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Rodent Models of Alcoholic Liver Disease: Of Mice and Men

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Abstract

Alcoholic liver disease (ALD) is a major cause of acute and chronic liver disease worldwide. The progressive nature of ALD is well described however the complex interactions under which these pathologies evolve remain to be fully elucidated. Clinically there are no clear biomarkers or universally accepted, effective treatment strategies for ALD. Experimental models of ALD are an important component in identifying underlying mechanisms of alcohol-induced injury to develop better diagnostic markers, predictors of disease progression, and therapeutic targets to manage, halt, or reverse disease progression. Rodents remain the most accessible model for studying ALD pathology. Effective rodent models must mimic the natural history of ALD while allowing examination of complex interactions between multiple hepatic, and non-hepatic, cell types in the setting of altered metabolic or oxidative/nitrosative stress, inflammatory responses, and sensitivity to cytotoxic stress. Additionally, mode and duration of alcohol delivery influences hepatic response and presents unique challenges in understanding disease pathology. This review provides an overview of rodent models of ALD, their strengths and weaknesses relative to human disease states, and provides insight of the potential to develop novel rodent models to simulate the course of human ALD.

INTRODUCTION

Alcoholic liver disease (ALD) is a significant cause of morbidity and mortality globally (Gao and Bataller, 2012; Beier et al., 2011; Jeong and Gao, 2008; Nath and Szabo, 2009), and refers to a spectrum of hepatic pathologies resulting from acute/binge or chronic alcohol exposure/abuse for which disease progression develops in a dose and time dependent manner (Diehl, 2002; Nath and Szabo, 2009). Alcohol abuse is a leading factor in mortality from liver disease and increases the risk for a wide range of adverse health effects (Gao and Bataller, 2012; Beier et al., 2011; Gerke et al., 1997; Siegmund et al., 2003). In the United States, the Centers for Disease Control and Prevention estimate that ≈50% of people aged 18 or older drink alcohol regularly, and of these, 5% are classified as heavy drinkers (≥30g alcohol/day) and 15% binge drink (≥5 drinks consumed on a single occasion) (CDC, 2009; Mann et al., 2003; Naimi et al., 2003).

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The toxic effects of alcohol are exerted on multiple organs; however, the liver, as the primary site of alcohol metabolism, is a major target of injury (Karinch et al., 2008; Lieber, 2000, 2005). Both chronic and acute drinking deliver unique pathological consequences that affect liver disease and injury, and almost half of all end-stage liver disease in the United States is attributed to alcohol abuse (Gao and Bataller, 2012; Beier et al., 2011). Currently there are no effective, universally accepted therapies for treatment of ALD (Arteel, 2010; Diehl, 2002). Understanding the pathology of ALD is impeded by the complexity of interactions between alcohol (including amount, duration, type consumed) and different hepatic cell types. The effect(s) of alcohol is/are further complicated by host genetics, variability in immunological and metabolic responses, nutritional status, and the presence/absence of comorbid factors such as smoking, and obesity (Nath and Szabo, 2009; Tsukamoto et al., 2009).

Significant progress has been made in developing animal models with which to investigate mechanisms of ALD initiation and progression. To be effective, ALD models should replicate the etiology and natural history of the human disease. In human ALD pathology the "*classic*" pattern of liver injury begins with hepatomegaly and alcoholic steatosis (fatty liver), an event that occurs in ≈90% of heavy alcohol users. Of the patients that develop alcohol-induced steatosis, 10–35% progress to steatohepatitis (fatty liver with inflammation), and a further 35–40% go on to develop fibrosis and cirrhosis (end-stage liver disease) (Arteel, 2010; Mann et al., 2003; Nath and Szabo, 2009; Sozio and Crabb, 2008; Szabo and Bala, 2010). Cirrhosis, the most serious form of ALD, is directly associated with duration and amount of alcohol consumed (estimated to be ≈30g/day for 10 years) (Mann et al., 2003), and is the leading risk factor for subsequent development of hepatocellular carcinoma (HCC) (McKillop and Schrum, 2009).

During early stages of ALD, alcohol consumption diverts metabolic pathways toward hepatic triglyceride accumulation (Lieber, 1991; Sozio and Crabb, 2008). Lipid accumulation leads to increased derangement of metabolic function and increases hepatic sensitivity to toxins (de la Hall et al., 2001; Siegmund et al., 2003). This is an important prerequisite during the development of inflammation (Ramaiah et al., 2004). Metabolic disturbances also contribute to impaired nutrient absorption and distribution, effects that are dependent on both amount of alcohol consumed, and patterns of consumption.

Chronic, long-term alcohol consumption and metabolism is associated with metabolic derangements and changes in nicotinamide adenine dinucleotide (NAD/NADH) ratios favoring accumulation of reducing equivalents in the liver (NADH). Changes in NAD/NADH ratio contribute to hepatic accumulation of triglycerides and depress the citric acid cycle. Alcohol also affects mitochondrial membrane function, metabolic demand, and generation of reactive oxygen species (ROS), a factor exacerbated by alcohol-dependent induction of cytochrome P450 2E1 (CYP2E1) (Lieber, 2005; Lu and Cederbaum, 2008). The toxic responses and injury associated with acute/binge drinking are mediated by amount and rate of alcohol consumption. During acute alcohol consumption, the majority of alcohol is metabolized by successive oxidation reactions, first *via* alcohol dehydrogenase (ADH) to acetaldehyde, which is in turn oxidized by acetaldehyde dehydrogenase (ALDH) to acetate and water (Lieber, 2005). Acetaldehyde is a toxic intermediate that is usually processed efficiently to prevent accumulation. However, chronic long-term alcohol ab(use) leads to CYP2E1 enzyme induction, which also generates acetaldehyde as the first metabolite during alcohol oxidation (Lieber, 2005; Lu and Cederbaum, 2008). Cytochrome P450 enzymes require oxygen for their catalytic function and reactions mediated by CYP2E1 are poorly coupled, leading to incomplete oxygen reduction and formation of oxyradicals and the partially reduced hydroxyethyl radical, thereby increasing oxidative stress responses (Albano, 2006; Lieber, 2005; Lu and Cederbaum, 2008). In addition, CYP2E1 is associated

with increased alcohol tolerance observed in individuals that chronically abuse alcohol and plays a significant role in activation of pro-carcinogens to carcinogens (Dey and Cederbaum, 2006; Lieber, 2005; Lu and Cederbaum, 2008).

In addition to changes in hepatic parenchymal cell (hepatocyte) physiology and function, human ALD is characterized by a state of chronic hepatic inflammation. Alcohol leads to alterations in the gastrointestinal mucosa by disrupting epithelial tight junctions allowing for bacterial endotoxin (lipopolysaccharide; LPS) translocation into the liver *via* the portal vein (Beck et al., 1986; Siegmund et al., 2003; Szabo and Bala, 2010). Hepatic response to gut derived endotoxin is a critical step in the development of ALD (Rao, 2009). Presence of LPS in the liver activates innate immune responses primarily *via* Kupffer cell sensitization (Szabo and Bala, 2010; Thurman, 1998; Yin et al., 1999) leading to intrahepatic inflammation and the production of tumor necrosis factor α (TNFα) and other pro-inflammatory cytokines (Jeong and Gao, 2008; Szabo and Bala, 2010). Additionally innate immune signaling is a mediator of tissue and organ homeostasis regulating proliferation and apoptosis of intestinal epithelial cells, and modulating liver regeneration after loss of liver mass (Seki and Schnabl, 2012). Aberrant regulation of immune system signaling may trigger harmful, inflammatory responses that contributes to tissue and organ injuries, fibrosis, and carcinogenesis (Seki and Schnabl, 2012). Collectively, these cytokine cascades combine to orchestrate liver injury, largely through neutrophil recruitment, although other inflammatory cell types (natural killer, natural killer T-lymphocytes, T-lymphocytes, and dendritic cells) also become activated, each serving unique roles in hepatic injury, repair and remodeling (Gao et al., 2011). Finally, prolonged alcohol-induced changes to liver function, hepatic circulation, and immune responses leads to hepatic stellate cell activation from a quiescent, lipid/vitamin A storing phenotype, to a pro-mitogenic, collagen producing state (de la Hall et al., 2001; Karaa et al., 2008). Initially, collagen deposition is localized to perivenular and pericellular regions, resulting in portal tract-septal fibrosis that surrounds apoptotic/necrotic hepatocytes (Michalak et al., 2003), and the eventual formation of fibrous septae and scar tissue that encompasses regenerative hepatocytes (Diehl, 2002; Karaa et al., 2008; McKillop et al., 2006; Ramaiah et al., 2004).

In developing effective models it is important to consider the goal of the intended study, the benefits to scientific research, and the potential limitations with respect to the contribution of the biochemical and pathophysiological consequences of acute or chronic alcohol consumption to organs, tissues and cells, and the role of genetic variability inherent to the population. As is evident from the preceding summary, while multiple factors contribute to ALD progression, there are three essential characteristics related to ALD pathology that must be considered in developing effective disease models. 1. Changes in hepatic metabolism, and particularly changes that lead to accumulation of lipids and/or depletion of essential nutrients, and enhanced hepatotoxicity of alcohol with formation of ROS. 2. Activation of innate inflammatory immune responses, Kupffer cell activation and associated induction of pro-inflammatory cytokines, and migration of infiltrating neutrophils. 3. Increased hepatic damage resulting from persistent inflammatory immune responses leading to stellate cell activation and collagen deposition, combined with compensatory hepatic regeneration.

In addition to these factors increasing evidence suggests other factors may be equally important in considering models of ALD. For example, alcohol induces changes in the gut microbiome that may influence innate and inflammatory immune responses (Seki and Schnabl, 2012; Son et al., 2010; Szabo and Bala, 2010; Yan et al., 2011). Indeed gut sterilization by antibiotics or alteration of gut microbiota using probiotics effectively reduces hepatic injury in experimental ALD (Miller and Spicer, 2012; Yan et al., 2011). Additionally, as the liver is a sexually dimorphic organ, the effect(s) of alcohol on liver

pathology in males and females is an important factor in hepatic disease pathology (Ellefson et al., 2011; Ramaiah et al., 2004). For instance, while females are more susceptible to initial injury following alcohol consumption they are less likely to progress to cirrhosis and HCC (Frezza et al., 1990; Iimuro et al., 1997; Ikejima et al., 1998; Saunders et al., 1981; Sorensen et al., 1984).

To date, no rodent model of ALD has been described that effectively replicates human alcoholic hepatitis with progression to fibrosis or cirrhosis without the addition of a secondary insult (e.g. iron, high fat diet, vitamin supplementation, LPS injection) (de la Hall et al., 2001; Karaa et al., 2008; Nanji and French, 2003). The aim of the current review is to provide a summary of the rodent models of ALD currently in use, the relative merits and potential drawbacks associated with these models, and the possible application of newer models being developed that may overcome some, or all, of these limitations.

ANIMAL MODELS OF ALCOHOL INGESTION

A number of species have been utilized to study ALD, the most common of which are rodents (mice, rats, hamsters), and primates (Batra et al., 1995; Ketcham et al., 1963; Lieber et al., 1985; Tsukamoto et al., 1984). Of these model organisms baboons maintained on alcohol drinking-water for 3–4 years develop ALD through all stages of progression and mostly closely resemble human ALD pathology (Lieber et al., 1985). However, cost and study duration renders primate use prohibitive for most research laboratories. As such, rodents (rats and mice) remain the most commonly used animal model. However, no comprehensive rodent model has been developed to date that accurately reflects the complete human pathology of ALD (de la Hall et al., 2001; Miller et al., 2011).

Several explanations as to why rodents do not develop ALD parallel to the human disease have been suggested. Rodents (generally) have a natural aversion to alcohol consumption, but will consume alcohol partially for its caloric value. However, unlike humans, consumption does not increase over time (Holmes et al., 1986). Additionally rate of alcohol catabolism is up to 5 times faster in rodents than humans, and rodents will stop consuming alcohol when blood acetaldehyde levels increase (Holmes et al., 1986). However, differences in alcohol catabolism between humans and rodents must also be considered within the context of higher basal metabolic rates in rodents (in general) as compared to humans (Ames et al., 1993; Austad, 2010). One can speculate as to the evolutionary and physiological factors contributing to these disparities, but they must be considered when determining efficacy of specific models. Rodents, as a scavenger species feeding on decomposing vegetation, would require the ability to metabolize alcohol. However, alcohol avoidance is most likely favored as a result of survival in which impaired neurological function from alcohol consumption would increase susceptibility to predator attack. Conversely, alcohol consumption and rate of metabolism could also be considered a factor in human "*social selection*" (Thomasson et al., 1993).

Inflammatory and innate immune responses, and how they are influenced by translocation of intestinal LPS, are major components of ALD that must be considered when evaluating rodent models, as these responses are markedly different between humans and rodents. For example, neutrophil infiltration is considered a critical aspect of human steatohepatitis and progressive injury during ALD. Human blood is neutrophil rich, 50–70% of leukocyte balance, compared to only 10–25% in mice, where lymphocytes comprise 75–90% of leukocytes (Mestas and Hughes, 2004; Ramaiah and Jaeschke, 2007). The consequences of this difference in leukocyte balance between humans and mice during progressive ALD remains unknown. However, rodents exhibit a higher (usually of the order of several degrees of magnitude) tolerance to LPS than humans (Ramaiah and Jaeschke, 2007) as demonstrated

in comparative studies reporting LPS induces rapid physiological responses in humans (fever, tachycardia, and slight hypotension) that are not detected in mice at comparable LPS doses (Copeland et al., 2005; Mestas and Hughes, 2004). On challenge with appropriate doses of LPS, both mice and humans respond with rapid (less than 2-hours) increases in TNFα and interleukin-6 production, effects that resolve by 24-hours (Copeland et al., 2005). However, significant differences were detected in the expression of other cytokines and chemokines, mice expressing more chemokines than humans, possibly as a result of differences in myeloid and lymphoid-derived cell populations (Copeland et al., 2005). However, this suggests immunological contributions to ALD may not occur in the same way or to the same extent in humans as they do in rodents.

Risk for developing ALD varies among individuals. While significant components of human ALD variability is attributed to diet, smoking, type of alcohol consumed, and other known risk factors for liver disease, underlying genotype also plays a significant role (Li, 2008; McKillop and Schrum, 2009). Polymorphisms in genes for alcohol metabolism (ADH, ALDH, and CYP2E1), methionine metabolism, oxidative stress (manganese superoxide dismutase), and immune response (TNFα) have been reported (DeNucci et al., 2010; Lieber, 2005; McKillop and Schrum, 2009; Ramaiah et al., 2004). Much like their human counterparts, rodents demonstrate similar, varying degrees of ALD susceptibility (DeNucci et al., 2010; Li, 2008; Tsuchiya et al., 2012). Inter-strain differences in ALD initiation and progression are reported for rats and mice, and thus become an important consideration in selecting species and strains for alcohol studies (DeNucci et al., 2010; Tsuchiya et al., 2012). For example, in a study using different strains of rat (Long Evans, Sprague Dawley, and Fisher 344) fed an isocalorically controlled liquid alcohol diet (8 weeks), all 3 strains demonstrated similar caloric intake and blood alcohol content (BAC). However, weight gain was notably different (Long Evans>Sprague Dawley>Fisher 344) and correlated to diminished hepatic function, including lipid metabolism, and increased hepatic damage (steatosis, lymphocyte infiltration, apoptotic and necrotic cell death, and altered hepatic architecture) (DeNucci et al., 2010). Of further note, variations in ethanol metabolizing enzyme (ADH 1, 3, and 7, ALDH 1, and CYP2E1) expression patterns were reported between the 3 strains, suggesting differences in alcohol metabolism efficiency may also contribute to inter-strain differences in alcohol-dependent liver injury (DeNucci et al., 2010). In humans polymorphisms in these genes can be correlated to risk of adverse effects from alcohol consumption, but have not been directly linked to disease progression (Garcia-Banuelos et al., 2012).

A comparable study of inbred mouse strains fed alcohol (up to 27mg/kg body weight/day) for 28 days using an intragastric, enteral feeding model, identified similar differences in susceptibility to alcohol–induced hepatic injury, and genetic variation in inbred mouse strains were similar to those reported in the human population (Roberts et al., 2007; Tsuchiya et al., 2012). As with rats, different mouse strains fed equal amounts of alcohol maintained comparable BAC, yet exhibited marked differences in sensitivity to the deleterious effects of alcohol (Tsuchiya et al., 2012). For example, while some mouse strains (C57B6/10J, NZW/LacJ, FVB/NJ, and KK/HIJ) exhibited a high degree of alcoholinduced liver injury, other strains (WSB/EiJ, PWSD/PhJ, 129S1Sv/ImJ, and AKRJ) were (relatively) resistant to alcohol-induced liver damage, despite exhibiting equivalent BACs (Tsuchiya et al., 2012). Of further interest, levels of adiponectin, a fat-derived hormone reported as protective against alcoholic fatty liver injury (You et al., 2005; You and Rogers, 2009), were depressed by alcohol consumption in all strains except those identified as (relatively) resistant to alcohol-induced liver damage (Qin and Tian, 2010; Tsuchiya et al., 2012; Xu et al., 2003). As with other studies of ALD, hepatic damage correlated with global changes in lipid synthesis pathways, increased endoplasmic reticulum stress, and disruptions to glutathione and methionine metabolism (Tsuchiya et al., 2012).

The amount of alcohol consumed and duration of consumption are the most significant factors to affect degree of hepatic dysfunction. However, means of alcohol delivery also plays an important role in determining rate and amount of alcohol ingested, and the pathological consequences. As previously discussed, (most) rodents demonstrate a natural aversion to alcohol consumption leading to a need for alternatives to chronic voluntary alcohol consumption. Alcohol administration by inhalation or intravenous/intraperitoneal injection has been widely reported, and has proven particularly useful in studying neurological effects of alcohol. Vapor inhalation of alcohol produces consistently higher BAC in rats (150–200mg/dL), bypassing limitations associated with oral consumption and overcoming problems associated with water/food intake (Gilpin et al., 2008). However, because these approaches do not mimic the effects of alcohol "*drinking*" on the gastrointestinal tract and liver, they are more commonly employed for addiction and behavioral studies than those addressing ALD (DiPadova et al., 1987; Guillaume et al., 1994; Ponnappa and Rubin, 2000; Slawecki et al., 2000).

Oral alcohol ingestion in rodents mimics many of the effects of human alcohol consumption. In humans, alcohol ingestion can be acute, short term (binge, ≈20–50g/day) consumed on a single occasion over the course of several hours), or long-term (chronic, ≥30g/day, for years/decades) (Mann et al., 2003; Naimi et al., 2003). Additionally, human patterns of consumption often include chronic consumption interspersed with episodes of binge alcohol, or patterns of relative abstinence (4–5 days) followed by 1–2 days of heavy, binge consumption, a practice that may be sustained for a number of years (i.e. chronic episodic binge) (Chen, 2004–2005).

Given the inherent aversion to alcohol consumption, several models have been developed to engage rodents in "*voluntary*" alcohol consumption. While these models offer distinct patterns of consumption, there is some overlap between them in alcohol-induced damage. As such, these models have been used in parallel and/or interchangeably to examine the effects of alcohol on specific pathways. For example, effects of alcohol on Kupffer cell activation, through translocation of intestinal LPS, have been well documented in both acute and chronic alcohol consumption models, and administration of antibiotics decreases alcohol mediated liver injury (Adachi et al., 1994; Adachi et al., 1995; Enomoto et al., 1999; Lu et al., 2010). Additionally, similar studies report that blocking TNFα signaling during acute or chronic alcohol can have similar inhibitory effects on ALD progression (Butura et al., 2009; Gao, 2012; Schwabe and Brenner, 2006; Yin et al., 1999). However, acute and chronic alcohol consumption produces many distinct patterns of injury that cannot be replicated in other models.

Lieber-DeCarli liquid diet

One of the first research based diets designed specifically for studying the effects of alcohol *in vivo* was the Lieber-DeCarli full liquid diet (LDLD). Early studies of alcoholic injury/ALD in rats suggested alcohol was not toxic *per se,* rather that alcohol-related liver injury was a consequence of nutritional deficiencies (Best et al., 1949). In these studies alcohol feeding was insufficient to cause liver injury unless essential nutrients were removed from the diet, a factor supported by evidence that nutritional deficits alone caused liver damage (Best et al., 1949). However this theory was dispelled by the seminal work of Lieber and DeCarli using rats exposed to increased levels of alcohol feeding in the presence of a nutritionally adequate diet (DeCarli and Lieber, 1967; Lieber and DeCarli, 1966, 1968; Lieber et al., 1965).

The LDLD is an isocalorically controlled liquid diet that derives up to 36% of its calories from alcohol. Pair-fed controls are maintained on a caloric and nutrition-matched diet, wherein alcohol is replaced with carbohydrates (maltose and dextrose) (DeCarli and Lieber,

1967; Lieber and DeCarli, 1982; Ramaiah et al., 2004). The LDLD is formulated from casein (18% of calories, with methionine and cysteine), a dextrin and maltose mixture (11% and 47% of calories respectively), fat (35% of calories, derived from olive, corn, and safflower oils), and supplemented with essential vitamins (A, D, E, K, and B), minerals and fiber (DeCarli and Lieber, 1967; Lieber and DeCarli, 1989). Animals are initiated on this diet and carbohydrates gradually replaced with increasing amounts of alcohol over a 7–10 day period. Once weaned onto the LDLD typical BAC levels reported are in the ≈100–160mg/dL range (Bradford et al., 2008; Leo and Lieber, 1983; Lieber and DeCarli, 1970, 1982, 1989). Chronic (4-week) alcohol feeding on the LDLD leads to CYP2E1 induction, generation of ROS, increased triglycerides, inflammatory cell infiltration, changes in iron homeostasis/anemia, and nutritional deficiencies (Best et al., 1949; Lieber, 2000; Lieber and DeCarli, 1982; Mueller et al., 2009). Of note, while increased triglycerides are measured after 4-weeks lesions other than steatosis have not been reported in rats maintained on this diet for up to 9-months (Leo and Lieber, 1983) The failure to progress beyond this stage of ALD is likely due to the comparatively low BACs achieved in this model when compared to other rodent models and the human disease state.

Despite limitations in severity of ALD progression, the LDLD in chronic rat models of alcohol feeding has proven extraordinarily useful in the study of early stages of ALD, and in particular the effect of alcohol on metabolic changes in the liver and other organs (Herrera et al., 2003; Lieber and DeCarli, 1989). The inclusion of a secondary hepatic stressor such as LPS, high fat diet, iron, hepatotoxins (e.g. carbon tetrachloride (CCl_4), acetominaphen), or viral proteins (HCV core protein or NS5A) have also been successfully employed with the LDLD to study the effect of alcohol on the initiation and progression of cirrhosis and HCC (Enomoto et al., 1998; Hall et al., 1991; Karaa et al., 2008; Koike et al., 2008; Lu and Cederbaum, 2008; Machida et al., 2009; McCuskey et al., 2005; Su, 2002; Tipoe et al., 2008; Tsukamoto et al., 1995; Tsukamoto et al., 2009).

Hospital admissions for acute steatohepatitis in chronic alcohol abusers are often preceded by episodes of heavy binge alcohol consumption, and this is a risk factor for ALD progression (Aroor et al., 2011; Mathurin and Lucey, 2007). To mimic human patterns of consumption and injury, models of binge and chronic alcohol have been developed in which a rat model of chronic alcohol feeding (5% *v/v,* 4-weeks in LDLD) is followed by either single (32% *v/v*) or repeat binge alcohol (32% *v/v*, 3 doses, 12-hour intervals) by intragastic infusion. Using this approach, Aroor *et al.* reported BAC was significantly increased over chronic alcohol alone (101.5 mg/dL), in single binge (175.7 mg/dL) and repeat binge (540.3 mg/dL) models and these increases were associated with augmented liver injury (Aroor et al., 2011). Of particular note this pattern of liver injury was achieved by alcohol consumption alone administered for a shorter duration than in previously reported models, at a lower percent alcohol, and without the need for a "*second hit*" (e.g. low-dose LPS) (Aroor et al., 2011). Mechanistically this chronic-binge alcohol feeding model also demonstrated significant increases in extracellular regulated kinase-1/2 activation, a finding that has potential implications in both ALD and HCC progression (Aroor et al., 2011; McKillop et al., 1997; McKillop et al., 1999; Schmidt et al., 1997).

Despite widespread use and reporting of the LDLD model, as with other model systems, there are important limitations that should be considered before undertaking these types of studies. For example, when studying nutritional-metabolic status it should be noted that rats maintained on LDLD consume the alcohol containing diet in smaller quantities over a prolonged period of time whereas pair-matched controls (restricted to calorically matched alcohol fed rats) consume the control diet over a shorter period of time (Lieber and DeCarli, 1989; Ramaiah et al., 2004). Indeed, the slow intake of the LDLD, in addition to CYP2E1 induction, are major reasons for the (relatively) low BAC levels achieved with the LDLD

model relative to the total amount of alcohol consumed. Control and alcohol fed animals must also be closely monitored (daily) throughout the course of what can be long experimental protocols (weeks-months) to ensure equal caloric intake.

As highlighted previously, choice of both species and strain are also important when designing experiments using the LDLD. Of note, mice tend to be more resistant to the LDLD diet requiring longer weaning periods, and with strain differences in susceptibility to alcohol-induced liver damage, care should be taken in selecting appropriate models, particularly when considering the use of genetic knock-out models and the background in which these mice are generated. One method used to overcome limits in achievable BAC, variation in consumption patterns, and diet resistance in mice has been to decrease the percentage of alcohol (3.5–6% *v/v*) used. As with the rat model, decreasing the percent of alcohol to 5% in mice leads to reproducible BACs comparable to rats maintained on traditional LDLD (Aroor et al., 2011). This lower (5% (*v/v*)) LDLD has also been employed in chronic-binge alcohol feeding models in mice in which increased markers of liver injury (alanine aminotransferase lipid deposition, and inflammatory cytokines) are reported (Ki et al., 2010) with BAC levels of 572.6 mg/dL (chronic-binge) compared to 193.9 mg/dL (chronic fed no binge) (Ki et al., 2010).

Ad libitum feeding

Administration of alcohol in drinking-water (A-DW) over the course of several hours, days or weeks has the advantage of being the simplest mode of alcohol feeding and mimics human behavior patterns of intermittent alcohol use and changes in dietary intake. The A-DW method involves rodents being gradually weaned onto increasing concentrations of ethanol (10–40% (*v/v*)) by supplementing (the only source of) available drinking water with increasing amounts of alcohol while allowing animals to feed on standard rodent chow diets *ad libitum* (Best et al., 1949; Brandon-Warner et al., 2012; Keegan et al., 1995). Using this approach animals develop significant hepatic steatosis and inflammation but do not progress to bridging hepatic fibrosis-cirrhosis (Brandon-Warner et al., 2012; Keegan et al., 1995).

As with the LDLD, the A-DW model has advantages and limitations. In addition to rats exhibiting a natural aversion to alcohol, they also have a (relatively) rapid metabolic rate (~4–5 fold higher than that of humans) that may prevent BAC from consistently reaching high enough levels to exert the levels of hepatic injury associated with ALD (Keegan et al., 1995; Yip-Schneider et al., 2011). Based on BAC measurements in mice maintained on an A-DW regime (10/20% alcohol (*v/v)*, alternating daily for 8-weeks) we observed BAC in males of 55–70mg/dL, while females had BAC of 50–65mg/dL (Brandon-Warner et al., 2012), amounts considered "*moderate consumption*" in humans (Miller and Spicer, 2012). Blood alcohol content in excess of 100mg/dL have been reported (Harrison-Findik et al., 2006). Unlike the LDLD, in which a major focus of the model is to regulate complete nutritional status in the presence and absence of alcohol, accurate assessment of nutritional status and alcohol intake is more challenging in the A-DW model.

In most studies, A-DW alone is sufficient to initiate steatosis, but in order to stimulate inflammatory and fibrotic changes, a secondary stressor, such as iron, LPS, high-fat diet, or diethylnitrosamine (DEN) (in drinking water or by intraperitoneal injection) must be included (Brandon-Warner et al., 2012; Dey and Cederbaum, 2006; Patek, 1979). Similarly, addition of increased poly-unsaturated fats administered concomitant with A-DW more closely approximated human ALD (Abraham et al., 2002; Lindros and Jarvelainen, 1998; Rouach et al., 1997; Walsh and Alexander, 2000).

Using the A-DW model, consistent long-term (months) alcohol feeding can be achieved; B6C3 mice maintained on this regime exhibiting increased CYP2E1 and markers of

oxidative stress while developing steatosis, mild fibrotic deposition, increased inflammatory cell infiltrates, increased hepatic injury, and depletion of cellular antioxidant capacity (Brandon-Warner et al., 2012). Finally, the A-DW approach has been used to provide alcohol intake at levels up to 40% (*v/v*) allowing development of more severe hepatic and systemic responses (Keegan et al., 1995). However, using higher alcohol contents is associated with increased mortality, and great care should be exerted in selecting the strain of mouse employed, the proposed study length, and the effect of other factors that may compromise liver function.

Tsukamoto-French (TF) intragastric feeding

To overcome limitations in BAC associated with oral alcohol administration a direct enteral feeding model, in which alcohol is administered through a surgically implanted intragastric cannula, was developed by Tsukamoto and French (TF Model) (Tsukamoto et al., 1984). This model of alcohol feeding has the advantage of circumventing the natural aversion rodents express toward alcohol by free choice, and limitations on the amount of alcohol that can/will be ingested. Alcohol intake is administered by liquid diet at a defined rate over a specified time course. Using this approach, carbohydrates can be substituted for alcohol, and caloric intake can be equalized for pair-matched animals (French, 2001; Nanji and French, 2003). The TF model also allows for manipulation of alcohol content, as well as that of other dietary additives and supplements. Animals established using intragastric feeding exhibit higher blood and urine alcohol content than animals fed alcohol using LDLD or A-DW approaches (Nanji and French, 2003; Wheeler et al., 2000).

Despite the need for an invasive surgical procedure, animals have been maintained using an intragastric feeding approach for 6 months, or more (Nanji and French, 2003). The TF intragastric feeding model also produces hepatic injury that more closely resembles advanced ALD, beginning with steatosis and progressing to necrosis and inflammation with the onset of mild fibrosis (French, 2001; Nanji and French, 2003; Tipoe et al., 2008). While the TF model appears to have many distinct advantages over other alcohol feeding models, it is not without drawbacks. The TF approach requires skilled surgical implantation of the intragastric cannula in combination with extensive animal monitoring. This is particularly evident at the site of cannula implant where the risk of infection and irritation exist, along with the need for monitoring physiological and pathological changes in animal health. While initially developed in the rat, the TF model has been established and characterized in mice (de la Hall et al., 2001; Kono et al., 2000), expanding its applicability to include a range of relevant genetic mouse models. However, employing this approach in mice requires an additional level of surgical competence in cannula placement based on the relative size of mice, procedures that may not be practical in all laboratory settings. As with other models of alcohol feeding in mice, the question of selecting appropriate strains for specific studies is raised. Finally, the TF model of direct alcohol delivery to the gut effectively bypasses the oral and upper gastrointestinal mucosa, eliminating the contribution of the effects of alcohol to those tissues.

In addition to the liver, acute and chronic alcohol consumption potentiate the development and progression of esophageal cancer and other malignancies of the upper gastrointestinal and respiratory tracts, in addition to pathological changes to the respiratory and cardiovascular systems (Liu et al., 2011; Siegmund et al., 2003). Increasing evidence also suggests the effects of alcohol on the bacterial flora in the oral cavity and gastrointestinal mucosa may alter pathological immune responses (Seki and Schnabl, 2012; Son et al., 2010). If this is the case, then selection of an experimental alcohol feeding model may significantly affect end-organ pathology. Interestingly, a study by Yan and colleagues report significant dysbiosis associated with the TF enteral feeding model in mice (Yan et al., 2011). In this case alcohol feeding was associated with changes in expression of bacteriocidal

peptides in the small intestine, and contributed to increased bacteria and changes in the ratio of bacterial populations allowing for the overgrowth of potentially harmful strains (Yan et al., 2011).

An alternative to TF intragastric feeding is direct gastric gavage in which alcohol is administered as a bolus dose *via* a blunt intragastric feeding needle inserted via the oral-pharynx (Seth et al., 2008). This approach is particularly well suited to acute/binge administration of alcohol in rodents and more closely resembles the route of alcohol intake in humans (Crabbe et al., 2011). As a result similar mucosal responses in the pharynx and esophagus occur following alcohol administration *via* gavage (Crabbe et al., 2011). However, this method has also been employed for studies of Kupffer cell activation during ALD in a chronic alcohol model (Enomoto et al., 2007).

Alcohol-preferring rodents (selectively bred)

A key factor in developing intragastic feeding models was the ability to increase amount of alcohol ingested/BAC to levels consistent with the human pathology of chronic alcohol abuse, thereby increasing hepatic injury beyond steatosis. While intragastric models bypass the natural aversion that rodents exhibit toward alcohol consumption, questions remain as to whether LDLD or enteral feeding regimes that provide alcohol within a single liquid food source adequately mimic human consumption with the inherent changes in dietary intake. Another means developed to overcome resistance to alcohol feeding has evolved from the selective breeding of rodents that demonstrate a preference for alcohol (Froehlich, 2010; Li et al., 1993; Li et al., 1979). In this model, selective breeding was started from a foundation stock of Wistar rats, and lines were generated to exhibit a (relatively) high or low preference for alcohol (Li et al., 1979). Rats were selected based on their voluntary preference for alcohol when given a choice of alcohol and non-alcohol containing solutions. This program produced distinct lineages, the alcohol preferring (P) rats, which voluntarily drink alcohol (6–8g/kg body weight, equivalent to 20–30% of total daily caloric intake), even in the presence of alternative non-alcohol containing liquids. Conversely, the non-preferring (NP) rat does not drink alcohol when provided with choice (Carr et al., 1998; Chester et al., 2004; Li et al., 1993; Li et al., 1988; Li et al., 1979; Yip-Schneider et al., 2011). Finally, an inbred preferring (iP) rat was generated through repeated brother-sister matings in which voluntary alcohol consumption occurs when provided with free choice, but at a level approximately half that measured in the P rat model. Blood alcohol contents for P rats have been reported as 40–70 mg/dL, but can rise to 260 mg/dL, rates comparable to those achieved in other alcohol feeding models, and (Table 1), and relevant to human alcoholics (Li et al., 1987; McBride and Li, 1998; Yip-Schneider et al., 2011). In comparison, human alcoholics frequently measure BAC 150–200 mg/dL, and rates can be higher during binge drinking, although loss of orientation, blackout and stupor can occur at levels >250–270 mg/dL, but many alcoholics have increased tolerance (Brick and Erickson, 2009; Urso et al., 1981). In rodent models BAC is frequently classified as low, <100 mg/dL, moderate, 100–200mg/dL, and high, >200mg/dL (Brick and Erickson, 2009; Keane and Leonard, 1989; Urso et al., 1981). Additional analysis of the P rat group reports these animals display 5 characteristics used to define human alcoholics (McMillen, 1997); 1) voluntary choice of alcohol, even when other palatable solutions are present (Lankford et al., 1991), 2) oral self-administration (Murphy et al., 1989), 3) consumption of alcohol for the reinforcing effects on the central nervous system, (Murphy et al., 1988), 4) increased tolerance for alcohol, and 5) development of physical dependence with chronic free-choice drinking (Kampov-Polevoy et al., 2000; Yip-Schneider et al., 2011).

To date these species have been extensively used to study the effects of alcohol on the nervous system, particularly in the field of addiction biology. The use of alcohol-preferring rats to study mechanisms of ALD has the advantage of oral "*self-administration*" of alcohol

mimics that of human alcoholics, while achieving similar, but lower BACs. This is of importance when considering that in the United States and Western Europe chronic alcohol exposure is the major underlying risk factor for development of HCC (McKillop and Schrum, 2009; Yan et al., 2011). One of the advantages to the self-administration of alcohol is that it overcomes the limitations of forced exposure and follows a pattern more closely associated with human consumption with bouts of alcohol intake and intermittent periods of liver recovery/regeneration (Yip-Schneider et al., 2011). When employed as a model to study alcohol as a risk factor for HCC, P rats develop neoplastic lesions when exposed to alcohol over an 18-month time course (Yip-Schneider et al., 2011). Tumor incidence, multiplicity and size were all increased in P rats compared to pair-matched rats on drinking water alone, and iP rats that consumed alcohol at a reduced rate (Yip-Schneider et al., 2011). This supports the relationship between quantity and duration of alcohol consumption and development of hepatic disease leading to HCC. However, despite P rats consuming alcohol over a prolonged period and expressing markers of ALD, animals did not develop hepatic fibrosis-cirrhosis (Yip-Schneider et al., 2011). Additionally, P rats used in this study did not begin alcohol regimes until they were 6 months of age suggesting that pre-neoplastic foci may have been present in the livers that were responsive to promotional effects of alcohol on HCC (Brandon-Warner et al., 2012; Yip-Schneider et al., 2011). This was supported by a similar study in which alcohol regimes were initiated in P rats at 6-weeks of age, and resulted in a tumor incidence of 50%, as compared to 83% in rats that began alcohol consumption at 6 months of age (Yip-Schneider et al., 2011).

Of further note, a similarly selectively-bred strain of high (HAP) and low (LAP) alcohol preference mice has been developed from a HS/ibg progenitor line (Brandish and Sheron, 2010; Matson and Grahame, 2011). Following selection, mice were cross-mated to non-sibling or first cousin pairs in order to "*fix*" alleles that may contribute to neurobehavioral characteristics of alcoholics (Brandish and Sheron, 2010). Mice offer the advantage of possessing well-characterized genomes, combined with lower purchase and animal husbandry costs. This HAP strain is reported to selectively prefer alcohol when given a choice between water alone, and water containing 10% (*v/v*) alcohol, consumes >20g alcohol/kg body weight/day, maintains a high BAC (reported 60–130 mg/dL) (Grahame and Grose, 2003; Matson and Grahame, 2011), and leads to significant hepatic CYP2E1 induction in the absence of changes in ALDH/ADH expression or liver histology after a 3–4 week experimental period (Matson et al., 2012). While these data indicate the HAP mouse model has great potential as a translational model of human alcoholism, it will be equally interesting to define the effects of longer-term alcohol feeding on liver pathology, when studied with alcohol alone, or concomitant with other factors that promote hepatic disease (Yip-Schneider et al., 2011, Brandon-Warner et al., 2012, Matson et al., 2012).

On the horizon - Humanized liver rodent models

The models described thus far exhibit both strengths and weaknesses in their use to study pathobiology of human ALD. Indeed, considerable controversy arises in the interpretation of data using these models based on the model selected and the pathological parameters being measured. As such, the key to fully elucidating the mechanisms of human ALD may depend on developing models that can better recreate the conditions and pathology of human disease. As previously discussed, xenobiotoic metabolism, including that of alcohol, predominately occurs in the liver and is influenced by a complex intersection of genetics, ingestion (both route of ingestion, how often, how much, and what type of alcohol), nutrition, and bioactivation of other substances that may affect absorption, distribution, metabolism, and excretion. In addition direct and indirect cellular effects that initiate inflammatory immune responses vary with model choice. Collectively this milieu of factors influences the pathogenesis of ALD, both directly and indirectly. A potential approach to

overcome disparities between humans and rodents in response to alcohol may lie in the development of rodents with "*humanized*" livers that express multiple human hepatic cell types combined with a human immune system for analysis of complex human diseases such as ALD.

Attempts to develop mice with humanized livers have taken various different approaches, beginning with studies in which isolated hepatocytes were injected directly into the hepatic portal vein (Matas et al., 1976; Shafritz and Oertel, 2011); to later studies which employ primary hepatocytes transplanted into the spleen that migrate to the liver and successfully engraft into the hepatic parenchyma (Gupta et al., 1987; Shafritz and Oertel, 2011). However, relatively little repopulation occurs under these conditions, and once these cells reached a density of ≈1–2% of total hepatic mass obstruction of liver sinusoids occurred resulting in portal hypertension and hepatic infarction (Shafritz and Oertel, 2011). This response was improved when repopulation was accompanied by a proliferative stimulus (partial hepatectomy) or toxic (CCl_4) hepatic injury (Shafritz and Oertel, 2011).

Hepatic cell transplantation has continued to evolve through the development of several mouse models that required selective conditions to enable successful hepatocyte repopulation (Shafritz and Oertel, 2011). The first, utilized transgenic expression of urokinase-type plaminogen activator directed by an albumin promoter that led to progressive destruction of hepatocytes, and was fatal in most transgenic mice (Braun and Sandgren, 1998; Sandgren et al., 1991). However, mice that survived underwent complete liver regeneration within 8–12 weeks, with repopulated hepatocytes exhibiting a full deletion of the transgene (Sandgren et al., 1991). Subsequent studies report that mice could be "*rescued*" from liver failure by transplantation of congenic or xenogenic hepatocytes (Rhim et al., 1995). The result of this startling observation was the development of chimeric mice with humanized livers. These chimeric "*humanized*" mice display human metabolic responses to a range of drugs and demonstrate a human hepatocyte repopulation index as high as 96% (typical range 80–90%) when treated with an inhibitor of human complement activity (Cheung and Gonzalez, 2008; Katoh et al., 2008; Tateno et al., 2004). A similar model utilized targeted disruption of fumarylacetoacetate hydrolase (Fah). The Fah gene encodes an enzyme utilized in the last step of tyrosine catabolism; in its absence there is an accumulation of intermediates that produce extensive persistent hepatic injury. Chemical inhibition of this pathway in Fah-null mice facilitates hepatic repopulation with wild-type hepatocytes (Lindstedt et al., 1992; Shafritz and Oertel, 2011). While these mice provide an exciting potential avenue for studying ALD, use of these animals can be impeded by variability of hepatocyte repopulation, technical difficulty in development-maintenance, combined with the immune (incompetent) status of the mice, and differences in basal metabolic rates between humans and rodents (Chen et al., 2011; Cheung and Gonzalez, 2008). Nevertheless, these animals have proved highly effective for studying hepatocyte metabolism and toxicology, with the caveat that they lack the presence of other hepatic cell populations and functional immune responses required to fully mimic human ALD pathology (Tateno et al., 2004).

The discovery that somatic cells can be reprogrammed to pluripotency as a foundation for organ regeneration without the need for immunosuppressant therapies raises new possibilities in the search for effective models of ALD, as well as a potential source for transplantable hepatocytes (Espejel et al., 2010). Human induced-pluripotent stem cell models are also being developed for use in studies of chemical or drug-induced hepatic toxicity (Sartipy and Bjorquist, 2011). Primary hepatocytes were often used to examine basic safety questions regarding drugs and toxicity, but they are largely ineffective in other aspects of pharmacology due to the loss of key metabolic and transporter functions, combined with the lack of integration with other cell types that are lost during culturing

(Sartipy and Bjorquist, 2011). The potential for creating a functional human liver with a complete complement of alternate hepatic cell types (endothelial, stellate, and Kupffer cells) in chimeric mice raise the possibility that mice can be engineered to more closely approximate human pathology, as well provide an opportunity to examine drug response and toxicity questions *in vivo*. As is expected, advances in the evolution of these models continue as attempts are directed toward developing models with both humanized livers and competent human immune systems. The success of this lies in merging human hematopoietic and immune system mice into a chimeric "*humanized*" liver models (Legrand et al., 2009). This may provide a valuable tool to study ALD, as well as other human hepatic diseases that can not be replicated in current mouse models, such as Hepatitis C and B viral infection, as well as the potential to evaluate potential therapeutics and toxicology studies (Dorner et al., 2011; Robinet and Baumert, 2011).

Another intriguing approach to humanized models involves the expression of human ectopic artificial livers (HEAL). Engineered HEAL's are polymer scaffolds that encapsulate primary human hepatocytes within a supportive microenvironment that are maintained by the murine circulation and perform human hepatic functions (Chen et al., 2011; Underhill et al., 2007). These HEAL's expressed 68 of 82 phase I and phase II detoxification enzymes, including cytochrome P450s, and transporters essential for biotransformation (Chen et al., 2011). These ectopically expressed human livers have the advantage of being generated in immunocompetent mice in the absence of liver injury, and may have particular application in elucidating pharmacological interactions and metabolism, but may also offer insights into human pathobiology.

While these models offer many interesting and novel approaches to study ALD, as with other models they are associated with important potential caveats that cannot be overlooked. As previously discussed, rodents exhibit notably higher basal metabolic rates compared to humans and, even though "*humanized*" models will begin to redress differences in alcohol metabolism between mice and humans, they do not necessarily overcome the balance of alcohol metabolism with overall metabolic status. Mice also exhibit a natural aversion to alcohol consumption, and it is uncertain as to what effect the humanized liver will exert on alcohol avoidance. Similarly, mice have evolved greater tolerance to LPS than humans, and it remains to be established how the effect of alcohol on, gut flora, gastrointestinal permeability, and systemic LPS levels may act on a humanized liver given the relatively higher sensitivity of humans to LPS compared to mice (Ramaiah and Jaeschke, 2007; Copeland et al., 2005; Mestas and Hughes, 2004)).

SUMMARY AND CONCLUSIONS

Globally, more than half the world's population consumes alcohol. Attempts to censor this behavior have historically proven unsuccessful; however, the health consequences associated with alcohol consumption remain a significant medical and financial burden. Major advances have been made in our knowledge and understanding of the mechanisms and pathology of ALD. Development of effective animal models is an essential tool in unraveling the interactions and roles of multiple cell types, immune modulation, and metabolic stresses within the hepatic milieu during ALD progression. While we are progressing toward development of rats and mice that can overcome some of the limitations, one stark difference is the time course from onset to diagnosis and progression of disease. In humans, progression of ALD can often be measured in decades; typical onset being between 40–60 years of age with cirrhosis developing as end-stage liver disease and an important precursor to development of HCC (McKillop and Schrum, 2009; Sofair et al., 2010). Humans who have been drinking for more than 10 years have been identified to be most likely to develop end-stage liver disease (Mann et al., 2003). Mice and rats rarely develop

fibrosis or cirrhosis, within the time constraints of experimental study without the addition of secondary hepatic insults (Karaa et al., 2008; McKillop and Schrum, 2009; Thompson et al., 2011; Tsukamoto et al., 2009). Much like their human counterparts, rodent-strains have differing sensitivity to alcohol-induced injury. While this suggests that these differences may offer an opportunity to investigate genetic and other environmental or dietary factors, it also highlights the importance of selecting appropriate rodent strains, and evaluating how closely they replicate disease pathology and progression.

Progress has been made in developing methods to better utilize rodent models. From early development of methods of oral or enteral feeding, which provided a means for effective delivery of alcohol, to ongoing efforts to generate "*humanized*" mouse models, with a full complement of hepatic cell types and immune function. Each of these methods affords different advantages and limitations, and the efficacy of individual methods is often dependent on the pathway(s) studied, and animal model(s) employed (Table 1). Of particular note, it is becoming increasing apparent that the gut microbiome plays an integral role in the pathobiology of ALD. While TF enteral feeding produces dysbiosis (Yan et al., 2011), the effects of other feeding methods (LDLD, *Ad libitum*, and alcohol preferring animals) with patterns of ingestion that more closely mimic human consumption have not been reported. Further work remains to fully understand relationship between the gut microbiome and initiation and maintenance of innate immune responses during ALD progression, and how these may be differently influenced by the mode of alcohol delivery.

The key to understanding the synergy between ethanol/ethanol metabolism, oxidative stress, and immune responses in the progression of ALD may depend on generating rodent models that can more effectively replicate the process as they occur in humans. In this regard, chimeric mice possessing human hepatocytes in concert with other hepatic cell types, combined with human immune cells, or mice ectopically expressing human liver tissue may provide new insights into the pathology of ALD. These *in vivo* and *ex vivo* models may overcome some of the inherent limitations that impede our ability to define the mechanisms of alcohol-induced injury in order to identify diagnostic markers, and develop therapeutic strategies to prevent disease progression. However, based on historical perspectives, caution should be applied in interpreting data from these models relative to the human disease state.

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ABBREVIATIONS

ALD	Alcoholic liver disease
HCC	Hepatocellular carcinoma
ROS	Reactive oxygen species
ADH	Alcohol dehydrogenase
ALDH	Acetaldehyde dehydrogenase
CYP2E1	Cytochrome P450 2E1
NAD/NADH	Nicotinamide adonine dinucleotide/reduced
LPS	Lipopolysaccharide
TNFα	Tumor necrosis factor-α
BAC	Blood alcohol content

LDLD	Lieber-DeCarli liquid diet
A-DW	Alcohol drinking water
TF	Tsukamoto-French enteral feeding model
HAP	High alcohol preferring mice
HEAL	Human ectopic artificial liver

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Table 1

Comparison of rodent models of alcohol feeding.

Model	Species	BAC	Pathology	Advantages/*Disadvantages*
Lieber-DeCarli oral liquid diet	Mice Rats	100–160 mg/dL	Animals develop steatosis, minor inflammatory infiltrates	• Oral delivery, strict nutritional equality with controls. • Activation of Kupffer cells by increased LPS • Can be combined with oral gavage to model chronic-binge patterns of alcohol consumption • Pathological changes do not progress beyond steatosis, metabolic oxidative stress in the absence of a secondary stress
Ad libitum oral alcohol in drinking water	Mice Rats	50–100 mg/dL	Animals develop steatosis, minor inflammatory infiltrates	• Mimics human consumption and delivery to the gastrointestinal tract. • Activation of Kupffer cells by increased LPS • Pathological changes do not progress beyond steatosis, metabolic, and oxidative stress in the absence of a secondary stress
Tsukamoto-French intragastic 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	• Enteral delivery, maintains nutritional equality with controls • Larger dosage of alcohol than oral feeding methods. • Progressive pathological changes including fibrosis with activation of Kupffer cells and inflammatory networks • Requires surgical expertise for insertion of cannula, which remains in place through duration of treatment. • Bypasses effects of alcohol on oral-pharyngeal mucosa and upper GI tract. • Contributes to dysbiosis and bacterial overgrowth in the GI tract • BAC must be closely monitored to avoid alcohol toxicity
Oral gavage	Mice Rats	Can be in excess of >500 mg/dL	Animals develop steatosis and mild inflammatory cell infiltrates	• Allows for administration of increased dosage of alcohol • Models binge drinking, more difficult for chronic consumption • Pathological effects when combined with a chronic oral ingestion mimic human pathology • Bypasses oral mucosa and upper GI • Stressful for animals, with risk of upper GI trauma • BAC must be closely monitored to avoid alcohol toxicity
Alcohol preferring rodents	(P, iP & NP rats) (HAP/LAP mice)	40–130 mg/dL can be in excess of 200 mg/dL	Animals do not develop cirrhosis	• Natural oral feeding model, mimics human behavior animals progressively develop HCC in the absence of cirrhosis (rats)

Model	Species	BAC	Pathology	Advantages/*Disadvantages*
				• Pathological progression from steatosis, oxidative stress, inflammation, necrosis and fibrosis are uncharacterized