

## Pathogenesis of Alcohol Induced Liver Injury and Established Animal Model

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### ABSTRACT

There are many factors related to alcoholic liver disease pathology which contribute to disease model development. First, modification of liver metabolism (i.e. storing of lipids and decreasing of essential nutrients) and increased hepatotoxicity of ethanol by formation of reactive oxygen species. Second, stimulation of innate inflammatory immune responses and kupffer cell which related to induction of proinflammatory cytokines. Finally, elevation of liver injury resulting from continued inflammatory immune responses bringing about activation of stellate cell which produces collagen within the liver<sup>(1)</sup>.

**Key words :** Animal model, alcohol, liver injury

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### Alcohol metabolism in the liver

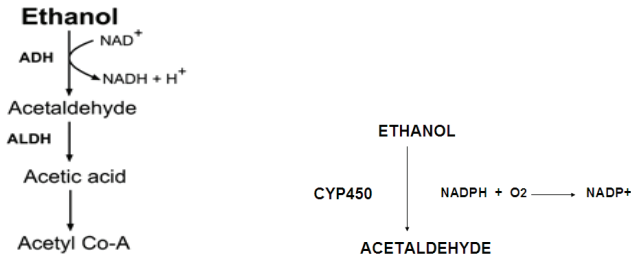
Alcohol metabolism after alcohol ingestion through the mouth, a large amount alcohol from the stomach and intestine is absorbed into the bloodstream. Then, it is transported to the liver, which is main site of alcohol metabolism, via portal vein<sup>(2)</sup>. In general, oxidative pathway achieves alcohol metabolism via alcohol dehydrogenase (ADH), cytochrome P450 (CYP2E1), and catalase enzymes. Firstly, alcohol dehydrogenase, which presents in the cytosol, is the main pathway of alcohol metabolism in the liver. This metabolism produces acetaldehyde that may cause tissue injury and the adduct formation; moreover, this process involves nicotinamide adenine dinucleotide (NAD<sup>+</sup>), an intermediate carrier of electrons, to from

reduced nicotinamide adenine dinucleotide (NADH), a byproduct which generates a highly reduced cytosolic state in liver cells. As a result, the liver is susceptible to injure from the byproducts of alcohol metabolism including acetaldehyde and free radicals. Secondly, cytochrome P450 isoenzymes (such as CYP2E1, 1A2, and 3A4), which present importantly in the microsomes of the endoplasmic reticulum, are enzymes of alcohol oxidation in the liver. Chronic alcohol intake induces CYP2E1 pathway, and CYP2E1 oxidation also generates acetaldehyde and ROS (such as hydroxyethyl, superoxide anion, and hydroxyl radicals) that lead to tissue injury. Finally, Catalase, located in peroxisomes, is a minor pathway of alcohol oxidation which also produces acetaldehyde. After, acetaldehyde

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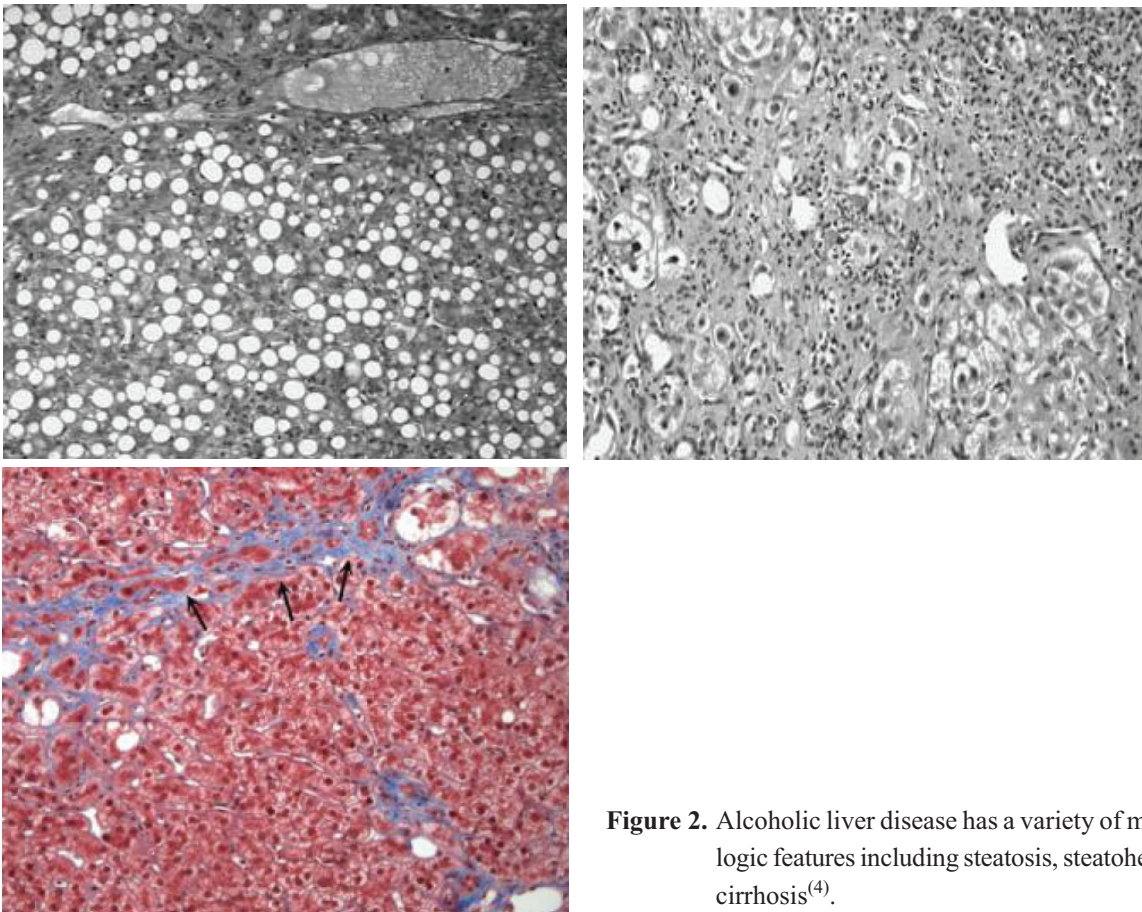
**Figure 1.** Oxidative pathways of alcohol metabolism: alcohol dehydrogenase pathway and microsomal ethanol oxidizing system (MEOS)<sup>(3)</sup>.

from oxidation pathway is metabolized in the mitochondria by aldehyde dehydrogenase 2 (ALDH2) to form acetate and NADH<sup>(3)</sup>; subsequently, acetate enters through the blood circulation to another parts of the body where it can enter other metabolic cycles to create energy or use molecules (Figure 1)<sup>(4)</sup>.

### Types of alcohol-induced liver injury

There are three type of alcohol-related liver injury. First, alcoholic fatty liver or steatosis: it results from heavy drinkers, but can be regressed by stopping

drink alcohol. In contrast, steatosis can bring about development of liver injury if continue drinking alcohol. In addition, it has no clinical symptoms in many cases unless for an enlarged liver or hepatomegaly<sup>(5)</sup>. Second, alcoholic hepatitis : characteristics of disease are disruption (including necrosis) and inflammation of liver tissue, and healthy liver tissue may be replaced by scar tissue known as fibrosis<sup>(4)</sup>. About 40% of patient will progress to cirrhosis if drinking alcohol continues. Simple clinical signs comprise nausea, vomiting, enlarged liver and abdominal pain<sup>(5)</sup>. Third, alcoholic cirrhosis: about 10-15% of the people who heavy drinker alcohol may develop to cirrhosis. In cirrhosis, normal liver tissues are replaced by scar tissue that leads to disrupting blood vessel and internal structure of the liver (Figure 2). It brings about malfunction that may affect functional impairment of other organs. There are many clinical signs of cirrhosis; for example, palmar erythema (capillary dilation), contractures which are caused by fibrous changes or toxic effects, clubbing finger, white nails, and liver inflammation or enlargement<sup>(4,5)</sup>.



**Figure 2.** Alcoholic liver disease has a variety of morphologic features including steatosis, steatohepatitis, cirrhosis<sup>(4)</sup>.

Table 1. Alcohol metabolites and adducts<sup>(3)</sup>.

Metabolites and Adducts	Source
Acetaldehyde	Alcohol metabolism
Malondialdehyde (MDA)	Lipid peroxidation of unsaturated fatty acids
4-hydroxynonenal (HNE)	Lipid peroxidation of long-chain polyunsaturated fatty acid
Malondialdehyde-Acetaldehyde Adduct (MAA)	Hybrid adducts with malondialdehyde and acetaldehyde
Hydroxyethyl radical (HER)	Alcohol oxidation in the presence of iron

### Pathogenesis of alcoholic liver injury

Alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1: especially once chronic alcohol consumption) are major enzymes to break down alcohol in the liver by changing it into a toxic substance, acetaldehyde. ADH pathway involves nicotinamide adenine dinucleotide (NAD<sup>+</sup>) to form reduced nicotinamide adenine dinucleotide (NADH) which leads to redox state; as a result, the liver is susceptible to injure from the byproducts of alcohol metabolism including acetaldehyde and free radicals<sup>(3)</sup>. In addition, the high level of NADH breaks down glucose production and enhance fat molecules production which promotes steatosis<sup>(2)</sup>. Acetaldehyde and reactive oxygen species (ROS), which are produced from alcohol metabolism, makes stable and unstable adducts by interaction with protein building block and other molecule<sup>(3)</sup>(Table 1) that causes DNA damage and lipid peroxidation in cellular membrane. These caused interfere with physiological processes and elevated oxidative stress in the liver<sup>(6)</sup>. In general, antioxidants, especially glutathione (GSH) eliminates ROS from the cells; however, alcohol consumption decreases glutathione levels in the liver cells by interruption with the transport of GSH through membranes which leads to glutathione depletion from mitochondria<sup>(4)</sup>.

Furthermore, alcohol increases permeability of intestine to enhance endotoxin (gram negative bacteria) from blood steam to the liver which activates kupffer cells. Then, kupffer cells generates tumor necrosis factor alpha (TNF-alpha) which in turn activates another type of liver cell such as the stellate cells to produce scar tissue formation causing fibrosis and other chemokines (e.g., IL-8) to attract inflammatory cells inducing liver inflammation<sup>(7)</sup>.

### Animal model of alcohol induced liver injury

Rodents and primates are the most common model to study alcoholic liver disease. Baboons model could develop all stages of ALD by providing alcohol via drinking-water for 3-4 years<sup>(8)</sup> similar to human alcoholic pathology. Although, rodents do not progress to ALD as human does. In general, rodents have detestation to alcohol intake, rodents not increased consume alcohol over time unlike humans<sup>(9)</sup>. In addition, rodents have rate of alcohol catabolism faster than humans (>5 times), and they will give up consumption when level of acetaldehyde elevate<sup>(9)</sup>. Rodents show a higher toleration to lipopolysaccharide than humans<sup>(10)</sup>. Rodents show inter-strain differences in disease progression; thus, selection species and strains are essential for alcohol studies<sup>(11,12)</sup>.

### Ad libitum feeding model

The simplest model of alcohol administration is add through drinking-water (A-DW); moreover, A-DW model mimics patterns of desultory alcohol use and changes in dietary consumption in human. A-DW methods are addition increasing concentrations of alcohol (10-40% (v/v)) into available drinking water while granting rodents to eat on standard diets ad libitum<sup>(13-15)</sup>. This model can develop fatty liver and hepatic inflammation but do not advance to liver fibrosis and cirrhosis<sup>(14,15)</sup>. The ad libitum feeding model has benefits and limitations. In fact, rodents exhibit aversion to alcohol, and they have rapid metabolic rate; thus, rats may protect BAC (blood alcohol concentration) from steadily and regularly reaching high enough levels to injure the liver<sup>(15,16)</sup> (Figure 3).

**Tsukamoto-French (TF) intragastric feeding model**

Tsukamoto and French (TF Model) was administered alcohol via a surgically implanted intragastric cannula<sup>(17)</sup>. TF Model has the advantage of circumventing the aversion animals' show toward alcohol by free alternative, and limitation of alcohol consumption. In addition, alcohol consumption is fed by liquid diet at an assigned rate over a designated time course; moreover, the TF model formulas use carbohydrates to replace alcohol, and pair-matched animals are fed equal caloric consumption<sup>(18,19)</sup>. Furthermore, Tsukamoto-French (TF) intragastric feeding model revealed higher blood and urine alcohol levels than providing alcohol by other models<sup>(19,20)</sup>. This model can develop liver injury which mimics advanced alcoholic liver disease including fatty liver, alcoholic hepatitis and mild liver fibrosis<sup>(18,19,21)</sup>. Disadvantage of TF Model is requires surgical expertise for implantation of the intragastric cannula, and it has adverse effect on oral pharyngeal mucosa and upper gastrointestinal tract (Figure 3).

**Lieber-DeCarli liquid diet model**

In many studies showed that only alcohol feeding was not sufficient to produce liver injury except

necessary nutrients were unstrapped from the diet<sup>(13)</sup>. On the other hand, Lieber and DeCarli showed that liver injury can be produced by increasing levels of alcohol consumption with a nutritionally adequate diet. Lieber and DeCarli developed a liquid diet model which was provided to rat. In alcohol group was fed 36% of calories from alcohol while control group was fed 36% calories from carbohydrates which replaced 36% calories from alcohol<sup>(22-24)</sup>. In brief, formulations are 18% from protein, 35% from fat, 11% (alcohol group) or 47% (control group) from carbohydrate<sup>(22,25)</sup>. After feeding rat with LDLD model for four weeks resulted in formation of ROS and inflammatory cell, CYP2E1 induction, increased triglycerides, changed in iron homeostasis and nutritional scarcity<sup>(23,26,27)</sup>. Moreover, after 4 weeks while elevation levels of triglycerides are measured, lesions beyond steatosis have not been showed in rats with LDLD model for up to nine months<sup>(28)</sup>. The failure to develop lesions in LDLD model resulted from low BACs (100-160 mg/dL range <sup>(23,25,28-30)</sup> when compared with other models; however, this model could use to study in early stage of ALD in particular the studies of alcohol on metabolic changes in the liver and other organs<sup>(25,31)</sup> (Figure 3).

Model	Species	BAC	Pathology	Advantages/disadvantages
Ad libitum oral alcohol in drinking water	Mice Rats	50-100 mg/dL	Animals develop steatosis, minor inflammatory infiltrates	<ul style="list-style-type: none"> <li>- Mimics human consumption and delivery to the gastrointestinal tract.</li> <li>- Activation of Kupffer cells by increased LPS</li> <li>- Pathological changes do not progress beyond steatosis, metabolic, and oxidative stress in the absence of a secondary stress</li> </ul>
Oral gavage	Mice Rats	Can be in excess of 500 mg/dL	Animals develop steatosis and mild inflammatory cell infiltrates	<ul style="list-style-type: none"> <li>- Allows for administration of increased dosage of alcohol</li> <li>- Models binge drinking, more difficult for chronic consumption</li> <li>- Pathological effects when combined with chronic oral ingestion mimics human pathology</li> <li>- Bypasses oral mucosa and upper GI</li> <li>- Stressful for animals, with risk of upper GI trauma</li> <li>- BAC must be closely monitored to avoid alcohol toxicity</li> </ul>

Figure 3. Rodents model of alcohol induced liver injury.

Model	Species	BAC	Pathology	Advantages/disadvantages
Tsakamoto-French intragastric cannulation, enteral feeding model	Mice Rats	As high as 500-600 mg/dL, depending on amount of alcohol. Average achieved is ~200 mg/dL with an oscillating pattern of high and low BAC	Animals develop steatosis, inflammatory cell infiltration, necrosis and fibrosis	<ul style="list-style-type: none"> <li>- Enteral delivery, maintains nutritional equality with controls</li> <li>- Larger dosage of alcohol than oral feeding methods</li> <li>- Progressive pathological changes including fibrosis with activation of Kupffer cells and inflammatory networks</li> <li>- Requires surgical expertise for insertion of cannula, which remains in place through duration of treatment.</li> <li>- Bypasses effects of alcohol on oralpharyngeal mucosa and upper GI tract.</li> <li>- Contributes to dysbiosis and bacterial overgrowth in the GI tract</li> <li>- BAC must be closely monitored to avoid alcohol toxicity</li> </ul>
Liebere-DeCarli oral liquid diet	Mice Rats	100-160 mg/dL	Animals develop steatosis, minor inflammatory infiltrates	<ul style="list-style-type: none"> <li>- Oral delivery, strict nutritional equality with controls.</li> <li>- Activation of Kupffer cells by increased LPS</li> <li>- Can be combined with oral gavage to model chronic-binge patterns of alcohol consumption</li> <li>- Pathological changes do not progress beyond steatosis, metabolic oxidative stress in the absence of a secondary stress</li> </ul>

Figure 3 (cont.). Rodents model of alcohol induced liver injury.

### CONCLUSION

Alcohol (ethanol) is directly hepatotoxic. Illness and death from liver disease throughout the world is caused by long term alcohol consumption<sup>(32,33)</sup>. Typically, alcohol-induced liver injury shows at the beginning as acute inflammation and next develops to steatosis (fatty liver); moreover, if alcohol intake is continued, fatty liver may advance to alcoholic hepatitis and finally to fibrosis, that may lead to cirrhosis<sup>(34)</sup>. Therefore, it is still a challenge to find more effective therapy with less adverse effect for alcoholic liver injury.

In rodent, the effects of oral alcohol administration mimic several of the effects of alcohol intake in human. Therefore, animal model was established to explain the pathogenesis of ALD and new management.

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