Animals Models of Gastrointestinal and Liver Diseases. Animal models of alcohol-induced liver disease: pathophysiology, translational relevance, and challenges

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Mathews S, Xu M, Wang H, Bertola A, Gao B. Animals Models of Gastrointestinal and Liver Diseases, Pathophysiology and Translational Relevance. Animal models of alcohol-induced liver disease: pathophysiology, translational relevance, and challenges. Am J Physiol Gastrointest Liver Physiol 306: G819–G823, 2014. First published April 3, 2014; doi:10.1152/ajpgi.00041.2014.—Over the past four decades, chronic ethanol feeding studies in rodents using either ad libitum feeding or intragastric infusion models have significantly enhanced our understanding of the pathogenesis of alcoholic liver disease (ALD). Recently, we developed a chronic plus binge alcohol feeding model in mice that is similar to the drinking patterns of many alcoholic hepatitis patients: a history of chronic drinking and recent excessive alcohol consumption. Chronic+binge ethanol feeding synergistically induced steatosis, liver injury, and neutrophil infiltration in mice, which may be useful for the study of early alcoholic liver injury and inflammation. Using this chronic+binge model, researchers have begun to identify novel mechanisms that participate in the pathogenesis of alcoholic liver injury, thereby revealing novel therapeutic targets. In this review article, we briefly discuss several mouse models of ALD with a focus on the chronic+binge ethanol feeding model.

In this review, we summarize the animal models currently in use, including their drawbacks and knowledge gained from each model, focusing on the recently developed chronic+binge ethanol feeding model. We also discuss the application of the chronic+binge ethanol feeding model to the study of ALD pathogenesis.

Animal Models of Alcoholic Liver Injury

Acute binge ethanol feeding model. An acute binge of one or multiple doses of ethanol has been used by many laboratories to study the pathogenesis of alcoholic liver injury [see review (22) and references therein]. The most commonly used doses are 4–6 g ethanol/kg body wt, and it has been shown that the acute gavage of ethanol significantly affects hepatic mitochondrial function, oxidative stress, and inflammatory responses. Despite the ability of a binge to induce a significant inflammatory response, the acute binge ethanol feeding model has not been widely used because it often results in only a mild elevation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Still, the model is beneficial for identifying mechanisms and targets that are distinct from those affected by chronic ethanol intake. For example, studies have suggested that acute ethanol binge induces autophagy in the liver (8), whereas chronic ethanol feeding may impair this process. More studies are needed to confirm the latter hypothesis.

Chronic ethanol feeding model (Lieber-DeCarli model). The earliest model of chronic alcohol consumption in animals relied on the administration of ethanol in the drinking water. Although this was an easy method of administering alcohol, the technique had several limitations due to the animals’ natural aversion to alcohol consumption. Dehydration, low blood alcohol level (BAL), and inadequate nutrition were a few of the drawbacks to using this model. Importantly, the ethanol in drinking water model did not induce significant liver injury and, therefore, has not been used for the study of ALD pathogenesis. To overcome the limitations of the ethanol in drinking water model, Dr. Lieber’s group developed a nutritionally competent liquid diet model that allowed for the administration of high concentrations of alcohol and the induction of significant liver injury (19). Liquid diet feeding has become a widely used model for studying ALD because of its ease of use and the ability to modify the dietary composition. Administration of the liquid diet for 4–12 wk, which mimics chronic drinking patterns in humans, induces mild liver injury, steatosis, and low-grade liver inflammation. Importantly, the Lieber-DeCarli liquid diet contains unsaturated fat, which has been shown to be required for the induction of liver injury in...
this model (33). On the basis of these improvements over the ethanol in drinking water model, the chronic ethanol feeding model is considered more appropriate for studying the early stages of ALD.

The chronic ethanol feeding model represents a great advancement over the ethanol in drinking water model; however, this model also has several drawbacks. Chronic ethanol feeding only induces mild elevations in serum ALT levels, mild steatosis, little inflammation, and no fibrosis (4, 14, 21). Additionally, this model does not completely circumvent the animals’ natural aversion to alcohol, since rodents on an ethanol liquid diet consume less than animals on a control diet. Indeed, these very limitations of the chronic ethanol feeding model led to the development of the intragastric feeding model by Tsukamoto et al. (26).

**Intragastric feeding model (Tsukamoto-French model).** To overcome the animals’ natural aversion to alcohol, Tsukamoto et al. (25) developed an enteral feeding model that enabled researchers to achieve high BALs while controlling the liquid diet volume and the nutrients administered to each rodent. This model was originally performed in rats (25) and was later used in mice (29). Mice on this feeding model developed steatosis, focal necrosis, inflammatory foci, and significantly elevated serum ALT levels (9, 29). A major advantage of this model is that it can be used to induce fibrosis (11) and cirrhosis (27) under certain conditions. Shortly after the development of the intragastric feeding model, researchers began introducing modifications to the diet in an attempt to increase liver injury. For example, French et al. (11) used a high-fat diet containing ethanol and found that significant fibrosis was induced in Wistar rats. To expand on those findings, Wistar rats intragastrically infused with a high-fat-ethanol diet containing carbonyl iron showed a twofold increase in ALT compared with rats that only received an ethanol diet, and micronodular cirrhosis was present in 17% of iron-supplemented rats. This increase in liver injury correlated with increased lipid peroxidation, which was induced by iron (27).

The intragastric feeding model has paved the way for more advanced research into the potential mechanisms of moderate to severe alcohol-induced liver injury. This feeding model affords the ability to modify various parameters, such as nutritional composition, while maintaining a high alcohol content, thus providing an attractive model for studying the symbiosis between alcohol and other nutritional or environmental factors. However, the intragastric infusion model has not been widely used because of its technical difficulty and the requirement for intensive medical care and expensive equipment (29).

**Second-hit or multiple-hits model.** Despite overcoming the limitations of chronic alcohol feeding, the intragastric feeding model does not employ the oral route of exposure or mimic the drinking pattern of humans. To circumvent these issues, investigators employ the “second-hit” model of ethanol feeding to mimic more advanced stages of disease (28). The second-hit model is based on the idea that consumption of alcohol alone only induces steatosis, and a second “hit” is required for progression to steatohepatitis and more advanced stages of ALD (28). The second hits that have been used include nutritional modifications, pharmacological agents (e.g., concanavalin A or carbon tetrachloride), hormones, cytochrome P-450 inducers, Toll-like receptor ligands, genetic manipulation (transgenic overexpression or genetic knockout), and viral infections [see review (28) and references therein]. The second hit or “multiple hits” models have been particularly useful for the study of alcoholic liver fibrosis and inflammation, as neither liver fibrosis nor significant inflammation is induced by chronic ad libitum ethanol feeding alone. Investigators using the second-hit model in their studies must be cautious when interpreting their results because it may be difficult to determine whether the observed mechanisms are a consequence of the ethanol feeding or the second hit.

**Chronic + binge ethanol feeding model (Gao-binge model).** Many alcoholic hepatitis patients have a history of chronic drinking combined with more recent periods of excessive alcohol consumption, a pattern that is not reproduced by any of the aforementioned ethanol feeding models. To address this, we recently developed a chronic + binge ethanol feeding model that closely mimics human consumption patterns and results in significantly elevated serum ALT levels, steatosis, and inflammation (5, 6). This model was originally named the NIAAA model (5), but it was recently suggested that this model be named the Gao-binge model (30). Rodents on the chronic + binge regimen are acclimated to the control liquid diet for 5 days. They then receive the ethanol liquid diet for 10 days followed by a single binge of ethanol (5 g/kg body wt). This simple chronic + binge ethanol feeding model achieves a high BAL, elevation of serum ALT and AST, and infiltration of neutrophils into the liver. Additionally, this model is an improvement over other models because it is less time consuming; this model can at least halve the procedure time compared to previous rodent models. This feeding protocol can also be extended to chronic feeding for longer periods of time, up to 8–12 wk, followed by single or multiple binges to induce more severe liver injury. An attractive feature of this model is that it may prove useful for studying the efficacy of pharmacological agents that putatively attenuate progression to chronic liver injury.

**Chronic + Binge Feeding Model: Pathogenesis and Translational Relevance**

It is clear that chronic + binge ethanol feeding induces higher levels of steatosis, serum ALT, and liver inflammation than ad libitum chronic ethanol feeding (Table 1) (5, 6). Another major difference between these two feeding models is that chronic feeding is associated with hepatic infiltration of macrophages, whereas neutrophil infiltration is a hallmark of chronic + binge ethanol feeding (6). This suggests that Kupffer cells/macrophages contribute to liver injury after chronic ethanol feeding, whereas neutrophils may play a more important role in promoting chronic plus binge ethanol-induced hepatocellular damage.

Accumulating data suggest that chronic alcohol consumption causes LPS-mediated Kupffer cell activation and monocye/macrophage infiltration, which is evidenced by increased hepatic gene expression of the macrophage marker F4/80 following chronic ethanol feeding (6). Interestingly, hepatic F4/80 expression was decreased in female C57BL/6J mice after gavage with a single dose of ethanol (6). This downregulation was likely due to macrophage apoptosis induced by acute ethanol exposure (23). The hepatic expression of F4/80 remained unchanged after the 10-day chronic + binge ethanol feeding, which may indicate that the 10-day chronic feeding-
induced upregulation of F4/80 cancelled out the binge-induced downregulation of F4/80 in female C57BL/6J mice (6). Interestingly, hepatic expression of F4/80 was upregulated by 2.5-fold after chronic-binge ethanol feeding in male C57BL/6J mice (15). These data suggest that the sex and strains of mice may affect hepatic macrophage infiltration after chronic-binge ethanol feeding. More studies using immunostaining and flow cytometric analyses are required to clarify the effects of chronic-binge ethanol feeding on hepatic macrophage infiltration.

Hepatic expression of the neutrophil marker Ly6G and the number of MPO+ neutrophils remained unchanged after chronic ethanol feeding but were markedly elevated after chronic-binge ethanol feeding (6). This hepatic neutrophil infiltration is partly due to the upregulation of hepatic E-selectin because chronic-binge ethanol feeding synergistically upregulated hepatic expression of E-selectin and the genetic ablation of E-selectin markedly reduced hepatic neutrophilia and liver injury after chronic-binge ethanol feeding (6). Importantly, antibody-mediated depletion of neutrophils also ameliorated chronic-binge ethanol-induced liver damage. Collectively, these data suggest crucial roles for neutrophils and E-selectin in chronic-binge ethanol-induced liver injury.

Several research groups have used the chronic-binge ethanol feeding model to identify novel mechanisms of alcoholic liver injury and novel therapeutic targets for the treatment of ALD. In addition to uncovering the critical role for E-selectin mentioned above, our own group has identified the hepatoprotective effect, and therefore therapeutic potential, of interleukin-22 in chronic-binge-induced liver injury (15). Recently, we also used the chronic-binge model to examine the effect of prednisolone on alcoholic liver injury (17). Prednisolone has been used for the treatment of acute hepatitis for more than four decades, but the results have been mixed. Prednisolone has been shown to exacerbate chronic-binge ethanol- or hepatotoxin CCl4-induced hepatitis (17), and the detrimental effects of prednisolone have been attributed to the inhibition of liver regeneration and the phagocytosis of neutrophils and macrophages. These findings suggest that prednisolone treatment may not be favorable for hepatotoxin-mediated liver injury. Conversely, treatment with prednisolone ameliorated T/NKT cell-mediated hepatitis induced by concanavalin A. Thus steroid therapy may have beneficial effects in tempering systemic inflammatory responses and improving the short-term survival of patients with severe acute hepatitis.

Osteopontin (OPN) is an extracellular matrix protein secreted by various tissues that has been shown to modulate inflammation, steatosis, immune responses, and tumor formation in several tissues, including the liver. Several studies, using both human and rodent models, have reported a role for OPN in driving inflammation and fibrosis in T cell-mediated and viral hepatitis, nonalcoholic fatty liver disease, and liver cancer. Importantly, OPN has also been identified as a contributor to alcohol-induced liver injury. Apte et al. (2) used a chronic ethanol feeding model followed by single injection of LPS to induce alcoholic steatohepatitis in rats and to elucidate the role of OPN during the early stages of ALD. Their results showed increased expression of OPN in ethanol-fed rats that correlated with neutrophilia, and these effects were enhanced by LPS (2). A recent study analyzed liver biopsies from patients with different forms of liver disease and revealed a significant increase in OPN mRNA and protein in cases of compensated cirrhosis and alcoholic hepatitis compared with normal livers (20). Importantly, patients with alcoholic hepatitis had the greatest increase in OPN, and the level of expression correlated with disease severity and mortality (20). In collaboration with Dr. Bataller’s group, we demonstrated that OPN-deficient mice were protected from chronic-binge ethanol-induced liver injury and inflammation (20), suggesting that OPN plays a role in promoting neutrophil infiltration and liver

### Table 1. Commonly used animal models for ALD

<table>
<thead>
<tr>
<th>Models</th>
<th>Characteristics</th>
<th>Mechanisms</th>
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<tbody>
<tr>
<td>Acute binge ethanol feeding model</td>
<td>· Mild elevation of serum ALT, AST</td>
<td>· Damages hepatocyte mitochondrial functions and produces oxidative stress</td>
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<tr>
<td></td>
<td>· Low levels of liver inflammation with a decrease in hepatic macrophages</td>
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<td></td>
<td>· Easy to perform</td>
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<tr>
<td>Chronic ethanol feeding (Lieber-DeCarli model)</td>
<td>· Mild elevation of serum ALT, AST</td>
<td>· Increases gut permeability and activates LPS-TLR4-Kupffer cells</td>
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<tr>
<td></td>
<td>· Low levels of liver inflammation with an increase in macrophages but not neutrophils</td>
<td>· Damages hepatocyte mitochondrial functions and produces oxidative stress</td>
</tr>
<tr>
<td></td>
<td>· Easy to perform</td>
<td>· Similar mechanisms as chronic ethanol feeding</td>
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<tr>
<td>Intra-gastric chronic ethanol feeding (Tsukamoto-French model)</td>
<td>· Moderate elevation of serum ALT, AST</td>
<td></td>
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<td></td>
<td>· Moderate liver inflammation with an increase in macrophages but low levels of neutrophils</td>
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<td>· Difficult to perform</td>
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<tr>
<td>“Second hit” or “Multiple hits” model</td>
<td>· Moderate to significant elevation of serum ALT, AST, and liver inflammation dependent on second hit</td>
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<td></td>
<td>· Easy to perform</td>
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<tr>
<td>Chronic+binge feeding model (Gao-binge model)</td>
<td>· Moderate elevation of serum ALT, AST</td>
<td>· Chronic ethanol feeding increases susceptibility of livers to second or multiple hit(s)-induced liver injury and inflammation</td>
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<td></td>
<td>· Moderate liver inflammation with an increase in neutrophils</td>
<td>· Increases hepatic neutrophil infiltration and subsequently induces liver injury</td>
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<td></td>
<td>· Easy to perform</td>
<td>· Damages hepatocyte mitochondrial functions and produces oxidative stress</td>
</tr>
<tr>
<td>Tsukamoto-hybrid model with high-cholesterol and high-fat diet plus chronic ethanol and binge ethanol feeding</td>
<td>· Significant elevation of serum ALT, AST</td>
<td>· Increases hepatic neutrophil and macrophage infiltration and subsequently induces liver injury</td>
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<tr>
<td></td>
<td>· Significant liver inflammation with an increase in neutrophils</td>
<td>· Damages hepatocyte mitochondrial functions and produces oxidative stress</td>
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<tr>
<td></td>
<td>· Mild liver fibrosis</td>
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<td></td>
<td>· Difficult to perform</td>
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ALD, alcoholic liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
injury in the early stages of ALD. In contrast, Dr. Nieto’s group revealed that OPN-deficient mice had a slightly increased or comparable rate of liver injury after chronic ethanol feeding or chronic+binge ethanol feeding (13). The difference between these two studies is unclear but may arise from the different feeding environments and feeding protocols. Interestingly, a recent study suggests that OPN does not play a role in inducing liver damage in severe alcoholic liver injury induced by intragastric ethanol feeding plus multiple binges (18).

Sirtuin 1 (SIRT1) is a NAD+-dependent protein deacetylase that regulates hepatic lipid metabolism by modifying histones and transcription factors. Dr. Min You’s group used the chronic+binge model in mice to demonstrate that ethanol-mediated impairment of the SIRT1-SFRS10-Lpin 1β/α axis is crucial to the development of alcoholic steatohepatitis (31). Additionally, the chronic+binge model has been used by many investigators to explore the roles of acetaldehyde dehydrogenase-2 (16), adipocytokine-specific lipin-1 (32), heat-shock protein gp96 (1), circadian oscillation (24), and fibroblast growth factor 21 (10) in the pathogenesis of ALD.

Conclusions and Challenges

In conclusion, compared with ad libitum chronic ethanol feeding models, the chronic+binge model has several advantages, such as inducing more severe steatosis, hepatocellular damage, and hepatic neutrophil infiltration. However, the hepatocellular damage and inflammation caused by short-term (10-day) chronic plus binge ethanol feeding are moderate and may only mimic early alcoholic steatohepatitis. Long-term chronic feeding and multiple binges may be required to induce more severe steatohepatitis and are currently under investigation. Recently, Dr. Tsukamoto’s group developed a hybrid model of chronic feeding with a Western diet high in cholesterol and saturated fat plus intragastric alcohol delivery with weekly binges of alcohol (18). This model induces much more severe liver damage and hepatic neutrophil infiltration. In summary, the chronic+binge model may represent moderate alcoholic steatohepatitis, whereas the hybrid model may represent severe alcoholic steatohepatitis. These models may be used to identify the underlying mechanisms that contribute to the pathogenesis of alcoholic steatohepatitis at different stages or severities.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

S.M., M.K., H.W., and B.G. drafted manuscript; A.B. and B.G. edited and revised manuscript; B.G. approved final version of manuscript.

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