[Frontiers in Bioscience, 4, d589-595, July 15, 1999]

ROLE OF KUPFFER CELLS IN THE ETHANOL-INDUCED OXIDATIVE STRESS IN THE LIVER

Abraham P. Bautista and John J. Spitzer

Department of Physiology and Alcohol Research Center, Louisiana State University Medical Center, New Orleans, Louisiana

TABLE OF CONTENTS

1. ABSTRACT

These studies test the hypothesis that acute and chronic alcohol intoxication stimulate the release of oxygen-derived radicals in the liver. Male Sprague-Dawley rats received an intravenous bolus followed by continuous infusion of ethanol to maintain blood alcohol level at about 175 mg/dl for 0-18 hr. They were then allowed to recover from this "alcohol binge" and the release of free radicals during the recovery phase was monitored. In the chronic alcohol intoxication model, rats were fed with 40% ethanol in agar blocks for 16 weeks. Acute ethanol intoxication induced two phases of hepatic superoxide release. The first phase peaked during the first 3 hr of alcohol intoxication, while the second phase reached its maximum at 6 hr of recovery following a 12 hr binge. The recovery period was also associated with elevated serum transaminase activity. Kupffer cells were largely responsible for hepatic superoxide release during the first phase, while both Kupffer and hepatic sinusoidal endothelial cells contributed to the second phase of free radical formation. Acute ethanol intoxication did not induce endotoxemia. During chronic alcohol intoxication, increased levels of serum endotoxin, TNF, IL-1, and transaminase were observed and hepatic superoxide anion release was present. Superoxide release by isolated Kupffer cells, blood and hepatic PMNs of alcoholic rats was also significantly enhanced in the chronic alcoholic rats. These data indicate that acute alcohol intoxication may directly stimulate the release of reactive oxygen intermediates, whereas chronic alcohol may elicit free radical generation through enhanced endotoxin influx and cytokine release. These studies further demonstrate that free radicals produced by hepatic non-parenchymal cells are likely to play an important role in the pathogenesis of hepatic injury in susceptible individuals with alcohol-related liver disorders.

2. INTRODUCTION

The liver is an important site of alcohol action as well as alcohol metabolism. It is also an organ containing several cell types. Although the metabolism of alcohol takes place primarily in the parenchymal cells, other cell types within the liver are also markedly affected by the consumption of ethanol. Thus, alterations in the functions of Kupffer cells, sinusoidal endothelial cells and stellate cells have been demonstrated following both the acute and chronic consumption of alcohol.

Oxygen-derived free radicals have been demonstrated to play an important role in a variety of conditions associated with cell injury, among them, alcohol intoxication, sepsis, endotoxemia and ischemia-reperfusion injury (1-5). Although the sources of free radicals in different tissues may vary, activated macrophages seem to play an important role. In the liver, Kupffer cells, the resident macrophages fulfill this function. Under normal resting conditions, the oxygen-derived free radicals produced by various cell types are counterbalanced by a variety of antioxidant agents, as indicated in figure 1. However, when Kupffer cells are primed and/or activated, and also when neutrophils (PMNs) infiltrate into the liver they are activated, the oxidant/antioxidant balance is upset and oxygen-derived free radical generation by the liver can be readily demonstrated. Acute endotoxemia (6-8) or acute ethanol intoxication (9) are good examples of conditions where hepatic superoxide release is evident. In addition to a binge-type stimulation of the Kupffer cells, recovery from the effects of alcohol, or chronic alcohol administration also alters Kupffer cell function (10).

The present work tested the hypothesis that Kupffer cell-derived free radicals play a role in the liver in

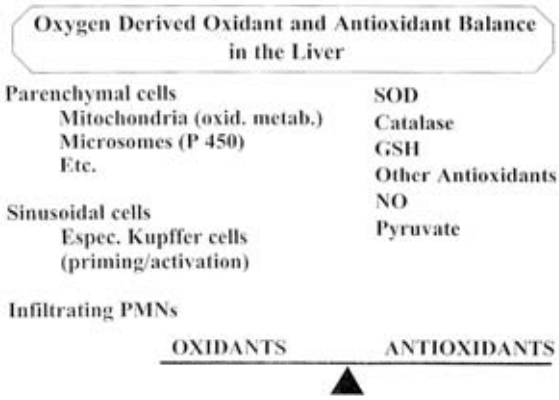

Figure 1: Oxygen derived oxidant and antioxidant balance in the liver.

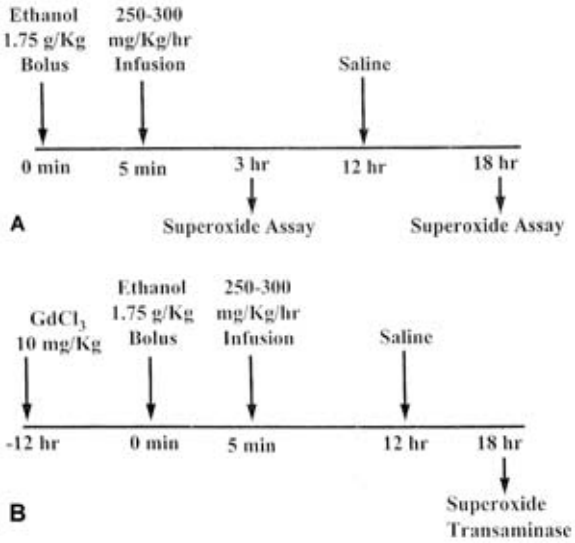

Figure 2: Experimental protocols used in acute alcohol binge (A) and alcohol withdrawal (B) studies.

causing an oxidative stress during and after a binge-type alcohol intake as well as following chronic alcohol administration.

3. MATERIALS AND METHODS

3.1. Experimental Design

Different experimental groups were set up as follows:

Acute ethanol administration (Alcohol-binge model). Male Sprague-Dawley rats (250-300 g, Hilltop Breeding Laboratories, Houston TX) received an ethanol bolus (20% v/v in sterile saline i.v.) at a dose of 1.75 g/Kg body weight followed by continuous infusion at a rate of 250-300 mg/Kg per hr for 0-18 hr.

Post-binge experiments. The rats received similar injection and infusion of ethanol as in A. After 12 hr of infusion, ethanol was replaced by saline and the infusion was continued for another 6 hr. Saline or ethanol only was infused to time-matched control groups. In another experimental groups, rats received an i.v. injection of $GdCl_3$ (10 mg/Kg) 12-21 hr before the start of infusion. This experiment was performed to test the hypothesis that selective depletion of Kupffer cells by $GdCl_3$ suppresses ethanol-mediated superoxide release. The experimental protocols are illustrated on figures 2A and 2B.

3.2. Chronic alcohol intoxication model

Specific pathogen-free male Sprague-Dawley rats (90-100 g, Charles River Breeding Laboratories, Cambridge MA) were maintained on an ethanol-containing agar block diet (40 % v/v, plus 0.5 g/Kg peanut butter) and ethanol-supplemented water (10% v/v) for 16 weeks. The animals were allowed free access to solid chow. Pair-fed rats were given similar amounts of agar block (without ethanol), solid chow and alcohol-free water. On the day of the experiments, there was no significant difference in body weight between pair- (625 ± 32 g) and ethanol-fed rats (575 ± 25 g). Blood ethanol level using this experimental condition was 125 ± 28 mg/dl.

All experimental animals used in this investigation received humane care according to the guidelines outlined in the "Guide for the Care and Use of Laboratory Animals" by the National Academy of Sciences (National Institutes of Health publication no. 80-23). The experimental protocol for the use of live animals in this study was approved by the Louisiana State University Institutional Animal Care and Use Committee.

3.3. Superoxide anion assay

At appropriate times indicated in Figure 2, the livers were perfused in situ for the determination of superoxide anion release and for cell isolation procedures. Superoxide anion release in the perfused liver and isolated hepatic cells was measured by means of superoxide dismutase-inhibitable reduction of cytochrome c as previously described (8,9,11).

Cell isolation procedure. Hepatic sinusoidal endothelial cells, Kupffer cells and hepatocytes were isolated after collagenase- perfusion of the liver and centrifugal elutriation as described previously (12,13). PMNs were separated from non-parenchymal cells and other blood elements by using Nycoprep (Accurate Chemicals, NJ) density gradient centrifugation.

Aspartate transaminase (ASAT), alcohol and endotoxin determinations. Serum ASAT and alcohol were measured using diagnostic kits from Sigma (St. Louis MO). Serum endotoxin was determined by using Endotoxin Chromogen (Pharmacia & Upjohn, Kalamazoo MI) and LAL (Sigma, St. Louis MO).

3.4. Cytokine assays

Tumor necrosis factor _ and interleukin-1ß were measured by ELISA kits (Biosource International, Camarillo, CA).

Data presented in this paper represent means Å SEM of 3-12 rats per treatment group. Statistical significance at P 0.05 was assessed by ANOVA, Student-t

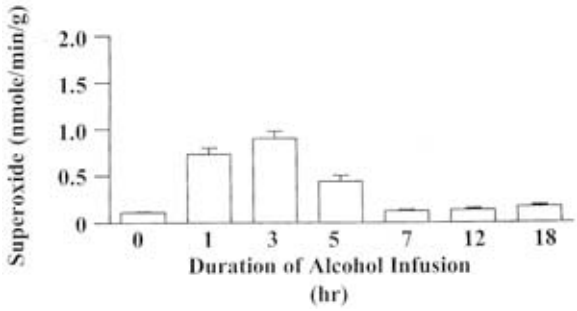

Figure 3: Effect of ethanol infusion on hepatic superoxide release.

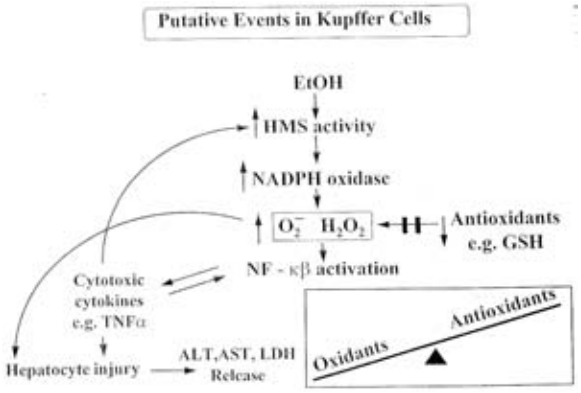

Figure 4: Schematic diagram showing the putative role of NFkappaB on cytokine and free radical generation

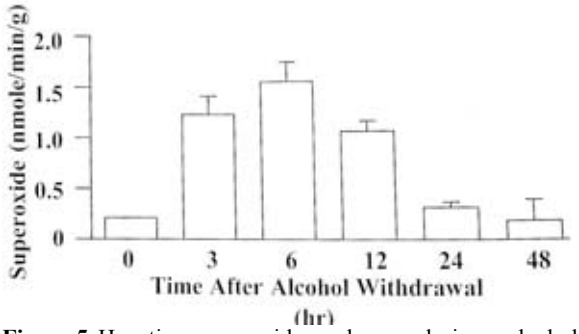

Figure 5: Hepatic superoxide release during alcohol withdrawal following a 12 hr binge.

and Student-Newman-Keuls multiple comparisons tests. Data that research statistical significance are indicated by different letters or asterisks.

4. RESULTS

4.1. Hepatic superoxide release during binge type-ethanol administration

When the blood ethanol concentration of approximately 175 mg/dl is maintained for varying periods of time and subsequently the animals' liver is perfused, superoxide release is demonstrable (7,9). This effect is time-dependent, showing a maximum release between 1-3 hr of ethanol infusion, and diminishing to virtually nondetectable levels after 7 hr of ethanol infusion (figure 3). As shown previously the activation of superoxide release by the liver is not dependent on the metabolism of the alcohol moiety, since the administration of 4-methylpyrazole, that inactivates alcohol dehydrogenase, does not alter the effect (9). We have further demonstrated that isolated hepatocytes or endothelial cells following ethanol infusion failed to release superoxides anion *in vitro*. However, isolated Kupffer cells following ethanol administration actively release SOD-inhibitable superoxide anions (9). The stimulatory effect of ethanol on different phagocytes have also been demonstrated by other investigators (14,15). Thus, short-term *in vitro* exposure of macrophages to low-dose ethanol enhanced superoxide anion production by these cells (14). *In vivo* ethanol treatment in mice also increases the phagocytic activity of Kupffer cells and the migration of neutrophils into the liver (16). While the exact mechanisms of action of ethanol in stimulating superoxide anion release from Kupffer cells is not completely understood, we postulate that the activation of the nuclear factor NFkappaB plays an important role. This is schematically illustrated in figure 4.

4.2. Post-binge Effects

The goal of this part of the studies, was to determine whether a binge-type alcohol intake would have any post -binge effects with regard to hepatic oxidative stress. Thus, rats were given a bolus injection of ethanol followed by a sustaining dose of infusion that lasted for twelve hours. This treatment elicited average ethanol concentrations of 143 mg/dl. At this time, ethanol infusion was discontinued and the rats were left to recover for a period of 3-18 hrs at which time, the animals were anesthetized and hepatic superoxide release was determined. By the 6 hr recovery period, alcohol was no longer detectable in the circulation. figure 2A depicts the experimental protocol for these studies.

As indicated above, ethanol infusion elicits superoxide release in the perfused liver that peaks at 3 and dissipates thereafter. Following withdrawal of ethanol, a second peak of superoxide release was demonstrable (figure 5). These data indicate that at the end of the 12 hr ethanol infusion, little or no hepatic superoxide anion release was demonstrable. However, at 3, 6 or 12 hrs post binge, the livers released significant quantities of superoxide anions which appear to peak at 6 hrs and diminish to barely detectable levels by 24 and 48 hrs post-binge (figure 5).

In the next group of experiments, using a similar protocol, Kupffer cells and hepatic endothelial cells were isolated at 6 hrs post binge and their *in vitro* superoxide release determined. Interestingly, both Kupffer cells and hepatic endothelial cells showed considerable superoxide anion release at this time point (table 1). This is in contrast to the effect of binge-type alcohol administration, where only Kupffer cells participated in a significant manner in the hepatic superoxide release (9,10). The participation of both hepatic cell types in this response, was also indicated by a different experimental approach. When $GdCl_3$ is given i.v. 24 hrs before the experiment, Kupffer cells are largely destroyed or inactivated (17). Thus, selective depletion of Kupffer cells prior to 12 hr infusion of ethanol

Table 1. Superoxide release by isolated Kupffer cells and endothelial cells after alcohol withdrawal following a 12 hr binge

Cell type	Superoxide	Release	nmol/10^6/hr
	Saline	Alcohol (12 hr + 6 hr)	Alcohol (18 hr)
Kupffer cells	0.2 ± 0.05	4.6 ± 0.3*	0.86 ± 0.05
Endothelial cells	0.11 ± 0.04	2.6 ± 0.1*	0.25 ± 0.1

- P < 0.001 vs saline and alcohol 18 hr. N = 5/group.

Table 2. Effect of gadolinium chloride ($GdCl_3$) in vivo on hepatic superoxide release following an alcohol binge and withdrawal

Experimental Protocol	Hepatic Superoxide	(nmol/min/g)
	$GdCl_3$	Saline
Alcohol Binge	0*	0.95 ± 0.20
Alcohol Withdrawal	0.78 ± 0.1*	1.54 ± 0.19

* P < 0.001 vs saline-treated; N = 5-7/group. $GdCl_3$ (10 mg/Kg) was injected intravenously at time 0. Twenty-one hr later the animals received ethanol as described in Fig. 2A. In another experimental group as shown on Fig. 2B, the rats received ethanol 12 hr after $GdCl_3$. The livers were perfused at 3 hr after an alcohol binge (24 hr after $GdCl_3$) and at 6 hr after alcohol withdrawal following a 12 hr alcohol binge (30 hr after $GdCl_3$).

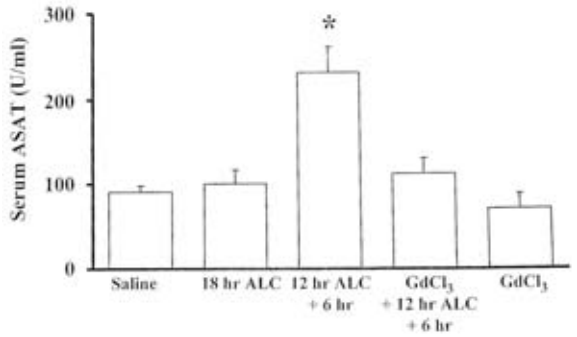

Figure 6: Effect of gadolinium chloride treatment on aspartate transaminase activity during alcohol withdrawal following 12 hr alcohol binge.

followed by 6 hrs of recovery, caused a significant, but not complete decrease in the elevated superoxide release by the liver (table 2). This suggests that along with Kupffer cells, other cell types also participate in this response. However, in the first phase of ethanol intoxication, Kupffer cells were largely responsible for hepatic superoxide release, because $GdCl_3$ completely abolished this response (table 2).

In the next phase of the study, we wished to ascertain whether the oxidative stress caused by the post-binge effect, had any demonstrable effect on the functional integrity of the liver. Thus, we determined serum aspartate transaminase (ASAT) activity, a measure of incipient hepatic function damage. Figure 6 shows that no increase in ASAT was demonstrated in the plasma of animals treated with alcohol alone. However, when 12 hrs alcohol treatment was followed by a 6 hr recovery, AST levels were significantly elevated and the elevation was suppressed by the prior administration of $GdCl_3$.

4.3. Hepatic superoxide release after long-term alcohol feeding

Alcohol feeding for 16 weeks was also associated with significant elevations in serum endotoxin and ASAT activity compared to pair-fed controls (table 3). At the same time, serum levels of TNF and IL-1 beta were significantly increased in chronic alcoholic rats compared to those of the pair-fed animals. Concomitantly, the release of superoxide anion in the perfused livers of alcohol-fed rats was enhanced (table 3). The likely sources of these radicals in the liver are the Kupffer cells and the sequestered PMNs in the liver. Table 4 shows that alcohol feeding enhanced the basal release of superoxide anion by isolated Kupffer cells, hepatic and blood PMNs.

5. DISCUSSION

These data demonstrate that acute or chronic alcohol intoxication elicits the release of oxygen-derived radicals in the liver. It is believed that the toxic effects of alcohol in a number of tissues are mediated through the production of cytotoxic free radicals. Thus, these radicals have been implicated in the pathogenesis of liver injury in several disorders, such as, alcoholic liver disease, endotoxemia, sepsis and hepatic ischemia followed by reperfusion (1-5).

The mechanism by which free radicals are formed during alcohol intoxication or alcohol binge is not fully understood. Others have demonstrated that ethanol stimulates membrane phosphatidyl inositol turnover in isolated hepatocytes (18,19) and alveolar macrophages (14). Enhanced phosphatidylinositol turnover is expected to induce the release of secondary messengers, which in turn modulate protein kinase C activity. As a result, protein kinase C induces the translocation of NADPH oxidase to the plasma membrane of phagocytic cells leading to superoxide anion generation. The involvement of NADPH oxidase and protein kinase C in the molecular activation of free radical release in mononuclear phagocytes is well defined (20).

As demonstrated in this study, there were two phases of free radical release during acute alcohol intoxication. The first phase was observed during the first 3 hr of acute alcohol intoxication, while the second phase was evident during the withdrawal state. During the latter stage, similar mechanism of free radical release may occur, i.e, phosphatidylinositol involvement. However, another possibility is that following alcohol withdrawal, enhanced intracellular influx of Ca^{++} into a number of cells, including Kupffer cells and endothelial cells may also manifest. It has been demonstrated that removal of alcohol, following ethanol intoxication enhances the influx of Ca^{++} in mononuclear cells (15). Ca^{++} is a known regulator of

Table 3. Effect of prolonged consumption of alcohol on serum endotoxin, transaminase, cytokines and hepatic superoxide release

Parameters	Pair-fed	Alcohol-fed
Serum endotoxin (pg/ml)	39 ± 12	192 ± 48*
Serum ASAT (U/L)	140 ± 76	843 ± 208**
Serum TNF (pg/ml)	6 ± 2	80 ± 17**
Serum IL-l beta(pg/ml)	38 ± 19	1646 ± 184**
Hepatic superoxide release (nmol/min/liver)	0.2 ± 0.10	7.14 ± 2.65**

Endotoxin and ASAT were measured by means of endotoxin chromogen (Pharmacia) and Limulus coagulation assay (Sigma) and UV-kinetic enzyme kit (Sigma), respectively. Cytokines were analyzed using ELISA kits from Biosource International, while hepatic superoxide was measured in the *in situ* perfused liver by superoxide dismutase-inhibitable reduction of cytochrome c.* $P < 0.05$ vs Pair-fed.** $P << 0.001$ vs Pair-fed. N = 3-12 per group.

Table 4. Effect of prolonged consumption of alcohol on superoxide release by isolated Kupffer cells, hepatic and blood PMNs.

Cell Type	Superoxide release (nmol/10^6 cells/hr)	
	Pair-fed	Alcohol-fed
Kupffer cells	0.3 ± 0.1	6.0 ± 0.8*
Blood PMNs	0.8 ± 0.2	3.8 ± 1.5 *
Hepatic PMN's	NT	8.0 ± 2.0

* $P < 0.001$ vs Pair-fed. N = 5 per group. NT = Not tested due to insufficient cell yield.

superoxide anion generation in phagocytes. It is therefore postulated that enhanced spontaneous production of free radicals by Kupffer cells and endothelial cells may be due to an upregulation of Ca^{++} influx in hepatic non-parenchymal cells. Another possibility is that enhanced glucose uptake by Kupffer cells and endothelial cells during the alcohol withdrawal state may also contribute to enhanced free radical generation in the liver (10). Elevated glucose uptake may regulate respiratory burst by phagocytic cells, by increasing hexose monophosphate shunt activity. Therefore, enhanced glucose use by Kupffer and endothelial cells may be an important factor in the upregulation of free radical formation by hepatic non-parenchymal cells during alcohol withdrawal.

This work demonstrates that Kupffer cells are the likely sources of free radicals in the liver during the early phase of acute alcohol intoxication and the recovery period. Endothelial cells seem to contribute significantly to hepatic superoxide release during the withdrawal phase but not in the early phase. These conclusions are supported by experiments whereby, the liver was depleted of Kupffer cells by $GdCl_3$ treatment. Selective depletion of Kupffer cells completely abolished free radical release during the first phase of acute alcohol intoxication and partially suppressed the release of these radicals during the alcohol withdrawal phase. Hepatocytes are not likely to release oxygen-free radicals in the extracellular environment. The enhanced production of superoxide anion by the liver following alcohol withdrawal is likely to contribute to the elevation of serum aspartate transaminase (ASAT) activity, suggesting that liver function was altered as a result of this condition.

The above studies suggest that acute alcohol intoxication may directly initiate the formation of oxygen-derived radicals in the liver. We have also demonstrated that metabolites of ethanol are not likely to contribute to this manifestation (9). In the chronic alcohol model, however, a more complex mechanism may exist. Results presented in this paper demonstrate that prolonged consumption of alcohol for 16 weeks enhanced the release of superoxide anion in the perfused liver compared to that of the pair-fed controls. The major sources of these radicals are the Kupffer, endothelial cells and infiltrated PMNs. Hepatic sequestration of PMNs during chronic alcohol intoxication has been documented (21,22). Elevated levels of cytokines and chemokines during chronic alcohol consumption are likely to contribute to the pathogenesis of alcoholic liver disease in humans (23-25) and animal models (1,12,26). However, acute alcohol intoxication has not been shown to induce the migration of PMNs into the liver.

The mechanism by which free radical formation is enhanced during chronic alcohol intoxication may involve endotoxins and cytokines. It has been demonstrated that chronic alcoholism is also associated with endotoxemia (27-29). Data presented in this paper show that alcohol-fed rats were significantly endotoxemic compared to non-alcoholic rats. It has been suggested that chronic alcohol consumption enhances the gut permeability which in turn induces the influx of endotoxin in the circulation (29). The increased concentration of endotoxin in sera of alcoholic rats may suggest enhanced LPS influx or reduced clearance of endotoxin by resident Kupffer cells in the liver. Kupffer cells are primarily responsible for clearance of particulate and soluble particles, including endotoxins. As a result, hepatic macrophages may become activated to produce a number of biologically active substances that include TNF, IL-1 and superoxide anion. Results show that prolonged consumption of alcohol was also associated with increased serum levels of TNF, IL-1 and hepatic superoxide release. TNF and IL-1 are potent immunomodulators that prime and activate mononuclear phagocytes and PMNs for enhanced respiratory burst and free radical release. We have also demonstrated that TNF primed the liver and hepatic non-parenchymal cells for enhanced superoxide anion release in vitro (30). These cytokines (31) are also known to enhance the expression of adhesion molecules and production of chemotactic factors that promote migration of inflammatory PMNs to the liver. We have demonstrated previously that chronic alcohol consumption enhances hepatic sequestration of PMNs (21,22). Enhanced migration of PMNs in the liver during chronic alcohol intoxication is likely to be induced by hepatic production of chemokines, i.e, macrophage inflammatory protein-2 and cytokine-induced chemoattractant, and upregulation of

CD18 and ICAM-1 expression (12,21,26). Thus, enhanced endotoxin influx and cytokine production during chronic alcohol intoxication are likely to contribute to the enhanced sequestration of PMNs in the liver.

Prolonged consumption of alcohol was also associated with increased ASAT activity in the serum suggesting hepatic injury. It has also been demonstrated that chronic alcohol intoxication induces histopathological changes in the liver, such as, sinusoidal swelling, inflammatory-like infiltrate in the interstitium and lipid deposition (12). It is also suggested that hepatic injury may result from an inflammatory-like reaction in the liver that may be induced directly by alcohol or indirectly through alcohol-induced endotoxemia. Oxygen-derived free radicals produced by inflammatory PMNs and activated Kupffer cells in the liver are likely to contribute to this phenomenon. The production of cytolytic proteases may also participate in this pathology (1).

In conclusion, these studies demonstrate that acute or chronic alcohol intoxication stimulates the release of hepatic oxygen-derived free radicals that are likely to contribute to the pathogenesis of alcoholic liver disease in susceptible individuals.

6. ACKNOWLEDGMENT

The authors are grateful to Scott M Sondes, Elise Arruebarrena, Sheesha Roy, Asya Shoichet, Adrian Dobrescu, Jean Carnal, Howard Blakesley for excellent technical assistance, and Anna May Hudson for preparing the manuscript. This work was supported by National Institute on Alcohol Abuse and Alcoholism grants AA 08846, AA 10466 (APB) and AA 09803 (JJS).

7. REFERENCES

1. Bautista AP: Role of Kupffer cells in the induction of immunosuppression and hepatotoxicity in chronic alcoholic rats, In Cells of the Hepatic Sinusoids, Vol. 5. Eds. Knook DE, Wisse E, Wake K, Kupffer Cell Foundation, Leiden, Netherlands 70-71(1995)

2. Cederbaum AI: Role of lipid peroxidation and oxidative stress in alcohol toxicity. *Free Rad Biol Med* 7, 537-539 (1989)

3. Jaeschke H, AP Bautista, S Spolarics & JJ Spitzer: Superoxide anion generation by Kupffer cells and priming of neutrophils during reperfusion after hepatic ischemia. *Free Rad Res Commun* 15,27-284 (1991)

4. Jaeschke H, A Farhood &, CW Smith: Neutrophils contribute to ischemia-reperfusion injury in rat liver in vivo. *FASEB J* 4,3355-3359 (1990)

5. Younes M & O Strubelt: Alcohol-induced hepatotoxicity: a role for oxygen-derived free radicals. *Free Rad Res Commun* 3,1- 5 (1987)

6. Bautista AP & JJ Spitzer: Acute endotoxin tolerance downregulates superoxide anion release by the perfused liver and isolated hepatic nonparenchymal cells. *Hepatol* 21,855-862 (1995)

7. Bautista AP & JJ Spitzer: Superoxide anion generation by in situ perfused liver: effect of *in vivo* endotoxin. *Am J Physiol* 259,G907-912 (1991)

8. Bautista AP, K Meszaros, J Bojta & JJ Spitzer: Superoxide anion production by the liver during the early stage of endotoxemia in rats. *J Leuko Biol* 48,123-128 (1990)

9. Bautista AP, Spitzer JJ: Acute ethanol intoxication stimulates superoxide anion production by in situ perfused rat liver. *Hepatol* 15,892-898 (1992)

10. Bautista AP & JJ Spitzer: Postbinge effects of acute alcohol intoxication on hepatic free radical formation. *Alcohol Clin Exp Res* 20,502-509 (1996)

11. Johnston RB, CA Godzik & ZA Cohn: Increased superoxide anion production by immunologically activated and elicited macrophages. *J Exp Med* 148,15-127 (1978)

12. Bautista AP: Chronic alcohol intoxication induces hepatic injury through enhanced macrophage inflammatory protein-2 (MIP_2) production and intercellular adhesion molecule-1 (ICAM-1) expression in the liver. *Hepatol* 25, 335-342 (1997)

13. Figdor CG, JMM Leemans, WS Bont & JE Vries: Theory and practice of centrifugal elutriation (CE): factor influencing the separation of human blood. *Cell Biophys* 5,105-112 (1983)

14. Dorio RJ, JB Hoek, R Rubin & E Rubin: Ethanol modulation of rat alveolar macrophage superoxide production. *Biochem Pharmacol* 37,3528-3531 (1988)

15. Nagy J, E Kornyey & A Orozs: Increased intracellular calcium level in peripheral blood mononuclear cells of alcoholic patients under withdrawal. *Drug Alcohol Depend* 32,107-111 (1993)

16. Zuiable A, E Wiener E & SN Wickramasinghe: In vitro effects of ethanol on the phagocytic and microbial killing activities of human monocytes and monocyte-derived macrophages. *Clin Lab Hematol* 14,137-147 (1992)

17. Husztik E, G Lazar & A Parducz: Electron microscopic study of Kupffer cell-phagocytosis blockade induced by gadolinium chloride. *Brit J Exp Pathol* 61,624-630 (1980)

18. Hoek JB, AP Thomas, R Rubin & E Rubin: Ethanol-induced mobilization of calcium by activation of phosphoinositide- specific phospholipase C in intact hepatocytes. *J Biol Chem* 262,682-691 (1987)

19. Taraschi TF, JS Ellingson , A Wu, R Zimmerman & E, Rubin: Phosphatidylinositol from ethanol-fed rats confers membrane tolerance to ethanol. *Proc Natl Acad Sci* 83,9398-9402 (1986)

20. Johnston RB & S Kitagawa: Molecular basis for the enhanced respiratory burst of activated macrophages. *Fed Proc* 4,2927- 2932 (1985)

21. Bautista AP: Chronic alcohol intoxication enhances the expression of CD18 adhesion molecules on neutrophils and the release of a chemotactic factor by Kupffer cells. *Alcohol Clin Exp Res* 19,285-290 (1995)

22. Bautista AP, NB D'Souza, CH Lang & JJ Spitzer: Modulation of f-met-leu-phe-induced chemotactic activity and superoxide production by neutrophils during chronic ethanol intoxication. *Alcohol Clin Exp Res* 16,788-794 (1992)

23. Hill DB, Marsano SL, McClain CJ: Increased plasma IL-8 concentration in alcoholic hepatitis. *Hepatol* 18,576-580 (1994)

24. McClain CJ & DA Cohen DA: Increased tumor necrosis factor production by monocytes in alcoholic hepatitis. *Hepatol* 9,349-351 (1989)

25. McClain CJ, DA Cohen, CA Dinarello & JG Cannon: Serum interleukin-1 activity in alcoholic hepatitis. *Life Sci* 39,1479- 1485 (1986)

26. Bautista AP: Production of macrophage inflammatory protein-2 (MIP_2) by Kupffer cells and upregulation of adhesion molecule expression promote hepatic injury in chronic alcoholics. In Cells of the Hepatic Sinusoid, Vol. 6. Eds. Wisse E, Knook DL, Balabaud C, Kupffer Cell Foundation, Leiden, Netherlands 272-275 (1997)

27. Bode C, V Kugler V & JC Bode: Endotoxemia in patients with alcoholic and non-alcoholic cirrhosis and in subjects with evidence of chronic liver diseases following acute alcohol excess. *J Hepatol* 4,8-14 (1987)

28. Liehr H, & M Grun: Clinical aspects of Kupffer cell failure in liver disease, In: Kupffer Cells and Other Sinusoidal Cells. Eds. Wisse E, Knook DL, Elsevier/North Holland Press, Amsterdam, the Netherlands 427-437 (1997)

29. Thurman RG, W Gao, HD Connor, Y Adachi, RF Stachlewitz , Z Zhong, KT Knecht, BU Bradford, RT Currin, RP Mason & JJ Lemasters: Role of Kupffer cells in liver transplantation and alcoholic liver injury: 1994 Update. In Cells of the Hepatic Sinusoid, Vol 5. Eds. Wisse E, Knook DL, Wake K, Kupffer Cell Foundation, Leiden, The Netherlands 219-227 (1995)

30. Bautista AP, A Schuler, Z Spolarics & JJ Spitzer: Tumor necrosis factor-_ stimulates superoxide anion generation by perfused rat liver and Kupffer cells. *Am J Physiol* 261,G891-G895 (1991)

31. Patarroyo M, J Prieto, J Rincon, T Timonen, C Lundberg, K Lindbom, B Sio & CG Gahmberg: Leukocyte-cell adhesion: A molecular process fundamental in leukocyte physiology. *Immunol Rev* 114,67-108 (1990)

Keywords: Endotoxin, Endothelial Cells, Neutrophils, Cytokines, Gadolinium Chloride, Transaminase, Rat, Review

Send correspondence to: Dr. Abraham P. Bautista, Department of Physiology, LSU-Medical Center, 1901 Perdido Street, New Orleans, LA 70112, Tel:504-568-6171 Fax:504-568-6158, E-mail:Barcdav@aol.com

Received: 5/1/99, Accepted: 5/20/99