“Hypothesis of Seven Balances”: Molecular Mechanisms behind Alcoholic Liver Diseases and Association with PPARα

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Abstract: “Hypothesis of Seven Balances”: Molecular Mechanisms behind Alcoholic Liver Diseases and Association with PPARα: Takashi Moriya, et al. Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine—Objectives: The purpose of this review to collate current leading scientific advances of molecular mechanisms in alcoholic liver diseases and to propose a working “hypothesis of seven balances” in relation to peroxisome proliferator activated receptor α (PPARα), which has important roles in fatty acid oxidation, oxidative stress, inflammatory responses, and possibly liver fibrosis. Methods: We conducted an extensive literature review of over a hundred publications and collated the findings with evidence generated in our laboratory. Results: Our research points to a working hypothesis of seven balances for alcoholic liver diseases consisting of: 1) ethanol oxidation balance in hepatocytes; 2) PPARα activities in liver; 3) fatty acid metabolism balance in hepatic mitochondria; 4) gastrointestinal response to ethanol, acetaldehyde and lipopolysaccharide (LPS); 5) Kupffer cells response to LPS, oxidative stress and inflammatory cytokines; 6) adiponectin levels in plasma interchangeably regulated by tumor necrosis factor-α (TNF-α); and 7) stellate cells response to all of the above promoting hepatic fibrosis. Cellular mechanisms behind alcoholic liver diseases reveal close temporal associations of PPARα, adiponectin, TNF-α, cellular inflammation, proliferation, and potentially fibrosis as illustrated in “the hypothesis of seven balances.” Conclusions: The regulation and adjustment of PPARα activation underlying the balance of molecular cascades might resolve the progression of alcoholic liver diseases by reducing oxidative stress and inflammatory effects induced by nuclear factor-xB as well as the associated adiponectin pathway. Further elucidation of these pathways would reveal exciting new prospects for treating alcoholic liver diseases and other related liver disorders. (J Occup Health 2009; 51: 391–403)

Key words: Adiponectin, Alcoholic liver diseases, Fibrosis, NF-κB, PPARα, TNF-α

Long-term intake and abuse of alcohol results in various liver abnormalities ranging from simple fatty liver or steatosis to steatohepatitis, fibrosis, cirrhosis and hepatocellular carcinoma1). Fatty liver is often undetected, is seldom fatal and is resolved within a few weeks stopping alcohol consumption1). However, alcohol abuse has become a social and clinical problem worldwide with nearly 20% of alcoholics developing fibrosis followed by cirrhosis, neither of which have an acceptable cure except liver transplantation2). In the United States, alcoholic liver disease patients are estimated to exceed 2 million persons3). Some patients with cirrhosis and superimposed alcoholic hepatitis have 65% mortality over a four-year period, with most of those deaths occurring in the first few months3). Furthermore, patients with similar backgrounds of occupation and lifestyle (for example, working men4), healthcare professionals4), or urban transit operators5)) tend to be more prone to developing alcoholic liver disease leading to alcohol-related deaths than others. To address these issues, new therapies for alcoholic liver diseases are in urgent need since no internationally approved treatment is available today3).

Chronic injury leading to liver diseases (such as hepatic inflammation, hepatitis, fibrosis and cirrhosis) occurs in response to various insults including alcohol abuse, drugs, metabolic diseases, viral hepatitis B and C, autoimmune attack of hepatocytes or bile duct epithelium, or congenital abnormalities6). Typically, injury is present for months to years before significant scar accumulates. Therefore, efforts to understand hepatic inflammation and fibrosis have primarily focused on events that lead to the early
accumulation of the scar in the hope of identifying therapeutic targets to hinder its progression \(^6\). In this review, we first summarize a comprehensive and holistic biological system into “seven balances” including current leading scientific advances in alcoholic liver diseases. Furthermore, this review describes the biological evidence associated with PPAR\(\alpha\) and hypothesizes a central and interactive role of PPAR\(\alpha\) within the “seven balances,” pointing to potential treatments for prevention of alcoholic liver disease progression.

**Molecular Mechanisms behind Alcoholic Fatty Liver and Progression to Inflammation and Fibrosis**

**Overview of alcohol effects in human and animals**

When human or animals consume alcohol, it becomes a substantial source of energy for the body. This is simply because ethanol has 7.1 kcal (29.7 kJ) per gram of energy content, an amount that exceeds that of carbohydrates or proteins\(^7\). Since the average alcoholic patient takes half of his/her calories from ethanol, it disturbs normal nutrient intake leading to malnutrition with deficiencies of folate, thiamine and other vitamins\(^7\). Secondary malnutrition also occurs from malabsorption due to pancreatic insufficiency and impaired hepatic metabolism of nutrients and vitamins\(^7,8\). Because malnutrition causes liver damage, some thought alcohol-induced liver disease, namely alcoholic fatty liver, could be prevented through nutritional therapy with enriched diets. However, this regimen did not prevent alcoholic fatty liver, which simply raised the question of the direct toxic effect of alcohol on the liver independent of malnutrition\(^7\). Through further investigations, Lieber and others established that even with an adequate diet, alcohol at a blood concentration that doesn’t induce intoxication in animal models can induce fatty liver\(^9\). A series of following studies proved that alcohol metabolism in the liver, particularly alcohol oxidation, is the key driver for fatty liver together with its direct toxicity\(^10,11\).

**Hepatic alcohol oxidation—The initial source of adverse effects in liver**

Oxidation of ethanol mediated by alcohol dehydrogenase (ADH) produces acetaldehyde, a toxic and reactive metabolite\(^7,12\). Acetaldehyde is further converted to a nontoxic form, acetic acid or acetate, by aldehyde dehydrogenase (ALDH)\(^7,12\). Both reactions reduce nicotinamide adenine dinucleotide (NAD\(^+\)) to its reduced form (NADH) (Fig. 1A. and D.)\(^7,12\). Another pathway of ethanol metabolism, the microsomal ethanol oxidizing system (MEOS), was shown to play an important role in the progression of liver diseases in baboons in 1974\(^13\). In MEOS, CYP2E1 is the primary enzyme involved in ethanol oxidation (Fig. 1B.)\(^7,14\). CYP2E1 was induced by ethanol with a corresponding 4- to 10-fold rise in mRNA in liver biopsy samples obtained from subjects who had recently drunk alcohol\(^15,16\). CYP1A2 and CYP3A4 were identified as additional players in MEOS enzymes in further studies (Fig. 1B.)\(^17\). Also, the combination of hydrogen peroxide generation from the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and catalase was revealed to account for microsomal ethanol oxidation in other prior studies (Fig. 1C.)\(^18,19\). In summary, the three ethanol oxidation pathways described above mainly generate acetaldehyde that causes further liver injury and
toxicity\textsuperscript{7, 12}.

The acetaldehyde derived from ethanol oxidation reactions has an impact on PPAR\textsubscript{α} activity. Acetaldehyde not only inhibits PPAR\textsubscript{α} activation of receptor genes but also directly impairs the ability of PPAR\textsubscript{α} and PPAR\textsubscript{α}/
retinoid X receptor (RXR) to bind to DNA\textsuperscript{20, 21}. Since the expression of PPAR\textsubscript{α}-regulated genes does not affect the activity of ADH and ALDH, the generation of acetaldehyde from ethanol oxidation reactions is further facilitated\textsuperscript{22}. This effect of acetaldehyde and ethanol retards homeostatic responses of hepatocytes to free fatty acids (FFA) regulated by PPAR\textsubscript{α}. Together with excess NADH formation inhibiting fatty acid oxidation, the effect of acetaldehyde and ethanol on PPAR\textsubscript{α} begins exacerbating a cascade of liver injury and toxicity which is discussed in the following sections.

**Fatty acid oxidation and oxidative stress—the important link of alcoholic liver diseases progression**

As a result of ethanol consumption and its oxidation, NADH is excessively produced in hepatocytes causing various metabolic disorders, such as inhibition of the Krebs cycle and of fatty acid oxidation\textsuperscript{8}. This suppression of fatty acid oxidation primarily drives steatosis by enhancing the synthesis of fatty acids\textsuperscript{7}. The impairment of PPAR\textsubscript{α} functions in hepatic homeostatic responses to acetaldehyde and ethanol underlies and aggravates fatty acids’ synthesis further\textsuperscript{20, 21}.

Fatty acids are a substrate of CYP2E1, and CYP2E1 activity induced by fatty acids is a major source of free radicals or reactive oxygen species (ROS) causing oxidative stress in the liver (Fig. IB.).\textsuperscript{5, 12, 14, 16} While different endogenous and exogenous sources of ROS exist\textsuperscript{22}, CYP2E1 releases oxygen radicals as a part of its normal operation. Two major roles of CYP2E1 are: 1) detoxification of xenobiotics and 2) glucose generation with ketone, which is derived from fatty acids, under fasting conditions\textsuperscript{7}. With excess ethanol and ketone supplied by fatty acids’ synthesis that is promoted by excess NADH, this defense mechanism of CYP2E1 inclines to adverse consequences—oxidative stress\textsuperscript{7}. Recent studies have uncovered subsequent cellular signaling associated with alcohol-induced oxidative stress\textsuperscript{13, 21}, although they are beyond the scope of this review.

The CYP2E1-induced increase in oxidative stress has implications for PPAR\textsubscript{α} activity. PPAR\textsubscript{α} activation is known to promote the expression of anti-oxidative molecules, such as catalase\textsuperscript{24} and Cu\textsuperscript{2+}, Zn\textsuperscript{2+}-superoxide dismutase (SODI)\textsuperscript{25}. However, acetaldehyde inhibits the intranuclear activity of PPAR\textsubscript{α}\textsuperscript{20, 21}, and the protective role of PPAR\textsubscript{α} against oxidative stress is consequently lost. Hence, oxidative stress becomes overwhelming and damages mitochondria in hepatocytes\textsuperscript{7, 14}. Liver damage is aggravated by depletion of mitochondrial glutathione due to acetaldehyde binding effects\textsuperscript{7, 14}, and in turn, this mitochondrial damage becomes a key component of alcohol-induced liver injury\textsuperscript{8, 14}. This interactive relationship among impairment of fatty acid oxidation, loss of PPAR\textsubscript{α}’s protective roles against oxidative stress, and increase of oxidative stress facilitated by CYP2E1 induction is a critical phenomenon that exacerbates liver disease progression.

**Intestinal endotoxemia—emerging cofactor of alcoholic liver diseases progression**

Gut-derived bacterial LPS or endotoxin has now been implicated as an important cofactor in the progression of alcohol-induced liver injury. In patients with alcoholic liver diseases, plasma endotoxin levels are high compared with those in normal subjects\textsuperscript{36–39} and patients with nonalcoholic cirrhosis\textsuperscript{41}. Human serum concentrations of TNF-\textalpha\textsuperscript{32, 33} and other TNF-\textalpha inducible cytokines, interleukin (IL)-1\textalpha, IL-6\textsuperscript{35} and IL-8\textsuperscript{36}, are initially enhanced in hospitalized patients with alcoholic steatohepatitis (ASH) and decrease later during recovery. Another study of patients with alcoholic liver diseases also showed a high correlation of serum levels of both TNF-\textalpha and soluble TNF receptors with degree of endotoxemia and stage of liver disease\textsuperscript{36}. In fact, patients with the highest serum cytokine concentrations showed the highest rates of in-hospital mortality\textsuperscript{32, 35}. Increased intestinal permeability from chronic ethanol exposure\textsuperscript{31, 37} must underlie such events. Indeed, gastrointestinal permeability to macromolecules is much higher in alcoholic patients than in normal healthy subjects and persists after two weeks of sobriety\textsuperscript{38, 39}. These aforementioned studies of human patients suggest that intestinally derived endotoxin and endotoxin-induced cytokines, such as TNF-\textalpha and others, are associated with the pathogenesis of steatohepatitis and alcoholic liver diseases.

Animal experimentations also support this notion since increased concentrations of plasma endotoxin is associated with alcohol-induced hepatitis in rats\textsuperscript{40, 41}. Many studies using animal models\textsuperscript{40, 42–45} have revealed that chronic and acute administration of ethanol increases gastrointestinal permeability to macromolecular markers (mannitol, lactulose, polyethylene-glycol, dextran and LPS). Close temporal associations among Kupffer cell activation, increased transcription of TNF-\textalpha and related cytokine promoter genes, hepatic inflammation, and liver cell death have also been reported\textsuperscript{37, 46}. In addition, treatments with poorly absorbed antibiotics and rats with intestinal sterilization showed virtually no growth of gram-negative bacteria in the intestinal lumen and alcohol-induced liver injury was prevented\textsuperscript{47, 48}, indicating gut-derived endotoxins play a role in such injury.

Furthermore, mounting evidence suggests that ethanol is oxidized into acetaldehyde in the gastrointestinal tract.
as illustrated in a review by Rao et al. in 2004\textsuperscript{50}, and acetaldehyde in “the gut” may also contribute to alcohol-related diseases\textsuperscript{49–51}. Alcohol ingested orally is transported to the colon by blood circulation and, after the distribution phase, intralocular ethanol reaches the same level as that in the blood\textsuperscript{49, 50}. A large amount of bacterial acetaldehyde production occurs in the gastrointestinal tract since it is the most richly bacterially colonized part of the human body\textsuperscript{50}.

In relation to this evidence, acetaldehyde has been shown to disrupt the intestinal epithelial tight junction and to enhance paracellular permeability by promoting redistribution of proteins specifically localized at tight junction\textsuperscript{52}. While detailed molecular mechanisms are discussed in various articles\textsuperscript{50, 53}, acetaldehyde modifies intracellular signal-transduction pathways to destabilize the tight junction protein complex leading to increased permeability to endotoxins\textsuperscript{50}. Generation and accumulation of acetaldehyde in the intestinal lumen may also play a crucial role in the onset of a cascade of cellular responses that ultimately lead to endotoxemia and liver injury\textsuperscript{30}. Therefore, the relationship among ethanol, acetaldehyde, LPS and Kupffer cells sensitization and activation facilitates the progression of the alcoholic liver diseases.

In relation to PPAR\textsubscript{\(\alpha\)}, it has not yet been elucidated whether or not accumulated acetaldehyde in the intestinal lumen is partially released to normal circulation, thereby reaching the liver. However, it is reasonable to expect that accumulated acetaldehyde in “the gut” is partially released into normal circulation due to increased intestinal permeability, thereby reaching the liver as a similar process occurs with LPS. If this were true, acetaldehyde originated from the gastrointestinal tract could partly inhibit the activities of PPAR\textsubscript{\(\alpha\)} in hepatocytes as described above\textsuperscript{20, 21}. Furthermore, it is important to stress here that gut-derived LPS reaching the liver cause Kupffer cell sensitization leading to production of TNF-\(\alpha\) and other cytokines known to be proinflammatory\textsuperscript{51}. This introduces PPAR\textsubscript{\(\alpha\)} to the progression of alcoholic liver diseases, since PPAR\textsubscript{\(\alpha\)} has well-known anti-inflammatory roles against cytokine-induced inflammation\textsuperscript{1, 22, 54–58}. Because the anti-inflammatory activity of PPAR\textsubscript{\(\alpha\)} is inhibited by acetaldehyde and ethanol, PPAR\textsubscript{\(\alpha\)} becomes a key factor in the progression of alcoholic liver as discussed in the following sections.

**Hepatic inflammation—a consequence of gut-derived endotoxins and oxidative stress**

Oxidative stress in the liver facilitates inflammation, which is exacerbated by a rise of proinflammatory cytokines (mainly TNF-\(\alpha\)) and reactive metabolites of oxygen (mainly superoxide) that are largely released by sensitized and activated Kupffer cells\textsuperscript{2, 7, 59, 60}. Although there is a difficulty in detecting ROS with very short lifetime in live-patients at the clinic, the notion of Kupffer cells’ response to LPS as generating cytokines and ROS is supported by the evidence that human serum concentrations of TNF-\(\alpha\)\textsuperscript{2, 33}, IL-1\(\beta\)\textsuperscript{14}, IL-6\textsuperscript{55} and IL-8\textsuperscript{56} are initially enhanced in hospitalized patients with ASH. A significant elevation of TNF-\(\alpha\), IL-1\(\beta\), and IL-6 is also observed in the fetus and pregnant mother when the mother has drunk moderate to heavy (chronic) amounts of alcohol during her pregnancy\textsuperscript{51}. Clinical data for patients suffering from chronic alcohol abuse further suggest LPS-stimulated production of ROS in Kupffer cells\textsuperscript{28, 62}.

In animal models, it is now clear that Kupffer cells are activated during early alcohol-induced liver injury, and the activation process is facilitated by gut-derived endotoxin\textsuperscript{1, 2, 53, 62–64}. First, LPS in the hepatic sinusoid binds to LPS binding protein (LPSB), which promotes the binding of LPS to its cell-surface protein receptor, CD14, which is found on mononuclear cells, including Kupffer cells\textsuperscript{2, 62, 65}. Next, LPS is transferred to the transmembrane signaling receptor toll-like receptor 4 (TLR4) and initiates various intracellular signaling pathways including protein kinase C (PKC), tyrosine kinases, NF-\(\kappa\)B and mitogen activated protein kinase (MAPK) family members in Kupffer cells\textsuperscript{2, 62, 65, 66}. While the signaling pathways of LPS and Kupffer cells and the temporal relationships with cytokines and ROS generation are rather complex, a number of studies have suggested that chronic ethanol exposure increases LPS-stimulated TNF-\(\alpha\), ROS, and NF-\(\kappa\)B generation as well as stimulating other signaling pathways modulated by Kupffer cells\textsuperscript{1, 2, 53, 60, 62–70}.

Among several cytokines in the liver, TNF-\(\alpha\) in particular has emerged as an important factor in pathophysiology of ASH\textsuperscript{1, 2, 53, 62}. TNF-\(\alpha\) is a pleiotropic regulatory peptide primarily existing as type II transmembrane protein, and large amounts of TNF-\(\alpha\) and related IL-1 and IL-6 cytokines are generated in response to physiologic and pathologic stimuli, such as LPS or other endotoxins\textsuperscript{1, 7, 8, 53, 59, 60, 68}. In mice models lacking TNF receptor 1 (TNF-R1, p55) or 2 (TNF-R2, p75), exposure to alcohol did not induce steatohepatitis in TNF-R1 knockout mice whereas it did in TNF-R2 knockout and wild-type mice, suggesting TNF-\(\alpha\) facilitates the development of early alcohol-induced liver injury via the TNF-R1 pathway\textsuperscript{71}. This constitutes good evidence that TNF-\(\alpha\) is a key pathogenic factor in alcohol-induced liver injury\textsuperscript{1, 71} regardless of the cause or source of TNF-\(\alpha\) production.

Other studies support this notion. Treatment of rats with TNF-\(\alpha\) antibody\textsuperscript{72} or agents such as poorly absorbed antibiotics\textsuperscript{73} or lactobacillus\textsuperscript{73}, that attenuates hepatic inflammation in mice liver induced by chronic ethanol exposure, prevented alcohol-induced liver injury. While TNF-\(\alpha\) and other IL-1 and IL-6 cytokines are produced
by lymphoid cells, mast cells, endothelial cells, fibroblasts, and neuronal cells. TNF-α produced by activated Kupffer cells plays a critical role not only in the production of inflammatory mediators, apoptosis and necrosis, cholestasis and fibrosis, but also in inducing hepatocyte proliferation and liver tissue regeneration. While there is a balance between proinflammatory and anti-inflammatory cytokine effects, chronic alcohol intake inclines it toward the proinflammatory axis, overwhelming anti-inflammatory effects which become unable to control hepatic inflammation and disease progression. Associations with proinflammatory cytokines and PPARα will be further discussed in the following sections.

Role of Adiponectin—emerging link with hepatic inflammation

Adiponectin is a hormone secreted by adipocytes or adipose tissues found beneath the skin, around the kidney and liver, and on the surface of the heart. It is also known as 30-kDa adipocyte complement-related protein (Acrp30). A small amount of globular adiponectin (gAd) circulates in plasma since full-length adiponectin undergoes proteolytic processing. Decreased circulating adiponectin concentrations have been revealed to be associated with various disease states, such as obesity, type II diabetes, atherosclerosis, inflammation, and animal models of alcoholic and non-alcoholic liver diseases. The levels of adiponectin concentration are closely tied to adipocyte differentiation and hypertrophy, and these are regulated by peroxisome proliferators-activated receptor γ (PPARγ) and other factors. In clinical settings, the results of a recent study support a relationship linking adiponectin, alcohol consumption and inflammatory cytokine TNF-α levels. In that study, moderate alcohol intake significantly increased plasma adiponectin concentrations both in healthy and in relatively insulin-resistant middle-aged men without affecting their plasma TNF-α concentrations.

Many animal studies have disclosed a complex mechanism involving interactions among adiponectin and other mediators. Two adiponectin receptors, AdipoR1 and AdipoR2, were cloned in 2003. AdipoR1 has a high affinity to globular adiponectin and a low affinity to full-length adiponectin, whereas AdipoR2 has an intermediate affinity to both forms of adiponectin. AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver. This means only full-length adiponectin is active in the liver, suggesting it may play a key role in liver disease. Another study has shown that the effects of adiponectin are largely facilitated by an increase in fatty acid oxidation associated with activation of AMP-activated protein kinase (AMPK) and PPARα pathways downstream of adiponectin receptors in the liver.

In March 2007, Yamauchi et al. revealed a strong association between AMPK activation and PPARα signaling pathways that are downstream of AdipoR1 and AdipoR2 in the liver, respectively. This brings us to an important relationship linking ethanol, adiponectin, TNF-α and PPARα. In 2003, Xu and others showed chronic consumption of a high-fat, ethanol-containing diet significantly reduced circulatory adiponectin concentrations by 30–40% after three to four weeks, and the decreased adiponectin levels closely correlated with the development of liver injury in mice. Treatment of these mice with recombinant adiponectin reduced hepatomegaly and steatosis (fatty liver) as well as significantly attenuating inflammation and elevating levels of serum alanine aminotransferase. Although chronic ethanol feeding significantly decreased the rate of hepatic fatty acid oxidation and reduced the activities of carnitine palmitoyltransferase (CPT), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), the adiponectin treatment restored all of these activities. This result suggests down-regulation of hepatic fatty acid oxidation during chronic ethanol intake is caused by the suppression of adiponectin levels by ethanol. Other studies have demonstrated similar results, that adiponectin activates PPARα and inhibits sterol regulatory element binding protein-1 (SREBP-1), suggesting the effect of adiponectin on alcoholic fatty liver is mediated by regulation of transcription factors controlling fatty acid synthesis and breakdown.

It is well known that adiponectin and TNF-α regulate each other’s production and antagonize each other’s biological effects in their target tissues. Interestingly, both hepatic expression and circulating levels of TNF-α increase after ethanol feeding, and adiponectin treatment suppresses the hepatic production of TNF-α as well as its plasma concentrations. Hence, ethanol could suppress adiponectin through its activation of TNF-α although humans may exhibit a slightly different response as suggested from the clinical evidence presented above. Although the protective role of adiponectin against liver injury has been clearly demonstrated, it could involve multiple mechanisms including antifibrogenic effects mediated by inhibition of hepatic stellate cell activation and fibrogenic cytokine secretion. Nevertheless, the anti-inflammatory effects of adiponectin through decreased TNF-α expression and its apparent relationship with PPARα bring a new insight to the complex regulation of the alcoholic and non-alcoholic liver disease progression.

Molecular control and cellular response of hepatic stellate cells upon liver injury

The normal liver contains hepatocytes, epithelial components and endothelial linings, which are...
distinguished by fenestrae or pores in the liver. It also contains tissue macrophages, such as Kupffer cells, and perivascular mesenchymal cells called stellate cells (formally known as Ito cells, lipocytes, perisinusoidal cells or fat-storing cells)\(^\text{80}\). Stellate cells are the primary fibrogenic cells and comprise 15% of the total number of resident liver cells\(^\text{81}\). In a normal liver, they act as a primary store for retinoid like vitamin A\(^\text{92}\). Stellate cells also produce extracellular matrix (ECM)\(^\text{89}\).

Following liver injury of any etiology, hepatic stellate cells undergo a cellular response known as “activation,” in which quiescent cells transform into proliferative, fibrogenic, and contractile myofibroblasts\(^\text{89}\). Stellate cell activation is a remarkably pleiotropic yet tightly programmed response occurring in a reproducible sequence\(^\text{41}\). Conceptually, its activation occurs in two phases, initiation and perpetuation, followed by resolution when the liver injury has been repaired\(^\text{41}\). These rather complex phenomena were described in an article by Friedman\(^\text{41}\), and he has illustrated it very clearly in his early review in 2000\(^\text{6}\). For the progression of liver disease from the inflammatory to the fibrogenic phase, TGF-\(\beta\) generated by Kupffer cells\(^\text{1}\) is considered a major facilitating factor. Therefore, the activity of hepatic stellate cells and their relationship with Kupffer cells are an important bridge between inflammation and fibrogenesis in the liver diseases.

Because PPAR\(\alpha\) mRNA is expressed neither in rodents and nor human stellate cells\(^\text{57}\), it has been difficult to establish a direct relationship between hepatic stellate cells and PPAR\(\alpha\). But, PPAR\(\gamma\) is known to be expressed in quiescent hepatic stellate cells, and its expression diminishes with the activation of hepatic stellate cells\(^\text{82}\). In addition, upregulation of PPAR\(\gamma\) and other adipogenic transcription factors, such as CCAAT/enhancer binding protein (C/EBP)\(\alpha\), liver X receptor \(\alpha\) (LXR\(\alpha\)) and sterol regulatory element-binding protein-1c (SREBP-1c), at hepatocytes are known to contribute and are believed to be required for the pathogenesis of fatty liver\(^\text{82}\). As alcohol-induced liver disease (including steatosis-fatty liver) progresses in hepatocytes with PPAR\(\gamma\) expression\(^\text{82}\), the same PPAR\(\gamma\) expression in hepatic stellate cells acts as a switch to transform them from their quiescence state to more proliferative and fibrogenic myofibroblasts\(^\text{6}\)\(^\text{84}\). While biological relationship of PPAR\(\gamma\) looks “paradoxical” as Tsukamoto said\(^\text{55}\), the change in PPAR\(\gamma\) expression may be caused by inflammatory cytokines generated in upstream hepatic responses. PPAR\(\gamma\) may be a factor linking steatosis and fibrosis although further research into the pathophysiological process is necessary. Moreover, stellate cells’ proliferation would be amplified by upstream events, such as enhanced oxidative stress caused by ethanol and acetaldehyde-inhibition of PPAR\(\alpha\)\(^\text{20}\)\(^\text{21}\), as well as Kupffer cell-derived TNF-\(\alpha\) in response to LPS and oxidative stress\(^\text{28}\)\(^\text{32}\)\(^\text{33}\)\(^\text{62}\). Thus, the response of hepatic stellate cells must partly occur as a downstream consequence of PPAR\(\alpha\) retardation in hepatocytes. Accordingly, PPAR\(\alpha\) activity should be considered as a factor influencing the biological state of stellate cells within the overall pathogenesis of hepatocytes.

**Discussion**

*Importance of inflammation and its relationship with PPAR\(\alpha\) in alcoholic liver disease progression*

In recent years, a link between the signal transduction of TNF-\(\alpha\) and NF-\(\kappa\)B, a heterodimer of p65 and p50, has been demonstrated. This conceptually advances our knowledge of not only the early stage of fatty liver disease but also the more advanced stages of liver injury due to regulation of hepatic stellate cell survival\(^\text{1}\)\(^\text{60}\)\(^\text{95}\). The involvement of tumor necrosis factor/nuclear factor-\(\kappa\)B (TNF/ NF-\(\kappa\)B) pathway is believed to play an key role in human liver disease, according to the evaluation of liver specimens taken from patients\(^\text{52}\)\(^\text{96}\)\(^\text{98}\).

In animal experiments, TNF-\(\alpha\) exerts its biological functions via interactions with two cognate membrane receptors, TNF-R1 (p55) and TNF-R2 (p75)\(^\text{1}\)\(^\text{60}\)\(^\text{95}\)\(^\text{99}\). TNF-\(\alpha\) acts as a potent activator of both proinflammatory and proapoptotic pathways at several levels in a complex network (Fig. 2.). While TNF-R1 is efficiently activated by soluble TNF-\(\alpha\) (sTNF-\(\alpha\), TNF-R2 activation requires the binding of membrane-bound TNF-\(\alpha\) (mTNF-\(\alpha\))\(^\text{59}\)\(^\text{60}\). After TNF-\(\alpha\) binding, TNF receptors undergo a conformational change allowing them to recruit adapter molecules (such as TNF receptor-associated protein with death domain (TRADD), TNF receptor-associated factor (TRAF2) and receptor-interacting kinase (RIP) that subsequently initiate the activation of intracellular signaling pathways (Fig. 2.). One of the pathways that activates inhibitor of NF-\(\kappa\)B kinase (IKK) leads to \(\kappa\)-B degradation and NF-\(\kappa\)B activation, exerting anti-inflammatory effects in the liver (Fig. 2.). Another pathway that activates c-Jun N-terminal kinase (JNK) eventually shifts the balance of hepatocytes toward cell death by inducing phosphorylation of E3 ligase (Ihch) and subsequent ubiquitination and degradation of the NF-\(\kappa\)B-regulated caspase 8 inhibitor (c-Flip) (Fig. 2.)\(^\text{59}\)\(^\text{60}\).

The JNK pathway leading to hepatocyte apoptosis can be a profibrogenic stimulus according to the study conducted by Ali Canbay, Scott Friedman and Gregory Gores\(^\text{100}\). Their observations clearly show the engulfment of apoptotic bodies, which were generated by hepatocyte apoptosis\(^\text{100}\), by stellate cells, leading to a fibrogenic response by elicitation of a kinase-signaling pathway\(^\text{100}\)\(^\text{102}\). These mechanisms together with other evidence show the importance of TNF-\(\alpha\) in the progression of hepatic inflammation and fibrosis.

Interestingly, the JNK pathway described above is also considered to be an important cascade in regulating
stellate cell survival\(^{60, 95, 103, 104}\). This cascade was disclosed by the role of NF-\(\kappa\)B while conducting investigations into the mechanism of the action of gliotoxin, a fungal metabolite that selectively facilitates stellate cell apoptosis, and is related to increased mitochondrial depolarization\(^{60, 103, 105, 106}\). Some pharmacological agents, including gliotoxin, sulfasalazine and specific NF-\(\kappa\)B antagonists (e.g. NF-\(\kappa\)B essential modulator (NEMO) and IKK inhibitors), are known to inhibit IKK and down-regulate NF-\(\kappa\)B activity followed by I\(\kappa\)B activation (Fig. 2.). Although NF-\(\kappa\)B has both proinflammatory and anti-apoptotic roles through the induction of inflammatory genes in activated stellate cells and of anti-apoptotic genes found in stellate cells, the apoptosis of hepatic stellate cells mediated by NF-\(\kappa\)B was shown to accelerate and reverse experimental fibrosis through clearance of activated stellate cells by its apoptosis\(^{103, 107}\). This evidence clearly suggests that NF-\(\kappa\)B plays an important role in the progression of liver disease.

The recent conceptual advance in understanding of the liver’s inflammatory response, which we have associated with TNF-\(\alpha\), NF-\(\kappa\)B, adiponectin, LPS, Kupffer cells and stellate cells, described above brings an interesting insight into the relationship between liver injury and with PPAR\(\alpha\), which has well known roles in the modulation of inflammatory responses, control of lipid homeostasis and metabolism of bioactive molecules\(^{22}\). In 2004, we presented studies that clarified the important relationship between the tumor necrosis factor/nuclear factor-\(\kappa\)B (TNF/ NF-\(\kappa\)B) pathway and PPAR\(\alpha\)\(^{22, 108}\), which had been suggested by other research groups earlier\(^{109–111}\). With the use of PPAR\(\alpha\) knockout mice, we demonstrated that those knockout mice fed a diet containing 4\% ethanol for six months showed remarkable hepatomegaly, hepatic inflammation, cell toxicity, fibrosis, apoptosis, and mitochondrial swelling\(^{22}\). PPAR\(\alpha\)-null mice particularly showed distinct molecular mechanisms through changes in ethanol and acetaldehyde metabolism, oxidative stress, inflammation, hepatocyte proliferation, fibrosis, and mitochondrial permeability transition activation\(^{22}\). These findings can be explained by two molecular mechanisms.

Fig. 2. Working schematic representation illustrating cell-signaling pathways involved in hepatic inflammation, proliferation, apoptosis, and PPAR\(\alpha\)'s transactivation and transrepression in hepatocytes.
of PPARα, transactivation and transrepression which were suggested in 1998 and 2000, respectively.

Transactivation of PPARα involves a direct inhibitory interaction with p65, one of two components of NF-κB, on NF-κB transcription with the introduction of a co-activator. Transrepression of PPARα means indirect inhibition of NF-κB transcription through induction of IκBα, which binds to NF-κB, a heterodimer of p65 and p50, interfering with translocation into the nucleus. Taken together with the evidence provided in previous sections, it led to our working hypothesis depicted in our Nakajima’s review in 2005.

We here summarize recent scientific understanding and advances in knowledge concerning seven critical components that play roles in alcoholic liver disease and its pathogenesis at molecular levels: 1) ethanol oxidation balance in hepatocytes; 2) PPARα activities in liver; 3) fatty acid metabolism balance in hepatic mitochondria; 4) gastrointestinal response to ethanol, acetaldehyde and LPS; 5) Kupffer cells response to LPS, oxidative stress and inflammatory cytokines; 6) adiponectin levels in plasma that are interchangeably regulated by TNF-α; and 7) stellate cells response to all of the above promoting hepatic fibrosis (Fig. 3.). From the various studies cited above, the activity of PPARα evidently affects and acts...
as an initial and central regulator leading to the other "six balances" either through proliferating or diminishing responses to ethanol. Key initiation factors were the oxidative stress caused by ethanol oxidation and ethanol and acetaldehyde-inhibition of PPARα. Oxidative stress in the liver must be the root-cause of alcoholic liver diseases. Therefore, the activation of PPARα in a well-controlled fashion, possibly with a selective and mild agonist, might resolve the root-causes of alcoholic liver diseases. Secondly, because PPARα is now considered to locate downstream of TNFα/ adiponectine signaling pathways through AdipoR2 in hepatocytes, and its activation by adiponectin exerts anti-inflammatory effects through inhibition of NF-κB in hepatocytes, the regulation of PPARα could prevent progression of liver injury and diseases. Finally, if the inhibition of NF-κB in hepatocytes were to promote apoptosis of hepatic stellate cells, it could also retard or possibly reverse liver fibrosis with PPARα activity acting as "a balance." All of these discussion above, therefore, led to our working "hypothesis of seven balances" (Fig. 3.) illustrating a holistic view of biology in animal and human homeostasis.

The study of alcoholic liver diseases and the biological roles of PPARα over the last 20 yr has uncovered many potential, novel therapeutic targets. With great advances in molecular biology, it seems we have plenty of targets to work on, and one of them is PPARα, although no good treatment is yet available for alcoholic liver diseases. We need to develop clinically useful products from the recent, vast accumulation of information in the field of biochemistry. Over the last decade, intensive efforts have been made by academia and the pharmaceutical industry for developing clinically useful products.

For alcoholic liver diseases, many academic institutions have attempted to develop beneficial treatments. For example, S-adenosylmethionine (SAMe) has shown efficacy against alcoholic liver diseases in both animal tests and clinical trials, including chronic active hepatitis and cirrhosis. Moreover, polyenylphosphatidylcholine (PPC) has been shown to prevent liver fibrosis by retarding stellate cell activation in both in vitro and in vivo experiments although PPC treatment did not affect progression of liver fibrosis in a two-year clinical trial. Some antioxidants such as alpha-tocopherol have been found to be effective in rodents, but have not produced good outcomes in human patients. While various efforts have been made by academia, a well-established clinical treatment for alcoholic liver diseases is not yet available.

On the other hand, by virtue of scientific advances, many molecular-target drugs, such as PPAR agonists and others, are being developed by the pharmaceutical industry particularly for cardiovascular and metabolic diseases. According to the Food and Drug Administration (FDA), 50 investigational new drugs (INDs) have been filed as agonists of the PPAR, including dual- and pan-agonists (details at http://www.fda.gov/cder/present/ DIA2006/). Fenofibrate, gemfibrozil and clofibrate are well known PPARα agonists that reduce low-density lipoprotein (LDL), very low density lipoprotein (VLDL), and cholesterol levels in blood, and are already available in the market. A few new drugs such as pioglitazone (Actos® developed by Takeda) and rosiglitazone (Avandia® developed by GlaxoSmithKline) are PPARγ agonists available for the treatment of diabetes mellitus type 2. A highly potent and selective PPARα agonist, LY518674, advanced to Phase-2 clinical studies for dyslipidemia and hypercholesterolemia in 2007 while many other molecular-targeted drugs are in the industry pipeline of clinical development. These efforts, nonetheless, are primarily directed toward the treatment of cardiovascular and metabolic diseases including non-alcoholic steatohepatitis (NASH), which has attracted great attention from both academia and industry. Although the molecular mechanisms behind NASH and ASH are considered very similar, less research and development investments have been made to ASH and alcoholic liver diseases.

Various different indications of PPAR agonists were discovered by Tsuchida et al. in 2005. Their study indicated PPARα agonist, Wy-14,643, upregulated expression of AdipoR1 and AdipoR2 and downregulated inflammation in white adipose tissue, while PPARγ agonist, rosiglitazone, increased serum adiponectin concentrations and the ratio of high molecular weight multimers of adiponectin to total adiponectin in rodents. This study, among many others, clearly suggests a relationship among adiponectin, PPARs and cellular inflammation and proliferation that would complement and support our working hypothesis shown in Figs. 2 and 3. While research and development into NASH, diabetes and cardiovascular diseases have drawn much investment worldwide, greater research efforts in parallel for different drug indications of similar molecular mechanisms (such as ASH and alcoholic liver diseases) would benefit more whose patients number is estimated to exceed 2 million. As we described above, transcriptional regulation and molecular cascades associated with PPARα clearly signify the importance of regulating the progression of alcoholic liver diseases. Continued research and development efforts and further elucidation of these pathways would give exciting new prospects for treating alcoholic liver diseases and other related liver disorders in the near future.

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