

Research Article

Chronic-Binge Model of Alcoholic Hepatitis in Long Evans Rats

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Abstract *Background.* Alcoholic hepatitis (AH) is largely a histopathologic diagnosis that is based upon the presence of significant inflammation, hepatocellular lipid accumulation, ballooned degeneration of hepatocytes, and Mallory-Denk bodies. Underlying biochemical and molecular abnormalities include increased oxidative and endoplasmic reticulum (ER) stress, mitochondrial dysfunction, insulin resistance, lipid dyshomeostasis, and cell death. Acute AH is often precipitated by binge drinking in the setting of chronic alcohol abuse, and is problematic due to its propensity to progress to chronic alcoholic liver disease with end-points of cirrhosis or liver failure. Improved understanding of AH pathogenesis could aid in the development of new treatments and preventive measures. *Study Design.* To mimic conditions leading to AH in humans, we generated a chronic-binge model of ethanol exposure in which Long Evans rats were fed with isocaloric liquid diets containing 0% or 37% ethanol (caloric content) for 5 weeks, and during the last 2 weeks, the ethanol diet fed rats were binged with 2 g/kg ethanol 3 times per week. Livers were used for histologic and electron microscopic studies. *Results.* Ethanol-exposed livers had severe panlobular steatohepatitis with disorganization of the chord-like architecture, foci of necrosis, apoptotic bodies, ballooned degeneration of hepatocytes, giant mitochondria, disrupted ER structure and relationship to mitochondria, and early fibrosis. These findings correspond well with those seen in human AH, except for the absence of Mallory-Denk bodies. *Conclusion.* The overall findings suggest that chronic-binge models of alcohol feeding produce pathologic lesions that correspond with AH in humans. This approach provides better tools for designing treatment and unraveling mechanisms by which AH progresses to chronic stages of liver disease.

Keywords alcoholic hepatitis; experimental model; binge drinking; electron microscopy

1. Introduction

Alcoholism is a costly public health problem that continues to grow, especially among women and young people [29]. On a global scale, alcohol abuse is a leading cause of liver-associated morbidity and mortality [23, 25, 29]. Acute alcohol-related liver injury mainly consists of steatosis, which is usually reversible. However, some individuals develop steatohepatitis which is problematic due to the increased risk of developing chronic progressive alcoholic

liver disease (ALD) that culminates in cirrhosis and liver failure [26]. The major cause of ALD is heavy drinking over prolonged periods [37].

Alcoholic steatohepatitis is diagnosed using standard clinical criteria together with histopathologic evidence of severe hepatic steatosis, acute inflammation, necrosis, ballooned degeneration of hepatocytes (keratin depletion), disorganization of the lobular architecture, megamitochondria, and presence of Mallory-Denk bodies [39, 44]. Mallory-Denk bodies are intracytoplasmic hyaline deposits of aggregated, misfolded, ubiquitinated proteins [2], including intermediate filament proteins [38]. With progression from acute to chronic stages of ALD, liver function deteriorates due to impairments of multiple interrelated pathophysiological processes, which together cause insulin resistance [10, 20, 28], cytotoxic and lipotoxic injury [7, 8, 12, 24], inflammation [7, 35], oxidative and endoplasmic reticulum (ER) stress [14, 18, 22, 27], metabolic/mitochondrial dysfunction [13, 30], decreased DNA synthesis [1, 28, 36], and increased cell death [12]. Progression of chronic ALD is marked by activation of profibrogenic pathways [7], setting the stage for eventual development of cirrhosis and liver failure [30, 41]. Improved understanding of the pathogenesis and factors leading to progression of alcoholic hepatitis could aid in the development of additional diagnostic and therapeutic tools that reduce morbidity and mortality from ALD.

The present study describes a new chronic-binge model of ALD that was generated in Long Evans rats. The objective was to produce a model that more closely mimics ALD in humans, since chronic ethanol feeding in rodents tends to cause relatively mild degrees of liver injury. The experimental design considered the fact that in humans, alcoholic hepatitis typically develops in chronic heavy

drinkers, with binge drinking variably contributing to disease pathogenesis. The Long Evans strain was used because in previous studies, we demonstrated it to be highly susceptible to alcohol-induced liver injury by chronic feeding with Lieber-deCarli ethanol-containing liquid diets (9% v/v) for 5–6 weeks [9,11,12,28,43]. The increased susceptibility to liver injury was linked to altered hepatic expression of alcohol metabolizing enzymes that could lead to acetaldehyde build-up, and higher basal levels of oxidative stress, mitochondrial dysfunction, p53 activation, and DNA damage in liver [12]. This suggests that endogenous host factors can modulate susceptibility to ALD. To mimic human clinical scenarios associated with alcoholic hepatitis and ALD, we modified our chronic exposure model by binge administering ethanol (2 g/kg, 3×/wk) during the 4th and 5th weeks of the experiment. Herein we report the histopathologic and ultrastructural features of this model which more closely mimics the pathology of human ALD. The study design was based on epidemiological evidence that acute alcoholic hepatitis often develops in the context of chronic heavy plus binge drinking, together with overwhelming experimental data showing that chronic ethanol feeding alone is not sufficient to produce significant ALD in most rodents.

2. Methods

2.1. Experimental model

Long Evans 4-week old male rats were pair-fed for 5 weeks with isocaloric liquid diets containing 0% or 9% (v/v) ethanol. Diet mixes were purchased from BioServ (Frenchtown, NJ, USA). The control and ethanol diets contained 151 kcal/L of protein and 359 kcal/L of fat. In addition, the control diet contained 490 kcal/L of carbohydrate while the ethanol diet had 135 kcal/L of carbohydrate and 355 kcal/L of ethanol (substituted for carbohydrates). Pair feeding was accomplished by balancing the volumes of supplied liquid diet such that equal amounts were consumed by each. After 3 weeks on the liquid diets, ethanol-fed rats were binge administered ethanol (2 g/kg-100 μ L) by intraperitoneal (IP) injection on Mondays, Wednesdays, and Fridays for the remaining 2 weeks of the experiment, while controls were injected with saline. At the end of the experiment, blood alcohol levels were measured at 7:00 AM (basal) and 30 min after bingeing. Rats were monitored daily to ensure adequate nutritional intake and maintenance of body weight. Rats were housed under humane conditions and kept on a 12 hour light/dark cycle with free access to the liquid diets and water. Our institutional guidelines cause us to maintain ambient temperature between 68 °F and 79 °F, and humidity between 30% and 70%. However, throughout the study, the ambient temperature was 70 °F–72 °F, and humidity ranged from 59% to 62%. All experiments were performed

in accordance with protocols approved by Institutional Animal Care and Use Committee at the Lifespan-Rhode Island Hospital, and guidelines established by the National Institutes of Health. Preset criteria for euthanasia included loss of 10% body weight, failure to self-groom, ruffled fur, or occurrence of seizures. All animals survived the experiments and none required euthanasia.

2.2. Liver tissue processing

Rats were deeply anesthetized with isoflurane and exsanguinated by cardiac puncture. Portions of liver were immersion fixed in 10% neutral buffered formalin. Fixed tissue was divided for paraffin embedding and cryosectioning. Adjacent paraffin-embedded histological sections (5 microns) were stained with Hematoxylin and Eosin (H&E) or Sirius Red (fibrosis). Formalin-fixed cryostat sections (10 μ m thick) of liver tissue were stained with Oil Red O to assess hepatic steatosis. For transmission electron microscopy (TEM), liver tissue was immersed in modified Karnovsky's fixative (2.5% glutaraldehyde, 4% paraformaldehyde in 0.15 M sodium cacodylate), post-fixed in osmium tetroxide, and embedded in Spurr's epoxy resin. Semithin (1 μ m thick) sections were stained with methylene blue-azure II to select regions for TEM. Ultrathin (50–60 nm) sections, contrasted with uranyl acetate and lead citrate were examined using a Morgagni 268 transmission electron microscope and photographed with an AMT Advantage 542 CCD camera.

3. Results

3.1. General features of the chronic-binge model

Over the course of the study, body weight gain was similar for the control and ethanol-exposed groups. In the control group, the initial and final mean body weights were 268.6 \pm 32.6 g and 388.4 \pm 45.6 g, and in the ethanol group, initial and final mean body weights were 259.9 \pm 29.8 g and 382.9 \pm 33.6 g. Alcohol levels were measured in tail blood using the Analox GM7 apparatus (Analox Instruments USA, Lunenburg, MA, USA) according to the manufacturer's protocol. At the end of the experiment, the mean basal and post-vehicle (mock binge) blood alcohol concentrations in control rats were 13.7 \pm 4.2 mg/dL and 19.7 \pm 7.9 mg/dL. In the ethanol group, basal and 30 min post binge blood alcohol concentrations were 80.2 \pm 8.5 mg/dL and 239.3 \pm 30.9 mg/dL ($P < .0001$ for both sets).

3.2. Histopathological features of experimental chronic-binge ethanol-induced steatohepatitis

H&E stained sections of control livers revealed the expected well-organized lobular architecture with hepatocytes arranged in chords alongside regularly aligned sinusoids. The hepatocytes were relatively uniform in size, and the livers had little or no chronic inflammation, necrosis,

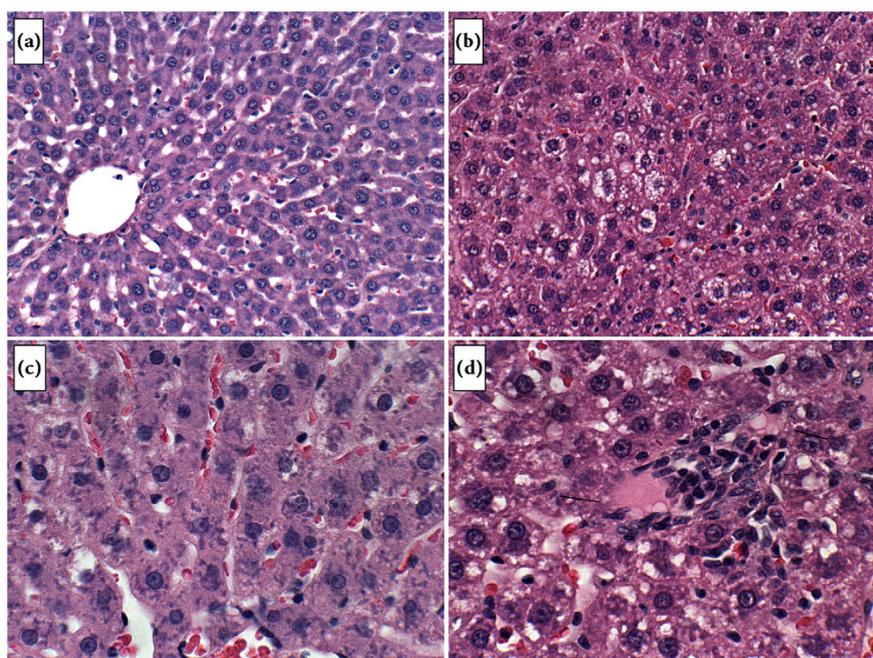


Figure 1: Effects of chronic-binge ethanol exposures on liver histology. Adult male Long Evans rats were chronically fed with isocaloric liquid diets containing (a), (c) 0% or (b), (d) 37% caloric (9% v/v) ethanol for 5 weeks. During the last 2 weeks of feeding, rats in the ethanol group were binged with 2 g/kg ethanol 3×/week (IP). Controls were injected with saline. Liver tissue samples were immersion fixed in 10% buffered formaldehyde solution, embedded in paraffin, and sectioned (5 microns thick) for H&E staining. Note regular chord-like architecture with no inflammation and minimal microvesicular lipid vacuoles in control liver shown at (a) low and (c) higher magnification, and (b), (d) marked disorganization of the lobular architecture, hepatocellular steatosis (clear cytoplasmic vacuoles in (b) and (d)), inflammation (d), and hepatocytes with ballooning degeneration (d) in the ethanol-exposed liver.

steatosis, or apoptosis (Figures 1(a) and 1(c)). Chronic-binge ethanol exposed livers had prominent microvesicular and macrovesicular steatosis, multiple foci of intralobular mixed, but mainly lympho-mononuclear cell inflammation, scattered apoptotic cells, foci of necrosis, increased variability in hepatocyte nuclear size, and lobular disarray with loss of the regular chord-like architecture (Figures 1(b), 1(d), and 2). In addition, scattered hepatocytes exhibited granular cytoplasmic ballooning degeneration (Figure 2). However, Mallory's hyaline was not detected.

3.3. Further analysis of chronic-binge ethanol effects on hepatic steatosis

In control livers, Oil Red O staining revealed moderate levels of cytoplasmic lipid in hepatocytes that were mainly distributed in Zone 3 (perivenous) (Figure 3(c)), but virtually absent staining of hepatocytes in Zones 1 (periportal; not shown) and 2 (midzonal) (Figure 3(a)). In contrast, ethanol-exposed livers had striking panlobular Oil Red O staining of hepatocytes, indicating that hepatic steatosis involved all three zones (Figures 3(b) and 3(d)). High magnification images demonstrated the expected punctate pattern of cytoplasmic Oil Red O staining (Figures 3(e) and 3(f)).

3.4. Effects of chronic-binge ethanol exposures on hepatic fibrosis

Sirius Red stains revealed minimal collagen fibril deposition in control livers (Figures 4(a) and 4(c)), and conspicuously increased levels and distribution of coarse and fine perihepatocyte and sinusoidal collagen deposits in livers from the chronic-binge ethanol exposure model (Figures 4(b) and 4(d)). In addition, ethanol exposure resulted in focal "chicken wire fibrosis" characterized by a partial trabecular or mesh-like pattern of collagen fibrils that surrounded individual hepatocytes (Figure 4(d)).

3.5. Fine histological features of chronic-binge alcohol-induced steatohepatitis

Semithin 1 μ M thick sections of control liver revealed relatively uniform hepatocyte cytology with prominent dense oval to round punctate bodies corresponding to mitochondria (Figures 5(a) and 5(c)). Ethanol-exposed livers exhibited increased microvesicular as well as macrovesicular cytoplasmic lipid droplets and coarse cytoplasmic granularity corresponding to giant mitochondria (Figures 5(b) and 5(d)). In addition, irregularly increased intercellular spacing was evident in ethanol-exposed livers.

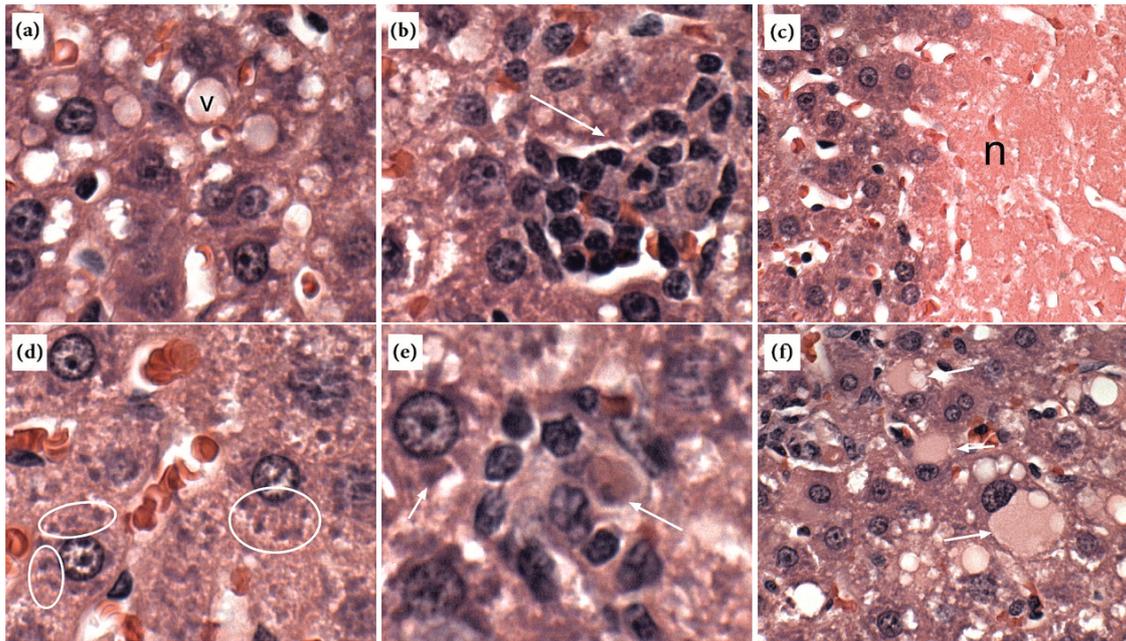


Figure 2: Detailed analysis of chronic-binge alcohol-induced steatohepatitis. H&E stained, formalin-fixed paraffin-embedded histological sections of liver revealed (a) microvesicular (small) and macrovesicular (large) steatosis (v = cytoplasmic vacuoles), (b) lobular inflammation (arrow), (c) foci of necrosis (n), (d) enlarged mitochondria (circled), (e) apoptotic bodies (arrows), and (f) granular ballooning cytoplasmic degeneration (arrows). Original magnification 400 \times .

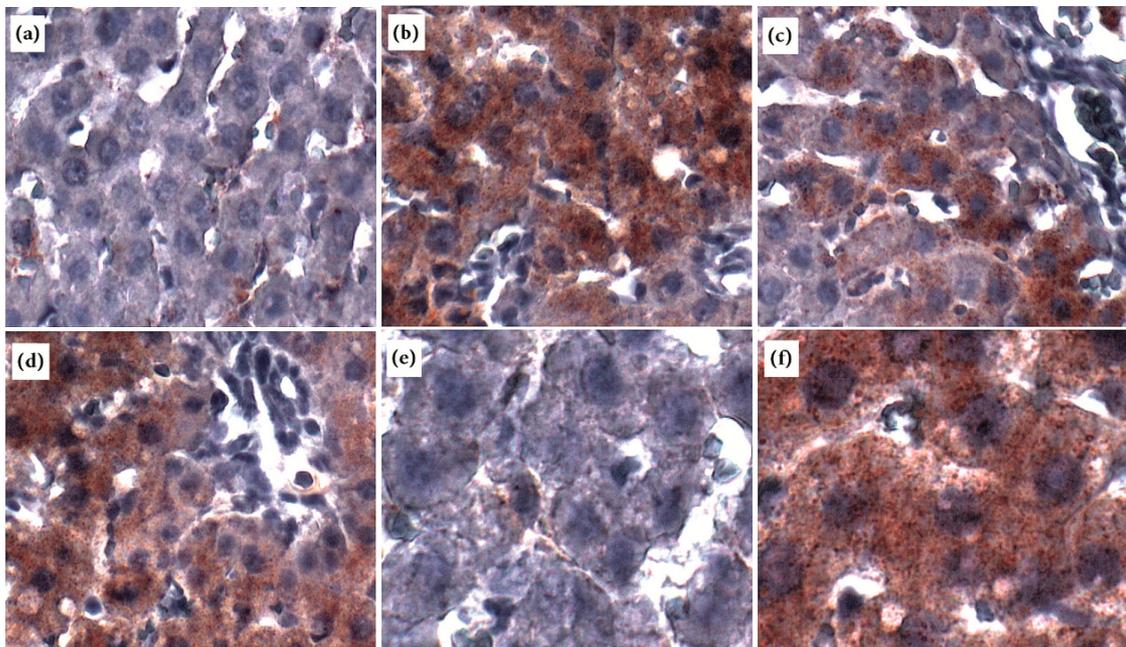


Figure 3: Alcohol-induced hepatic steatosis demonstrated by Oil Red O staining. Formalin-fixed, frozen sections of (a), (c), (e) control and (b), (d), (f) chronic-binge ethanol exposed Long Evans rat livers were stained with Oil Red O (ORO-red) and counterstained with hematoxylin (blue). Control livers had (a) low levels of ORO staining in Zone 2 and (c) moderate ORO staining of hepatocytes in centrilobular regions (Zone 3). In contrast, ethanol exposed livers had intense panlobular ORO staining ((b) = Zone 2; (d) = Zone 3). (e), (f) Higher magnification images of hepatocytes in Zone 2. Note punctate ORO staining in (f). Original magnifications, (a)–(d) = 200 \times ; (e), (f) = 400 \times .

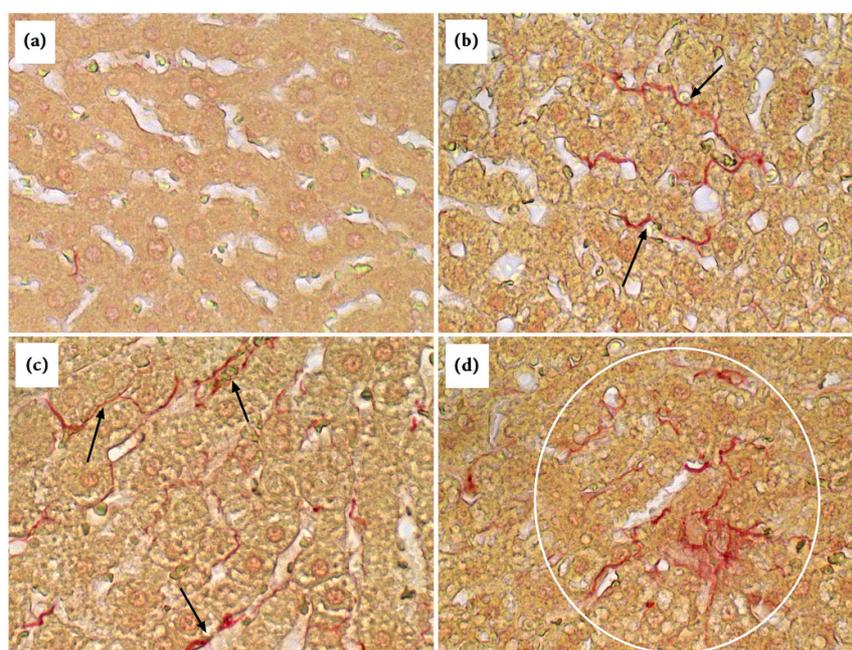


Figure 4: Effects of chronic-binge ethanol exposures on hepatic fibrosis. Formalin-fixed paraffin-embedded histological sections were stained with Sirius Red (red) to detect collagen deposition and fibrosis. (a), (c) Control liver showing minimal staining with Sirius Red (arrows in (c)). (b), (d) Ethanol-exposed liver with early fibrosis characterized by prominent linear labeling and focal chicken wire collagen deposition (arrow in (b) and circle in (d)). Original magnification 400 \times .

3.6. Ultrastructural features of chronic-binge ethanol-induced steatohepatitis

TEM studies focused on the effects of alcohol on the structure and spatial distribution of ER and mitochondria. Relatively low magnification images (7,100 \times) revealed abundant mitochondria that were uniform in size and shape and had complex matrix/cristae. Although mitochondria were distributed throughout the cytoplasm, they were also conspicuously localized at the periphery of rough ER (RER) cisternae (flattened/narrowed, elongated tubules) that were stacked in parallel arrays (Figures 6(c) and 7(a)). Glycogen granules were diffusely distributed in cytoplasm and lipid droplets were scarce.

Chronic-binge ethanol feeding increased nuclear swelling, abundance of lipid droplets and glycogen granules, mitochondrial pleomorphism, and RER dispersal (Figures 6(b), 6(d), 7(b)–7(d)). Lipid droplets were distributed throughout the cytoplasm, interrupting the normal subcellular mitochondria-RER organization. Mitochondrial pleomorphism was manifested by increased variability in mitochondrial size and shape with irregular and striking enlargement of the organelles (megamitochondria); however, the cristae and matrix were relatively preserved. In contrast to control samples, mitochondria in ethanol-exposed livers were distributed amongst the RER rather than at the perimeters of stacked cisternae (Figures 6(d), 7(b), and 7(c)). RER structure was highly irregular due to loss or

shortening (also possibly fragmentation) of the normal cisternae, focal swellings (dilated tubules), and irregular spacing of ribosomes. In addition, chronic-binge ethanol exposures produced regionally severe disorganization of the RER with loss of the tubular and saccular architecture, and irregular aggregations of ribosomes (Figure 7(d)).

4. Discussion

This work describes a new model of experimental alcohol-induced steatohepatitis that is based on the concept that in humans, acute alcoholic hepatitis occurs in the context of binge drinking superimposed on chronic alcohol abuse. The present model differs from the chronic-binge model reported earlier [5,6] in that it utilizes rats instead of mice, the period of chronic ethanol exposure that we used was more prolonged (5 weeks instead of 10 days), and alcohol bingeing was repeated (3 \times per week for 2 weeks) and injected rather than administered in a single gavage. Our alternative methodological approach produced steatohepatitis that has many features shared in common with human ALD. The advantage of the longer term model is that it provides opportunity to assess efficacy of therapeutic intervention at various time points in relation to chronic and binge alcohol exposures.

Previous work using a Long Evans rat model demonstrated that chronic ethanol feeding produces steatohepatitis with persistent inflammation, hepatocellular apoptosis, and

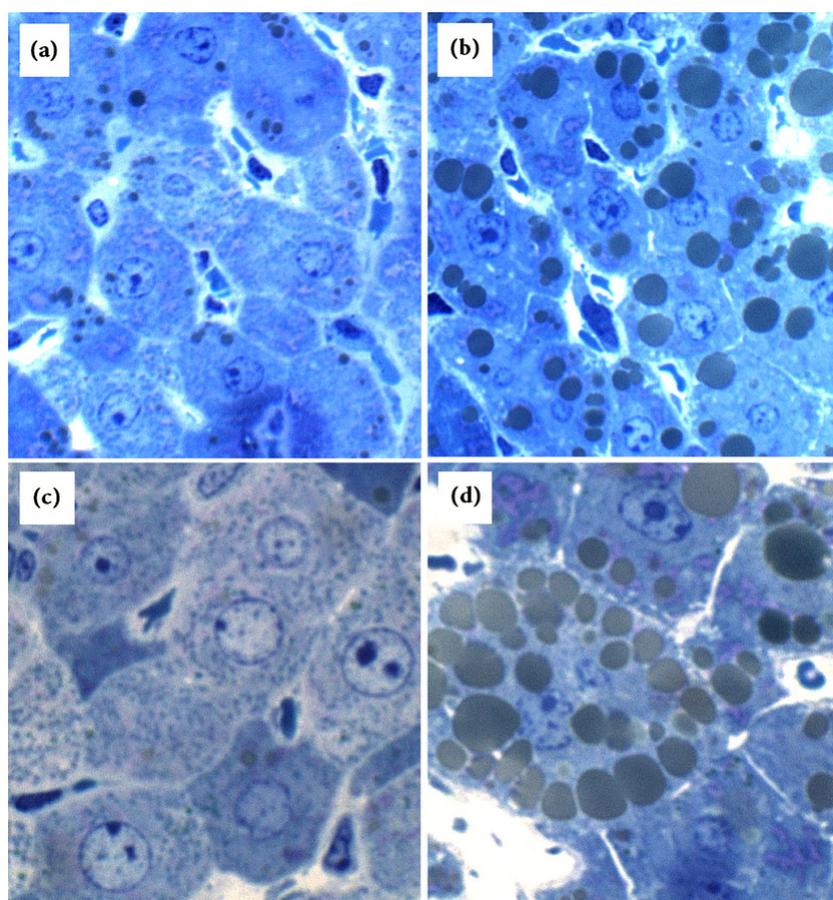


Figure 5: Fine histological features of chronic-binge alcohol-induced liver disease. Liver tissue was embedded in Spurr's epoxy resin. $1\ \mu\text{m}$ thick sections were stained with Toluidine blue. Note uniform cytoplasm with minimal lipid droplets in (a), (c) control and (b), (d) prominent lipid droplets (greenish-gray) and (d) megamitochondria (coarse cytoplasmic granularity) in ethanol-exposed livers. Original magnifications: (a)–(b), $200\times$; (c)–(d), $400\times$.

increased cell turnover, and disorganization of the lobular architecture [31]. The present work shows that superimposed binge alcohol exposures worsen the pathology by producing conspicuous foci of nonzonal necrosis, severe panlobular steatosis, hepatocellular ballooning degeneration, and increased inflammation. Although the inflammatory infiltrates were mainly lymphomononuclear, mixed populations, including occasional neutrophils, were observed, whereas with the short-term model, hepatic neutrophil infiltrates were more abundant, possibly due to differences in the route of administration (gavage vs. IP) and binge dose ($5\ \text{g/kg}$ vs. $2\ \text{g/kg}$) [5, 19]. On the other hand, the additional histopathological features observed in our chronic-binge versus chronic ethanol exposure model most likely correspond to direct hepatotoxic effects of acute high-level (i.e., binge) alcohol exposures. In contrast, the early fibrosis observed in the chronic-binge model (Sirius Red staining) was similar to the effects of chronic ethanol feeding [31]. Therefore, fibrosis is more likely the result of chronic liver injury rather than repeated binge alcohol exposures.

In humans with acute alcoholic hepatitis, hepatocellular steatosis, which reflects mainly increased storage of triglycerides, is typically severe and associated with tissue necrosis, apoptosis, and inflammation. In the chronic-binge exposed livers, steatosis was panlobular, and foci of lobular inflammation or necrosis were distributed throughout the liver without a zonal pattern, similar to human disease. Ballooning degeneration of hepatocytes, manifested by cytoplasmic enlargement and granular pallor with nuclear pyknosis or karyorrhexis, corresponds to degeneration or apoptosis occurring in the context of steatosis. Although not specific for ALD, ballooning degeneration is one of the signature features of ALD in humans. This chronic-binge model produces hepatocellular ballooning degeneration, distinguishing it from the effects of chronic ethanol feeding and likening it to human disease.

Although livers from rats subjected to chronic-binge ethanol exposures shared a number of histopathological features in common with human ALD, notable differences included the nature of the lobular inflammatory cell

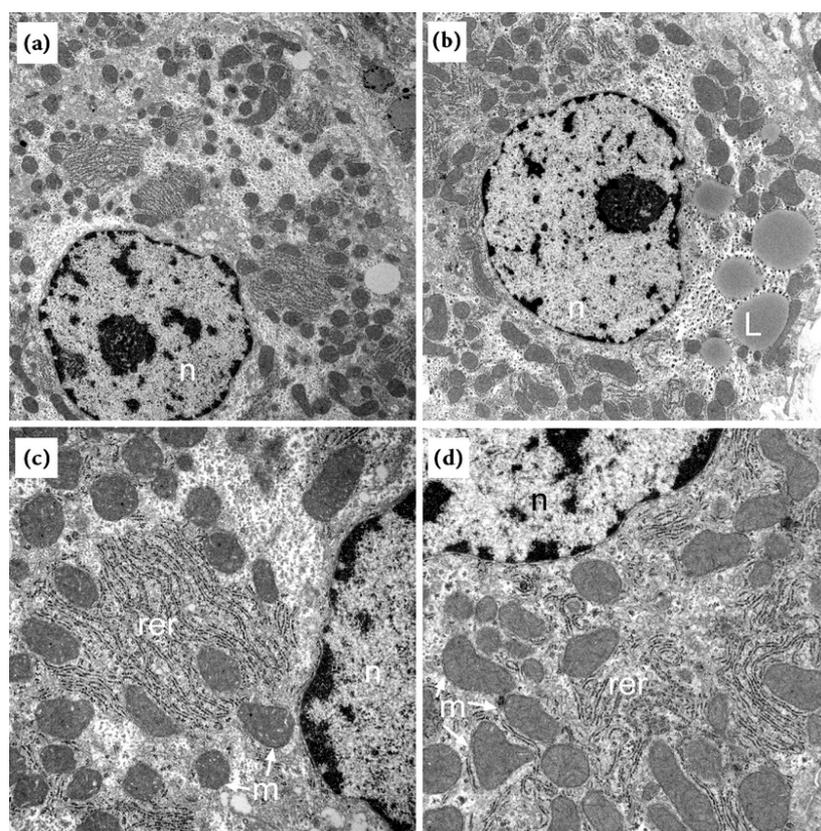


Figure 6: Ultrastructural features of chronic-binge alcohol-induced liver injury. Spurr's resin-embedded, 50–60 nm thick sections of liver were contrasted with uranyl acetate and lead citrate and examined by transmission EM. (a), (b) Control sample showing abundant relatively uniform mitochondria (m), many localized at the periphery of parallel stacks of RER (rer). (c), (d) Ethanol-exposed sample exhibiting nuclear swelling and pallor due to increased glycogen, irregularly enlarged mitochondria (m), conspicuous lipid droplets (L) among free ribosomes, and disorganized RER profiles. (Original magnifications: (a), (b) = 7100 \times ; (c), (d) = 18,000).

infiltrates and absence of Mallory-Denk bodies (Mallory's hyaline). In humans, a distinct feature of acute alcoholic hepatitis is the presence of polymorphonuclear neutrophils. In our model, only rare scattered inflammatory cells had trilobate nuclei corresponding to the morphology of neutrophils, whereas the vast majority of infiltrating inflammatory cells were lymphocytes and macrophages. It is not entirely clear why in humans, acute alcoholic hepatitis is associated with robust infiltrates of polymorphonuclear neutrophils [4], but a major area of investigation pertains to the role of increased gut permeability with release of bacterial toxins and attendant activation host inflammatory responses [4]. Bertola et al. developed a different chronic-binge model in which mice were fed with ethanol-containing liquid diets for 10 days and binged once by gavage [6]. That protocol results in steatohepatitis with infiltrating neutrophils [6], although the severity of acute inflammation did not reach the levels seen in human biopsies of acute alcoholic hepatitis. Nonetheless, the robust liver injury marked by serum transaminase

elevations, and proinflammatory cytokine responses suggest a role for systemic inflammation mediated by increased gut permeability. Our model was designed to examine direct effects of alcohol in relation to liver injury, and avoid contributions of enteric endotoxin release and sepsis. Future studies could be designed to address the specific mediators of neutrophil recruitment to the liver in the context of chronic-binge alcohol exposures.

Mallory-Denk bodies are intracytoplasmic hyaline deposits that correspond to aggregates of misfolded ubiquitinated proteins [15]. Protein misfolding and aggregation are common themes of cellular and tissue degeneration and chronic disease. In particular, Mallory-Denk bodies contain aggregates of ubiquitinated, cytokeratin intermediate filaments [38], and are detectable in advancing stages of steatohepatitis caused by various factors, including ALD, nonalcoholic fatty liver disease, hepatocellular carcinoma, and primary biliary cirrhosis [3]. The absence of Mallory-Denk bodies in our model could indicate that additional factors are needed to drive the unfolded protein response

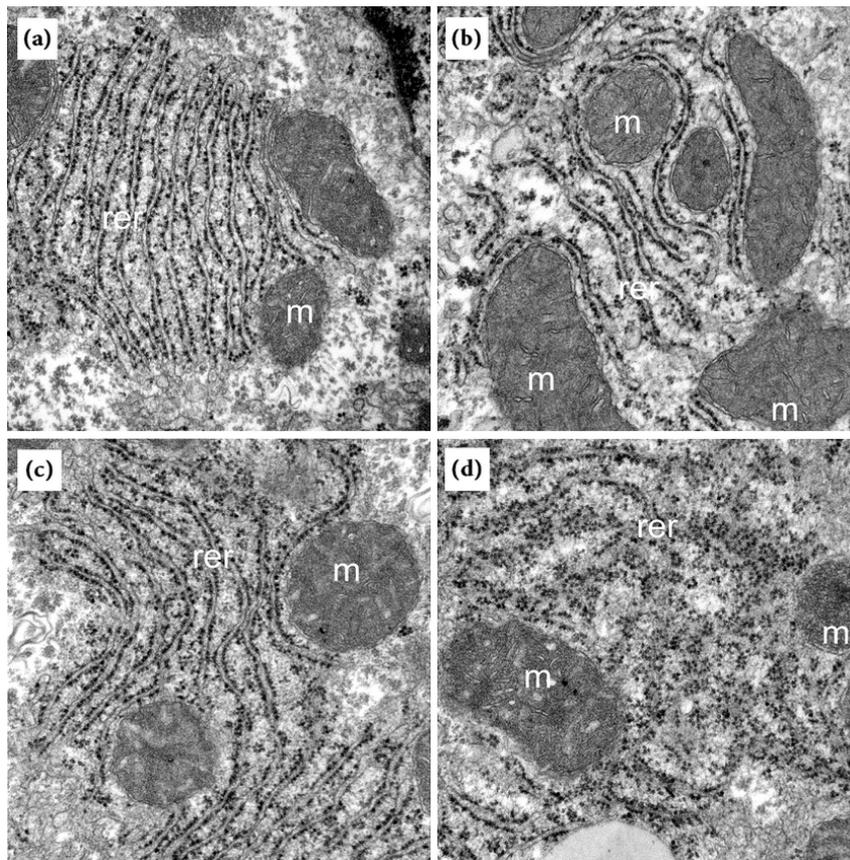


Figure 7: Chronic-binge ethanol effects on mitochondrial and RER structure. Spurr's resin-embedded, 50–60 nm thick sections of liver were contrasted with uranyl acetate and lead citrate and examined by transmission EM. (a) Control livers had relatively uniform size and shape of mitochondria (m) with complex matrix/cristae and uniform parallel stacks of RER. (b), (c) Ethanol exposed livers had irregularly shaped mega mitochondria that displaced and distorted the RER. (d) In addition, ethanol exposure was associated with focal disruption of the RER with loss of organized structure. Original magnifications, 44,000 \times .

and ER stress in liver. Considerations include the need for higher levels and a more acute nature of inflammation, or extended chronicity of the alcohol exposures. Potential contributions of endotoxin and/or sepsis are also possible given the fact that Mallory-Denk bodies accumulate in concert with other cellular degenerative changes that result in keratin depletion, including apoptosis and hepatocellular ballooning degeneration [21].

Examination of 1 micron thick sections by light microscopy and ultrathin sections by TEM demonstrated abundant mitochondrial pleomorphism with megamitochondria, abundant cytoplasmic lipid droplets, and pronounced disorganization and disruption of RER tubules in chronic-binge alcohol exposed livers. These findings are reminiscent of the abnormalities reported in human ALD [16,17,32,33,34,40,42]. The 1 micron thick sections demonstrated ethanol-induced variability in hepatocyte size, abundant lipid droplets, and chaotic hepatocellular arrangements with large gaps between cells. Ultrastructural studies revealed

substantial alterations in mitochondria and ER caused by ethanol. These abnormalities probably contribute to cell injury and death mediated by mitochondrial dysfunction and ER stress, which are well-documented features of ALD [20].

In summary, we describe histopathologic and ultrastructural features in livers from rats subjected to chronic-binge ethanol exposures, and demonstrate that this model has a number of similarities and overlapping features with human ALD. The model described herein should aid in the design of new treatments and approaches to prevent ALD progression.

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References

- [1] K. Banerjee, L. Mohr, J. R. Wands, and S. M. de la Monte, *Ethanol inhibition of insulin signaling in hepatocellular carcinoma cells*, *Alcohol Clin Exp Res*, 22 (1998), 2093–2101.

- [2] F. Bardag-Gorce, *Effects of ethanol on the proteasome interacting proteins*, World J Gastroenterol, 16 (2010), 1349–1357.
- [3] M. Basaranoglu, N. Turhan, A. Sonsuz, and G. Basaranoglu, *Mallory-denk bodies in chronic hepatitis*, World J Gastroenterol, 17 (2011), 2172–2177.
- [4] A. P. Bautista, *Neutrophilic infiltration in alcoholic hepatitis*, Alcohol, 27 (2002), 17–21.
- [5] A. Bertola, S. Mathews, S. H. Ki, H. Wang, and B. Gao, *Mouse model of chronic and binge ethanol feeding (the NIAAA model)*, Nat Protoc, 8 (2013), 627–637.
- [6] A. Bertola, O. Park, and B. Gao, *Chronic plus binge ethanol feeding synergistically induces neutrophil infiltration and liver injury in mice: A critical role for E-selectin*, Hepatology, 58 (2013), 1814–1823.
- [7] J. I. Cohen and L. E. Nagy, *Pathogenesis of alcoholic liver disease: interactions between parenchymal and non-parenchymal cells*, J Dig Dis, 12 (2011), 3–9.
- [8] S. M. de la Monte, L. Longato, M. Tong, S. DeNucci, and J. R. Wands, *The liver-brain axis of alcohol-mediated neurodegeneration: role of toxic lipids*, Int J Environ Res Public Health, 6 (2009), 2055–2075.
- [9] S. M. de la Monte, M. Pang, R. Chaudhry, K. Duan, L. Longato, J. Carter, et al., *Peroxisome proliferator-activated receptor agonist treatment of alcohol-induced hepatic insulin resistance*, Hepatol Res, 41 (2011), 386–398.
- [10] S. M. de la Monte, J. E. Yeon, M. Tong, L. Longato, R. Chaudhry, M. Y. Pang, et al., *Insulin resistance in experimental alcohol-induced liver disease*, J Gastroenterol Hepatol, 23 (2008), e477–e486.
- [11] S. M. Denucci, M. Tong, L. Longato, M. Lawton, M. Setshedi, R. I. Carlson, et al., *Rat strain differences in susceptibility to alcohol-induced chronic liver injury and hepatic insulin resistance*, Gastroenterol Res Pract, 2010 (2010), Article ID 312790.
- [12] Z. Derdak, C. H. Lang, K. A. Villegas, M. Tong, N. M. Mark, S. M. de la Monte, et al., *Activation of p53 enhances apoptosis and insulin resistance in a rat model of alcoholic liver disease*, J Hepatol, 54 (2011), 164–172.
- [13] W. X. Ding, S. Manley, and H. M. Ni, *The emerging role of autophagy in alcoholic liver disease*, Exp Biol Med (Maywood), 236 (2011), 546–556.
- [14] A. E. Feldstein and S. M. Bailey, *Emerging role of redox dysregulation in alcoholic and nonalcoholic fatty liver disease*, Antioxid Redox Signal, 15 (2011), 421–424.
- [15] S. Hanada, P. Strnad, E. M. Brunt, and M. B. Omary, *The genetic background modulates susceptibility to mouse liver Mallory-Denk body formation and liver injury*, Hepatology, 48 (2008), 943–952.
- [16] O. A. Iseri, C. S. Lieber, and L. S. Gottlieb, *The ultrastructure of fatty liver induced by prolonged ethanol ingestion*, Am J Pathol, 48 (1966), 535–555.
- [17] J. Junge, T. Horn, and P. Christoffersen, *Megamitochondria as a diagnostic marker for alcohol induced centrilobular and periportal fibrosis in the liver*, Virchows Arch A Pathol Anat Histopathol, 410 (1987), 553–558.
- [18] N. Kaplowitz and C. Ji, *Unfolding new mechanisms of alcoholic liver disease in the endoplasmic reticulum*, J Gastroenterol Hepatol, 21 (2006), S7–S9.
- [19] S. H. Ki, O. Park, M. Zheng, O. Morales-Ibanez, J. K. Kolls, R. Bataller, et al., *Interleukin-22 treatment ameliorates alcoholic liver injury in a murine model of chronic-binge ethanol feeding: role of signal transducer and activator of transcription 3*, Hepatology, 52 (2010), 1291–1300.
- [20] L. Longato, K. Ripp, M. Setshedi, M. Dostalek, F. Akhlaghi, M. Branda, et al., *Insulin resistance, ceramide accumulation, and endoplasmic reticulum stress in human chronic alcohol-related liver disease*, Oxid Med Cell Longev, 2012 (2012), 479348.
- [21] M. V. Machado and H. Cortez-Pinto, *Cell death and nonalcoholic steatohepatitis: where is ballooning relevant?*, Expert Rev Gastroenterol Hepatol, 5 (2011), 213–222.
- [22] H. Malhi and R. J. Kaufman, *Endoplasmic reticulum stress in liver disease*, J Hepatol, 54 (2011), 795–809.
- [23] A. J. McCullough, R. S. O’Shea, and S. Dasarthy, *Diagnosis and management of alcoholic liver disease*, J Dig Dis, 12 (2011), 257–262.
- [24] B. L. McVicker, D. J. Tuma, J. L. Kubik, P. L. Tuma, and C. A. Casey, *Ethanol-induced apoptosis in polarized hepatic cells possibly through regulation of the Fas pathway*, Alcohol Clin Exp Res, 30 (2006), 1906–1915.
- [25] A. Miller, N. Horiguchi, W. Jeong, S. Radaeva, and B. Gao, *Molecular mechanisms of alcoholic liver disease: Innate immunity and cytokines*, Alcohol Clin Exp Res, 35 (2011), 787–793.
- [26] R. S. O’Shea, S. Dasarthy, A. J. McCullough, Practice Guideline Committee of the American Association for the Study of Liver Diseases, and Practice Parameters Committee of the American College of Gastroenterology, *Alcoholic liver disease*, Hepatology, 51 (2010), 307–328.
- [27] S. J. Pandol, F. S. Gorelick, A. Gerloff, and A. Lugea, *Alcohol abuse, endoplasmic reticulum stress and pancreatitis*, Dig Dis, 28 (2010), 776–782.
- [28] M. Pang, S. M. de la Monte, L. Longato, M. Tong, J. He, R. Chaudhry, et al., *PPAR δ agonist attenuates alcohol-induced hepatic insulin resistance and improves liver injury and repair*, J Hepatol, 50 (2009), 1192–1201.
- [29] H. Paula, S. K. Asrani, N. C. Boetticher, R. Pedersen, V. H. Shah, and W. R. Kim, *Alcoholic liver disease-related mortality in the United States: 1980–2003*, Am J Gastroenterol, 105 (2010), 1782–1787.
- [30] V. Purohit, B. Gao, and B. J. Song, *Molecular mechanisms of alcoholic fatty liver*, Alcohol Clin Exp Res, 33 (2009), 191–205.
- [31] T. Ramirez, L. Longato, M. Dostalek, M. Tong, J. R. Wands, and S. M. de la Monte, *Insulin resistance, ceramide accumulation and endoplasmic reticulum stress in experimental chronic alcohol-induced steatohepatitis*, Alcohol Alcohol, 48 (2013), 39–52.
- [32] P. Rømert and M. E. Matthiessen, *Alcohol-induced injury of mitochondria in hepatocytes of mini-pig fetuses*, Virchows Arch A Pathol Anat Histopathol, 399 (1983), 299–305.
- [33] P. Rømert and M. E. Matthiessen, *Ethanol-induced changes of granular endoplasmic reticulum in hepatocytes of mini-pig fetuses*, Acta Pathol Microbiol Immunol Scand A, 91 (1983), 413–417.
- [34] P. Rømert and M. E. Matthiessen, *Fine structure of hepatocytes from mini-pigs and mini-pig fetuses exposed to alcohol (ethanol) in vivo*, Acta Anat (Basel), 120 (1984), 190–195.
- [35] M. J. Ronis, A. Butura, S. Korourian, K. Shankar, P. Simpson, J. Badaeux, et al., *Cytokine and chemokine expression associated with steatohepatitis and hepatocyte proliferation in rats fed ethanol via total enteral nutrition*, Exp Biol Med (Maywood), 233 (2008), 344–355.
- [36] Y. Sasaki, N. Hayashi, T. Ito, H. Fusamoto, T. Kamada, and J. R. Wands, *Influence of ethanol on insulin receptor substrate-1-mediated signal transduction during rat liver regeneration*, Alcohol Alcohol Suppl, 29 (1994), 99–106.
- [37] D. Seth, P. S. Haber, W. K. Syn, A. M. Diehl, and C. P. Day, *Pathogenesis of alcohol-induced liver disease: Classical concepts and recent advances*, J Gastroenterol Hepatol, 26 (2011), 1089–1105.
- [38] P. Strnad, K. Zatloukal, C. Stumtner, H. Kulaksiz, and H. Denk, *Mallory-denk-bodies: Lessons from keratin-containing hepatic inclusion bodies*, Biochim Biophys Acta, 1782 (2008), 764–774.
- [39] A. Tannapfel, H. Denk, H. P. Dienes, C. Langner, P. Schirmacher, M. Trauner, et al., *Histopathological diagnosis of non-alcoholic*

- and alcoholic fatty liver disease*, *Virchows Arch*, 458 (2011), 511–523.
- [40] T. Uchida, H. Kao, M. Quispe-Sjogren, and R. L. Peters, *Alcoholic foamy degeneration – a pattern of acute alcoholic injury of the liver*, *Gastroenterology*, 84 (1983), 683–692.
- [41] M. Vidali, S. F. Stewart, and E. Albano, *Interplay between oxidative stress and immunity in the progression of alcohol-mediated liver injury*, *Trends Mol Med*, 14 (2008), 63–71.
- [42] T. Wakabayashi, M. Horiuchi, M. Sakaguchi, H. Onda, and M. Iijima, *Induction of megamitochondria in the rat liver by N-propyl alcohol and N-butyl alcohol*, *Acta Pathol Jpn*, 34 (1984), 471–480.
- [43] J. E. Yeon, S. Califano, J. Xu, J. R. Wands, and S. M. de la Monte, *Potential role of PTEN phosphatase in ethanol-impaired survival signaling in the liver*, *Hepatology*, 38 (2003), 703–714.
- [44] L. Yerian, *Histopathological evaluation of fatty and alcoholic liver diseases*, *J Dig Dis*, 12 (2011), 17–24.