Thai Journal of Physiological Sciences
Vol. 20 No. 1 Mar.–Sept. 2007

Original Article

Factors Influencing a Rat Model of Alcoholic Liver Disease for Research Study

Samuhasaneeto S*, Thong-Ngam D*, Klaikeaw N†

ABSTRACT *Aims:* **To establish a simplified and reliable animal model of alcoholic liver disease (ALD) and find the factors influencing this condition.**

Materials and Methods: **Male and female Sprague-Dawley rats were kept in Macrolon cages in a room temperature (25 ˚C) and humidity (55%), and a 12/12-hr light/dark cycle. The rats were randomly divided into 6 experimental groups; Group 1 : Feeding with 7 g/kg of ethanol in male rats, Group 2 : Feeding with 7 g/kg of ethanol + high fat-low protein diet in male rats, Group 3 : Feeding with 5 g/kg of ethanol in male rats, Group 4 : Feeding with 7 g/kg of ethanol in female rats, Group 5 : Feeding with 7 g/kg of ethanol + high fat-low protein diet in female rats, Group 6 : Feeding with 5 g/kg of ethanol in female rats. At the end of study (3-18 weeks), rats were sacrificed and livers were removed for grading of steatosis, inflammation and necrosis.**

Results: **Liver sections from rats fed only ethanol (5g/kg or 7g/kg) showed no steatosis, inflammation and necrosis at 3 or more weeks. Steatosis, inflammation and necrosis were inclusively presented in group 2 and 5. The degree of steatosis and necroinflammation was associated with longer period of ethanol and high fat-low protein diet treatment. However, there was no different liver histopathology results between gender (male and female).**

Conclusion: **We establised a simplified and feasible animal model of ALD in which protein-malnutrition with high fat intake contributing to it's progression. This model can be useful for future research study.**

INTRODUCTION

Alcoholic liver disease (ALD) remains to be one of the most common etiologies of liver disease and is a major cause of morbidity and mortality worldwide[1]. The pathologic stages of ALD comprises of steatosis, steatohepatitis, and fibrosis/cirrhosis[2]. Alcoholic steatosis is the initial stage of ALD and consists of fat accumulation in hepatocytes. Alcoholic steatohepatitis (ASH), the second and rate-limiting step in the progression of ALD, is characterized by hepatic fat accumulation, hepatocellular necrosis, the presence of Mallory bodies, and surrounding infiltrate composed of polymorphonuclear leukocytes[3]. With prolonged alcohol abuse, there is progressive fibrosis. This is most frequently in the form of sinusoidal and perivenular fibrosis that splits apart the parenchyma[2]. Various animal models of ALD have been developed that they have significantly increased our understanding of

** Department of physiology, † Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.*
Address for correspondence:
Correspondence to: Duangporn Thong-Ngam, M.D. Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. E-mail: thongngam007@yahoo.com
Tel 662-2564267 Fax 662-2564267

how alcohol causes damage to the liver and helped to determine new way of preventing or treating ALD. However several animal models of ALD are limited in their applicability[4]. In 1989, Lieber and co-workers developed oral liquid diets (Lieber-DeCarli Diets) that contained 35.5 percent energy from ethanol[5]. They observed only steatosis, no inflammation and fibrosis. Ethanol containing liquid diets and ethanol feeding via surgically implanted gastrostomy tubes (Tsukamoto-French model) have used to overcome these problems and have produced liver injury in the rat[6,7]. These models are either inconvenient or difficult to use, especially over prolonged periods. Models based on the administration of ethanol in drinking water have an advantage of relative simplicity but are limited by inherent difficulties in assessing and controlling the nutritional effects of ethanol consumption. More recently, Enomoto and colleagues (1999) reported a new rat model in which female wistar rats received 5 g/kg alcohol intragastrically every 24 hours[8]. After 4 weeks, this treatment induced fat accumulation, inflammation, and necrosis in the liver. However, other researchers still must reproduce and confirm this model to establish its validity. According to Lieber-DeCarli Diets, Tsukamoto-French model, and Enomoto model, animals were given higher fat diet than standard diet (chow diet). Therefore, our objectives were to establish a simplified and feasible animal model of ALD and factors influence such as nutrition and sex.

MATERIALS AND METHODS

Animals

Male and female Sprague-Dawley rats were obtained from The Salaya research animal center, Mahidol University, Bangkok, Thailand. The experimental protocol was approved by the Ethical Committee of Pharmacology Faculty, Chulalongkorn University, Thailand. The animals were kept in Macrolon cages in a room temperature (25 ˚C) and humidity (55%), and a 12/12-hr light/dark cycle.

Experimental design

The rats were randomly divided into 6 experimental groups.

Group 1 (Male+Ethanol I); Male rats were given 40 percent ethanol (7 g/kg), orally, using an intragastric tube and fed standard diet.

Group 2 (Male+Ethanol I+High fat-low protein); Male rats were given 40 percent ethanol (7 g/kg), orally, using an intragastric tube and fed a high fat-low protein diet with 58.82 percent of energy from fat, 2.35 percent from protein and 38.83 percent from carbohydrate.

Group 3 (Male+Ethanol II); Male fed rats were given 40 percent ethanol (5 g/kg), orally, using an intragastric tube and fed standard diet.

Group 4 (Female+Ethanol I); Female rats were given 40 percent ethanol (7 g/kg), orally, using an intragastric tube and fed standard diet.

Group 5 (Female+Ethanol I+High fat-low protein); Female rats were given 40 percent ethanol (7 g/kg), orally, using an intragastric tube and fed a high fat-low protein diet with 58.82 percent of energy from fat, 2.35 percent from protein and 38.83 percent from carbohydrate.

Group 6 (Female+Ethanol II) ; Female rats were given 40 percent ethanol (5 g/kg), orally, using an intragastric tube and fed standard diet.

All rats were fed ad libitum diets and weighed weekly.

Histopathology

At the end of each time (Table 1.) rats were sacrified using intraperitoneal injection overdose of sodium pentobarbital 45 mg/kg BW. The livers were removed and then fixed in 10 percent formalin solution at room temperature. They were processed by standard method, tissues were embedded in paraffin, sectioned at 5 (m, and stained with hematoxylin-eosin (H&E) and then picked up on glass slides for light microscopy. An experienced pathologist performed a blinded evaluation for all samples. Each liver section was examined for grading of steatosis, inflammation and necrosis according to Korourian et al. criteria(9).

Steatosis was scored as the percentage of parenchymal cells containing fat (micro- or macrosteatosis) as < 25% = 1, 25% to 50% = 2, 50% to 75% = 3, and <75% = 4.

The presence of inflammation, based on infiltration by polymorphonuclear leukocytes and mononuclear cells, was evaluated using a scale where no polymorphonuclear leukocytes and mononuclear cells present = 1; occasional foci of inflammatory cells = 2; widely dispersed,

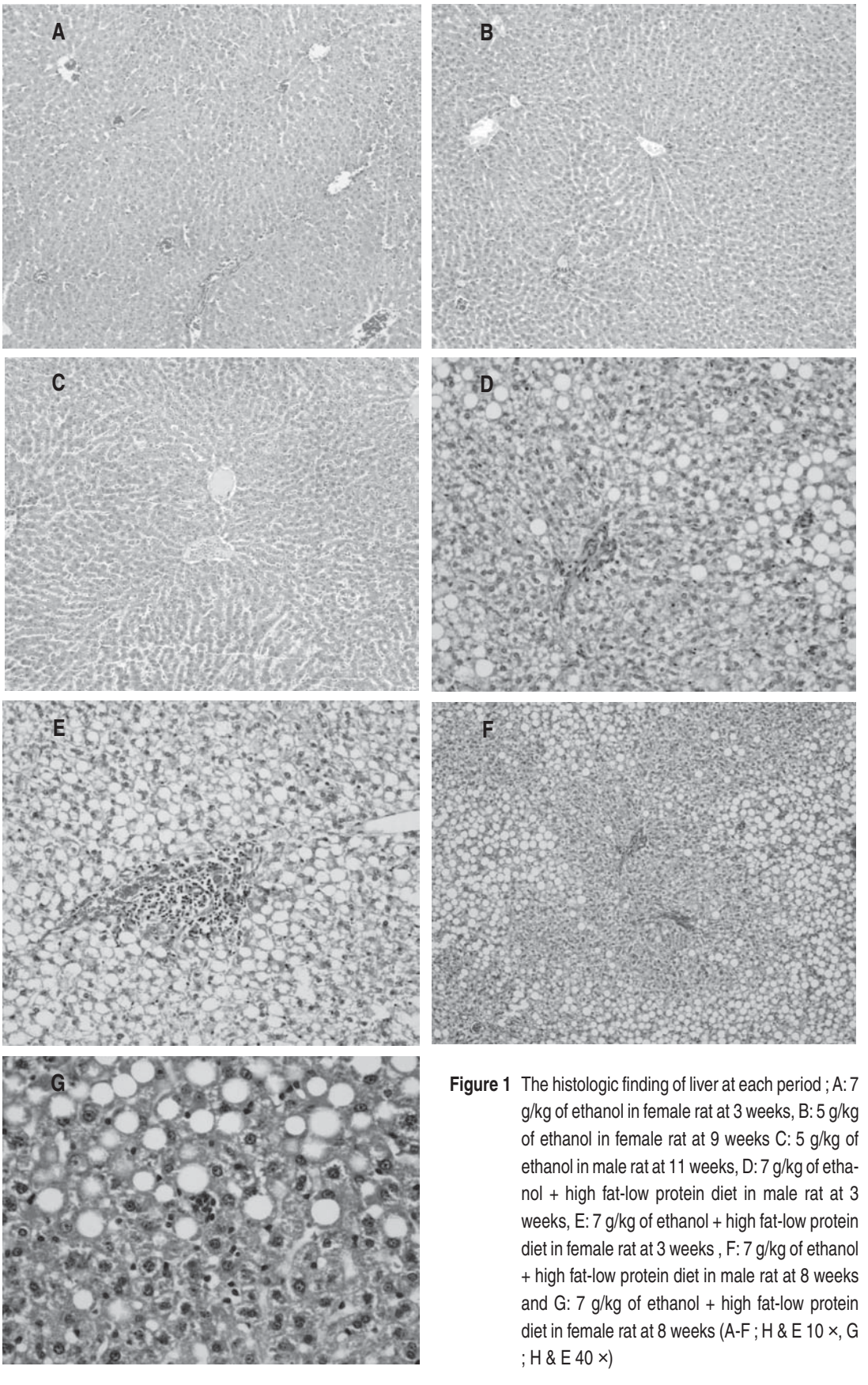

Figure 1 The histologic finding of liver at each period ; A: 7 g/kg of ethanol in female rat at 3 weeks, B: 5 g/kg of ethanol in female rat at 9 weeks C: 5 g/kg of ethanol in male rat at 11 weeks, D: 7 g/kg of ethanol + high fat-low protein diet in male rat at 3 weeks, E: 7 g/kg of ethanol + high fat-low protein diet in female rat at 3 weeks , F: 7 g/kg of ethanol + high fat-low protein diet in male rat at 8 weeks and G: 7 g/kg of ethanol + high fat-low protein diet in female rat at 8 weeks (A-F ; H & E 10 ×, G ; H & E 40 ×)

Table 1 Summarized the scores of steatosis, inflammation and necrosis levels

Group+time	N	Steatosis					Inflammation				Necrosis				
		0	<25%	25-50%	50-75%	>75%	1 (no)	2	3	4	Negative	1	2	3	4
Group 1 at 11 weeks	1	-	1	-	-	-	1	-	-	-	1	-	-	-	-
Group 2 at 3 weeks	2	1	-	-	1	-	1	1	-	-	1	1	-	-	-
Group 2 at 5 weeks	3	-	3	-	-	-	2	1	-	-	2	1	-	-	-
Group 2 at 8 weeks	1	-	-	-	-	1	-	1	-	-	-	1	-	-	-
Group 2 at 10 weeks	1	-	-	-	-	1	-	-	-	1	-	-	-	-	
Group 3 at 11 weeks	2	2	-	-	-	-	2	-	-	-	2	-	-	-	-
Group 3 at 14 weeks	1	-	1	-	-	-	1	-	-	-	1	-	-	-	-
Group 3 at 18 weeks	1	-	1	-	-	-	1	-	-	-	1	-	-	-	-
Group 4 at 3 wees	2	-	2	-	-	-	2	-	-	-	2	-	-	-	-
Group 4 at 5 weeks	1	-	1	-	-	-	1	-	-	-	1	-	-	-	-
Group 4 at 8 weeks	1	1	-	-	-	-	1	-	-	-	1	-	-	-	-
Group 5 at 3 weeks	1	-	-	1	-	-	-	1	-	-	-	-	1	-	-
Group 5 at 8 weeks	1	-	-	1	-	-	1	-	-	-	1	-	-	-	-
Group 6 at 9 weeks	1	1	-	-	-	-	1	-	-	-	1	-	-	-	-

Group 1 = Male+Ethanol 7 g/kg
Group 2 = Male+Ethanol 7 g/kg+High fat-low protein
Group 3 = Male+Ethanol 5 g/kg
Group 4 = Female+Ethanol 7 g/kg
Group 5 = Female+Ethanol 7 g/kg+High fat-low protein
Group 6 = Female+Ethanol 5 g/kg
*Grading of steatosis, inflammation and necrosis according to Korourian et al. criteria(9).
Steatosis was scored as the percentage of parenchymal cells containing fat (micro- or macrosteatosis) as < 25% = 1, 25% to 50% = 2, 50% to 75% = 3, and <75% = 4.

Inflammation was based on infiltration by polymorphonuclear leukocytes and mononuclear cells, no present = 1; occasional foci of inflammatory cells = 2; widely dispersed, organized foci of inflammatory cells = 3, and frequently occurring, large foci of inflammatory cells = 4.

Necrosis was assessed, occasional (<1%) necrotic hepatocytes = 1, frequent (5-10%) necrotic hepatocytes = 2, small foci of necrosis (clusters >10 necrotic hepatocytes) = 3, and extensive areas of necrosis (>25% of the lobular unit) = 4.

organized foci of inflammatory cells = 3, and frequently occurring, large foci of inflammatory cells = 4.

Necrosis was assessed using a scale of 1 to 4 as follows; occasional (<1%) necrotic hepatocytes = 1, frequent (5-10%) necrotic hepatocytes = 2, small foci of necrosis (clusters >10 necrotic hepatocytes) = 3, and extensive areas of necrosis (>25% of the lobular unit) = 4.

RESULTS

Change on liver histology

Liver sections from rats fed only ethanol (5g/kg or 7g/kg) showed no steatosis, inflammation and necrosis at 3 or more weeks (Figure 1A, 1B.). Steatosis, inflammation and necrosis were presented in group 2 and 5. The degree of steatosis and necroinflammation was associated with period of ethanol and diet treatment (Figure 1C, 1D.). However, there was no difference between male and female. The pathological grading for steatosis, inflammation and necrosis was summarized in Table 1.

DISCUSSION

The present study describes the animal model of alcohol-related injury and the effect of alcohol administration on the liver under dependent factor that may facilitate further studies of this condition. Only ethanol feeding was observed only mild steatosis, no inflammation and necrosis. Similarly, The liquid diet model : ethanol as a part of nutritionally defined liquid diet was ad-

ministered to rats. Even though fatty liver was induced, advanced liver lesions such as liver necrosis and fibrosis did not occur[10]. This probably is rapid metabolism of ethanol which associated with a specific group of liver enzymes that metabolize alcohol and that are activated after chronic drinking[11,12]. Enzyme activation increases alcohol degradation and reduces the time during which alcohol is active in the body, thereby reducing the duration of alcohol's intoxicating effects.

Malnutrition is a critical factor for alcohol-related liver damage in several experiments. Rao and co-workers concluded from a critical review of pubished studies on alcohol consumption and nutritional conditions in the monkey and man, that alcohol requires a nutritional co-factor to exhibit hepatotoxicity[13]. Recent studies by French and co-workers have addressed the issue of malnutrition as a critical factor for alcohol-related liver damage in experiments with dietary manipulations that are comparable to those applied in our present study[14,15-17]. However, their model is rather artificial in that it involves administration of both ethanol and food through permanent intra-gastric catheters. In addition, unscheduled mortality in this model is high, both as a result of surgical complications, and through ethanol intoxication due to very high blood ethanol levels[16]. In the present study, we exhibited factors influencing a rat model of alcoholic liver disease for research sudy. The greater severity of histopathology in liver was found in ethanol+high fat and low protein groups, whereas the ethanol groups showed only mild steatosis. The quality and composition of diet are known to have a major influence on the histology of ethanol-treated rat livers. The supply of lipotropes and the proportions of dietary fat and protein are particular importance[6,7,15,18-19]. It seems likely that both alcohol and nutritional play a part in alcohol hepato-toxicity.

Another factor in development of alcoholic liver damage is sex. Women appear to have serious forms of alcoholic liver disease more frequently than men and after consumption of lower amount of alcohol. The point was the possible reasons by gender difference in response to alcohol, including alterations in absorption, disposition, and metabolism[3]. However, there was no clear sexual difference in liver histology of ethanol-treated rats[4] that male and female rats showed similar histopatholog in our study.

Therefore, we demonstrated a simplified and feasible animal model of ALD that protein-malnutrition with high fat intake contributes to the progression of alcoholic liver disease. This model can be useful for future research study.

REFERENCES

1. Ramaiah SK, Rivera C, Arteel GE. Early-phase alcoholic liver disease: an update on animal models, pathology, and pathogenesis. Int J Toxicol 2004; 23: 217-31.
2. Kumar V, Abbas AK, Fausto N. Robbins and cotran pathologic basis of disease. 2005; China: 904-7.
3. Kaplowitz N. Liver and biliary diseases. 1992; The United States: Williams & Wilkins: 377.
4. Keegan A, Martini R, Batey R. Ethanol-related liver injury in the rat: a model of steatosis, inflammation and pericentral fibrosis. J Hepatol 1995; 23: 591-600.
5. Lieber CS, DeCarli LM. Liquid diet technique of ethanol administration. Alcohol Alcoholism 1989; 24: 197-211.
6. Lieber CS, DeCarli LM, Sorrell MF. Experimental methods of ethanol administration. Hepatology 1989; 10: 501-10.
7. Tsukamoto H, French SW, Benson N, Delgado G, Rao GA, Larkin EC, Largman C. Severe and progressive steatosis and focal necrosis in rat liver induced by continuous intragastric infusion of ethanol and low fat diet. Hepatology 1985; 5: 224-32.
8. Enomoto N, Yamashina S, Kono H. Development of a new, simple model of early alcohol-induced liver injury based on sensitization of Kuffer cells. Hepatology 1999; 29: 1680-9.
9. Korourian S, Hakkak R, Ronis MJ, Shelnutt SR, Waldron J, Ingelman-Sunberg M, Badger TM. Diet and risk of ethanol-induced hepatoxicity: carbohydrate-fat relationships in rats. Toxicol Sci 1999; 47: 110-7.
10. Lieber CS, Jones DP, DeCarli LM. Effects of prolonged ethanol intake : Production of fatty liver despite adequate diets. J Clin Invest 1965; 44: 1009-21.
11. Lieber CS. Metabolism of ethanol and associated hepatotoxicity. Drug Alcohol Rev 1991; 10: 175-202.
12. Lieber CS. The microsomal ethanol oxidizing system : Its role in ethanol and xenobiotic metabolism. Biochem Soc Trans 1988; 16: 232-9.
13. Derr RF, Porta EA, Larkin EC, Rao GA. Is ethanol per se hepatotoxic? (Review). J Hepatol 1990; 10: 381-386.
14. Tsukamoto H, Gaal K, French SW. Insights in the pathogenesis of alcoholic liver necrosis and fibrosis : status report. Hepatology 1990; 12: 599-608.
15. Tsukamoto H, Towner SJ, Ciofalo LM, French SW. Ethanol-induced fibrosis in the rat : role of the amount of dietary fat. Alcohol Clin Exp Res 1986; 10: 13S-19S.
16. French SW, Miyamoto K, Tsukamoto H. Ethanol-induced hepatic fibrosis in the rat : Role of the amount of dietary fat. Clin Exp Res 1986; 10: 13S-19S.
17. French SW, Miyamoto K, Wong K, Jui L, Briere L. Role of Ito

cell in liver parenchymal fibrosis in rats fed alcohol and a high fat-low protein diet. Am J Pathol 1988; 132: 73-85.

18. Porta EA, Gomez-Dunn CLA. A new approach in the study of chronic alcoholism : Effects of high alcohol intake in rats fed a commercial laboratory diet. Lab Invest 1968; 18: 352-364.
19. Gomez-Dunn CLA, Porta EA, Hartroft WS, Koch OR. A new experimental approach in the study of chronic alcoholism : Effects of high alcohol intake in rats fed diets of various adequacies. Lab Invest 1968; 18: 365-378.