Published in final edited form as:
Biochem Biophys Res Commun. 2011 June 10; 409(3): 406–411. doi:10.1016/j.bbrc.2011.05.007.

Upregulation of Kupffer cell α_{2A}–adrenoceptors and downregulation of MKP-1 mediate hepatic injury in chronic alcohol exposure

Michael Ajakaiye, **Asha Jacob**, **Rongqian Wu**, **Mian Zhou**, **Youxin Ji**, **Weifeng Dong**, **Zhimin Wang**, **Xiaoling Qiang**, **Wayne W. Chaung**, **Jeffrey Nicastro**, **Gene F. Coppa**, and **Ping Wang**
Center for Immunology and Inflammation, The Feinstein Institute for Medical Research, Manhasset, NY & Department of Surgery, North Shore University Hospital and Long Island Jewish Medical Center, Manhasset, NY

Abstract

Alcohol-induced liver disease is associated with unacceptable morbidity and mortality. When activated, Kupffer cells (KCs), the resident macrophages in the liver, release proinflammatory cytokine TNF-α, a key mediator of hepatic damage. Although chronic alcohol causes increase in norepinephrine (NE) release leading to hepatic dysfunction, the mechanism of NE-induced hepatic injury in chronic alcohol exposure has not been elucidated. This study was conducted to determine whether chronic alcohol exposure increases NE and upregulates KC α_{2A}-adrenoceptors (α_{2A}-AR) to cause TNF-α release. We also examined the role of mitogen activated protein kinase (MAPK) phosphatase-1 (MKP-1) in this process. Male adult rats were fed the Lieber-DeCarli liquid diet containing alcohol as 36% of total calories. The animals were sacrificed after 6 weeks and blood and liver samples were harvested for further analysis. KCs from healthy male rats were cultured with alcohol for 7 days, and cells then harvested for RNA and protein analyses. Chronic alcohol exposure resulted in hepatic damage. Alcohol caused a 276% increase in circulating NE and 86% increase in TNF-α in the liver. There was a 75% and 62% decrease in MKP-1 mRNA and protein levels in the liver, respectively. In-vitro experiments revealed 121% and 98% increase in TNF-α and α_{2A}-AR mRNA levels with alcohol exposure, respectively, and a 32% decrease in MKP-1 mRNA compared to controls. In summary, chronic alcohol exposure elevates NE and upregulates KC α_{2A}-AR to release TNF-α. Alcohol induced downregulation of MKP-1 leads to further release of TNF-α and hepatic injury.

Keywords

chronic alcohol; α_{2A}-adrenoceptor; MKP-1; liver injury

Please address correspondence, proofs, and reprint requests to: Ping Wang, MD, Center for Immunology and Inflammation, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, USA, Tel: (516) 562-3411, Fax: (516) 562-1022, pwang@nshs.edu.

1. Introduction

Alcohol-related diseases constitute a severe health burden in the United States and the world. Strong epidemiological data have proven a definite link between prolonged excessive alcohol intake and the development of liver diseases. Alcoholic liver disease covers a wide spectrum of histopathological changes with varying clinical manifestations. The multi-factorial molecular mechanisms underlying these changes involve several mediators operating through different processes including the generation of harmful metabolites and oxygen species in the hepatic milieu [1, 2]. Kupffer cells (KCs) are the resident hepatic macrophages. Physiologically, they maintain maximal liver function by phagocytosing bacteria and foreign materials. They are quiescent in the absence of stimulatory agents, and have been shown to be activated by various stimuli including endotoxins and norepinephrine (NE) [3, 4]. Activated KCs release a myriad of inflammatory cytokines including tumor necrosis factor-α (TNF-α), transforming growth factor-α (TGF-α), and interleukins 1 (IL-1) and IL-6. Clinical and animal studies have implicated these cytokines, especially TNF-α, as major mediators in the pathogenesis of alcoholic liver disease [5, 6].

We have previously shown a correlation between α_{2A}-adrenoceptor (α_{2A}-AR) expression and TNF-α release by KC. Intra-portal infusion of NE increased TNF-α release which was inhibited by co-infusion with yohimbine, a non-specific antagonist of the α-AR [7], or with the specific α_{2A}-AR antagonist, BRL-44408 maleate [8]. Furthermore, upregulation of α_{2A}-AR gene expression was demonstrated in sepsis, and the increase in plasma TNF-α and its gene expression was abolished by BRL-44408 maleate treatment [8]. We therefore postulated that the increased TNF-α release and consequent hepatic damage associated with catecholaminergic activity is mediated by the action of NE on KC α_{2A}-AR and that the intracellular pathway of the NE-mediated response would involve the utilization of mitogen-activated protein kinases (MAPKs) cascades. MAPKs are a group of serine/threonine protein kinases that have been widely described across eukaryotic species. The MAPK family, including ERK, JNK, and p38 MAPK, plays prominent roles in the immune system [9–11]. MAPK can alter mRNA stability, transport and translation of its targets. This signaling has been linked to the pathology of many diseases involving immune dysfunction and the overproduction of pro-inflammatory cytokines [12–16]. Studies have demonstrated that p38 MAPK regulates TNF-α transcription in macrophages and that approximately one-third of the TNF-α-induced genes are regulated by this signal pathway [17]. The MAPK activity is inhibited by MAPK phosphatases (MKPs). The archetypal dual specificity phosphatase, MAPK phosphatase -1 (MKP-1), dephophosphorylates phosphorylated serine/threonine and tyrosine residues on activated MAPKs [18–20]. MAPK pathways are activated through phosphorylation, therefore dephosphorylation of these kinases by MKP-1 is a rapid and highly energy-efficient mode of deactivation and consequent negative regulation of these pathways [19]. In fact, we have previously shown that MKP-1 is significantly downregulated in the liver during sepsis as well as in NE treated KC, suggesting that increased MAPK activation leads to increased TNF-α production [21].

The purpose of this study was to ascertain the role of chronic alcohol exposure on circulating NE and the interaction of NE and alcohol on KC α_{2A}-AR stimulated release of TNF-α in the etiology of alcohol-induced hepatic injury. We explored the direct action of alcohol on KC α_{2A}-AR and examined the role of MKP-1 in the release of inflammatory cytokines, particularly TNF-α.

2. Materials and Methods

2.1. Animal model of chronic alcohol exposure

Male Sprague-Dawley rats (275–325g, Charles River Laboratories, Wilmington, MA) were housed in a temperature-controlled room on a 12-h light/dark cycle and fed the Lieber-DeCarli liquid diet containing alcohol as 36% of total calories for 6 weeks [22]. Control animals were kept under the same conditions and fed on a standard Purina rat chow diet and allowed water *ad libitum*. The animals were sacrificed after 6 weeks and blood and liver samples were collected and analyzed. The experiments were performed in accordance with the National Institutes of Health guidelines for the use of experimental animals. This project was approved by the Institutional Animal Care and Use Committee of The Feinstein Institute for Medical Research.

2.2. In-vitro KC culture

Male adult Sprague-Dawley rats were anesthetized with isoflurane inhalation and the inferior vena cava (IVC) was cannulated with PE50 tubing. The liver was perfused first with Hank's balanced salt solution (HBSS) and then with type IV collagenase. KCs were isolated by Percoll gradient centrifugation as described previously by us [23, 24]. After isolation and counting, KCs were plated at a density of 1 million cells/well in 12 well culture plates with DMEM containing 10% FBS and incubated overnight at 37°C and cultured for 7 days. All media were supplemented with 10 mM HEPES, pH 7.4, 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin.

2.3. Determination of Kupffer cell viability

KCs were isolated as previously outlined and plated at a density of 2×10^5 cells/ well in 96 well culture plates with culture medium and alcohol treatment as above. [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, a validated calorimetric method of determining cell viability in culture [25, 26], was performed after 7 days culture using the CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison WI) according to the manufacturer's instructions. Briefly, MTS and PMS were combined in volumetric ratio of 20:1. 20 μl of the resulting solution was pipetted into each well of the 96 well plate containing 100 μl of Kupffer cells in culture medium. The plates were then incubated for 3 hours at 37°C in a humidified, 5% CO2 atmosphere. The absorbance was read at 490nm with Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek, Winooski, VT).

2.3. Alcohol exposure

For alcohol groups, appropriate volumes of 200 proof (100%) ethanol (PHARMCO-AAPER) were diluted in the media resulting in alcohol concentrations of 25 mM and 100 mM respectively for each of the 2 groups. Treatment was commenced 24 hours after initial plating to allow undisturbed attachment of KCs. The media were replaced daily with fresh alcohol supplemented medium for 7 days. KCs were processed 24 hours after the last media change. Control KCs were simultaneously cultured in identical conditions but without the addition of alcohol to the medium. KCs were then processed either for RNA or protein and analyzed.

2.4. Determination of circulating liver enzymes

Blood samples were centrifuged at 4,000 *g* for 10 min to collect serum and stored at −80°C. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total and direct bilirubin were measured by using commercial assay kits according to the manufacturer's instructions (Pointe Scientific, Lincoln Park, MI).

2.5. Measurement of serum norepinephrine

Plasma samples were quantified by the use of commercially obtained ELISA kit specific for NE (IBL-America Inc., Minneapolis, MN). The assay was carried out according to manufacturer's instructions as previously described [27].

2.5. Determination of hepatic TNF-α

Snap frozen tissue were homogenized in lysis buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM EGTA, , 2 mM sodium orthovandate, 0.2 mM phenylmethyl sulfonyl fluoride, 2 μg/ml leupeptin, 2 μg/ml aprotinin,1.0 % Triton-X-100). Tissue lysates were prepared by sonication and centrifugation at 10,000 *g* at 4°C for 15 min. Tissue levels of TNF-α were determined by using commercially obtained enzyme linked immunoabsorbent assay (ELISA) kits specific for rat-TNF-α (BioSource International, Camarillo, CA). The assay was carried out according to the instructions provided by the manufacturer.

2.6. Total RNA extraction and real-time PCR

Total RNA was extracted from the liver and KC by Tri-Reagent (Molecular Research Center, Cincinnati, OH). 5μg of RNA from each sample was reverse-transcribed and 1 μl cDNA was amplified with 0.15 μM each of 3′ and 5′ primers, specific for the rat α_{2A}-AR, TNF-α and MKP-1. Rat glyceraldehyde 3-phosphate dehydrogenase (G3PDH) was used as the housekeeping gene. The primers are as follows: 5′-CGT GTT CGT GGT GTG TTG GT -3′ (forward) and 5′-GCA GCC GAC CGC TAT GAG -3′ (reverse) for α_{2A}-adrenoceptor (NM_012739), 5′-TGA TCG GTC CCA ACA AGG A -3′ (forward) and 5′-GGG CCA TGG AAC TGA TGA GA -3′ (reverse) for TNF-α (NM_012675), 5′-GCG CGC TCC ACT CAA GTC-3′ (forward) and 5′-GGG CAG GAA GCC GAA AAC-3′ (reverse) for MKP-1 (NM_0503769) and 5′-TGA AGG TCG GTG TCA ACG GAT TTG GC-3′ (forward) and 5′-CAT GTA GGC CAT GAG GTC CAC CAC-3′ (reverse) for G3PDH (M17701). Each cycle consisted of 30 seconds at 94°C, 30 seconds at 60°C, and 45 seconds at 72°C.

2.7. MKP-1 Western immunoblotting

Liver tissue lysates (100 μg) were electrophoresed on NuPAGE 4–12% Bis-Tris gels and transferred to 0.22 μm nitrocellulose membrane and blocked in TBS-T (10 mM Tris-HCl, 150 mM NaCl, 0.1% Tween-20) containing 5% non-fat milk for 1 h at room temperature. Blots were incubated overnight at 4°C with 1:500 dilution of rabbit polyclonal IgG (Santa Cruz Biotech., Santa Cruz, CA) for MKP-1, reacted with 1:5,000 dilution of HRP labeled anti-rabbit IgG for 1 h at room temperature and detected with chemiluminescence (ECL; Amersham Biosciences, Inc.). The same blot was stripped and reprobed with anti-β-actin antibody (Sigma-Aldrich, St. Louis, MO). All membranes were exposed to X-ray film and the band densities were quantitated using the Bio-Rad image system (Hercules, CA). The data are represented as the ratio between MKP-1 and β-actin protein expression.

2.8. Statistical analysis

All data are expressed as mean ± SE and analyzed by Student's *t*- test for two groups. One-way analysis of variance (ANOVA) and Student Newman Keuls test was used for multiple groups. The differences in values were considered significant if $P<0.05$.

3. Results

3.1. Effect of chronic alcohol exposure on markers of hepatic damage

Rats fed with the liquid Lieber-DeCarli alcoholic diet have been shown signs of hepatic injury [22]. Serum AST and ALT levels increased by 40% and 17%, respectively, when

compared to standard rat chow fed (control) rats (Figs. 1A–B). There was a 276% rise in direct bilirubin after alcohol exposure for 6 weeks (Fig. 1C).

3.2. Effects of chronic alcohol exposure on NE and TNF-α

We have previously shown that increased circulating NE results in the release of TNF-α by KC [4]. Therefore, we measured NE from the circulation and TNF-α protein levels in the liver tissues of chronic alcohol exposed rats. A 276% rise in circulating NE and a significant 86% increase in TNF-α protein in the liver were observed after chronic alcohol exposure (Figs. 2A–B).

3.3. Effect of chronic alcohol exposure on MKP-1

MKP-1 has been postulated as an upstream regulator of TNF-α. This inhibition results in increased production of TNF-α. In the liver, there was a 75% decrease in MKP-1 mRNA and a corresponding 62% reduction in the protein levels after chronic alcohol exposure (Figs. 3A–B).

3.4. Effects of chronic alcohol exposure on cultured KC

KCs are implicated as the major secretors of cytokines. We postulate that KCs are activated after alcohol exposure, leading to the elaboration of TNF-α. In the presence of 100 mM ethanol for 7 days, there was a 121% increase in KC TNF-α mRNA levels as compared to control samples (Fig. 3C). Previously we demonstrated that there was an upregulation of KC α_{2A}-AR in sepsis, which is positively correlated with increased TNF-α production by KC [7]. We hypothesized that chronic alcohol leads to an upregulation of KC α_{2A}-AR and that this is acted on by NE in producing the observed increase in TNF-α. To ascertain this, we determined the expression of KC α_{2A}-AR with and without chronic alcohol treatment. KC treated with 100 mM alcohol showed a significant 98% increase in α_{2A}-AR mRNA expression when compared to controls (Fig. 3D). MKP-1 is a regulator of the MAPK cascades that produce TNF-α and other inflammatory mediators through the activation of transcription factors. In vivo experiments with chronic alcohol exposure showed a negative correlation between liver MKP-1 and TNF-α production (Figs. 2B and 3A–B). Similar to the in vivo data, KC treated with 100 mM alcohol showed a 32% decrease in MKP-1 mRNA expression (Fig. 3E).

3.5. Confirmation of cultured cell viability

To determine KC viability at the time of analysis, MTS assay was performed as described in the methods section. Treatment with alcohol at 7 days had a minimal impact in cell viability (8.9% decrease in absorbance compared to control) (Fig. 4A). There was no decrease in MTS absorbance in either control or alcohol treated cells compared to the first day of culture.

Discussion

Alcohol-induced hepatic diseases are associated with high morbidity and mortality, but the underlying mechanisms have not been fully elucidated. TNF-α is implicated as a major mediator of the damage seen in hepatic injury [5, 28, 29]. Continuous TNF-α stimulation may cause reorganization of the hepatic cytoarchitecture due to excessive fibrous deposition and inadequate attempts at healing the injured tissue. The role of TNF-α in the etiology of liver disease has been corroborated in animal [28, 29] and human studies [6, 30]. Furthermore, Neumann et al. demonstrated the role of cytokines in alcohol-induced cytotoxicity on Hep G2 cells and correlated alcohol-induced damage with increased TNF-α

production [31]. TNF-α, largely produced by macrophages, has been identified as a major mediator of alcohol induced damage in the liver.

To elucidate the mechanism responsible for alcohol-induced hepatic damage via increase in TNF-α, we employed both in vivo and in vitro approaches. We fed animals with alcohol and also conducted in vitro studies in alcohol-treated cultured KC to determine the effect of chronic alcohol and accompanying systemic NE release and upregulation of α_{2A}-AR in relation to the release of TNF-α from the liver. Our results showed that chronic alcohol exposure significantly increased hepatic injury markers, AST, ALT, and direct bilirubin. Furthermore, chronic alcohol significantly increased circulating NE levels and caused a corresponding increase in TNF-α in the liver. Our findings from in-vitro culture of alcohol-treated primary KC clearly showed that alcohol acts directly on KC to upregulate α_{2A}-AR. A positive correlation existed between increased expression of KC α_{2A}-AR and TNF-α, which corresponded to the increase in TNF-α observed in the liver. Thus, we have observed a clear relationship among chronic alcohol, upregulation of α_{2A}-AR, and TNF-α production.

We postulate that the increase in α_{2A}-AR mRNA expression observed in our study could be due to an increase in transcription of the α_{2A}-AR gene acting via protein kinase C (PKC). Alcohol is a known activator of PKC in various cell types[32–35] and PKC has been implicated in the induction of the α_{2A}-AR gene transcription [36, 37]. As reported by Venkataraman *et al*., [37] the increase in α_{2A}-AR mRNA resulting from the exposure of rat C6 glioma cells to phorbol- 12, 13-myristate acetate (PMA) was mediated by PKC through the AP-2 element of the α_{2A}-AR promoter. PMA, like alcohol, is an activator of PKC [38, 39].

To further our understanding of the possible mechanisms underlying the relationship between KC α_{2A}-AR and TNF-α production in chronic alcohol exposure, we investigated the response of the dual specificity phosphatase MKP-1, a protein that deactivates MAPKs [9, 40]. Our analysis of the MAPK negative regulator MKP-1 revealed a reduction of its expression and protein levels in hepatic tissues of chronic alcohol exposed rats. Furthermore, MKP-1 was significantly downregulated in cultured KC treated with alcohol. This decrease in MKP-1 expression was positively correlated with an increased expression of TNF-α in the hepatic tissues as well as in rat KC. The sequence of events leading to KC TNF-α release thus involves the increased activity of MAPK due to the inhibition of MKP-1 which culminates in increased hepatic TNF-α. We postulate that chronic alcohol exposure, through the increased expression of α_{2A}-AR shifts the balance of KC response to NE towards the α_{2A}-AR mediated pathway and associated increased TNF-α production. Alcohol mediated upregulation of α_{2A}-AR correlates with reduced expression of MKP-1 and leads to KC TNF-α production probably through the MAPK pathway.

Although the role of TNF-α in the etiology of alcoholic liver disease has been demonstrated in animal models [41], clinical attempts to treat patients with infliximab (an antibody to TNF-α) have generally been unfavorable [42–44]. This may be because physiologically, TNF-α is required in the liver for the general maintenance of immunity and for hepatocyte regeneration [45]. It is therefore advisable to modulate, rather than completely block TNF-α production, with a view to optimizing its levels. This would minimize hepatic damage while mitigating the effects of other mediators of hepatic dysfunction and injury. Systemic inhibition of α_{2A}-AR is, however, unlikely to mitigate alcoholic liver injury as these receptors occur ubiquitously with widely varying effects on stimulation. Thus, interventions that selectively target KC α_{2A}-AR would be a viable means of modulating KC production of TNF-α in response to chronic and excessive alcohol exposure.

In summary, we have demonstrated that alcohol acts directly on KC to increase α_{2A}–AR and that this enhances its sensitivity to NE. Furthermore, chronic alcohol exposure leads to elevated levels of NE and this act on the already upregulated KC α_{2A}-AR and leads to increased secretion of TNF-α. Alcohol induced reduction in MKP-1 expression in the liver leads to increased MAPK activity and increased TNF-α production. Taken together, upregulation of α_{2A}-AR with the concomitant reduction of MKP-1 in the liver leads to increased production of TNF-α, and subsequent TNF-α-mediated hepatic damage (Figure 4). The KC thus plays a central role in the evolution of hepatic damage from chronic alcohol exposure. The identification of this pathway is promising for the development of novel interventions in the management of alcoholic liver disease.

Acknowledgments

This work was supported by the National Institutes of Health (NIH) grants, R01 GM053008 and R01 GM057468 (P.W.).

List of Abbreviations

KC	Kupffer Cell
TNF-α	tumor necrosis factor-α
NE	norepinephrine
α_{2A}-AR	alpha2A-adrenoceptor
MAPK	mitogen activated protein kinase
MKP-1	MAPK phosphatase-1
IL-1	interleukin-1
IL-6	interleukin-6
TGF-β	transforming growth factor-β
ERK	extracellular receptor kinase
JNK	jun kinase
ALT	alanine aminotransferase
AST	aspartate aminotransferase

References

1. Mann RE, Smart RG, Govoni R. The epidemiology of alcoholic liver disease. Alcohol Res Health. 2003; 27:209–219. [PubMed: 15535449]
2. Miranda-Mendez A, Lugo-Baruqui A, Armendariz-Borunda J. Molecular basis and current treatment for alcoholic liver disease. Int J Environ Res Public Health. 2010; 7:1872–1888. [PubMed: 20622998]
3. Wheeler MD. Endotoxin and Kupffer cell activation in alcoholic liver disease. Alcohol Res Health. 2003; 27:300–306. [PubMed: 15540801]
4. Yang S, Koo DJ, Zhou M, et al. Gut-derived norepinephrine plays a critical role in producing hepatocellular dysfunction during early sepsis. American Journal of Physiology Gastrointestinal and Liver Physiology. 2000; 279:G1274–1281. [PubMed: 11093951]
5. Fukui H. Relation of endotoxin, endotoxin binding proteins and macrophages to severe alcoholic liver injury and multiple organ failure. Alcohol Clin Exp Res. 2005; 29:172S–179S. [PubMed: 16344605]

6. Vidali M, Hietala J, Occhino G, et al. Immune responses against oxidative stress-derived antigens are associated with increased circulating tumor necrosis factor-alpha in heavy drinkers. Free Radic Biol Med. 2008; 45:306–311. [PubMed: 18460346]
7. Zhou M, Yang S, Koo DJ, et al. The role of Kupffer cell alpha(2)-adrenoceptors in norepinephrine-induced TNF-alpha production. Biochimica Biophysica Acta. 2001; 1537:49–57.
8. Miksa M, Das P, Zhou M, et al. Pivotal role of the alpha(2A)-adrenoceptor in producing inflammation and organ injury in a rat model of sepsis. PLoS One. 2009; 4:e5504. [PubMed: 19430535]
9. Chi H, Flavell RA. Acetylation of MKP-1 and the control of inflammation. Sci Signal. 2008; 1:pe44. [PubMed: 18922786]
10. Dickinson RJ, Keyse SM. Diverse physiological functions for dual-specificity MAP kinase phosphatases. J Cell Sci. 2006; 119:4607–4615. [PubMed: 17093265]
11. Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. Annu Rev Immunol. 2002; 20:55–72. [PubMed: 11861597]
12. Darieva Z, Lasunskaia EB, Campos MN, et al. Activation of phosphatidylinositol 3-kinase and c-Jun-N-terminal kinase cascades enhances NF-kappaB-dependent gene transcription in BCG-stimulated macrophages through promotion of p65/p300 binding. J Leukoc Biol. 2004; 75:689–697. [PubMed: 14742634]
13. Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev. 2001; 81:807–869. [PubMed: 11274345]
14. Park SJ, Jeong KS. Cell-type-specific activation of mitogen-activated protein kinases in PAN-induced progressive renal disease in rats. Biochem Biophys Res Commun. 2004; 323:1–8. [PubMed: 15351692]
15. Wang X, Meng X, Kuhlman JR, et al. Knockout of Mkp-1 enhances the host inflammatory responses to gram-positive bacteria. J Immunol. 2007; 178:5312–5320. [PubMed: 17404316]
16. Zakkar M, Chaudhury H, Sandvik G, et al. Increased endothelial mitogen-activated protein kinase phosphatase-1 expression suppresses proinflammatory activation at sites that are resistant to atherosclerosis. Circ Res. 2008; 103:726–732. [PubMed: 18723442]
17. Zer C, Sachs G, Shin JM. Identification of genomic targets downstream of p38 mitogen-activated protein kinase pathway mediating tumor necrosis factor-alpha signaling. Physiol Genomics. 2007; 31:343–351. [PubMed: 17652167]
18. Cuadrado A, Nebreda AR. Mechanisms and functions of p38 MAPK signalling. Biochem J. 2010; 429:403–417. [PubMed: 20626350]
19. Liu Y, Shepherd EG, Nelin LD. MAPK phosphatases--regulating the immune response. Nat Rev Immunol. 2007; 7:202–212. [PubMed: 17318231]
20. Ma FY, Ikezumi Y, Nikolic-Paterson DJ. Macrophage signaling pathways: a novel target in renal disease. Semin Nephrol. 2010; 30:334–344. [PubMed: 20620676]
21. Jacob A, Rajan D, Pathickal B, et al. The inhibitory effect of ghrelin on sepsis-induced inflammation is mediated by the MAPK phosphatase-1. Int J Mol Med. 2010; 25:159–164. [PubMed: 19956915]
22. DeCarli LM, Lieber CS. Fatty liver in the rat after prolonged intake of ethanol with a nutritionally adequate new liquid diet. J Nutr. 1967; 91:331–336. [PubMed: 6021815]
23. Wu R, Cui X, Dong W, et al. Suppression of hepatocyte CYP1A2 expression by Kupffer cells via AhR pathway: the central role of proinflammatory cytokines. Int J Mol Med. 2006; 18:339–346. [PubMed: 16820944]
24. Wu R, Zhou M, Wang P. Adrenomedullin and adrenomedullin binding protein-1 downregulate TNF-alpha in macrophage cell line and rat Kupffer cells. Regul Pept. 2003; 112:19–26. [PubMed: 12667621]
25. Buttke TM, McCubrey JA, Owen TC. Use of an aqueous soluble tetrazolium/formazan assay to measure viability and proliferation of lymphokine-dependent cell lines. J Immunol Methods. 1993; 157:233–240. [PubMed: 8423368]
26. Cory AH, Owen TC, Barltrop JA, et al. Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture. Cancer Commun. 1991; 3:207–212. [PubMed: 1867954]

27. Wu R, Zhou M, Das P, et al. Ghrelin inhibits sympathetic nervous activity in sepsis. Am J Physiol Endocrinol Metab. 2007; 293:E1697–1702. [PubMed: 17911350]
28. Roychowdhury S, McMullen MR, Pritchard MT, et al. An early complement-dependent and TLR-4-independent phase in the pathogenesis of ethanol-induced liver injury in mice. Hepatology. 2009; 49:1326–1334. [PubMed: 19133650]
29. Yee SB, Ganey PE, Roth RA. The role of Kupffer cells and TNF-alpha in monocrotaline and bacterial lipopolysaccharide-induced liver injury. Toxicol Sci. 2003; 71:124–132. [PubMed: 12520082]
30. Bird GL, Sheron N, Goka AK, et al. Increased plasma tumor necrosis factor in severe alcoholic hepatitis. Ann Intern Med. 1990; 112:917–920. [PubMed: 2339855]
31. Neuman MG, Shear NH, Bellentani S, et al. Role of cytokines in ethanol-induced cytotoxicity in vitro in Hep G2 cells. Gastroenterology. 1998; 115:157–166. [PubMed: 9649471]
32. Cosen-Binker LI, Lam PP, Binker MG, et al. Alcohol-induced protein kinase Calpha phosphorylation of Munc18c in carbachol-stimulated acini causes basolateral exocytosis. Gastroenterology. 2007; 132:1527–1545. [PubMed: 17408632]
33. Crabbe JC, Phillips TJ, Harris RA, et al. Alcohol-related genes: contributions from studies with genetically engineered mice. Addict Biol. 2006; 11:195–269. [PubMed: 16961758]
34. Funk CK, Dohrman DP. Chronic ethanol exposure inhibits dopamine release via effects on the presynaptic actin cytoskeleton in PC12 cells. Brain Res. 2007; 1185:86–94. [PubMed: 17996852]
35. Wallace MJ, Newton PM, Oyasu M, et al. Acute functional tolerance to ethanol mediated by protein kinase Cepsilon. Neuropsychopharmacology. 2007; 32:127–136. [PubMed: 16541084]
36. Overland AC, Kitto KF, Chabot-Dore AJ, et al. Protein kinase C mediates the synergistic interaction between agonists acting at alpha2-adrenergic and delta-opioid receptors in spinal cord. J Neurosci. 2009; 29:13264–13273. [PubMed: 19846714]
37. Venkataraman V, Duda T, Sharma RK. alpha2D/A-adrenergic receptor gene induction in the retina by phorbol ester: involvement of an AP-2 element. Genes Cells. 1999; 4:161–173. [PubMed: 10320481]
38. Castagna M, Takai Y, Kaibuchi K, et al. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J Biol Chem. 1982; 257:7847–7851. [PubMed: 7085651]
39. Stoeck M, Lees R, Szamel M, et al. Comparison of phorbol-12-myristate-13-acetate and dioctanoyl-sn-glycerol in the activation of EL4/6.1 thymoma cells. J Cell Physiol. 1989; 138:541–547. [PubMed: 2784444]
40. Wang X, Liu Y. Regulation of innate immune response by MAP kinase phosphatase-1. Cell Signal. 2007; 19:1372–1382. [PubMed: 17512700]
41. Thurman RG. II. Alcoholic liver injury involves activation of Kupffer cells by endotoxin. Am J Physiol. 1998; 275:G605–611. [PubMed: 9756487]
42. Addolorato G, Russell M, Albano E, et al. Understanding and treating patients with alcoholic cirrhosis: an update. Alcohol Clin Exp Res. 2009; 33:1136–1144. [PubMed: 19389182]
43. Boetticher NC, Peine CJ, Kwo P, et al. A randomized, double-blinded, placebo-controlled multicenter trial of etanercept in the treatment of alcoholic hepatitis. Gastroenterology. 2008; 135:1953–1960. [PubMed: 18848937]
44. Naveau S, Chollet-Martin S, Dharancy S, et al. A double-blind randomized controlled trial of infliximab associated with prednisolone in acute alcoholic hepatitis. Hepatology. 2004; 39:1390–1397. [PubMed: 15122768]
45. Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. N Engl J Med. 2000; 343:1467–1476. [PubMed: 11078773]

Research Highlights

- Alcohol-induced liver disease is associated with high morbidity and mortality.
- Although chronic alcohol exposure causes norepinephrine (NE) release leading to hepatic injury, the mechanism has not been fully elucidated.
- We induced chronic alcohol conditions in vivo and in vitro and examined the α_{2A}-adrenoceptor mediated hepatic damage.
- Our results showed that chronic alcohol exposure elevates NE and upregulates Kupffer cell α_{2A}-adrenoceptors to release TNF-α.
- Alcohol induced downregulation of MKP-1 leads to further release of TNF-α and hepatic injury.

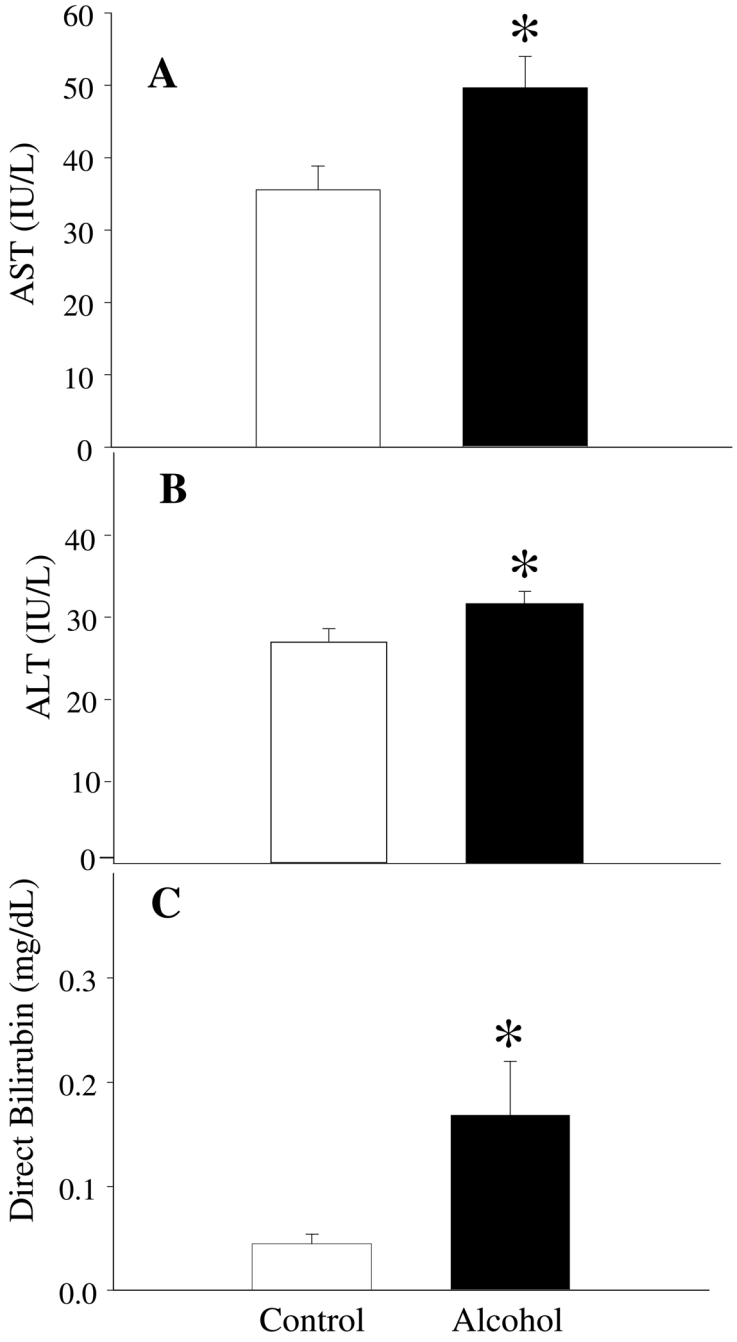


Figure 1. Alterations in hepatic injury markers after chronic alcohol exposure

Serum samples from control fed (control) and six weeks alcohol fed rats (Alcohol) were analyzed for (A) aspartate transaminase (AST) and (B) alanine transaminase (ALT) and (C) direct bilirubin. Data are presented as mean ± SE (n=4–6) and compared with Student's *t*-test. $^{*}P<0.05$ vs. Control.

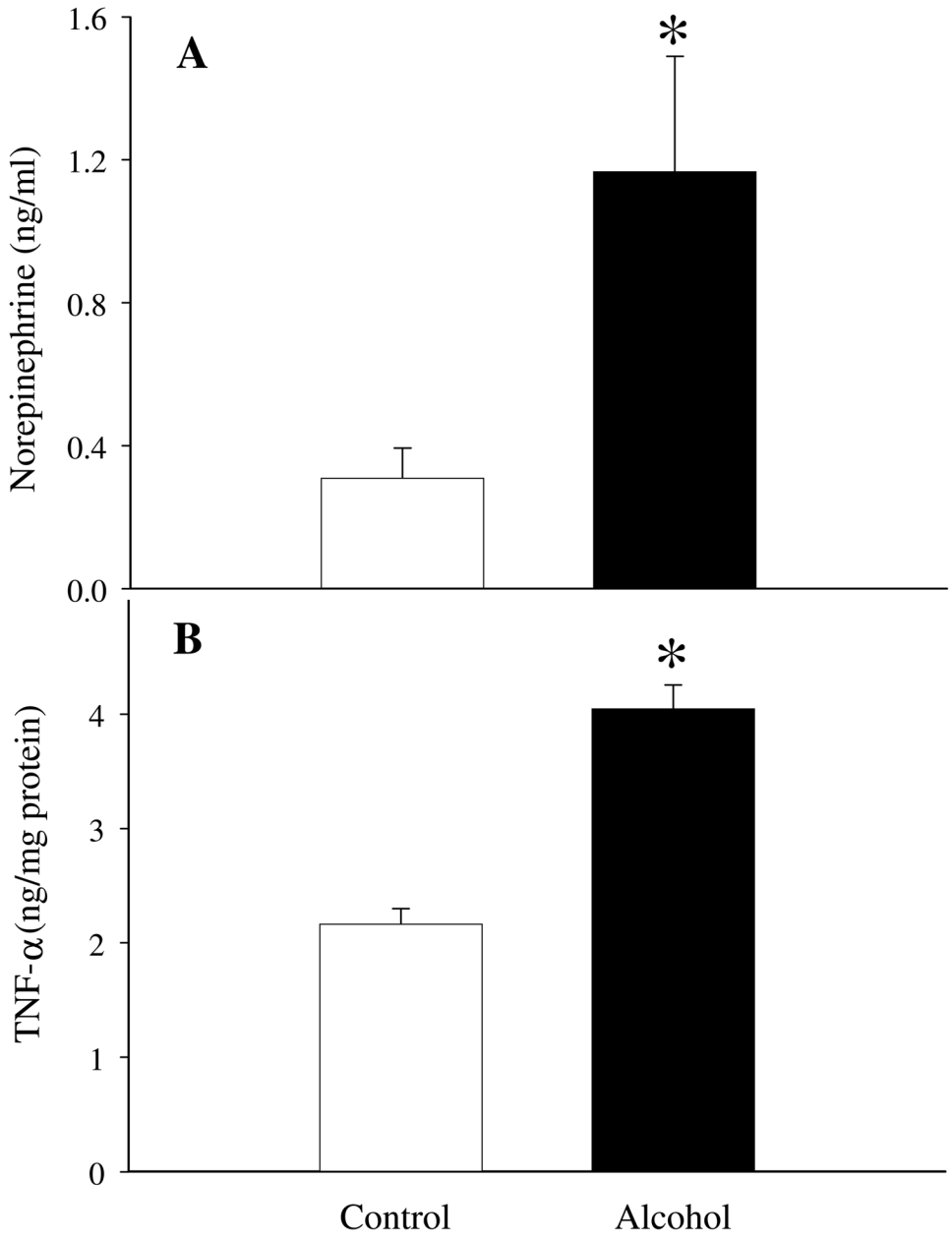
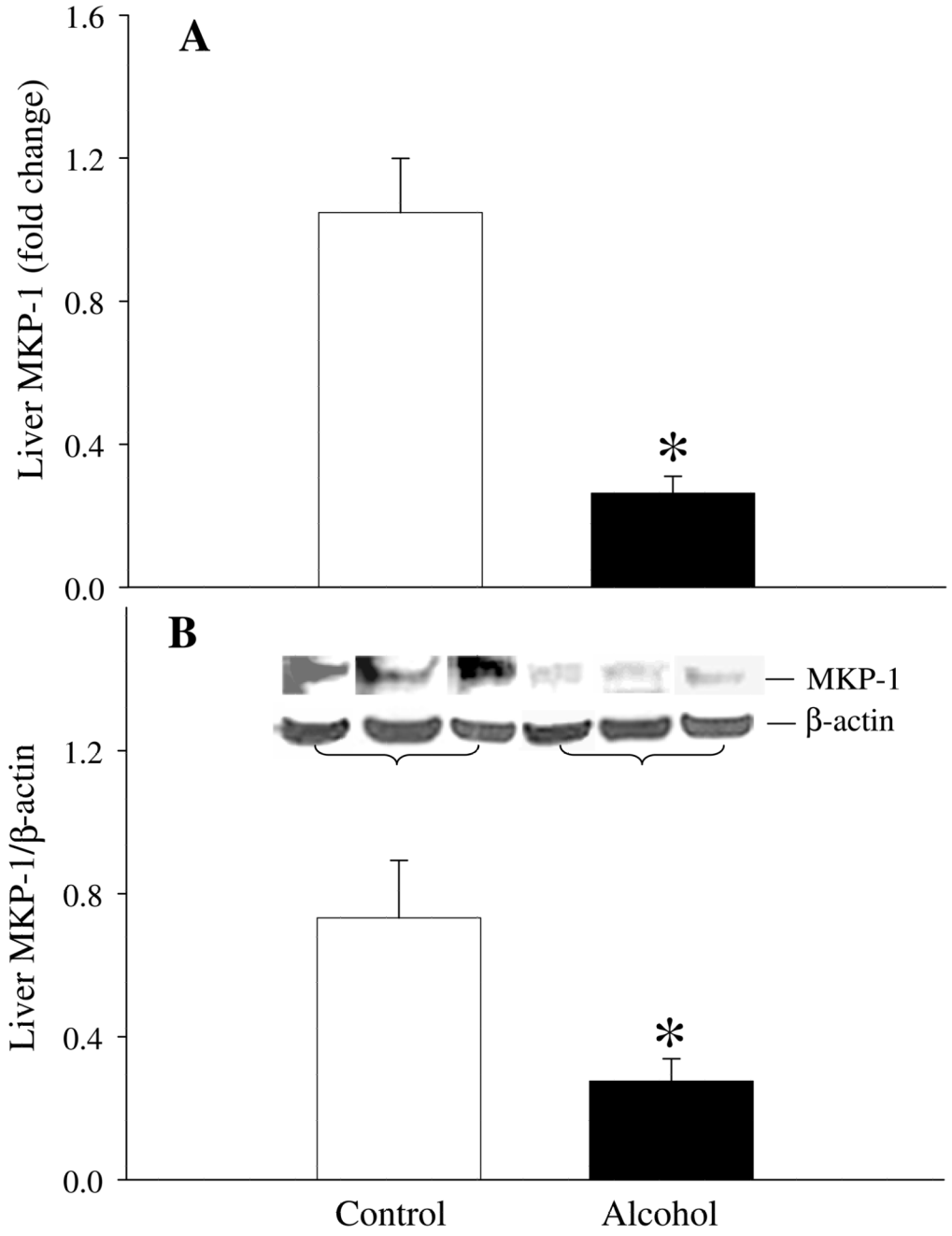


Figure 2. Changes in serum NE and liver TNF-α after chronic alcohol exposure

Serum samples from control fed (control) and six weeks alcohol fed rats (Alcohol) were analyzed for (A) NE. Protein extracted from hepatic tissues was assessed for (B) TNF-α. Data are presented as mean ± SE (n=5–6) and compared with Student's *t*-test. $^{*}P<0.05$ vs. Control.



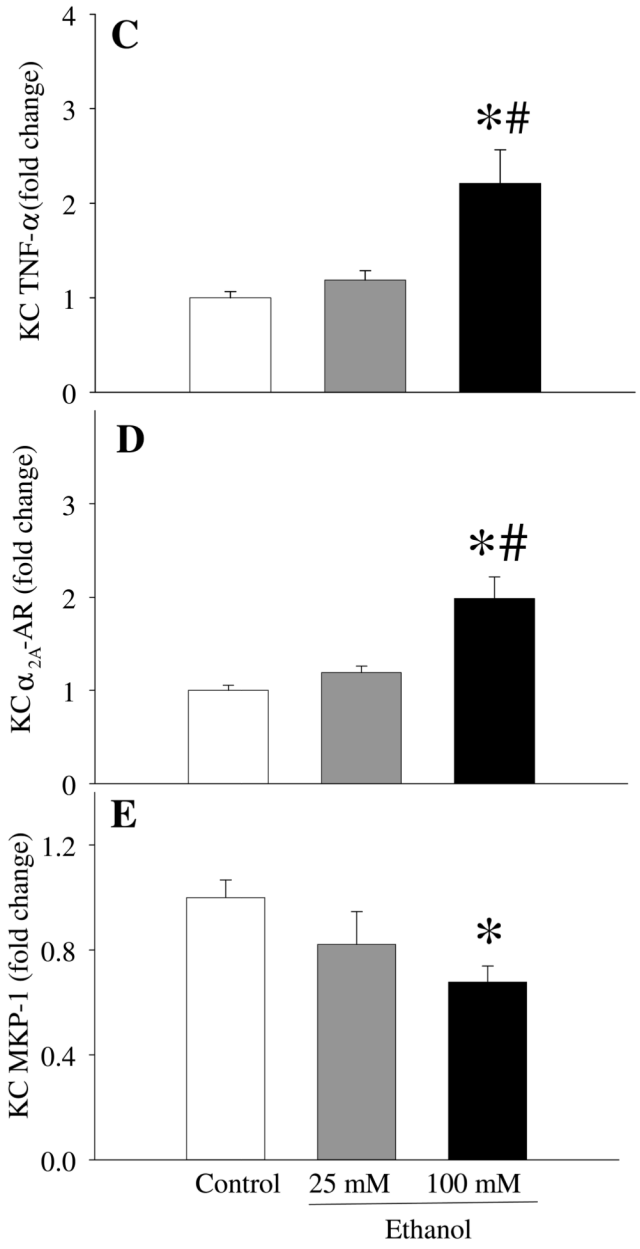


Figure 3. Changes in Liver and KC TNF-α, α_{2A}-AR, and MKP-1 after chronic alcohol exposure

Hepatic tissues from control fed (control) and six weeks alcohol fed rats (Alcohol) were analyzed for MKP-1 (A) mRNA (B) protein. A representative autoradiograph of the Western blot is also shown. RNA was extracted from cultured KC treated with different concentrations of alcohol for 7 days and analyzed for (C) TNF-α and (D) α_{2A}-AR, (E) MKP-1 mRNA, by quantitative real-time PCR. RNA expression is shown as fold change from G3PDH. Protein levels are expressed as the ratio between MKP-1 and β-actin. Data are presented as mean ± SE (n=5) and compared with Student's *t*-test for two groups and Student Newman Keuls test by ANOVA for more than two groups. $^{*}P<0.05$ vs. Control; $^{\#}P<0.05$ vs. 25 mM alcohol.

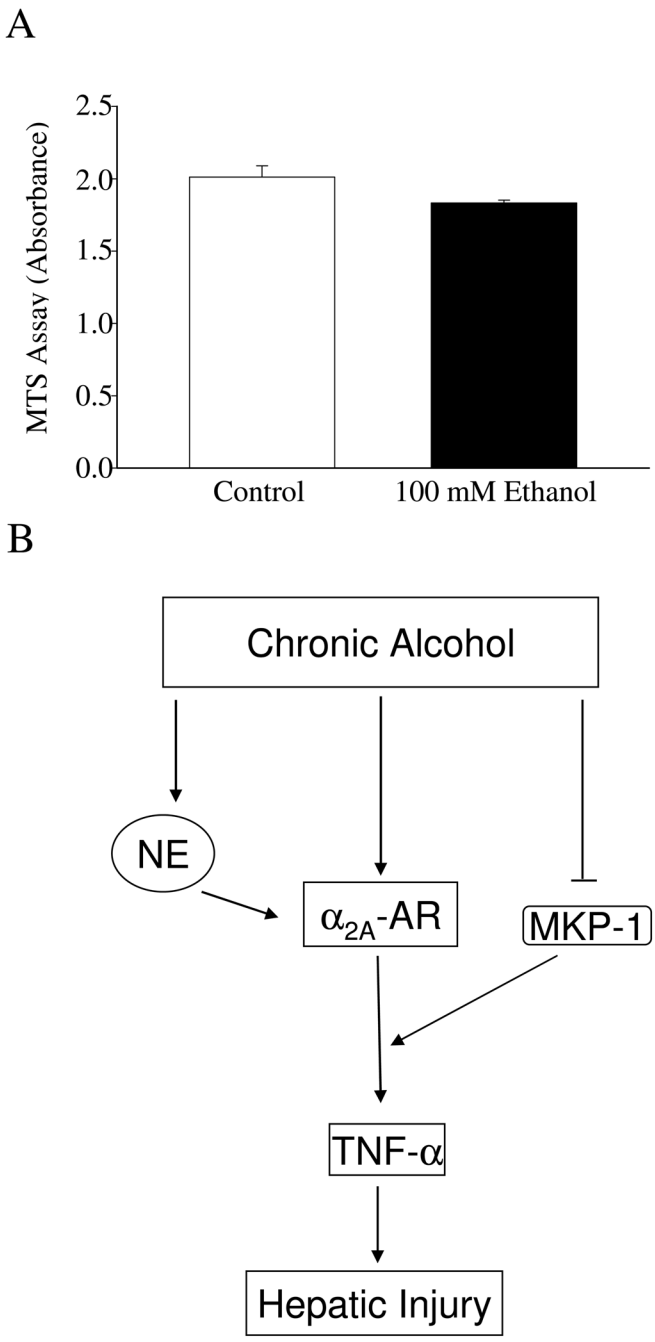


Figure 4.

(A) *Determination of Kupffer cell viability following 7 days alcohol exposure*: Kupffer cells were isolated and treated with 100 mM ethanol for 7 days. Afterwards, MTS assay was performed and data are shown as the absorbance at 490 nm. (B). *Diagram representing potential mechanism of chronic alcohol exposure leading to hepatic damage*: Chronic alcohol exposure elevates NE release, upregulates α_{2A}-AR on KC and leads to hepatic TNF-α production. Alcohol induced downregulation of MKP-1 further causes increased production of TNF-α. Taken together, chronic alcohol induced upregulation of α_{2A}-AR with the concomitant reduction of MKP-1 on KC results in increased TNF-α and subsequent hepatic dysfunction.