched in subjects with NAFLD compared to controls, and both haplotypes were significantly associated with steatosis and fibrosis [51]. As the carriers of the 12Ala variant have been reported to have increased resistance to oxidative stress [55], and since smoking increases the production of reactive oxygen species, Yang et al. explored the influence of the Pro12Ala SNP on the risk of NAFLD and determined whether this polymorphism and smoking showed a synergistic effect on the development of NAFLD in middle-aged and older Chinese people (considering 436 NAFLD patients and 467 controls). The 12 Pro/Pro genotype and smoking were significant independent risk factor for NAFLD. In addition, the higher risk group (smokers with the 12 Pro/Pro genotype) showed 3.75 times higher risk of NAFLD than the low-risk group (nonsmokers with the 12 Pro/Ala genotype). However, no relationship between the PPARγ gene and grading for steatohepatitis was observed. They hypothesized a possible synergistic effects of genotype and smoking in the development of NAFLD by aggravating oxidative stress [54]. Zhou et al. investigated the association of seven candidate SNPs with susceptibility to NAFLD in 117 Chinese patients and matched controls and found that the genotypic distributions and allelic frequencies of the PPARγ gene −161 C/T polymorphism in the NAFLD group were significantly different from those in the control group suggesting that the C/T variant increased the susceptibility to NAFLD [56]. Finally, very recently Bhatt et al. have investigated the associations of polymorphisms C161T and Pro12Ala of PPARγ with clinical and biochemical parameters in 162 Asian patients with ultrasonographically diagnosed NAFLD and 173 controls. They found that the Pro12Ala polymorphism was associated with significantly higher serum TG, alkaline phosphatase, and waist-hip ratio, whereas the C161T polymorphism with increased TG and total cholesterol. At multivariate analysis, NAFLD was associated with these two polymorphisms [57].

8. A Meta-Analysis of Available Studies on the Association between PPAR Pro12Ala Variant and NAFLD

In view of the still controversial evidence concerning the association between PPARγ genotype and NAFLD susceptibility mentioned above, we decided to estimate from the available literature the strength of the effect of Pro12Ala variant of PPARγ gene on NAFLD across different populations. In contrast, due to the heterogeneity of genetic markers evaluated in previous studies, it was not possible to conduct a meta-analysis of PPARα studies. For the electronic searches, published studies were found through PubMed at the National Library of Medicine (http://ncbi.nlm.nih.gov/entrez/query/) for the query NAFLD, PPARγ polymorphism, and Pro12Ala variant (rs1801282). References list in relevant publications was also considered. The literature search was done on studies up to 2012, written in English and for which were available abstracts and complete article. For the meta-analysis we considered five papers, which are presented in Table 2. There were not country restrictions. The presence of NAFLD was diagnosed by biopsy or ultrasound. All the studies were population-based case-control studies. Complete or partial information about liver biopsy was available in four studies, and data about fatty liver was analyzed in 1238 subjects with NAFLD. Genotyping for rs18012282 was carried out using TaqMan allelic discrimination in three studies [52–54] and by polymerase chain reaction (PCR) and restriction analysis in two studies [25, 52]. The calculations were performed using the free meta-analysis REV Manager 5.0 Software Informer (http://ims.cochrane.org/revman/). Results of the meta-analysis are presented in Figure 1. This meta-analysis, by summarizing the amount of evidence, failed to detect a significant association between the Pro12Ala SNP in the PPARγ gene and NAFLD, highlighting at the same time a significant heterogeneity among the published studies.

In line with this result, no genome-wide association studies found an association between genomic variants in PPARs genes and NAFLD. Moreover, the majority of studies indicate that the Pro12Ala variant is especially involved in
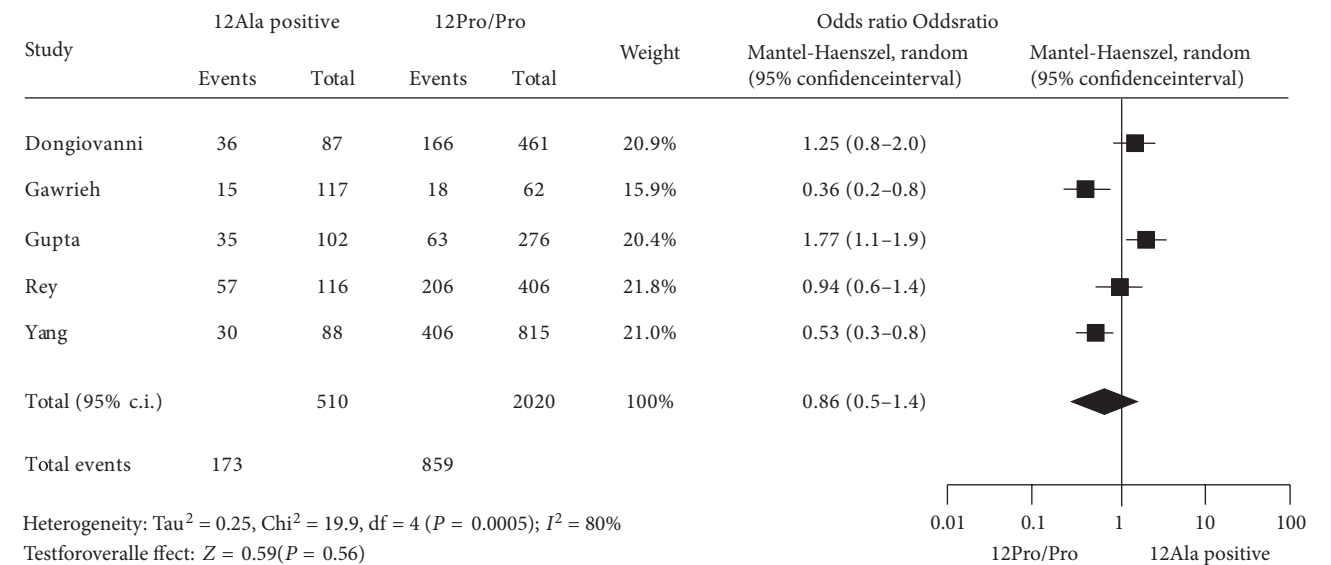
Study	12Ala positive		12Pro/Pro		Weight	Odds ratio Oddsratio Mantel-Haenszel, random (95% confidenceinterval)	Mantel-Haenszel, random (95% confidenceinterval)
	Events	Total	Events	Total			
Dongiovanni	36	87	166	461	20.9%	1.25 (0.8–2.0)	
Gawrieh	15	117	18	62	15.9%	0.36 (0.2–0.8)	
Gupta	35	102	63	276	20.4%	1.77 (1.1–1.9)	
Rey	57	116	206	406	21.8%	0.94 (0.6–1.4)	
Yang	30	88	406	815	21.0%	0.53 (0.3–0.8)	
Total (95% c.i.)		510		2020	100%	0.86 (0.5–1.4)	
Total events	173		859				

Heterogeneity: Tau^2 = 0.25, Chi^2 = 19.9, df = 4 (P = 0.0005); I^2 = 80%
Testforoveralle ffect: Z = 0.59(P = 0.56)

FIGURE 1: Meta-analysis of the effect of the Pro12Ala PPARγ variant on the risk of NAFLD in published studies. The odds ratio (ORs) and the corresponding 95% confidence interval (c.i.) limits (lower and upper) are calculated by random effects meta-analysis (Mantel-Haenszel; M-H) for nonalcoholic fatty liver disease (NAFLD) according to the Pro12Ala variant (12Ala positive versus 12Pro/Pro genotype). In the "Study" is cited the first author of the study. "Events" indicate the number of patients with NAFLD who carry a genotype (e.g., 36 positive for 12Ala allele) while "Total" indicates the sum of patients and controls with the same genotype (e.g., 87 is the sum of NAFLD patients and controls who carry the 12 allele). In the graph, numbers indicate OR and filled diamond express random effect. The symbol size is proportional to the weight of the study.

the development of type 2 diabetes. However, it is possible that the Pro12Ala polymorphism in the PPARγ gene may contribute to the pathogenesis of NAFLD in the presence of other genetic variants or in the presence of environmental risk factors, such as obesity. Therefore, future studies should be conducted in larger series of well-characterized patients with a homogenous clinical subphenotype of NAFLD (e.g., in severely obese patients) and should be controlled for other major risk factors for NAFLD, such as the I148 M PNPLA3 variant. In conclusion, the Pro12Ala variant cannot be considered a clinically relevant marker for NAFLD, at least when evaluated alone in the overall population. Despite this, as we are moving towards individualized medicine, these data could provide the basis to design pharmacogenetic studies to address whether therapeutic efficacy of PPARγ agonists in NAFLD patients is affected by the 12Ala SNP alone or in combination with other SNPs of genes involved in lipid metabolism, and whether PPAR SNPs may modify NAFLD risk in specific populations.

9. PPARδ

PPARδ is ubiquitously expressed, most highly in brain, macrophages, lung, adipose tissue, and skeletal muscle [26, 58] and is activated by fatty acids and components of very-low-density lipoprotein (VLDL) [28, 59]. PPARδ activation enhances fatty acids transport and oxidation, improves glucose homeostasis via the inhibition of hepatic glucose output, reduces macrophages inflammatory responses, and increases HDL levels [60]. PPARδ knockout mice die in midgestation.

Surviving mice show markedly decreased adipose tissue suggesting a requirement for PPARδ in peripheral tissues [61]. Further support for a role of PPARδ in lipoprotein metabolism results from studied exploring the activity of the PPARδ specific synthetic agonist GW501516. Treatment of animals including primates with GW501516 significantly increases HDL, lowers triglycerides, and LDL and decreases fasting insulin levels [62, 63]. Synthetic PPARδ agonists have proven to be effective also in preclinical model of diabetes and dyslipidemia, and preliminary results are also available for steatosis. Results of a two-week phase II study in patients with dyslipidemia demonstrated that total cholesterol, LDL cholesterol, triglycerides, and nonesterified fatty acids were significantly lowered by GW501516 [64]. The impact on the regulation of lipid and carbohydrate metabolism observed for PPARδ has led to the hypothesis that genetic variation within the human PPARδ gene may be associated with human disease such as the metabolic syndrome and/or coronary heart disease. The +294 T/C polymorphism in exon 4 of the PPARδ gene seems to influence binding of Sp-1 resulting in higher transcriptional activity for the rare C allele than the common T allele [65]. Skogsberg et al. observed in 543 healthy, middle-aged men that the C genotype was associated with elevated levels of LDL cholesterol and ApoB [66]. In 580 male subjects with hyperlipidemia recruited from the West of Scotland Coronary Prevention Study (WOSCOPS) carriers of the C allele had significantly lower HDL plasma concentrations [67], whereas Aberle et al. found a highly significant association between the rare C allele and lower plasma HDL concentrations in 967 females with mixed hyperlipidemia [68]. Robitaille et al. identified 15 variants

in the PPARδ gene and found that another polymorphism (-87 T $>$ C) was associated with a lower risk to exhibit the metabolic syndrome and that this association was influenced by dietary fat intake [69]. Andrulionyte et al. found that SNPs of the PPARδ gene may modify the conversion from IGT to type 2 diabetes particularly in combination with Gly482Ser SNP of the PPARγ coactivator-1A (PGC-1A) and Pro12Ala SNP of PPARγ2 [70]. Grarup et al. investigated variation in PPARδ gene in 6071 Danish white subjects of whom 4543 had NGT, 503 had IFG, 693 had IGT, and 352 had diabetes. They concluded that common variation in PPARδ does not affect the risk of metabolic disease in the population studied [71]. However, no published study specifically addressed the role of PPARδ SNPs in the susceptibility to NAFLD.

10. Conclusions

Available studies do not provide sufficient evidence for a significant evidence for an association between PPARα and PPARγ SNPs, and the risk of NAFLD. In particular, our meta-analysis of the effect of the Pro12Ala PPARγ2 SNP, the best studied genetic factor to date, and NAFLD did not provide conclusive results. However, most of the studies were underpowered, the definition of the NAFLD phenotype was rather heterogeneous (histological versus ultrasonographic versus based on liver enzymes), the analyses were conducted in ethnically diverse population, and most studies were not controlled for other genetic risk factor for NAFLD such as the PNPLA3 I148 M SNP, so that the patients included were not phenotypically homogeneous. Furthermore, even scarcer data are available for the association of PPARs variant with the progression of liver damage, and variants of PPARδ, another nuclear receptor involved in IR and lipid metabolism, were not assessed. Most importantly, PPARs are promising targets for NASH, but no study has yet assessed the effect of genetic variants in PPARs genes and the effect of therapy.

The evaluation of the impact of PPAR variants on (1) the susceptibility to NASH in specific subgroup of patients such as severely obese subjects in adequately powered studies and (2) on the response to drugs targeting PPARs (such as glitazones or PPARα/δ agonists, which are under study in NASH patients), represent promising new areas of investigation.

Abbreviations

AFLD: Alcoholic fatty liver disease
BMI: Body mass index
FFAs: Free fatty acids
IR: Insulin resistance
NAFLD: Nonalcoholic fatty liver disease
NASH: Nonalcoholic steatohepatitis
PNPLA3:   Patatin-like phospholipase domain-containing 3
PPARs: Peroxisome proliferator-activated receptors
RXR: Retinoid X receptor
SNP: Single nucleotide polymorphism
VLDL: Very low-density lipoproteins.

Conflict of Interests

There is no conflict of interests to disclose.

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Review Article

PPAR Could Contribute to the Pathogenesis of Hepatocellular Carcinoma

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Viral hepatitis with hepatitis C virus or hepatitis B virus and chronic liver disease such as alcoholic or nonalcoholic steatohepatitis are critical factors in the development of hepatocellular carcinoma (HCC). Furthermore, diabetes is known as an independent risk factor for HCC. Peroxisome proliferator-activated receptor (PPAR) is known to have an important role in fatty liver, and the mechanism of carcinogenesis has been clarified. PPAR controls ligand-dependent transcription, and three subtypes (α, δ, and γ) in humans are known. PPARs could contribute to the mechanisms of cell cycling, anti-inflammatory responses, and apoptosis. Therefore, to clarify the pathogenesis of HCC, we should examine PPAR signaling. In this paper, we have summarized the relevance of PPARs to the pathogenesis of HCC and cancer stem cells and possible therapeutic options through modifying PPAR signaling.

1. Introduction

Worldwide, the mortality from hepatocellular carcinoma represents one-third of all cancer deaths with more than 1 million a year [1]. In the early stages of hepatocellular carcinoma, when patients maintain a hepatic functional reserve, local treatment such as hepatic resection or radio-frequency ablation is relatively effective [2]. However, many patients die from repeated recurrence. Hepatic arterial infusion chemotherapy (HAIC) [3, 4] for advanced hepatocellular carcinoma is only sometimes effective. Also, sorafenib (VEGF-2/PDGFR-beta inhibitor) [5], which has come in to use recently, has not shown satisfactory results [6, 7]. Therefore, finding a new therapeutic target molecule has become very important.

Viral hepatitis with HCV or HBV and chronic liver disease such as alcohol or nonalcoholic steatohepatitis (NASH) [8] are critical factors in the development of hepatocellular carcinoma. Furthermore, diabetes is known as an independent risk factor for hepatocellular carcinoma [9, 10]. In addition, the hepatitis C is known to become fatty liver at a high rate [11, 12]. Peroxisome proliferator-activated receptor (PPAR) [13, 14] plays an important role in fatty liver, and its involvement in carcinogenesis has been clarified. PPAR controls ligand-dependent transcription, and three subtypes (α, δ, and γ) in humans are known. PPARα [14] is present in liver, kidney, heart, and small intestine and has an important role in the regulation of lipid metabolism [15]. PPARγ is expressed in adipose tissue and macrophages. It is involved in adipose cell differentiation and lipid uptake and has anti-inflammatory effects. In addition, PPARγ expression is induced in the liver in a hypernutrition state such as fatty liver. PPARδ is expressed universally. It is involved in fatty acid metabolism and the induction of energy in skeletal muscle and adipose tissue.

PPARs have roles regulating the cell cycle and metabolism and have been reported to be involved in carcinogenesis. As the organ that controls metabolism, the liver in particular shows the strong involvement of PPARs. It is not clear whether each subtype of PPARs works to promote or inhibit cancer. In this paper, we describe the associations between PPAR and HCC.

2. Relevance of PPARα and the Pathogenesis of HCC

PPARα expression has a major impact on the maintenance of mitochondrial beta-oxidation [15]. The ligand in the natural

product of PPARα assumes the form of a fatty acid, and fenofibrates that reduce triglycerides act as a PPARα agonist [16]. It has been controversial whether it promotes or suppresses cancer growth. Several reports have described that it has an inhibitory effect on cancer [17–20]. PPARα agonist suppressed the inhibition of angiogenesis via excess production of thrombospondin (TSP)-1. In addition, PPARα acts as a master regulator of inflammation, showing an anti-inflammatory action in suppressing interleukin-1β, TNF-α, and ICAM-1 [21]. The antiangiogentic and anti-inflammatory effects promote the suppression of tumor growth by improving microenvironment.

On the other hand, hepatocellular carcinoma or hepatomegaly has been known to occur when PPARα agonists were administered for a long time to mice or rats [22]. Peters et al. [23] employed fenofibrates in PPARα knockout mice and wild-type mice to investigate the cell cycle regulatory proteins. The expression levels of cell cycle regulatory proteins did not change significantly between the knockout mice and wild-type mice in the steady state. On the other hand, the expressions of cyclin D1, cyclin E, cyclin-dependent kinase 2, CDK4, and proliferating cell nuclear antigen increased remarkably in wild-type mice that were administered fibrates. However, their expression levels did not change in the knockout mice. In this study, it was revealed that PPARα was involved in the regulation of the cell cycle. In addition, HCV core transgenic mice showed a high rate of hepatocellular carcinoma from fatty liver and hepatomegaly [24]. In this model, by knocking down PPARα, the development of hepatocellular carcinoma was suppressed [25]. In brief, HCV core, which can lead to carcinoma, is abnormally sustained by PPARα activation. In this mouse model, the overexpression of several genes related to fat was observed. PPARα leads to carcinoma from fatty liver through these genes (fatty acid translocase (FAT) and fatty acid transport protein (FATP)). Appropriate stimulation of PPARα suppresses the cancer through the microcirculation. On the other hand, continuous abnormal stimulation promotes the cancer.

3. Relevance of PPARγ and the Pathogenesis of HCC

PPARγ expression is observed in adipose cells and macrophages. Furthermore, PPARγ is expressed in the liver in a hypernutrition state such as fatty liver [26]. The expression of PPARγ varies in hepatocellular carcinoma and is reported to be at the same level [27], a higher level [28], or a lower level [29] in comparison with normal liver. It has been reported that PPARγ inhibits hepatocellular carcinoma [28, 30, 31] and other carcinomas [32–35] in many *vitro* studies. These control epithelial-mesenchymal transition (EMT) and prevent the invasion and metastasis of carcinoma. The over-expression of PPARγ inhibits the metastasis of carcinoma by increasing E-cadherin through TIMP3 [36]. PPARγ has been also revealed to be involved in cell cycle arrest [36]. These mechanisms have been reported to act through p21 and p53 [37]. Additionally, the pathway of p27 has been reported to

be independent [29]. Furthermore, PPARγ induces apoptosis directly through Fas, resulting in an inhibitory effect on carcinoma [31].

4. PPARδ and HCC

PPARδ is expressed universally. PPARδ plays an important role in lipid and glucose metabolism and has been implicated in obesity-related metabolic disease. The involvement of PPARδ in colon cancer has been reported. It is reported that PPARδ in the cells indicates extreme malignancy in a colon cancer cell [38]. PPARδ is a gene derived from TCF/β-catenin pathway. However, there are few reports on the association between PPARδ and HCC. EpCAM is a useful cancer stem cell marker in HCC. Activation of the Wnt/β-catenin signaling in the nucleus causes it to migrate along with β-catenin, FHL2, and intracellular domain (EpICD) of EpCAM [39]. The association between PPARδ and HCC through Wnt/β-catenin signaling should be clarified in the future.

5. PPARs and Cancer Stem Cell

The cancer stem cell theory has been proposed in recent years to be applicable to many types of cancer [40–43]. In this theory, there are low-frequency cancer cells that have the potential for self-renewal, pluripotency, tumorigenicity, and asymmetric division like bone marrow stem cells and progenitor cells in normal tissue. The cancer stem cell theory itself was first suggested in the 1970s [44], and since then, it has been difficult to be confirmed experimentally. However, it was reported that there is a high tumorigenic fraction, CD34$^+$CD38$^-$, in human acute myeloid leukemia in 1997 [40]. The presence of cancer stem cells was subsequently reported in various types of cancer. Now, this concept has been established by many studies. PPAR is involved in the control of cancer because it acts in the control of the cell cycle. Therefore, it seems that some link exists between PPAR and cancer stem cells. However, there are still few reports concerning cancer stem cells [45]. Chearwae and Bright [46] reported that PPARγ agonists inhibited growth and expression of brain tumor stem cells by inhibition of EGF/bFGF signaling through Tyk2-Stat3 pathway and the expression of PPARγ. It is also notable that there is a relationship between cancer stem cells and EMT. Mani et al. [47] reported that EMT-generating cells had the properties of stem cells. If PPARγ is involved in EMT, it is possible that PPAR has a role in the mechanism of metastasis of the cancer stem cells. PPARγ agonists might regulate cancer stem cells. However, cancer stem cells have reduced the expression of PPARγ, allowing cells to escape from the control of the normal cell cycle [45].

6. PPARs and Treatment

PPARs may become the target of cancer treatment. Particularly, PPAR has an important role in carcinogenesis from fatty liver cells. It is expected that the thiazolidinediones

(TZDs), which act on PPARγ, can be used to treat hepatocellular carcinoma. TZDs are expected to inhibit the proliferation of hepatocellular carcinoma. Troglitazone was the first TZD to become clinically available. However, troglitazone produced liver damage at a high rate [48] and therefore can no longer be used. Today, we can only use pioglitazone in Japan. Pioglitazone is reported to have a certain therapeutic effect for NASH. Although it has been widely used in clinical practice, sufficient effectiveness has not been reported fore hepatocellular carcinoma. However, aggressive usage has become more difficult, and some reports suggested that pioglitazone caused weight gain and edema as a side effect, and also bladder cancer [49]. However, PPARγ activity exists in other drugs and natural products. For example, telmisartan [50], a kind of angiotensin II receptor blockers that has some PPARγ activity, and side effects such as weight gain do not occur. Chearwae and Bright [46] reported PPARγ agonist, and all-*trans* retinoic acid combination therapy had strong inhibitory effects on brain tumor stem cells. All-*trans* retinoic acid is used for acute promyelocytic leukemia (APL). Such use of retinoic acid is a differentiation induction therapy used only in a cancer. APL is caused by abnormal molecules called PML/RARα made by translocation t(15; 17).

With PPAR agonists alone, it is difficult to treat cancer. However, there is a possibility for its use in the treatment of HCC in the future, such as in combination with molecular target drugs like retinoic acid. Therefore, curative effects for HCC are expected in the future.

7. Conclusion

PPARs play an important role in the generation of fatty liver. *Abnormal* stimulation of PPARα generates HCC through fatty liver. Particularly, infection of HCV causes abnormal stimulation of PPARα. In HCC, it is not clear whether PPARγ promotes cancer or can control it. At present, PPARγ suppresses cancer *in vitro*. Some reports describe that PPARγ affects control of cancer stem cells. In cancer stem cell theory, it is thought that cancer stem cells participate in chemoresistance and recurrence. Accordingly, it is important to induce cancer stem cells to become noncancer stem cells (mature cancer cells). Nuclear receptor agonists like PPARs might be the key for differentiation therapy. PPARs might be useful to target cancer stem cells in inducing the differentiation of HCC, because the expression of PPARs has been implicated in the regulation of cell cycle of hepatocytes and adipocytes in the liver.

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Review Article

Misregulation of PPAR Functioning and Its Pathogenic Consequences Associated with Nonalcoholic Fatty Liver Disease in Human Obesity

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Nonalcoholic fatty liver disease in human obesity is characterized by the multifactorial nature of the underlying pathogenic mechanisms, which include misregulation of PPARs signaling. Liver PPAR-α downregulation with parallel PPAR-γ and SREBP-1c up-regulation may trigger major metabolic disturbances between *de novo* lipogenesis and fatty acid oxidation favouring the former, in association with the onset of steatosis in obesity-induced oxidative stress and related long-chain polyunsaturated fatty acid n-3 (LCPUFA n-3) depletion, insulin resistance, hypoadiponectinemia, and endoplasmic reticulum stress. Considering that antisteatotic strategies targeting PPAR-α revealed that fibrates have poor effectiveness, thiazolidinediones have weight gain limitations, and dual PPAR-α/γ agonists have safety concerns, supplementation with LCPUFA n-3 appears as a promising alternative, which achieves both significant reduction in liver steatosis scores and a positive anti-inflammatory outcome. This latter aspect is of importance as PPAR-α downregulation associated with LCPUFA n-3 depletion may play a role in increasing the DNA binding capacity of proinflammatory factors, NF-κB and AP-1, thus constituting one of the major mechanisms for the progression of steatosis to steatohepatitis.

1. Introduction

1.1. Epidemiologic Aspects. Nonalcoholic fatty liver disease (NAFLD) is considered the hepatic manifestation of metabolic syndrome (MetS) and has emerged as the most frequent cause of chronic liver disease worldwide, becoming the third most common indication for liver transplantation in order to rescue patients with end-stage liver disease [1, 2]. NAFLD encompasses a wide disease spectrum ranging from simple triacylglycerol (TAG) accumulation in hepatocytes (hepatic steatosis), which is defined by accumulation of liver fat (>5% per liver weight) in the presence of <20 g of daily alcohol consumption, to steatosis with inflammation (nonalcoholic steatohepatitis, NASH), fibrosis, and cirrhosis [2, 3]. Liver biopsy is the gold standard for diagnosis and has the additional benefit of distinguishing between NASH and simple steatosis, thus allowing for the staging of the degree of fibrosis [4]. NAFLD affects 17 to 33% in the general populations, whereas that of NASH affects 2% to 3% of the population [2, 5]. In obese subjects, NAFLD incidence reaches 60% to 90% and for NASH and hepatic cirrhosis 20% to 25% and 2% to 8%, respectively. In subjects with MetS, the prevalence of NAFLD is increased fourfold compared with those without the disease, and 30% of NAFLD subjects have MetS [6, 7]. In children population, an autopsy study found that 9.6% of the American population aged 2 to 19 years has NAFLD, and this figure increased to 38% among those who were obese [8].

Obesity is a state of chronic low-grade inflammation accompanied by excess fat storage deposited in tissues other than adipose tissue, including liver and skeletal muscle, which may lead to local insulin resistance (IR) and may

stimulate inflammation, as in NASH [9]. Therefore, obesity and IR, both key features of the MetS, are intimately linked and strongly associated with NAFLD progression [3, 10].

1.2. Etiopathology of NAFLD. The primary metabolic abnormalities that lead to hepatic steatosis involve a lipotoxic response with an oxidative-stress component, nutritional factors, and alterations in the lipid metabolism of the liver, which result from the development of IR [3]. Hepatic fat accumulation, secondary to IR, develops when there is an imbalance in which fatty acid uptake and *de novo* synthesis exceed oxidation and secretion [11]. In this respect, the sources that contribute to fatty liver are (i) delivery of dietary fat to the liver (contribution to liver fat ∼5%); (ii) delivery of extrahepatic nonesterified fatty acids (NEFAs) to the liver (contribution to liver fat ∼60%); (iii) the remainder of liver fat accumulation is related to hepatic *de novo* lipogenesis, which is increased in obese patients [12].

The retention of FAs and TAGs within the hepatocytes that depends on IR and hyperinsulinemia leads to the production of free radicals at a mitochondrial level, capable of inducing lipid peroxidation, cytokine production, and hepatocyte necrosis [13], which may trigger NAFLD progression to the more severe state of NASH [2, 3].

The regulation of hepatic lipogenesis and FA oxidation is under rigorous control that involves a complex network of nuclear receptors, which regulate the expression of enzymes that participate in the lipid metabolism in a coordinated manner [11].

1.3. PPARs. The ligand-activated transcription factors belonging to the peroxisome proliferators-activated receptors (PPARs) are a subfamily of the steroid/thyroid/retinoid receptors superfamily. PPARs act as fatty acid sensors to control many metabolic programs that are essential for systematic energy homeostasis, including adipocyte differentiation, inflammation and energy homeostasis, lipoprotein metabolism, and FA oxidation, representing an important target for NAFLD [9, 14, 15]. The PPAR family consists of three members, namely, PPARα (NR1C1 according to the unified nomenclature system for the nuclear receptor superfamily), PPARβ/δ (NR1C2), and PPARγ (NR1C3), with two forms, γ1 and γ2, with differing amino termini, each encoded by different genes [14]. Similar to most members of the superfamily, all PPAR isoforms have a highly conserved structure. They are composed of five different domains, (i) an aminoterminal A/B domain involved in ligand-independent transactivation, which in other cases can regulate DNA binding, (ii) a two zinc-finger DNA-binding domain (DBD) responsible for half-site specificity of target gene recognition, (iii) a hinge region, (iv) a carboxy-terminal ligand-binding domain (LBD) with 60∼70% homology between the subtypes, and (v) a transactivation domain, called AF2 (activation function 2) [16–18]. To control gene expression, PPARs heterodimerize with 9-cisRXR, which bind to peroxisome proliferator response elements (PPRE) located in the promoters of their targets genes. The canonical PPRE consists of two direct

repeats AGGTCA separated by a single nucleotide so-called DR-1 element [14]. Activation of target gene transcription depends on the binding of the ligand to the receptor. Ligand binding induces a conformational change in the LBD of the receptor that facilitates recruitment of coactivator molecules. Unliganded nuclear receptors recruit corepressors N-CoR and SMRT. For PPAR:RXR heterodimer, binding of the ligand of either receptor can activate the complex, but binding of both ligands simultaneously is more potent [17, 19]. In this context, several studies have identified a series of endogenous and synthetic ligands for PPARs such as unsaturated fatty acids, oxidized low-density lipoproteins (LDL-ox), VLDL, metabolites derived from linoleic acid, fibrates, and thiazolidinediones [14, 20].

1.4. PPAR-α. Liver plays a pivotal role in lipid metabolism by upregulating the expression of numerous genes involved in FA uptake through membranes, FA activation, intracellular FA trafficking, FA oxidation, and ketogenesis, in addition to TAG storage and lipolysis. Furthermore, PPAR-α also governs the metabolism of glucose, lipoprotein, and amino acids besides inflammatory processes, mainly by downregulating gene expression via a transrepression mechanism [9, 21] (for a detailed review see [21]). PPAR-α is well expressed in metabolically active tissues including liver, heart, kidney, intestine, macrophages, and brown adipose tissue, and it has mostly been studied in the context of liver parenchymal cells, where it is highly expressed [21]. Although the functionality of PPAR-α was initially questioned due it lower expression compared with mouse liver [22], a recent study showed that in liver tissue and primary hepatocytes, PPAR-α expression levels in mice are similar to humans [23]. However, in this context, it has to be considered the presence of both a truncated splice variant of human PPAR-α that negatively interferes with wild-type PPAR-α activity [24] and polymorphic variants in the functional coding sequence of human PPAR-α, val227ala, and Leu162Val, which are implicated in NAFLD and IR but not with liver damage, respectively [25, 26]. Natural ligands of PPAR-α include a variety of FAs as well as numerous FA derivatives and compounds showing structural resemblance to FAs, including acyl-CoAs, oxidized FAs, eicosanoids, endocannabinoids, and phytanic acid [27–29]. Synthetic PPAR-α ligands include fibrates such as gemfibrozil, bezafibrate, clofibrate, fenofibrate, and Wy14643, drugs that are used in the treatment of dyslipidemia primarily associated with type 2 diabetes mellitus [21].

1.5. PPAR-γ. PPAR-γ is the master regulator in the control of genes involved in lipogenic pathways of adipocytes, promoting the uptake of FAs and the differentiation of the adipocyte, with the consequent increase in the cellular content of TAGs and reduction in the FA delivery to the liver [17]. Target genes of PPAR-γ are involved in adipocyte differentiation, lipid storage, and glucose metabolism and include lipoprotein lipase, CD36, adipocyte FA binding protein aP2, FA transport protein, acyl-coA synthetase, phosphoenolpyruvate carboxykinase, aquaporin 7, and citrate carrier [9, 30, 31]. PPAR-γ also confers sensitization to insulin through

the transcriptional activation of the adiponectin gene in adipocytes, up-regulating its expression [32]. Ligands for PPAR-γ include specific polyunsaturated fatty acid (PUFA) metabolites, several eicosanoids, and synthetic compounds with very high (nanomolar) affinity such as thiazolidine-diones [17, 29].

Increased PPAR-γ expression is a feature of the steatotic liver and several studies attribute a causal role of PPAR-γ in steatosis development by mechanisms involving activation of lipogenic genes and *de novo* lipogenesis [33]. In humans, PPAR-γ is much more abundant in adipose cells; yet reasonable levels of PPAR-γ mRNA can also be found in other organs including skeletal muscle, colon, lung, and placenta. In contrast to adipose tissue, liver and heart express very little PPAR-γ; however, under certain pathological conditions, these tissues can express considerable amounts of PPAR-γ that have significant impacts on metabolic homeostasis and tissue function [34].

Studies addressing the expression of PPAR-γ in obese subjects revealed an increased adipose tissue expression of the splice variants PPAR-$\gamma1$ and PPAR-$\gamma2$, compared with lean subjects, suggesting that under pathological conditions and different nutritional situations, regulation of the human PPAR-γ expression may change [35]. In some infectious diseases such as hepatitis B and C viruses, multiple observations suggest that liver steatosis is a common histological characteristic, in which an increase in the expression and/or activity of PPAR-γ could contribute to the regulation of lipid synthesis [36–38]. Furthermore, similar to PPAR-α, it has to be considered PPAR-γ variants, considering that Pro12Ala and C1431T polymorphisms alter the susceptibility to hepatic steatosis, lobular inflammation, and fibrosis in humans with NAFLD. It was suggested that subjects with a haplotype containing both minor Pro12Ala and C1431T alleles are at reduced risk for NAFLD, and its histological features are associated with NASH [39]. Similar results have been found in Chinese population [40], which is in agreement with previous results associating Pro12Ala variant with increased insulin sensitivity, lower body mass and protection from type 2 diabetes [41–43].

1.6. PPAR-δ. Due to its ubiquitous expression profile, much less is known about PPAR-δ compared to PPAR-α and PPAR-γ in relation to human obesity and NAFLD. Studies from a decade ago showed that insulin-resistant obese rhesus monkeys normalized fasting glucose and insulin, increased high-density lipoprotein-cholesterol, and reduced low-density lipoprotein cholesterol after treatment with the potent and specific PPAR-δ agonist GW501516, which is approximately 1200 times more selective for PPAR-δ than the α and γ receptors [44]. Studies in an animal model of adenovirus-mediated hepatic PPAR-δ overexpression showed that PPAR-δ regulates lipogenesis and glucose utilization for glycogen synthesis. These effects could result in hepatic protection from free FA-mediated damage, possibly due to the generation of protective mono-unsaturated FA and lowering lipotoxic saturated FA levels [45]. Overweight and obese men subjected to PPAR-δ agonists GW501516 or

MBX-8025 improved insulin sensitivity and decreased fasting plasma TAGs, NEFAs, apoB-100, and LDL-cholesterol concentrations, with diminished liver fat content quantified by magnetic resonance imaging (MRI) [46–49]. Furthermore, recent studies showed that enhanced inflammation in visceral adipose tissue (VAT) is accompanied by a reduction in SIRT1 protein levels and PPAR-δ activity, in association with NF-κB activation, in morbidly obese IR patients compared with normal and overweight subjects, suggesting interplay between PPAR-δ and NF-κB [50]. However, this contention and the mechanisms underlying PPAR-δ effects remain to be studied in the liver of obese patients.

Collectively, discussed data point to various molecular mechanisms underlying NAFLD, some of which are modulated by PPARs. The aim of this work is to review the alterations of PPAR functioning and its pathogenic consequences associated with NAFLD in human obesity.

2. The Role of PPAR-α Downregulation in Liver Steatosis

Simple TAG accumulation in hepatocytes or steatosis is an early hallmark in NAFLD associated with obesity that is characterized by the multifactorial nature of the underlying pathogenic mechanisms, including the development of oxidative stress and insulin resistance [3, 52, 53], which provides the setting for further hepatic injury [54]. In this respect, the concept of nutritional or dietary oxidative stress has been introduced to denote an imbalance between the prooxidant load and the antioxidant defence, resulting from excess oxidative load or inadequate supply of the organism with nutrients [55]. Prolonged consumption of calorie-enriched diets stimulates fatty acid (FA) synthesis from glucose, and FAs in excess are converted into TAGs and store as lipid droplets within hepatocytes (Figure 1) [56]. FA overloading in the liver may favour high rates of FA oxidation due to substrate pressure, with consequent reactive oxygen species (ROS) generation [3]. This contention is supported by studies in J774.2 macrophages, which upon TAG overload generate ROS at mitochondrial complex I of the respiratory chain, coupled to higher FA β-oxidation, with concomitant induction of cellular necrosis, features that are diminished by antioxidants [13]. In agreement with these views, the liver of obese NAFLD patients with steatosis exhibits major changes in oxidative stress-related parameters. These include (i) a diminished antioxidant potential (glutathione (GSH) depletion and reduced superoxide dismutase (SOD) activity) [57]; (ii) an increased free-radical activity (higher lipid peroxidation) [57–59], protein oxidation [57], and 3-nitrotyrosine reactivity [60]; (iii) Kupffer-cell activation (increased lipid peroxidation potential and superoxide radical ($O_2^{\bullet-}$) generation, implying NADPH oxidase (NOX2) activation) [61]; (iv) a consequent reduction in the systemic antioxidant capacity of plasma [57] with higher lipid peroxidation indicators [62], thus evidencing the onset of oxidative stress (Figure 1A). Overnutrition-induced ROS generation might represent a triggering mechanism for the onset of insulin resistance [63, 64], in addition to the accumulation of lipids
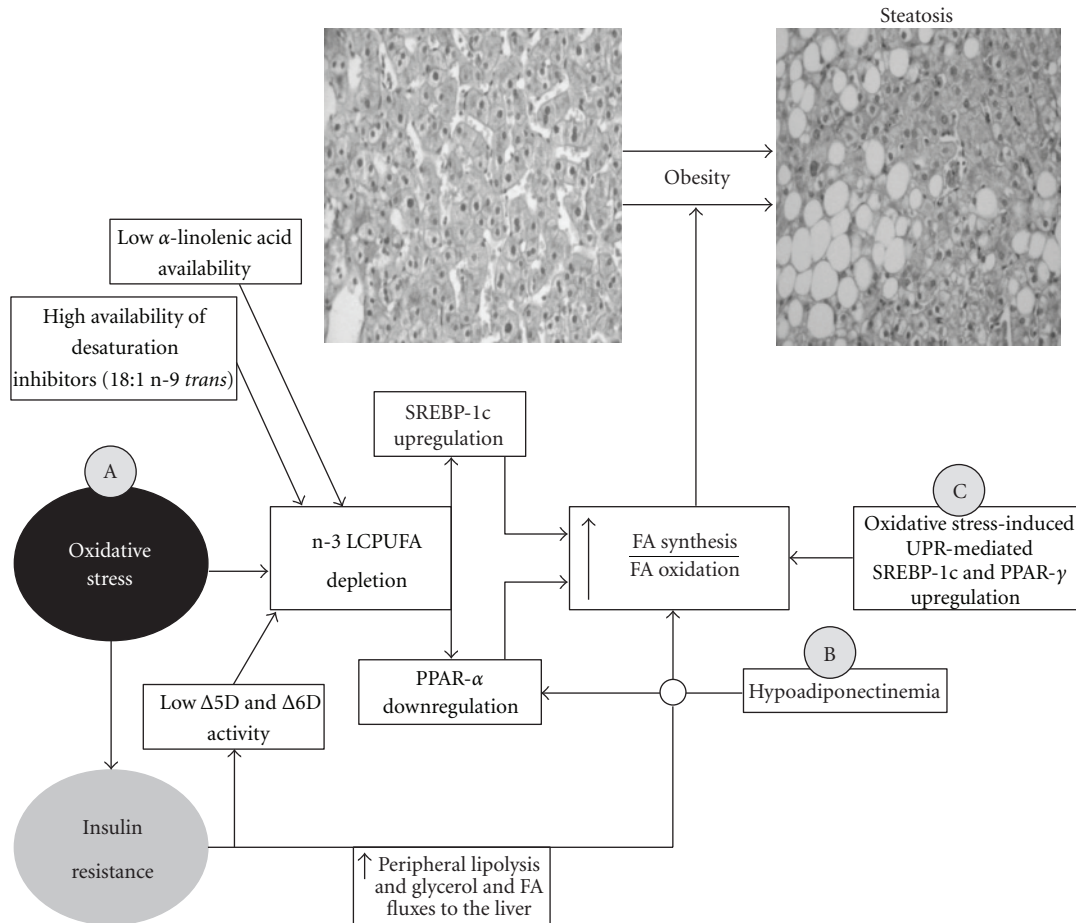
FIGURE 1: Obesity-induced liver oxidative stress (A), hypoadiponectinemia (B), and endoplasmic reticulum stress (C) as factors leading to hepatic steatosis in nonalcoholic fatty liver disease. *Abbreviations:* $\Delta5(6)$D: delta-5(6) desaturase; FA, fatty acid; LCPUFA: long-chain polyunsaturated fatty acid; PPAR-$\alpha(\gamma)$: peroxisome proliferator-activated receptor-$\alpha(\gamma)$; SREBP-1c: sterol regulatory element binding protein-1c; UPR: unfolded protein response.

such as free FAs (FFAs) [65, 66]. This proposal points to the activation of several stress-sensitive serine/threonine kinases by ROS and FFAs, which upon phosphorylation of the insulin receptor and/or the insulin receptor substrate proteins, achieve derangement of insulin-stimulated tyrosine phosphorylation resulting in insulin resistance [63–66].

Development of cellular oxidative stress leads to the production of oxidized products of biomolecules such as DNA bases, aminoacid residues in proteins, and PUFAs in membrane phospholipids [67]. In the latter case, long-chain PUFAs (LCPUFAs) of the n-3 series, namely, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), are the most susceptible to free-radical attack, considering that their respective rate constants for lipid peroxidation initiation are about 7- to 10-fold higher than that for linoleic acid (LA, 18:2n-6) taken as unity [68]. Assessment of the FA pattern of the liver of obese NAFLD patients revealed a significant depletion of LCPUFA n-3 (EPA plus DHA) levels [51, 69, 70]), a parameter that correlates with the levels of LCPUFA n-3 in erythrocytes [69] and that significantly recovers after weight loss [71]. Liver LCPUFA n-3 depletion in obesity may be related to higher utilization

due the prevailing high oxidative stress status [57, 72] (Figure 1A), a contention that is supported by the significant inverse correlation established between liver phospholipid LCPUFA n-3 content and serum F_2-isoprostane levels, as index of free-radical activity (Figure 2(a)). Under these conditions, the nonenzymatic oxidative decomposition of LCPUFA n-3 to J_3-isoprostane derivatives [73] can occur; however, utilization of LCPUFA n-3 by cyclooxygenase-2/5-lipoxygenase pathway and/or the cytochrome P450 NADPH-dependent epoxygenase system [74] cannot be discarded. In addition to enhanced liver LCPUFA n-3 utilization, depletion of LCPUFA n-3 in NAFLD is associated with defective hepatic capacity for desaturation of the LCPUFA n-3 essential precursor α-linolenic acid (α-LA, 18:3n-3). (i) Livers from NAFLD patients show a significant diminution in the hepatic activity of Δ-5 and Δ-6 desaturases (Δ-5D and Δ-6D) [75] and in the (20:5 + 22:6)n-3/18:3n-3 product/precursor ratio [51]. These parameters exhibit inversed correlations with the HOMA index [75], pointing to coordinate down-regulation of Δ-5D and Δ-6D expression by insulin resistance (Figure 1A) [76, 77]. (ii) Dietary imbalance, as determined by the abdominal adipose tissue PUFA levels as biomarker
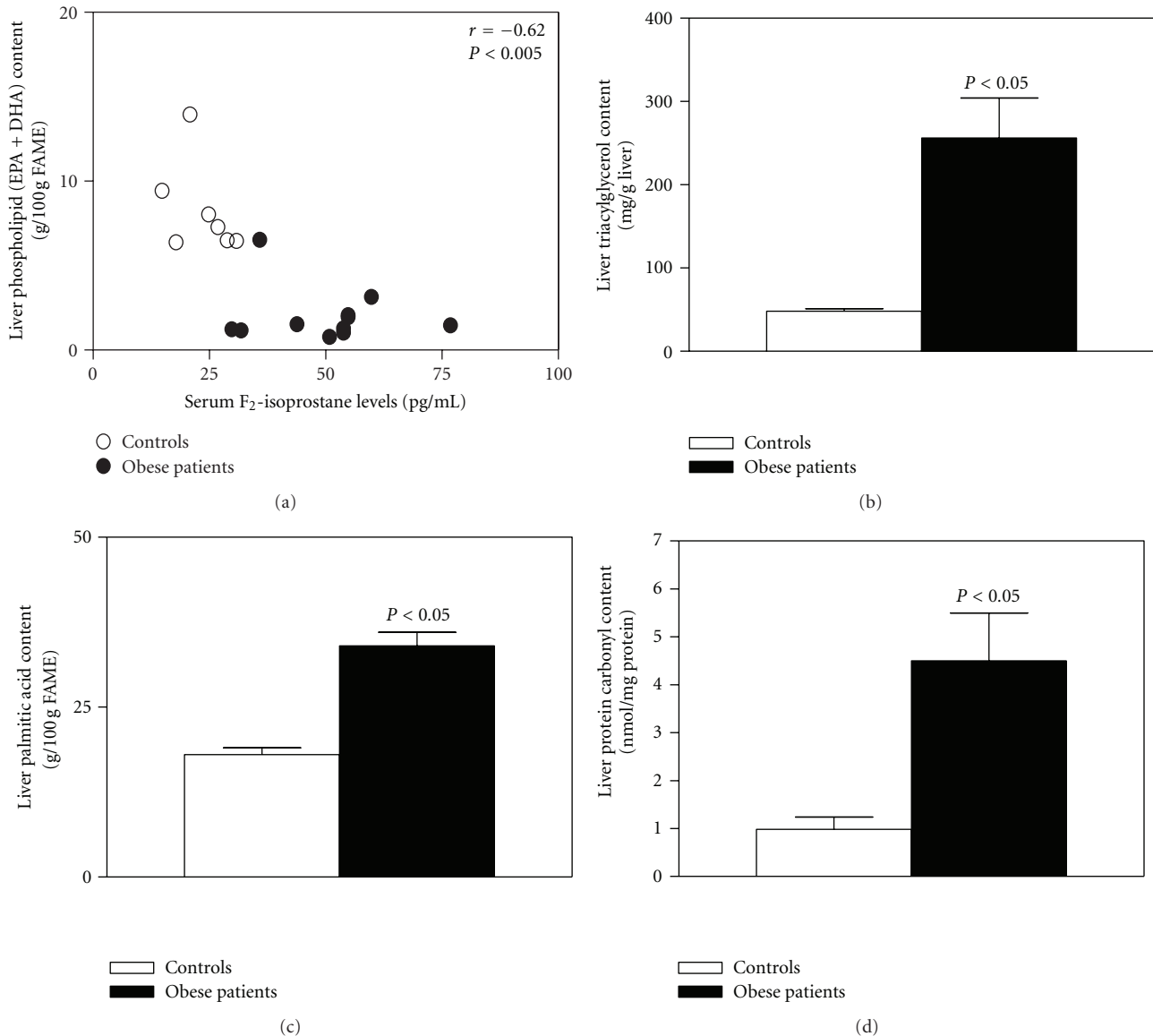
(a)

(b)

(c)

(d)

FIGURE 2: Correlation between liver phospholipid content of LCPUFA n-3 and F_2-isoprostane levels in serum as index of oxidative stress (a) and contents of liver triacylglycerols (b), palmitic acid (c), and protein carbonyls (d) in control subjects and obese patients with steatosis. LCPUFA n-3 content corresponds to eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA), expressed as g/100 g fatty acid methyl esters (FAME). Correlation in (a) was carried out by Spearman rank order correlation coefficient (unpublished data). Data (means ± SEM; 10 controls and 8 obese patients with steatosis) presented in (b), (c), and (d) were adapted from Araya et al., 2004 [51].

of dietary intake [78], involves decreased consumption of α-LA and higher-than-normal intake of *trans* FAs (elaidic acid, 18:1n-9 *trans*), effective Δ-6D inhibitors (Figure 1A) [51].

Under physiological conditions, LCPUFAs n-3 and/or their oxidized metabolites regulate hepatic lipid metabolism acting as (i) ligands of PPAR-α promoting the expression of genes encoding for proteins involved in FA oxidation at mitochondrial, peroxisomal, and microsomal levels, FA binding in cells, and lipoprotein assembly and transport [20] and (ii) downregulators of the lipogenic transcription factor SREBP-1c expression and activation [79–81]. Therefore, LCPUFA n-3 depletion in the liver of obese NAFLD patients might favour FA and TAG synthesis over FA oxidation,

promoting hepatic steatosis (Figure 1A), with major changes in the mRNA expression of transcription factors controlling liver lipid metabolism. The latter view is evidenced by the increased mRNA expression of SREBP-1c inducing lipogenic genes such as fatty acid synthase (FAS), the concomitant reduction in that of PPAR-α controlling FA oxidation (carnitine palmitoyltransferase-1a; CPT-1a), with the consequent enhancement in the hepatic SREBP-1c/PPAR-α ratios denoting a prolipogenic status [70]. This condition may also involve diminution in TAG export from the liver via very-low density lipoprotein (VLDL) due to decreased production of apolipoprotein B-100 [82], which is upregulated by LCPUFA n-3 and PPAR-α activation [83, 84]. The above contention

is further strengthened by the substantial enhancement in the LCPUFA n-6/n-3 ratio observed in liver phospholipids [73, 85], considering that LCPUFA n-3 are more effective PPAR-α activators than LCPUFA n-6 [79]. In agreement with these findings obtained in the liver, of obese patients, nutritional disequilibrium at the expense of PUFA n-3 in mice subjected to a PUFA n-3 depleted diet-induced hepatic SREBP-1c and lipogenesis up-regulation, with significant depression of FA oxidation and steatosis development [86].

In addition to the prosteatotic mechanism underlying NAFLD with development of oxidative stress and LCP-UFA n-3 depletion triggering liver SREBP-1c upregulation and PPAR-α downregulation (Figure 1A), alterations in the signaling pathway of adiponectin may also play a role [70, 87] (Figure 1B). Adiponectin, an adipokine secreted by adipocytes in reverse proportion to the body mass index [88], exerts beneficial effects through actions on several tissues, leading to reduction of body fat, improvement of hepatic and peripheral insulin sensitivity, and increased FA oxidation [32, 89]. In the liver, adiponectin binds to the integral membrane proteins AdipoR1 and AdipoR2 acting as receptors for the globular and full-length forms of the adipokine [89]. Although the signaling pathway triggered by adiponectin is not completely understood, current views suggest that most of its cellular effects are mediated by the activation of AMP-activated protein kinase (AMPK) [90]. This is achieved by APPL1 (adaptor protein containing phosphotyrosine binding, pleckstrin homology domains, and leucine zipper 1) that couples adiponectin receptors to AMPK activation [91], with the sequential activation of p38 mitogen-activated protein kinase (p38 MAPK) [92] that phosphorylates PPAR-α, thus increasing its association with PPAR-α coactivator-1α and the transcriptional activity of PPAR-α [93]. Consequently, the expression of PPAR-α target genes encoding for acyl-CoA oxidase, CPT-1a, and fatty acid binding protein 3 is upregulated [91]. Therefore, diminution in the circulating levels of adiponectin [79, 87, 94] and in the hepatic expression of adiponectin and AdipoR2 [95] observed in obese NAFLD patients might contribute to liver PPAR-α downregulation (Figure 1B), representing an alternate reinforcing prolipogenic mechanism in addition to that related to LCPUFA n-3 depletion (Figure 1A).

3. Liver PPAR-fl Upregulation as a Steatotic Signaling Mechanism

The specific PPAR subtype PPAR-γ is mainly expressed in the white and brown adipose tissue [96], where it controls the expression of genes related to lipogenesis, promoting cell differentiation, FA uptake, and TAG accumulation, which reduces FA delivery to the liver [97]. In the human liver, PPAR-γ is expressed at a level that is 9–12% of that of adipose tissue [35]; however, enhanced expression levels are associated with induction of PPAR-γ-responsive genes related to lipid metabolism [98]. These include (i) lipoprotein lipase, (ii) proteins involved in FA uptake and intracellular binding and transport, such as FA translocase (FAT/CD36), FA binding proteins 1, 4, and 5 (FABP1, FABP4, and FABP5),

and FA transport proteins 2 and 5 (FATP2 and FATP5), and (iii) liver X receptor, which favours both PPAR-γ and FAT/CD36 expression [14, 99]. Studies in the liver of obese NAFLD patients revealed significant upregulation of PPAR-γ mRNA levels over those in lean control subjects [94], in agreement with data assessing the PPAR-γ2 isoform [100]. Furthermore, liver PPAR-γ upregulation coincided with that of SREBP-1c, parameters that showed a significant direct correlation and that constitute a reinforcing lipogenic mechanism [94, 101]. This contention is supported by the differential lipogenic gene expression pattern exhibited by both transcription factors. Under condition of insulin resistance, higher mobilization of nonesterified FAs from peripheral tissues to the liver occurs [102, 103], which may be efficiently taken up and subjected to intracellular trafficking for metabolic processing, due to PPAR-γ-dependent upregulation of liver FAT/CD36 and FATP5, respectively [102]. Thus, enhancement in de novo TAG biosynthesis can be achieved [12, 104], which may be contributed by de novo FA biosynthesis due to SREBP-1c-dependent induction of acetyl-CoA carboxylase, FAS, and stearoyl-CoA desaturase-1 observed [94, 105].

Upregulation of liver PPAR-γ can be achieved by a ligand-dependent process including LCPUFA n-3 binding [106]; however, this mechanism does not seem to play a role in obesity-induced PPAR-γ activation due to the substantial depletion of LCPUFA n-3 reported [51, 69, 70]. Although development of insulin resistance is likely to involve loss of the regulatory actions of insulin on hepatocellular carbohydrate, protein, and lipid anabolism, FA and TAG biosynthesis is preserved [53, 107]. It is therefore likely that other mechanisms may play a role in the prolipogenic status observed in obese, insulin-resistant, hyperinsulinemic individuals involving PPAR-γ and SREBP-1c [70, 92]; the endoplasmic reticulum (ER) stress is one of them [108]. The ER is the cellular compartment for protein synthesis, folding, assembly, and trafficking, as well as for TAG, phospholipid, and sterol biosynthesis [108, 109]. Under several stress conditions, accumulation of abnormally folded proteins triggers the unfolded protein response (UPR), to relieve the ER from the accumulation of misfolded proteins and avoid loss of protein function [108, 109]. A short-lasting UPR reestablishes folding capacity; however, under prolonged or sustained conditions, ER stress changes from cellular survival promotion to liver injury development [108, 110]. The UPR is mediated by three ER transmembrane proteins, namely, (i) double-stranded RNA-activated protein kinase (PKR-) like endoplasmic reticulum kinase (PERK); (ii) inositol requiring enzyme 1 (IRE1); (iii) activating transcription factor 6 (ATF6) [108, 111, 112]. These UPR transducers are normally inhibited by the ER chaperone BiP/Grp78 (binding immunoglobulin protein/glucose regulated protein 78) [113], which upon accumulation of misfolded proteins in the ER lumen dissociates from the luminal domains of PERK, IRE1, and ATF6 allowing their activation [108]. UPR is induced by several stress conditions, including reduced capacity for protein glycosylation or disulfide bond formation, nutrient deprivation, viral infections, and increased FA availability or ROS generation, which led to abnormal

protein folding [108, 114]. As already discussed in Section 2, human obesity is characterized by TAG (Figure 2(b)) and saturated FA (palmitic acid; Figure 2(c)) overload in the liver, determining high rates of FA oxidation and ROS generation [3], which is associated with 4-fold increase in hepatic protein carbonylation (Figure 2(d)), as a measure of protein oxidation by ROS [115]. Protein damage by ROS is complex, irreversible and involves various oxidative modifications of amino acid residues in proteins, which may lead to protein unfolding and rapid degradation [115–117]. Thus, under conditions of hepatic palmitate overload (Figure 2(c)) and ROS-dependent protein carbonylation (Figure 2(d)), ER stress is likely to be induced in the liver of obese NAFLD patients. This contention is in agreement with the elevated hepatic levels of BiP/Grp78 and of phosphorylated eukaryotic translation-initiation factor 2α (eIF2α) as components of the PERK signaling pathway [118, 119], which are significantly diminished after weight loss [118]. In addition to the liver, adipose tissue from obese patients also exhibits increased parameters related to ER stress, evidencing the activation of the PERK [118, 120], IRE1 [111, 118], and TAF6 [120] signaling pathways. These findings suggest the involvement of the UPR in lipogenesis leading to hepatic steatosis (Figure 1C), in addition to obesity-induced oxidative stress-related LCPUFA n-3 depletion, insulin resistance (Figure 1A), and hypoadiponectinemia (Figure 1B). Interestingly, ER stress has been associated with ROS generation [121] that may contribute to the oxidative stress status developed in the liver of obese patients [3, 57, 72]. The proposed mechanisms involving ER stress induced (i) sustained Ca^{2+} release from the ER and mitochondrial Ca^{2+} accumulation with promotion of ROS production [114, 121], and (ii) oxidative folding of nascent proteins by protein disulfide isomerase (PDI) coupled to ER-oxidoreductin 1 (Ero1) operation [114, 121, 122]. However, several important mechanistic questions remain to be addressed regarding the role of UPR in obesity-related liver disease [114] and oxidative stress development [121].

4. Liver PPAR-α Downregulation: Proinflammatory Connotations

Liver oxidative stress status, a major mechanism associated with the pathogenesis of steatosis (Figure 1), is exacerbated in obese patients with steatohepatitis (Figure 3). This is evidenced by (i) diminution of hepatic catalase activity, in addition to SOD reduction and GSH depletion already observed in steatosis [57]; (ii) upregulation of the cytochrome P450 2E1 isoform (CYP2E1) and higher *in vivo* chlorzoxazone hydroxylation catalyzed by CYP2E1, changes that are not observed in steatosis [123]; (iii) further increases in liver nitrotyrosine immunoreactivity [60], hepatic 4-hydroxynonenal (marker of lipid peroxidation) and 8-hydroxydeoxyguanosine (marker of oxidative DNA damage) immunostaining [124], Kupffer-cell-dependent O$_2^{\bullet-}$ generation, and lipid peroxidation extent [61] (Figure 3). These changes observed in the liver of steatohepatitis subjects are paralleled by a further decrease in the antioxidant capacity

of plasma over that in steatosis [57], which correlates with higher systemic levels of lipid peroxidation products [62, 125–127]. Liver oxidative stress in steatohepatitis is related to several contributory factors, including upregulation of the highly prooxidant CYP2E1 [58, 123, 128], hepatic mitochondrial dysfunction [129, 130], and mixed inflammatory-cell infiltration and Kupffer-cell activation, involving upregulation of NOX2 [61]. The high prooxidant status attained in steatohepatitis was observed concomitantly with significant enhancement in the DNA binding capacity of hepatic nuclear factor-κB (NF-κB) [131, 132] and activating protein 1 (AP-1) [131], redox-sensitive transcription factors that upregulate the expression of proinflammatory mediators at the Kupffer-cell level (Figure 3) [3]. These parameters were not modified in patients with simple steatosis, in relation to controls [131].

ROS activate NF-κB through the classical or canonical inhibitor of κB (IκB) kinase (IKK) complex pathway, which depends on NF-κB essential modulator (NEMO) or IKKγ, IKKβ activation, nuclear localization of p65-p50 dimers and is associated with inflammation (Figure 3A) [133, 134]. In addition, AP-1 signaling requires *de novo* synthesis of c-Jun and c-Fos proteins, followed by phosphorylation of the c-Jun component by activated c-Jun N-terminal kinase (JNK) (Figure 3B), which requires ROS-mediated inhibition of JNK-inactivating phosphatases [135]. At the nuclear level, both NF-κB and AP-1 may form heterodimers with PPAR-α, leading to the formation of the transcriptionally inactive complexes p65-PPAR-α and c-Jun-PPAR-α [53]. Thus, obesity-induced diminution in both liver PPAR-α expression and PPAR-α activation related to LCPUFA n-3 depletion may be considered as a proinflammatory mechanism [70], due to the reduced antagonizing action of PPAR-α downregulation on NF-κB and AP-1 activation. This contention is supported by the significant inverse correlations established for liver NF-κB and AP-1 DNA binding with PPAR-α mRNA levels observed in control subjects and obese NAFLD patients with steatohepatitis [136]. Furthermore, significant 7.8-fold and 15.1-fold enhancements in the hepatic NF-κB/PPAR-α and AP-1/PPAR-α ratios are observed in steatohepatitis over control values, respectively, pointing to a major disturbance in signaling pathways triggering a proinflammatory status in the liver of obese patients (Figure 3) [136]. The latter state may be reinforced by three additional mechanisms, namely, (i) TNF-α up-regulation [61, 137–139] in response to the initial NF-κB activation, which signals through the TNF-α receptor 1 and the canonical pathway and/or by TNF-α-induced ROS production at the mitochondrial level that activates JNK, enhancing AP-1 DNA binding capacity [133, 134]; (ii) development of endotoxemia [140], with increasing plasma levels of lipopolysaccharide (LPS) triggering toll-like receptor 4 (TLR4) [141], recruitment of several adaptor molecules, and activation of transforming growth factor β-activated kinase 1 (TAK1) leading to IKK and JNK phosphorylation and NF-κB and AP-1 activation [141, 142]; (iii) induction of the ER stress, with upregulation of both the PERK/eIF2α pathway [118, 119] achieving NF-κB activation [143] and the IRE1 pathway leading to JNK/AP-1 activation [111, 118, 119]. Although ER stress can activate NF-κB and JNK/AP-1, activation by other mechanisms is also possible,
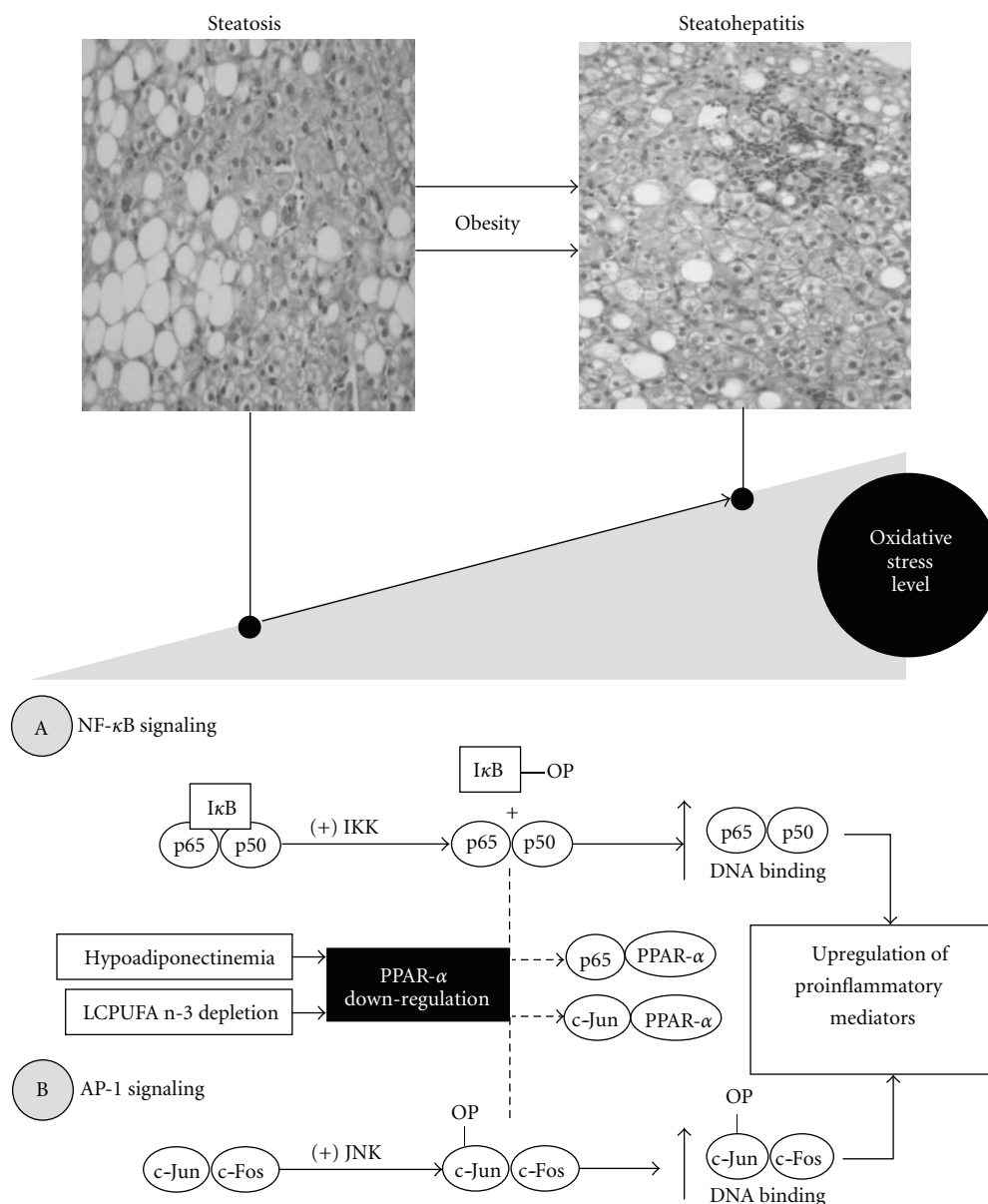
FIGURE 3: Interrelationships between the level of oxidative stress and PPAR-α downregulation in the progression of steatosis to steatohepatitis associated with obesity involving NF-κB (A) and AP-1 (B) signaling. *Abbreviations:* AP-1: activating protein-1 (c-Jun-cFos; c-Jun-OP, phosphorylated c-Jun); IκB: inhibitor of κB (IκB-OP, phosphorylated IκB); IKK: IκB kinase; JNK: c-Jun N-terminal kinase; LCPUFA, long-chain polyunsaturated fatty acid; NF-κB: nuclear factor-κB (p65-p50); PPAR-α: peroxisome proliferator-activated receptor-α. Solid arrows indicate enhanced contribution, whereas broken arrows indicate reduced contribution.

and further studies are needed to establish their relative importance in the development of steatohepatitis in human obesity.

5. Conclusions

Prolonged liver oxidative stress in human obesity is associated with development of a wide disease spectrum ranging from steatosis to steatosis with inflammation, fibrosis, and cirrhosis (nonalcoholic steatohepatitis), a redox imbalance showing a functional interdependency with insulin resistance [3]. Disease mechanisms might involve (i) the initial ROS production due to lipotoxicity with the onset of steatosis (Figure 1); (ii) exacerbation of ROS generation due to concurrence of mitochondrial dysfunction, microsomal CYP2E1 induction, and inflammatory-cell activation (Figure 3). Misregulation of inflammatory cytokine, adipokine, and chemokine signaling may reinforce the initial mechanisms of ROS production and insulin resistance, representing determinant factors in the progression of steatosis to steatohepatitis. In this setting, alterations in the expression and/or activation in hepatic PPARs may play crucial pathogenic roles, considering their importance in energy homeostasis and inflammation [106].

Liver PPAR-α downregulation and substantial enhancement in the hepatic SREBP-1c/PPAR-α mRNA content ratio represent major metabolic disturbances between *de novo* lipogenesis and FA oxidation favouring the former, as a central issue triggering liver steatosis in obesity-induced oxidative stress and insulin resistance. The prosteatotic action of PPAR-α downregulation may be reinforced by PPAR-γ upregulation favouring hepatic FA uptake, binding, and transport, representing a complementary lipogenic mechanism to SREBP-1c induction leading to *de novo* FA synthesis and TAG accumulation. In addition, PPAR-α downregulation may play a significant role in enhancing the DNA binding capacity of proinflammatory factors NF-κB and AP-1 in the liver of obese patients, thus constituting one of the major mechanisms for the progression of simple steatosis to steatohepatitis. In the past, PPARs have been studied as drug targets for the management of NAFLD in obesity and the broader MetS [53]. However, PPAR-α agonists such as fibrates used to diminish steatosis and inflammatory scores in human steatohepatitis revealed poor effectiveness, thiazolidinediones have weight gain limitations, whereas that of dual PPAR-$\alpha\gamma$ agonists has safety concerns [53]. Considering the negative correlation established between liver SREBP-1c/PPAR-α ratios and LCPUFA n-3 levels in control and obese subjects [70], which points to liver LCPUFA n-3 depletion as a major factor directing FAs toward TAG storage, LCPUFA n-3 supplementation was used as PPAR-α targeting. Supplementation with either fish oil, seal oil, or purified LCPUFA n-3 diminished steatosis scores, as evidenced by ultrasonography [144–147] or by determination of lipid content in posttreatment liver biopsies [148]. Furthermore, improvement in liver function markers [144–148], TAGs [145, 146] and TNF-α [145] levels in serum were observed after LCPUFA n-3 administration. These data were recently included in a larger meta-analysis comprising nine studies involving 355 individuals, which concluded that LCPUFA n-3 supplementation in human NAFLD patients is associated with a positive effect on liver fat [149]. In addition, positive anti-inflammatory outcome is also observed [144–148], which may include (i) PPAR-α activation and further inhibitory action on NF-κB and AP-1 signaling [150]; (ii) EPA and DHA metabolism by the cyclooxygenase-2/5-lipoxygenase pathway to produce E(D) resolvins and protectin D1 as anti-inflammatory mediators [151]; (iii) EPA and DHA oxygenation by cytochrome P450 NADPH-dependent epoxygenases, with production of epoxy derivatives with potent anti-inflammatory effects [151]. LCPUFA n-3 effects on liver inflammation and fibrosis are being currently addressed by several clinical trials [152].

Conflict of Interests

The authors have declared that no conflict of interests exists.

Acknowledgment

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Research Article

Peroxisome Proliferator-Activated Receptor Gamma Exacerbates Concanavalin A-Induced Liver Injury via Suppressing the Translocation of NF-κB into the Nucleus

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Peroxisome proliferator-activated receptor-γ (PPARγ) has been reported to reduce inflammation and attenuate fibrosis in the liver. In this study, we investigated the effects of PPARγ on the liver injury induced by 20 mg/kg Concanavalin A (Con A). The mice were administered one of the three types of PPARγ ligands (pioglitazone, ciglitazone, and troglitazone) for 1 week, and the serum alanine aminotransferase (ALT) levels at 20 h after Con A injection were significantly elevated in the PPARγ ligand-treated mice. Furthermore, the serum ALT levels after Con A injection in the PPARγ hetero-knock-out mice (PPAR$\gamma^{+/-}$ mice) were lower than those in the wild-type mice (WT mice). Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) revealed extensive liver damage induced by Con A in the pioglitazone-treated mice. Electrophoresis mobility shift assay (EMSA) revealed that activation of translocation of nuclear factor- (NF-) κB, which is a suppressor of apoptosis, in the nucleus of the hepatocytes was suppressed in the pioglitazone-treated mice after Con A injection. In this study, we showed that PPARγ exacerbated Con A-induced liver injury via suppressing the translocation of NF-κB into the nucleus, thereby inhibiting the suppression of liver cell apoptosis.

1. Introduction

PPARs are members of the nuclear receptor superfamily [1]. Three isotypes designated PPARα, PPARβ/δ, and PPARγ have been described in mammals [2]. The PPARs form heterodimers with the retinoid X receptor (RXR), and the PPAR-RXR heterodimers, when bound to a ligand, change their conformation and bind to the DNA at the PPAR response elements, which results in gene transcription [3, 4]. PPARγ is expressed in adipose tissue, heart, kidney, skeletal muscle, liver and other organs PPARγ ligands improve insulin resistance and inflammation by increasing serum adiponectin levels [5–7]. Thus, thiazolidinediones (TZDs), which are PPARγ ligands, are widely used in the treatment of type 2 diabetes mellitus (DM).

Liver injury is caused by various factors such as viral infections, autoimmune reactions, and metabolic disorders. Recently, PPARγ agonists have received attention in relation to the treatment of liver diseases. PPARγ has been reported to reduce hepatic inflammation by decreasing the expression of tumor necrosis factorα (TNF-α) [8], and suppressing the translocation of NF-κB into the nucleus [9]. Furthermore, the PPAR pathway inhibits the fibrogenic actions in hepatic stellate cells and attenuates liver fibrosis *in vivo* [10, 11]. PPARγ agonists have been reported to be useful in mice and humans with NAFLD [12–14], as PPARγ promotes adipocyte differentiation [15], increases triglyceride storage in adipocyte, and reduces delivery of fatty acids to the liver [9]. However the effect on other liver diseases has not yet been investigated.
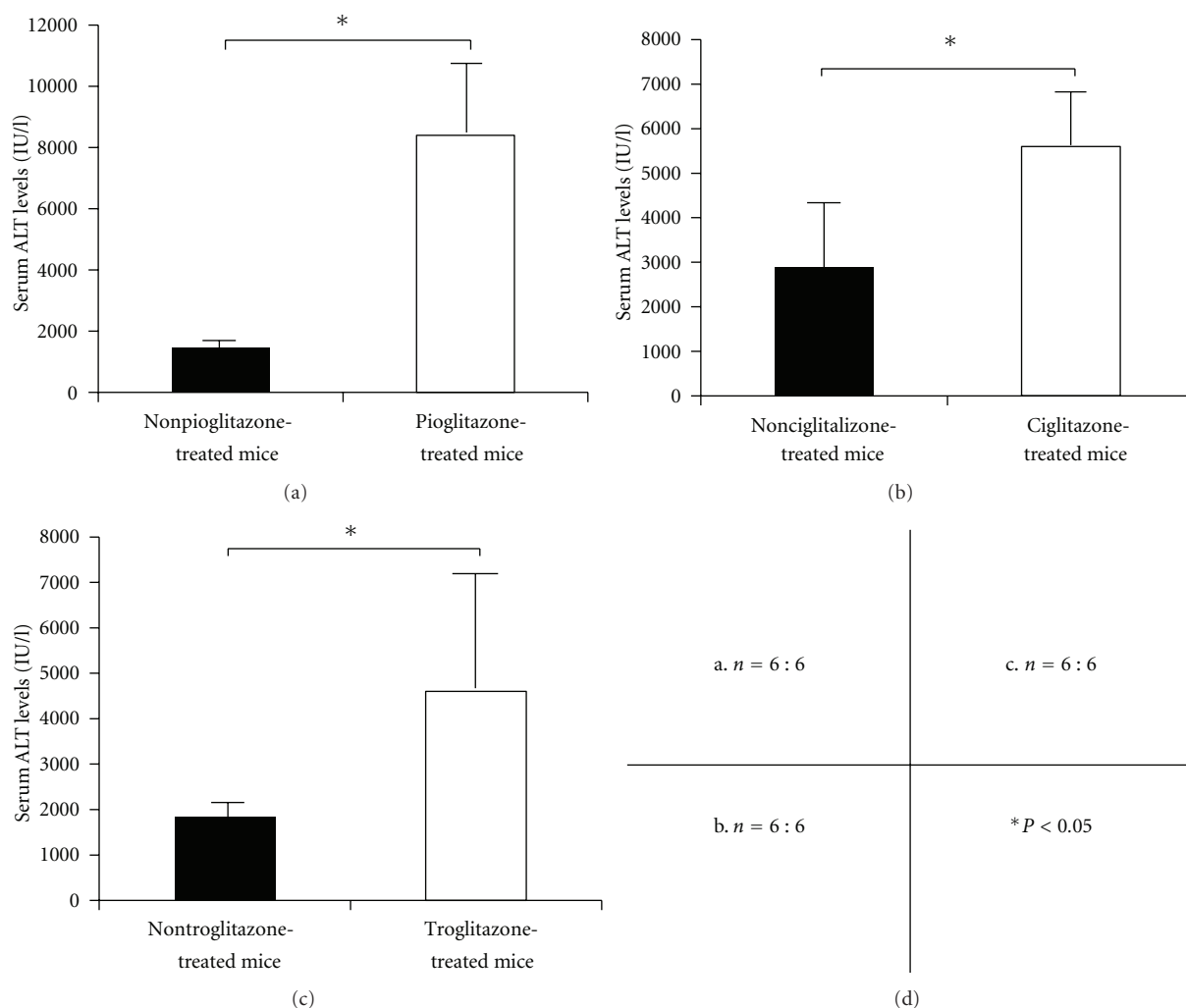
(a)

(b)

(c)

a. $n = 6 : 6$

b. $n = 6 : 6$

c. $n = 6 : 6$

$*P < 0.05$

(d)

FIGURE 1: At 20 h after Con A injection, the serum ALT levels in the mice treated with one of the three types of PPARγ ligands (pioglitazone (a), troglitazone (b), ciglitazone (c)) were significantly higher as compared with those in the non-PPARγ-treated mice ($*P < 0.05$).

In this study, PPARγ ligands and PPAR$\gamma^{+/-}$ mice were used to confirm the effects of PPARγ on the liver injury induced by Con A. Con A induces serious hepatitis in mice by activating T cells and triggering apoptosis [16, 17].

2. Materials and Methods

2.1. Animal Experiments. Eight-week-old male WT BALB/c mice and eight-week-old male PPAR$\gamma^{+/-}$ mice on a BALB/c background were purchased from CLEA Japan, Inc. and Jackson Laboratory (Bar Harbor, ME, USA), respectively. All the mice were maintained in filter-topped cages on auto-claved normal chow diet containing 22% protein, 6% fat, and 47% carbohydrate. In the Con A-induced hepatitis model, Con A (Sigma Aldrich, St. Louis, MO, USA; 20 mg/kg) was injected intravenously (i.v.) into mice. First, WT mice ($n = 6$ mice) were fed either a control chow or chow supplemented with one of the two types of PPARγ ligands (ciglitazone (100 mg/kg) and troglitazone (150 mg/kg)) *ad libitum* for 1 week and sacrificed at 20 h after the Con A injection.
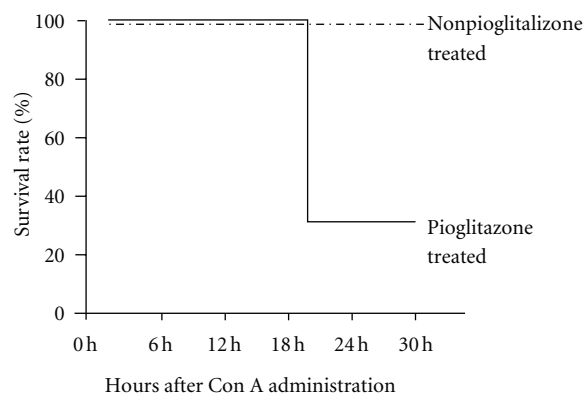

FIGURE 2: At 20 h after Con A injection, there were no cases of fatality in the nonpioglitazone-treated group of mice, whereas the fatality rate was 70% in the pioglitazone-treated mice.

These doses and duration of treatment with the PPARγ agonists were selected based on the efficacy demonstrated in
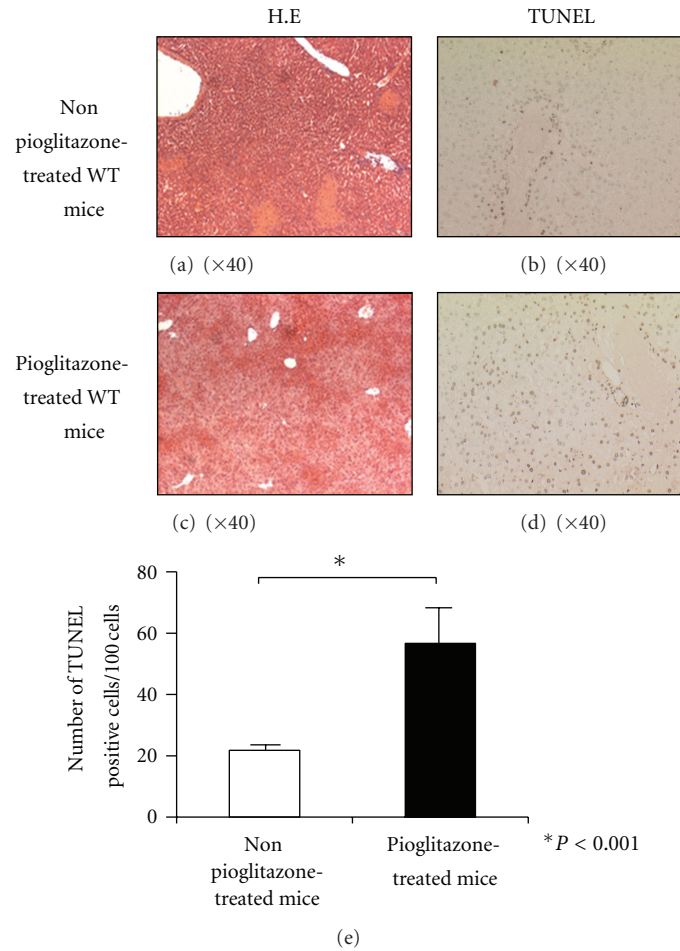
FIGURE 3: Histopathological examination of liver sections stained with H-E at 20 h after Con A injection revealed more extensive liver necrosis in the pioglitazone-treated mice (c) in comparison with that in the nonpioglitazone-treated mice (a). TUNEL assay at 20 h after Con A injection revealed more extensive liver apoptosis in the pioglitazone-treated mice (d) as compared with that in the nonpioglitazone-treated mice (b). The number of TUNEL-positive cells/100 cells was three-times higher in the livers of the pioglitazone-treated mice as compared with that in the livers of the nonpioglitazone-treated mice (56 ± 8.01 versus 21 ± 1.83, $P < 0.001$).

pilot experiments (data not shown). Subsequently, PPAR$\gamma^{+/-}$ mice were treated with either control chow or pioglitazone-supplemented chow and sacrificed at 20 h after the Con A injection. Finally, to investigate the effect of PPARγ on the activation of NF-κB induced by Con A, control or pioglitazone-supplemented chow was administered to the WT mice and sacrificed at various time points (0.5 h, 1 h, 3 h, 6 h, and 8 h) after the Con A injection, to obtain nuclear protein. The animal protocols were approved by the Yokohama City University Medical School Guidelines for the Care and Use of Laboratory Animals.

2.2. Biochemistry. Serum alanine aminotransferase (ALT) levels were measured by a local laboratory for clinical examinations (SRL Co, Ltd., Tokyo, Japan).

2.3. Liver Histology. Liver specimens were fixed overnight in buffered formaldehyde (10%) and embedded in paraffin. Paraffin sections were prepared at 5 μm thickness and stained with hematoxylin and eosin (H-E).

2.4. Assay for Apoptosis. The apoptotic tumor cells were stained using a TUNEL staining kit, according to the manufacturer's instructions (Wako Pure Chemical, Osaka, Japan). In brief, paraffin sections were digested with 20 μg/mL of proteinase K (Takara, Shiga, Japan) for 15 min at room temperature and reacted with terminal deoxynucleotidyl transferase enzyme for 60 min at 37°C. The sections were then incubated with antidigoxigenin conjugate at room temperature for 30 min, followed by incubation with diaminobenzidine solution.

2.5. Electrophoretic Mobility Shift Assay (EMSA). NF-κB binding was determined by EMSA. We collected liver tissue specimens at various time points (0.5, 1, 3, 6, and 8 h) after the Con A injection. Nuclear protein extracts (10 μg) were prepared using Nuclear Extraction kit (BizScience, Osaka,

Japan), according to the manufacturer's instructions. The probe oligonucleotide was 22 bp, double-stranded (5′-GCCTGGGAAAGTCCCCTCAACT-3′) and endlabeled with biotin (Sigma Chemical, St. Louis, MO). DNA-protein complexes were resolved at 80 V for 1 h in a taurine-buffered, native 6% polyacrylamide gel (4% for supershift) and blotted onto a positively charged nylon membrane (Sigma Chemical, St. Louis, MO). Transferred DNA was immediately cross-linked to the membrane on an ultraviolet transilluminator equipped with 312 nm bulbs and detected using horseradish peroxidase-conjugated streptavidin (Light-Shift Chemilu-minescent EMSA kit), according to the manufacturer's instructions.

2.6. Statistical Analysis. Data are presented as means ± SD. Differences between the two groups were assessed using the unpaired two-tailed Student's t-test; P values of <0.05 were considered to denote significance. All statistical analyses were performed using Microsoft Excel and the SPSS 16.0 statistical package (SPSS, Chicago, IL)

3. Results and Discussion

To assess the degree of liver injury, we analyzed the time course of changes of the serum ALT levels after the Con A injection. Unexpectedly, the serum ALT levels in the piogli-tazone- (30 mg/kg) treated mice were significantly higher in comparison with that in the nonpioglitazone-treated mice at 20 h after Con A injection (Figure 1(a)). The survival rate of the nonpioglitazone-treated mice was 100%, while that of the pioglitazone- (30 mg/kg) treated mice was 30% at 20 h after Con A injection (Figure 2). Subsequently, we conducted a histological examination to assess the degree of Con A-induced liver injury at 20 h after Con A injection in the mice treated and not treated with 30 mg/kg of pioglitazone. Histopathological examination of tissue sections stained with H-E revealed that the liver damage was more extensive in the pioglitazone-treated mice as compared with that in the nonpioglitazone-treated mice (Figures 3(a) and 3(c)). To determine the presence and extent of apoptotic cells, we performed TUNEL assay. More TUNEL-positive hepatocytes could be detected in the liver sections of the pioglitazone-treated mice than in those of the control mice (Figures 3(b) and 3(d)). The number of TUNEL positive cells/100 cells in the livers of the pioglitazone-treated mice was three-times higher as compared with that in the livers of the nonpioglitazone-treated mice (56.2 ± 8.0 versus 21.0 ± 1.8, $P < 0.001$). From these results, we hypothesized that PPARγ might actually exacerbate Con A-induced liver injury by intensifying hepatocyte apoptosis. Then, we used two other PPARγ ligands (ciglitazone and troglitazone) to confirm the effect of PPARγ. All of the three PPARγ ligands produced a significant increase of the serum ALT levels in the treated mice as compared with the levels in the untreated mice (Figures 1(a), 1(b), and 1(c)). This result indicates that PPARγ ligands exacerbate Con A-induced liver injury regardless of the kinds.

To evaluate the effect of PPARγ on liver injury, we used PPAR$\gamma^{+/-}$ mice. The reason for using the heterologous
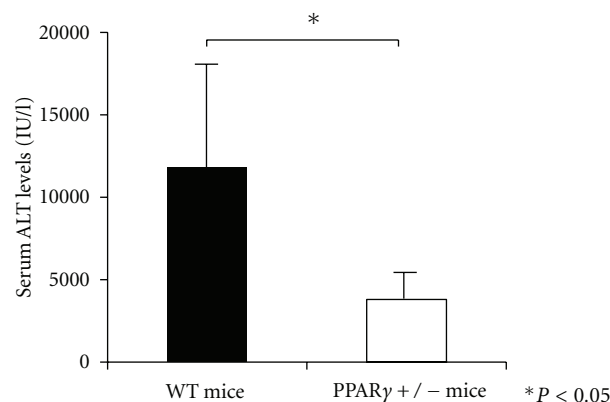

Figure 4: At 20 h after Con A injection, the serum ALT levels in the WT mice were significantly higher than those in the PPAR$\gamma^{+/-}$ mice.

PPAR$\gamma^{+/-}$ mice was that double knock-out of this gene results in embryonic lethality. Con A-induced liver injury was less extensive in the PPAR$\gamma^{+/-}$ mice as compared with that in the WT mice (Figure 4). This result suggests the possible involvement of the endogenous PPARγ-mediated pathway in the exacerbation of Con A-induced liver injury.

To confirm the apoptosis in the pioglitazone-treated and nonpioglitazone-treated mice after Con A-injection, we ana-lyzed the expression of NF-κB, which is a known suppressor of apoptosis. To determine the quantity of activated NF-κB, we performed EMSA. Activation of NF-κB in the hepatocyte nuclei after Con A-injection was suppressed in the livers of the pioglitazone-treated mice as compared with that in the livers of the nonpioglitazone-treated mice (Figure 5). This result suggests that PPARγ suppresses the translocation of NF-κB into the nucleus, thereby inhibiting the suppression of liver cell apoptosis. Maeda et al. reported that hepatocyte-specific IKKβ knockout mice exhibit little NF-κB activity and are highly susceptible to liver apoptosis of Con A-induced liver injury [18].

From this study, suppression of PPARγ, such as using PPARγ antagonists may potentially reduce the extent of liver injury.

4. Conclusion

In this study, we showed that PPARγ ligands exacerbate Con A-induced liver injury via suppressing the translocation of NF-κB into the nucleus. Con A-induced liver injury in PPARγ-treated mice represents an intensified apoptosis. PPARγ antagonists may be considered as novel candidates for the therapy of liver injury in an intensifying apoptosis model.

Abbreviations

PPARγ: Peroxisome proliferator-activated receptor-γ
NAFLD: Nonalcoholic fatty liver disease
Con A: Concanavalin A
ALT: Alanine aminotransferase
WT mice: Wild-type mice

1. Positive control
2. Cold competitor

Pioglitazone treatment　　+　+　+　+　+　−　−　−　−　−　　2　1

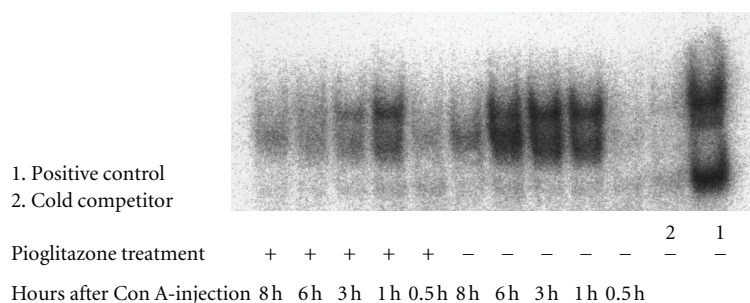Hours after Con A-injection　8 h　6 h　3 h　1 h　0.5 h　8 h　6 h　3 h　1 h　0.5 h

FIGURE 5: NF-κB binding was determined by EMSA. We collected liver tissue specimens at various time points (0.5, 1, 3, 6, and 8 h) after Con A injection. At 1 h after the Con A injection, NF-κB was activated in the nonpioglitazone-treated mice, whereas the NF-κB activation was suppressed in the pioglitazone-treated mice.

TUNEL:　Terminal deoxinucleotidyl transferase dUTP nick end labeling
EMSA:　Electrophoresis mobility shift assay
NF:　Nuclear factor
RXR:　Retinoid X receptor
TZDs:　Thiazolidinediones
DM:　Diabetes mellitus
TNF-α:　Tumor necrosis factor α
H-E:　Hematoxylin and eosin.

Acknowledgment

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Review Article

Antioxidant Stress and Anti-Inflammation of PPARα on Warm Hepatic Ischemia-Reperfusion Injury

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Hepatic ischemia-reperfusion (IR) injury is a serious clinical problem. Minimizing the adverse effect of ischemia-reperfusion injury after liver surgery or trauma is an urgent need. It has been proved that besides the effect of regulating the lipid and lipoprotein metabolism, PPARα also undertakes the task of organ protection. In this paper, related literature has been summarized and we come to the conclusion that administration of PPARα agonists can strengthen the antioxidant and anti-inflammation defense system by the upregulation of the expression of antioxidant enzymes and inhibition of NF-κB activity. This may provide a potential clinical treatment for hepatic ischemia-reperfusion injury.

1. Introduction

Hepatic ischemia reperfusion (IR) injury is an important clinical problem complicating liver surgery and transplantation [1]. Depending on whether the ischemia occurs in a warm setting, such as surgical resection or trauma surgery, or cold ischemia, as occurs during liver transplantation, it can be categorized into warm IR and cold storage reperfusion injury [2]. In warm IR, the pathophysiology underlying the injury of hepatic ischemia-reperfusion is complex encompassing a number of mechanisms including oxidant stress, inflammation, and apoptosis. Recently, some researchers have focused on the use of peroxisome proliferator-activated receptor alpha (PPARα) agonist to ameliorate this injury [3, 4]. In this paper, the mechanisms of hepatic ischemia-reperfusion injury, the characteristics of PPARα, and the role of PPARα in warm hepatic ischemia-reperfusion injury have been discussed in the following sections.

2. Mechanisms of Warm Hepatic Ischemia-Reperfusion Injury

With the growth in the field of hepatobiliary surgery, the technique of partial or total vascular occlusion in room temperature has been adapted, and it has enabled surgeons to perform complex procedures such as large liver resections and repairs that otherwise would have resulted in massive hemorrhage and certain death. Apart from the apparent superiority of the technique, there are still some limitations that can cause substantial morbidity and mortality named warm hepatic ischemia-reperfusion injury. Warm hepatic ischemia-reperfusion injury is a complex cascade of events involving a multitude of pathophysiological processes, more than 50% of hepatocytes and sinusoidal endothelial cells (SEC) that formerly considered to undergo apoptosis during the first 24 hours of reperfusion [5, 6]; however, work done by team of Jaspreets Gujral suggested that apoptotic cell death, if it occurs at all, is a very minor aspect of the entire cell death [7, 8]. Based on it we can conclude that the oxidant stress and inflammation are the most critical mechanisms which contribute to the organ pathophysiology after warm hepatic ischemia reperfusion. Work done by Jaeschke et al. [9–12] indicated that there are two distinct phases of liver injury after warm ischemia and reperfusion. The initial phase of injury (<2 hours after reperfusion) is characterized by Kupffer cells activated, and the activated Kupffer cells are a primary source of reactive oxygen-derived free radicals [10, 13]. These free radicals and reactive oxygen species (ROS) are generated to create a severe enough disturbance of the cellular homeostasis. Mitochondria must be a primary

target, and its dysfunction may impair the electron flow and enhance superoxide formation [14, 15]. All these will eventually trigger mitochondrial dysfunction and oxidant stress and eventually kill the cell [16, 17]. Studies have shown that it attenuates early hepatocellular injury after hepatic IR that Kupffer cells activity is suppressed by gadolinium chloride or methyl palmitate in mice [18]. Conversely, chemically upregulating Kupffer cell activation aggravates cellular injury and production of reactive oxygen species [19]. In addition, complement is a key factor that contributes to the early activation of Kupffer cells after IR [20]. Kupffer cell generation of superoxide has been shown to be a decisive factor in the injury observed in the early reperfusion period [20, 21]. In addition to Kupffer cell-induced oxidant stress, with increasing length of the ischemic episode, intracellular generation of reactive oxygen by xanthine oxidase and, in particular, mitochondria [22] may also lead to impaired adenosine triphosphate (ATP) production and acidosis result in liver dysfunction and cell injury during reperfusion [23]. Nevertheless, liver architecture assessed histologically shows only minor changes during the period of ischemia and early reperfusion. In the late phase of injury (>6 hours after reperfusion), events occurring during the initial phased serve to initiate and propagate a complex inflammatory response that culminates with the hepatic accumulation of neutrophils [24]. Kupffer cells which can not only directly activate and recruit neutrophils but also serve as the principal source of the oxidant stress during the first period phase of reperfusion injury, the production, and the release of reactive oxygen species can lead to an oxidative shift in the hepatic redox state [10, 11, 25], that is thought to activate redox-sensitive transcription factor NF-κB, which provides the signal for activation of proinflammatory genes, such as IL-12 and TNF-α [26–29]. Productions of these mediators lead to inducing the expression of secondary mediators, including neutrophil-attracting CXC chemokines and endothelial cell adhesion molecules which mediate the adhesion and transmigration of neutrophils from the vascular space into the hepatic parenchyma [30–32]. Neutralizing antibodies to CXC chemokines proven to be effective against neutrophil-induced liver injury during ischemia reperfusion [33] and partial hepatectomy [34]. The priming of neutrophils during this time may be an important factor for the later neutrophil-induced injury phase [11]. Activated neutrophils generate two major cytotoxic mediators, that is, reactive oxygen species and proteases [21]. In addition to the NADPH oxidase-derived superoxide and its dismutation product hydrogen peroxide, data from Tadashi Hasegawa and his co-workers provide a direct evidence for a specific neutrophil-mediated oxidant stress [hypo-chlorite (HOCl)-modified epitopes] during reperfusion when a relevant number of neutrophils have extravasated into the parenchyma from sinusoids [21]. HOCl, generated only from H_2O_2 and Cl^- by myeloperoxidase (MPO), can diffuse into hepatocytes and cause formation of chloramines, which are potent oxidants and cytotoxic agents involved in hepatocytes killing and responsible for maintaining the inflammatory response [35]. In addition, neutrophils store various proteases in granules and can release these proteolytic enzymes during activation.

Protease inhibitors are shown to attenuate neutrophil-induced liver injury [36]. Moreover, reactive oxygen species are indispensable for a protease-mediated injury mechanism under in vivo conditions. Therefore, accumulated neutrophils release oxidants and proteases that directly injure hepatocytes and vascular endothelial cells and may also obstruct hepatic sinusoids resulting in hepatic hypoperfusion [37]. During the second phase of reperfusion injury, neutrophils work as the most acute cytotoxic inflammatory cells activated and recruited, and the damage caused by neutrophils is recognized as a major mechanism of during reperfusion [24, 38, 39]. A recent study by Beraza et al. has suggested that the hepatic inflammatory response to IR is driven largely by NF-κB activation in hepatocytes [40]. Nuclear factor (NF)-κB is a broad term used to describe a number of dimeric combinations of proteins of the Rel family [41, 42]. In unstimulated cells, NF-κB is sequestered in the cytoplasm by inhibitors of κB (IκB) proteins which prevent nuclear localization of NF-κB by masking its nuclear localization signal peptide and block NF-κB from binding to DNA by allosteric inhibition [43]. Once is freed from IκB, NF-κB translocates to the nucleus where it initiates the transcription of target genes such as tumor necrosis factor-a (TNF-a), interleukin (IL)-1b, and IL-6 [24, 44, 45]. The NF-κB inhibitory protein A20 demonstrates hepatoprotective abilities through curtailing inflammation by inhibiting NF-κB activation [46]. On the contrary, A20 knockout mice are born cachectic and die within 3 weeks of birth as a result of unfettered liver inflammation [47]. Therefore, above the literature provides compelling evidence that inhibition of oxidant-stress and inflammation in hepatocytes during IR injury is an essential mechanism of protection.

3. Characteristics of PPARα

PPAR is originally identified by Issemann and Green [48] after screening the liver cDNA library with a cDNA sequence located in the highly conserved C domain of nuclear hormone receptors. A notability subtype of PPAR will be discussed here is PPARα. In human body, PPARα gene which spans ~93.2 κb is located on chromosome 22q12-q13.1 and encodes a protein of 468 amino acids [49]. While in mice, PPARα gene is located on chromosome 15E2, and it also encodes a protein of 468 amino acids [50]. PPARα contains four major functional domains, which are the N-terminal ligand-independent transactivation domain (A/B domain), the DNA binding domain (DBD or C domain), the cofactor docking domain (D domain), and the C-terminal E/F domain (including the ligand binding domain (LBD) and the ligand-dependent transactivation domain (AF-2 domain) [51, 52]. The divergent amino acid sequence in the LBD of PPARα is thought to provide the molecular basis for ligand selectivity. A large ligand-binding pocket (1300 Å) exists in PPARα, allowing diverse and structurally distinct compounds access to the LBD [51] and enabling PPARα to sense a broad range of endogenous substances, including fatty acids and their derivatives, or exogenous ligands, such as fibrates, Wy14643 [53, 54], and so on.

Analogous with several other nuclear hormone receptors, PPARα also is a ligand-activated transcription factor which upon heterodimerization with the retinoic X receptor (RXR), recognizes PPAR response elements (PPRE), located in the promoter of target genes [55]. PPARα is highly expressed in the liver, kidney, and heart muscle, which are all organs that possess high mitochondrial and β-oxidation activity. PPARα basically function, as sensor for fatty acid derivatives and controls essential metabolic pathways involved in lipid and energy metabolism, and it also plays a significant role in various pathophysiologic conditions, such as inflammation and apoptosis caused by injury [56]. Our group and others have demonstrated that PPARα is an important regulator of postischemic liver injury [3, 4].

4. Roles of PPARα on Warm Hepatic Ischemia-Reperfusion Injury

Reactive oxygen species and inflammation factors are critical mediators that exert a toxic effect during warm hepatic ischemia-reperfusion injury. Therefore, the most significant mechanisms of PPARα hepatoprotective abilities have been demonstrated through antioxidant stress and anti-inflammation functions (Figure 1).

4.1. Antioxidant Stress. Interruption of blood flow to liver and subsequent reperfusion lead to an acute oxidant stress response that may cause significant cellular damage and organ dysfunction. Hepatocellular injury during both the initial and later phases of reperfusion is caused in large part by reactive oxygen species including hypochlorite (HOCl) [10, 11, 25]. Accordingly, antioxidant therapy can limit the ischemia-reperfusion injury [57, 58]. The discovery of the colocalization of catalase with H_2O_2-generating oxidases in peroxisomes is the chief indication of their involvement in the metabolism of oxygen metabolites [59]. Peroxisomes, which are subcellular organelles within the hepatocyte, contain a battery of antioxidant enzymes and may help protect hepatocytes from oxidative damage. Catalase is the classical marker enzyme of peroxisomes metabolizing both H_2O_2 and a variety of substrates such as ethanol, methanol, phenol, and nitrites by peroxidatic activity [60], so it plays an important protective function against the toxic effects of peroxides and removes them with high efficiency [61]. PPARα stimulation by Wy14643 induces expression and activation of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione (GSH), which protects hepatocytes against hepatic IR injury mice model in vivo [3]. From the vitro experimental results of our group, the protective effect of Wy14643 is demonstrated herein by reducing ALT, AST, ROS levels and ameliorating ultrastructure alterations of hepatocytes; this protection is associated with an inhibition of oxidative stress and upregulation of hepatocytes PPARα-mRNA expression [62]. PPARα has also been implicated in the expression or activation of antioxidant enzymes such as catalase and Cu_2^+, Zn_2^+ superoxide dismutase (SOD1) [63]. Work done by Tetsuya Toyama has indicated that PPARa ligands (WY14643) play an antifibrotic action through

disrupting the vicious cycle between hepatic damage and oxidative stress by activating antioxidant enzymes such as catalase, and resolving the oxidative stress in the rat TAA model of liver cirrhosis [63]. The PPARα agonist is also highly effective in the treatment of dietary steatohepatitis in mice which results from the action of ROS on accumulated lipids and excessive formation of lipoperoxides in the liver [64]. Simultaneously, the improved antioxidant defense system after PPARα activation can thus additionally protect against neutrophil cytotoxicity, and the related mechanism can be concluded that formation of ROS plays a key role in leading to hepatocytes necrosis mediated by neutrophil. After mice with 90 min ischemia and 8 h reperfusion, Tomohisa Okaya and Alex B. Lentsch have suggested that PPAR$\alpha^{-/-}$ mice have augmented hepatocellular injury compared with wild-type mice, which is proved to be associated with a marked increase in the amount of neutrophils recruited to the liver, because at this time point much of the injury to hepatocytes is thought to be due to reactive oxygen species and proteases released from recruited neutrophils. On the contrary, treatment of C57BL/6 mice with 10 mg/kg iv WY-14643 1 h before ischemia resulted in a modest but significant reduction in hepatocellular injury [3]. Furthermore, antioxidants and other interventions directed toward detoxification of reactive oxygen species also attenuated inflammatory liver injury [12, 65–67]. The mechanism of PPARα response to ROS is much more complex that it requires further research. At the same time, the regulation of antioxidant enzymes is closely related to apoptosis signaling [68]. Therefore, elevation of anti-oxidative enzymes can suppress apoptosis and this can also promote carcinogenesis [69].

4.2. Anti-Inflammation. Neutrophils are recruited from the vascular space through a complex series of events that involve upregulated expression of cellular adhesion molecules on hepatic vascular endothelial cells and increased production of CXC chemokines [31, 70]. Accumulated neutrophils release oxidants and proteases that directly and drastically injure hepatocytes and vascular endothelial cells [24]. The first evidence indicating a potential role for PPARα in the inflammatory response is the demonstration that leukotriene B4 (LTB4), a proinflammatory eicosanoid, binds to PPARα and induces the transcription of genes involved in ω- and β-oxidation which leads to the induction of its own catabolism [71]. In this respect, the activation of PPARα by leukotriene B4 serves to limit the inflammatory process, providing a physiological mechanism to stop the damaging effects associated with inflammation [72]. Numerous recent studies have been aimed at delineating the cellular and molecular mechanisms explaining the control of the inflammatory response by PPARα in hepatic ischemia-reperfusion injury. Primarily according to previous studies by our group, the protective effects of PPARα agonists (WY14643) in postischemic liver injury are possibly associated with reductions in neutrophil accumulation, oxidative stress, and tumor necrosis factor (TNF) and interleukin-1 (IL-1) expression in livers during IR [4]. The results also have been supported by additional experimental results [38, 63]. Furthermore, works done by
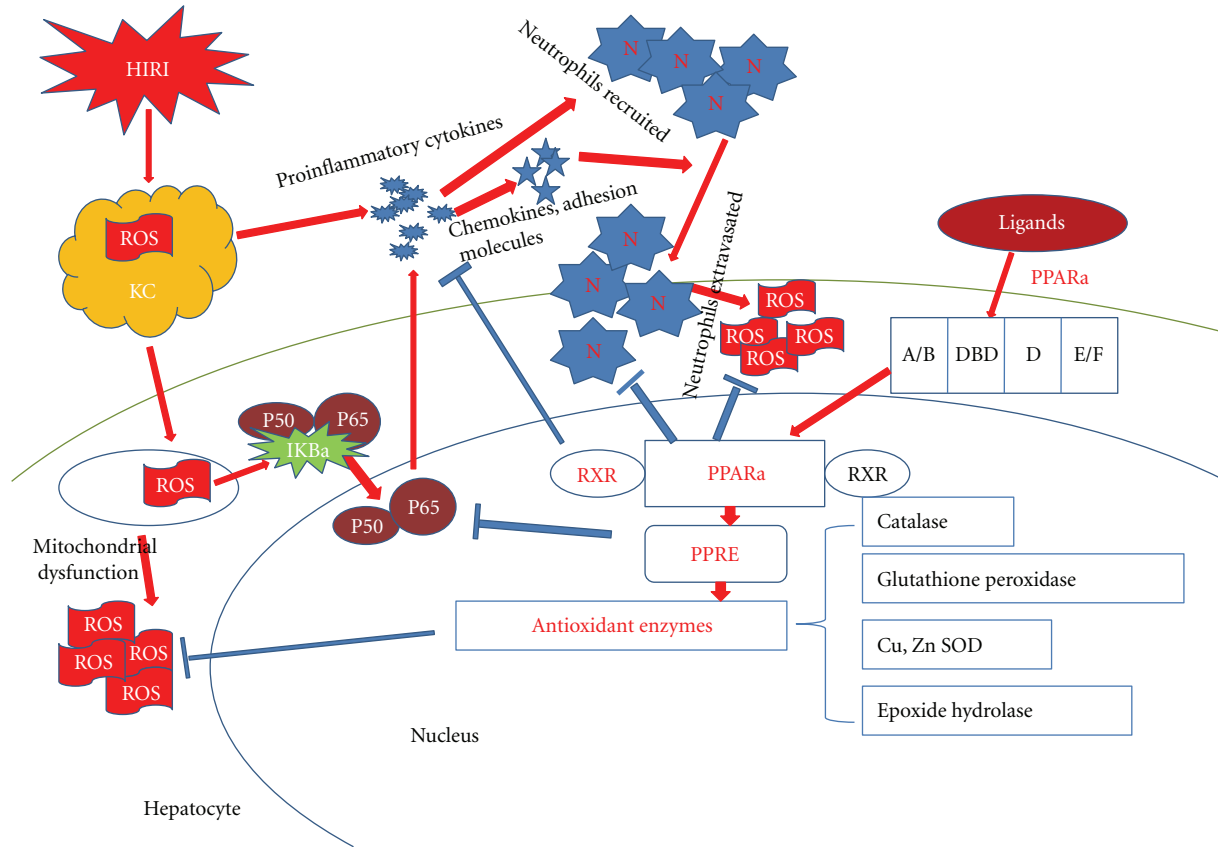
FIGURE 1: Protection mechanism of PPARα in the liver during IR injury. Ischemic stress results in the generation of reactive oxygen species (ROS) in Kupffer cells. ROS activates NF-κB and induces mitochondrial dysfunction in neighboring hepatocytes. Activation of NF-κB consequences in the production of proinflammatory cytokines, chemokines and adhesion molecules which can recruit neutrophils and propagate the inflammatory response. This vicious circle is broken by PPARα which is a ligand-activated transcription factor that upon heterodimerization with the retinoic X receptor (RXR), recognizes PPAR response elements (PPRE), located in the promoter of target genes. Abbreviations: Neutrophil (N), Kupffer cell (KC).

Tomohisa Okaya and Alex B. Lentsch suggest that livers from PPAR$\alpha^{-/-}$ mice have significantly more postischemic injury compared with those from wild-type mice. A possible reason may be the augmented liver neutrophil accumulation and the modest increases in activation of the transcription factor NF-κB. Treatment of cultured murine hepatocytes with WY-14643, a specific agonist of PPARα, protected cells against oxidant-induced injury. However, there are no differences in proinflammatory mediator production between PPAR$\alpha^{-/-}$ and wild-type mice. These data suggest that PPARα is an important regulator of the hepatic inflammatory response to ischemia reperfusion in a manner that is independent of proinflammatory cytokines [3]. What's more, evidence from in vitro experiments for an anti-inflammatory action of PPARα in endothelial cells and monocytes also demonstrate, that PPARα ligands inhibit cytokine-induced genes, such as expression of vascular cell adhesion molecule-1 and tissue factor by downregulating the transcription of these genes [73–75]. Researches address the molecular mechanisms of this anti-inflammatory action demonstrate that PPARα negatively regulate the transcription of inflammatory response genes by antagonizing the nuclear factor-κB (NF-κB) signaling pathway [76]. In addition to the antagonistic

action on NF-κB signalling, PPARα activators are known to induce inhibitor of NF-κB, IκB-α, in primary smooth muscle cells and hepatocytes, which is associated with reduced NF-κB DNA binding triggered by PPARa [58]. N-3 polyunsaturated fatty acids (n-3 PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are considered as PPARα agonists and can also decrease the expression of proinflammatory genes by preventing IκB phosphorylation and NF-κB translocation into the nucleus [77]. Recent studies have suggested that liver preconditioning against IR injury by n-3PUFA supplementation is mediated by PPARα diminishing NF-κB DNA binding through direct protein-protein interaction with NF-κB subunit p65, leading to the recovery of NF-κB signalling activity and reestablishment of inflammatory cytokine homeostasis [78]. The zinc finger protein A20 [79], which is an intracellular ubiquitin-editing enzyme, plays a significant role in the negative feedback regulation of NF-κB activation in response to a diverse range of stimuli [80]. In lethal liver ischemia-reperfusion injury model, the survival rate of mice treated with A20 reached 67% compared with 10%–25% of control mice injected with saline. This major survival advantage in A20-treated mice is associated with protecting against liver IR injury

by increasing hepatic expression of PPARα. A20-mediated protection of hepatocytes from hypoxia/reoxygenation and H$_2$O$_2$-mediated necrosis is reverted by pretreatment with the PPARα inhibitor MK886 [81]. However, the reverse evidence published by Yu et al. [82] reported that inhibition of NF-κB activation by A20 aggravated partial hepatic ischemia-reperfusion injury. According to this report, Xu et al. suggested that NF-κB inactivation in hepatocytes switches the TNF-α response from proliferation to apoptosis, so decreased NF-κB activity sensitizes hepatocytes to TNF-α-induced cytotoxicity and may contribute to increased liver dysfunction [83]. In nonalcoholic steatohepatitis (NASH) and simple steatosis, besides reducing steatosis by regulation lipid and lipoprotein metabolism, treatment of mice with the PPARα activator Wy14643 protects steatotic livers against IR injury, with the benefits of this treatment potentially occuring through dampening vascular cellular adhesion molecule-1 and cytokine responses and activation of NF-κB and IL-6 production [84]. The former contradictory results have suggested that the role of NF-κB activation during hepatic ischemia-reperfusion injury is controversial because it induces both protective and proinflammatory genes [85] and NF-κB inactivation either protects against hepatic IR injury [86–88] or aggravates such injury [45, 89, 90]. Studies done by Nozomu Sakai and Heather have indicated that activation of NF-κB in Kupffer cells promotes inflammation through cytokine expression, whereas activation in hepatocytes may be cell protective, based on the fact that they further proved that exogenous administration of receptor activator of NF-κB ligand (RANKL) reduces liver injury in a manner associated with increased hepatocyte NF-κB activation [91]. Supplementary works needed to be completed to explore how the PPARα plays a role in directing a clinical outcome which may lead to better prospects of more rational approaches to reduce postischemic liver injury. In addition to all of the mentioned above, PPARα is also a target of the hypothalamic hormone signaling as it plays an important role in the anti-inflammatory action of glucocorticoids [92].

5. Perspectives

As outlined in this paper, there is ample evidence for a critical involvement of oxidant-stress and inflammatory response in various animal models of hepatic ischemia-reperfusion injury. ROS are generated during initial reperfusion, where the initial cell damage triggers an inflammatory response with activation of tissue macrophages and recruitment of neutrophils both of them cause cell death and liver injury. Growing evidences for a role of PPARα in a variety of physiological and pathological processes, particularly the participation in the pathophysiology of inflammation and the protective role of hepatic ischemia-reperfusion injury via limiting oxidative injury as well as inhibiting inflammation response, has emerged in prevenient researches. However, species-specific differences in response to PPARα activators still exist between human and animals. Rats and mice are highly susceptible to peroxisome proliferation and are

susceptible to hepatocarcinogenesis due to the antiapoptosis of PPARα activators. Whereas, PPARα agonists are clinically and functionally relevant as fibrate therapeutics against hyperlipidemia and agents for reducing the complications of peripheral vascular disease in diabetic patients [93]. Yet, there are no sufficient evidence to show that hepatic cancer, hypertrophy, or peroxisome proliferation is relevant to it. On the contrary, PPARα receptor activation can interrupt the development process of chronic hepatitis C and NASH to liver cancer by regulating the lipid and lipoprotein metabolism and enhancing the antioxidant stress. This is because obesity-related metabolic abnormalities [94], especially insulin resistance, may be a decisive factor in the pathogenesis of chronic hepatitis C as recently suggested in nonalcoholic steatohepatitis (NASH), along with impairment in lipid metabolism [95]. And the second reason is that oxidative stress not only damages hepatocytes and increases the rate of hepatocyte death, but also inhibits the replication of mature hepatocytes [96]. In order to balance the decreased replication capacity of mature hepatocytes, hepatic progenitors accumulate in the liver; this abnormal regenerative process may contribute to Hepatocellular Carcinoma.

One crucial difference is that the level of expression of PPARα in the human liver is lower than that found in rats and mice [97]. It is also worth pointing out that long term exposure to these drugs can result in oxidative DNA damage, among other effects [92, 98]. So despite their potentially beneficial roles, PPARα agonists should be used judiciously.

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