III. 研究成果の刊行に関する一覧表

主任研究者　石井裕正
書籍：なし
雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Ishii H	Common pathogenic mechanisms in ASH and NASH	Hepatol Res	28	18-20	2004
Nakamura Y, Yokoyama H, Ishii H, et al.	Acetaldehyde accumulation suppresses Kupffer cell release of TNF-Alpha and modifies acute hepatic inflammation in rats	J Gastroenterol	39	140-147	2004
Tomita K, Azuma T, Ishii H, et al.	Pioglitazone prevents alcohol-induced fatty liver in rats through up-regulation of c-Met	Gastroenterology	126	873-885	2004
Konishi M, Kato S, Ishii H, et al.	Ethanol upregulates pro-fibrogenic connective tissue growth factor (CTGF) gene expression in HepG2 cells via cytochrome P450 2E1-mediated ethanol oxidation	Heoatol Res	28	102-108	2004
Adachi M, Higuchi H, Ishii H, et al.	Bax interacts with the voltage-dependent anion channel and mediates ethanol-induced apoptosis in rat hepatocytes	Am J Physiol Gastrointest Liver Physiol	287	G695-705	2004
Yamagishi Y, Horie Y, Ishii H, et al.	Hepatocellular carcinoma in heavy drinkers with negative markers for viral hepatitis	Hepatol Res	28	177-183	2004
Saito H, Ishii H	Recent understanding of immunological aspects in alcoholic hepatitis	Hepatol Res	30	193-198	2004
Kato S, Ishii H	Lifestyle guidance for patients with chronic liver diseases; information provision via educational classes on liver diseases	Hepatol Res	30 S	81-85	2004
Fukuda M, Yokoyama H, Ishii H, et al.	Kupffer cell depletion attenuates superoxide anion release into the hepatic sinusoids after lipopolysaccharide treatment	J Gastroenterol Hepatol	19	1155-1162	2004
Saito H, Tada S, Ishii H, et al.	Efficacy of non-invasive elastometry on staging of hepatic fibrosis	Hepatol Res	29	97-103	2004
Kaneko F, Saito H, Ishii H, et al.	Down-regulation of matrix-invasive potential of human liver cancer cells by type I interferon and a histone deacetylase inhibitor sodium butyrate	Int J Oncol	24	837-845	2004
Horie Y, Han JY, Ishii H, et al.	Herbal cardiotonic pills prevent gut ischemia/reperfusion-induced hepatic microvascular dysfunction in	World J Gastroenterol	11	511-515	2005

	rats fed ethanol chronically				

分担研究者　鈴木一幸
書籍　なし
雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
加藤章信、岩井正勝、鈴木一幸	意識障害	肝胆膵	50	263-269	2004
岩井正勝、加藤章信、鈴木一幸、他	肝硬変における肝実質機能評価法としての 13C-フェニルアラニン呼気テストの有用性	消化器科	39	164-168	2004
Sawara K, Kato A, Suzuki K, et al.	Brain glutamine and glutamate levels in patients with liver cirrhosis: assessed by 3.0-T MRS	Hepatol Res	30	18-23	2004
Kato A, Kato M, Suzuki K, et al.	Development of quantitative neuropsychological tests for diagnosis of subclinical hepatic encephalopathy in liver cirrhosis patients and establishment of diagnostic criteria-multicenter collaborative study in Japanease	Heoatol Res	30	71-78	2004

分担研究者　幕内博康
書籍　なし
雑誌　なし

分担研究者　福井　博
書籍　なし
雑誌　なし

分担研究者　森脇久隆
書籍　なし
雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Moriwaki H, Miwa Y, et al	Branched-chain amino acids as a protein-and energy-source in liver cirrhpsis	BBRC	313	405-409	2004
Shiraki M, Shimomura Y, Moriwaki H, et al.	Activation of hepatic branched-chain α-keto acid dehydrogenase complex by tumor necrosisfactor-α in rats	BBRC	327	973-978	2005

分担研究者　市田隆文
書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版年	ページ
市田隆文	肝硬変	小俣政男	シャーロック肝臓病学翻訳	西村書店	2004	315-328
市田隆文、川崎誠治	肝細胞癌	各務伸一	コンセンサス2004肝疾患治療	アークメディア	2004	57-74

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Takeishi T, Sato Y, Ichida T,	Rapid progressive hepatitis C after liver transplantation: a case report	Transplant Proc	36	2304-2304	2004
市田隆文、鳩田裕慈	肝細胞癌に対する肝移植 Explant liver と画像所見の一致率	肝胆膵	49	575-579	2004
Ikai I, Arii S, Ichida T, et al.	Reevaluation of prognostic factors for survival after liver resection in patients with hepatocellular carcinoma in a Japanese nationwide survey	Cancer	101	796-802	2004
市田隆文	肝移植より変貌を遂げた肝臓病診療	新潟医学会誌	118	327-332	2004
市田隆文、他	原発性胆汁性肝硬変	外科	66	1055-1060	2004
市田隆文、島田裕慈、他	原発性硬化性胆管炎と原発性胆汁性肝硬変の肝移植と再発問題	肝胆膵	49	251-255	2004
市田隆文、他	生体肝移植ドナーが肝不全に陥った事例の検証と再発予防への提言	移植	39	47-55	2004

分担研究者　沖田　極
書籍　なし
雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Yamamoto N, Terai S, Okita K, et al.	A subpopulation of bone marrow cells depleted by a novel antibody, anti-Liv8,is useful for cell therapy to repair damaged liver	BBRC	313	1110-1118	2004
Sakaida I, Hironaka K, Okita K, et al.	Herbal medicine Sho-saiko-to (TJ-9) increases expression matrix metalloproteinases (MMPs) with reduced expression of tissue	Life Sci	74	2251-2263	2004

	inhibitor of metalloproteinases (TIMPs) in rat stellate cell				
Yokoyama Y, Kuramitsu Y, Okita K, et al.	Proteomic profiling of proteins decreased in hepatocellular carcinoma from patients infected with hepatitis C virus	Proteomics	4	2111-2116	2004
Sakaida I, Terai S, Okita K, et al.	Transplantation of bone marrow cells reduces CCl4-induced liver fibrosis in mice	Hepatology	40	1304-1311	2004
Omori K, Terai S, Okita K, et al.	Molecular signature associated with plasticity of bone marrow cell under persistent liver damage by self-organizing-map-based gene expression	FEBS Lett	578	10-20	2004
山崎　隆弘、木村　輝昭、沖田極、他	肝細胞癌に対する新バルーンマイクロカテーテルを用いたリピオドール併用肝動脈バルーン閉塞下ラジオ波凝固療法	肝臓	45	505-506	2004
寺井　崇二、大森薫、沖田極、他	自己骨髄細胞に対する肝硬変症に対する肝臓再生療法	治療学	38	114-115	2004
寺井　崇二、石川剛、沖田極、他	骨髄細胞から肝細胞への分化制御機構の解析とその臨床応用	GI Resaerch	12	117-125	2004
寺井　崇二、坂井田功、沖田　極	肝硬変治療の新たなストラテジー ー自己骨髄細胞を用いた肝臓再生療法ー	炎症と免疫	12	66-73	2004

分担研究者　齋藤英胤
書籍　なし
雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Kaneko, H. Saito, et al.	Down-regulation of matrix-invasive potential of human liver cancer cells by type I interferon and a histone deacetylase inhibitor sodium butyrate	Int J Oncol	24	837-845	2004
Kumagai N,. Takahashi M, Saito H, et al.	Polymorphisms of NS5B protein relates to early clearance of hepatitis C virus by interferon plus ribavirin: a pilot study	J Viral Hepatit	11	225-235	2004
Saito H, et al.	Efficacy of non-invasive elastometry on staging of hepatic fibrosis	Hepatol Res	29	97-103	2004
Saito H et al.	Recent understanding of immunological aspects in alcoholic hepatitis	Hepatol Res	30	193-198	2004
Wakabayashi	Gene expression associated with the	Int J Oncol	26	233-239	2005

K, Saito H, et al.	reduction of malignant phenotype in a human liver cancer cell line HCC-T following stimulation with a histone deacetylase inhibitor				
Takahashi M, Saito H, et al.	Benefit of hepatitis C virus core antigen assay in prediction of therapeutic response to interferon and ribavirin combination therapy.	J Clin Microbiol	43	186-191	2005
多田慎一郎、齋藤英胤	肝線維化の定量におけるエラストメトリーの有用性	医学のあゆみ	208	692-693	2004
齋藤英胤	最近の NASH に対する臨床試験	医学のあゆみ	209	381-382	2004
北村公美、多田慎一郎、齋藤英胤、他	ペグインターフェロンα-2a	日本病院薬剤師会雑誌	40	737-742	2004
齋藤英胤、多田慎一郎、他	原発性硬化性胆管炎の診断—当科の症例を中心に—	消化器科	39	103-107	2004
齋藤英胤、多田慎一郎、他	高齢者に対する低侵襲性診断：肝線維化のエラストメーターによる測定	日本高齢消化器医学会議会誌	6	54-57	2004

分担研究者　栗山茂樹
書籍　なし
雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Watanabe S, Kurokohchi K, Kuriyama S, et al.	Enlargement of thermal ablation zone by the combination of ethanol injection and radiofrequency ablation in excised bovine liver	Int J Oncol	24	279-284	2004
Jin Y, Masaki T, Kuriyama S, et al.	Identification of p46 Shc expressed in the nuclei of hepatocytes with high proliferating activity: Study of regenerating rat liver	Int J Mol Med	13	721-728	2004
Kurokohchi K, Masaki T, Kuriyama S, et al.	Percutaneous ethanol and lipiodol injection therapy for hepatocellular carcinoma	Int J Oncol	24	381-387	2004
Kita Y, Masaki T, Kuriyama S, et al.	Expression of G1 phase-related cell cycle molecules in naturally developing hepatocellular carcinoma of Long-Evans Cinnamon rats	Int J Oncol	24	1205-1211	2004
Funakoshi F, Masaki T, Kuriyama S, et al.	Proliferative capability of hepatocytes and expression of G1-related cell cycle molecules in the development of liver cirrhosis in rats	Int J Mol Med	13	779-787	2004
Yoshiji H, Kuriyama S,	Halting the interaction between vascular endothelial growth factor	Hepatology	39	1517-1524	2004

et al.	and its receptors attenuates liver carcinogenesis in mice				
Yoshiji H, Kuriyama S, et al.	Involvement of the vascular endothelial growth factor receptor-1 in murine hepatocellular carcinoma development	J Hepatol	41	97-103	2004
Tanaka M, Watanabe S, Kuriyama S, et al.	Fulminant hepatic failure caused by malignant melanoma of unknown primary origin	J Gastroenterol	39	804-806	2004
Morishita A, Masaki T, Kuriyama S, et al.	Reduced expression of cell cycle regulator $p18^{INK4C}$ in human hepatocellular carcinoma	Hepatology	40	677-686	2004
Tominaga K, Kuriyama S, et al.	Repeated adenoviral administration into the biliary tract can induce repeated expression of the original gene construct in rat livers without immunosuppressive strategies	Gut	53	1167-1173	2004
Yoshida S, Masaki T, Kuriyama S, et al.	Enhanced expression of adaptor molecule p46 Shc in nuclei of hepatocellular carcinoma cells: Study of LEC rats	Int J Oncol	25	1089-1096	2004
Yoshiji H, Kuriyama S, et al.	Angiotensin-I converting enzyme inhibitors as potential anti-angiogenic agents for cancer therapy	Current Cancer Drug Targets	4	555-567	2004
Kurokohchi K, Masaki T, Kuriyama S, et al.	Efficacy of combination therapies of percutaneous or laparoscopic ethanol-lipiodol injection and radiofrequency ablation	Int J Oncol	25	1737-1743	2004
Kuriyama S, et al.	Adenovirus-mediated gene transfer into rat livers: Comparative study of retrograde intrabiliary and antegrade intraportal administration	Oncol Rep	13	69-74	2005
Kurokohchi K, Masaki T, Kuriyama S, et al.	Successful laparoscopic radiofrequency ablation of hepatocellular carcinoma adhered to the mesentery after transcatheter arterial embolization	Oncol Rep	13	65-68	2005
Kimura Y, Selmi C, Kuriyama S, et al.	Genetic polymorphisma influencing xenobiotic metabolism and transport in patients with primary biliary cirrhosis	Hepatology	41	55-63	2005

分担研究者　渡辺　哲
書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版年	ページ
Okazaki I, Watanabe T, et al.	Reversibility of Liver fibrosis: Role of Matrix	Mohammed S. Razzaque	Fibrogenesis: Cellular and Molecular	Kluwer Academic/ Plenum	2005	143-159

	Metalloproteinases		Basis	Publishers		

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Sugioka Y, Watanabe T, et al.	c-Jun NH_2-terminal kinase pathway is involved in constitutive matrix metalloproteinase-1 expression in a hepatocellular carcinoma-derived cell line	Int J Cancer	109	867-874	2004
Ito S, Furuta H, Watanabe T, et al.	Comparative socio-cultural analysis of smoking behavior and difficulty of quitting smoking in Japan and Thailand	Jpn J Public Health	51	975-985	2004

分担研究者　小林廉毅
書籍　なし
雑誌　なし

分担研究者　森實敏夫
書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版年	ページ
森實敏夫	入門医療統計学	森實敏夫	入門医療統計学	東京図書	2004	全体
森實敏夫	わかりやすい医学統計学	森實敏夫	わかりやすい医学統計学	メディカルトリビューン	2004	全体

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
森實敏夫.	診療ガイドラインおよびMindsにおける医学関連文献の評価選定基準について	医学図書館	51	51-56	2004
森實敏夫.	肝疾患の予後	医学書院	41	1603-1605	2004

IV. 研究成果の刊行物・別刷

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Hepatology Research 28 (2004) 18–20
Hepatology Research
www.elsevier.com/locate/ihepcom

Editorial

Common pathogenic mechanisms in ASH and NASH

Ludwig et al. [1] introduced the term nonalcoholic steatohepatitis (NASH) to describe a form of liver disease that is histologically indistinguishable from alcoholic hepatitis but occurs in persons who do not consume excessive alcohol. Nonalcoholic fatty liver disease (NAFLD) is an increasingly recognized condition that may progress to end-stage liver disease. Current best estimates make the prevalence of NAFLD (approximately 20%) and of NASH (2–3%) in the general population and NAFLD is perhaps the most common of all liver disorders [2]. The similar histological features and natural histories of alcoholic and nonalcoholic fatty liver suggest that common pathogenic mechanisms might be involved in these two conditions. In contrast to NASH, the pathogenesis of alcoholic steatohepatitis (ASH) have been well studied. Understanding the pathogenesis of alcoholic fatty liver disease (AFLD) should contribute to elucidating the pathogenesis of NAFLD.

It has been shown that the induction of cytochrome P4502E1 (CYP2E1) plays a key role in the pathogenesis of ASH because of the oxidative stress it generates [3]. The most significant role of CYP2E1 is its adaptive response to high blood ethanol levels with a corresponding acceleration of ethanol metabolism. The associated free radical production contributes to liver injury in alcoholics [3]. Furthermore, CYP2E1 activates a score of xenobiotics to highly hepatotoxic compounds. CYP2E1 induction also results in strikingly increased oxidation of ethanol to acetaldehyde, a highly reactive and toxic compound [4,5]. Thus, CYP2E1 is a major microsomal source of oxidative stress, and it has been explored as a candidate in the pathogenesis of NASH because CYP2E1 is also up-regulated in other clinical settings that are associated with NASH, such as diabetes mellitus and obesity [3]. Besides being highly inducible by ethanol, CYP2E1 is upregulated in the aberrant nutritional states of fasting, diabetes, and obesity as well as by a high-fat/low-carbohydrate diet. The increased circulating levels of ketone bodies and fatty acids observed in these seemingly disparate conditions may be directly involved in CYP2E1 induction [3,10]. In the review article of this issue [36], Lieber described clearly and comprehensively pathophysiological role of CYP2E1 in ASH and NASH.

Weltman et al. showed that rats fed with a methionine- and choline-deficient diet develop liver injury resembling NASH in which the extent and lobular distribution of CYP2E1 expression are closely related to the distribution of steatosis and inflammation [6]. They also reported that hepatic CYP2E1 was significantly increased in patients with NASH compared with controls. The increased expression and lobular distribution of hepatic CYP2E1 in NASH resembled that seen in ASH as well as in an animal nutritional model of steatohepatitis [7]. It has been recently shown that hepatic CYP2E1 activity is significantly higher in nondiabetic patients with NASH compared with age- and gender-based BMI-matched controls [8]. Morbid obesity is frequently associated with NAFLD. Hepatic CYP2E1 activity was up-regulated in morbidly obese subjects [9] with a positive association between the degree of steatosis and CYP2E1 activity. These results support the idea that CYP2E1 plays a role in the pathogenesis of NASH.

One of the key pathogenic factors for NASH may be insulin resistance, and insulin resistance is thought to be common in patients with NAFLD and NASH [11]. Insulin resistance could contribute to hepatic steatosis by favoring peripheral lipolysis with an increased hepatic uptake of fatty acids. It may also be the reason for an increased expression of CYP2E1, thereby contributing to the production of prooxidants in fatty liver.

The investigators surmised that the increased expression of CYP2E1 in patients with NASH may result in the production of free oxygen radicals capable of inducing lipid peroxidation of hepatocyte membranes. In a study of a mouse model of NASH, hepatic CYP2E1 was up-regulated and was associated with a dramatic increase in total lipid peroxide levels in the liver that were substantially inhibited by anti-CYP2E1 antibody [12]. Oxidative cellular damage has also been frequently detected in livers with NAFLD [13].

Although CYP2E1 plays an important role in pathophysiology of NASH, induction of CYP2E1 probably is not the only cause of lipid peroxidation and oxidative stress in NASH, and cannot also be the only factor required for the development of NASH because it is a common phenomenon during intake of drugs such as isoniazid that do not cause NASH [14]. Lipid peroxidation is probably a key pathogenic mechanism in NASH, but other factors may also contribute to the development of NASH, either by enhancing lipid peroxidation or by directly stimulating fibrogenesis and inflammatory response characteristic of NASH.

1386-6346/$ – see front matter © 2003 Published by Elsevier B.V.
doi:10.1016/j.hepres.2003.11.001

Endotoxin has been implicated in the pathogenesis and progression of AFLD. Endotoxemia is often observed in patients with AFLD, and animal experiments have shown plasma endotoxin levels to be higher in rats fed with ethanol chronically than in control rats. Long-term ethanol feeding also increases intestinal permeability to and absorption of endotoxin, which can evoke Kupffer cell activation [15]. The level of gut-derived endotoxin in portal blood increases, the LPS then binds to LBP, and the LPS–LBP complex binds to CD14 on Kupffer cells. Toll-like receptor 4 (TLR4) associates with CD14 on the cell surface, mediating LPS-induced signal transduction, including NFκB activation and subsequent enhancement of proinflammatory agents such as TNFα and COX-2 in Kupffer cells, leading to a liver injury [16]. Furthermore, oral administration of antibiotics prevents ethanol-induced increases in plasma endotoxin levels and progression of liver injury [17].

Recently, an increased production of endogenous ethanol by the intestinal microflora has been demonstrated in genetically obese ob/ob mice, a model for NAFLD, even in the absence of ethanol ingestion [18]. Treatment with antibiotics has also been reported to improve NAFLD histology and reduce serum ALT levels in ob/ob mice [19]. Indeed, a subsequent pilot study of patients with NASH demonstrated increased breath ethanol concentrations among obese females with this condition, confirming the suspicion that increased intestinal production of ethanol occurs in some humans with NAFLD [20]. Surgical procedures to treat obesity such as jejunoileal bypass and gastroplasty have been suggested to be risk factors for NAFLD, associated with intestinal bacterial overgrowth [21]. Advanced alcoholic liver disease was much more frequently observed among individuals possessing the T allele at the −159 position of the promoter region of the CD14 gene, Kupffer cell receptor [22]. Homozygosity for the same allele was also significantly higher in NASH patients [23]. These results suggest some commonality with the pathogenesis of AFLD and NAFLD; bacterial overgrowth and increased endogenous production of ethanol in the intestinal flora, subsequently activated Kupffer cells, and hepatotoxic cytokines, such as TNFα, released from Kupffer cells [24].

Hepatic TNFα is known to play an important role in the pathogenesis of AFLD. Yin et al. reported that ethanol-induced liver injury was minimized in mice lacking TNF receptor 1 [25]. This study showed an essential role of TNFα in alcohol-induced liver injury. TNFα plays a central role in both proinflammatory and apoptotic responses to endotoxin and can transduce its signal by binding to one of the two cellular receptors: types I and II. Activation of TNF receptors by TNFα causes the release from mitochondria of reactive oxygen species, such as superoxide anion, as well as cytochrome c oxidase, which induce apoptosis and necrosis [26]. Treatment with anti-TNFα monoclonal antibody has been recently shown to be effective in patients with severe alcoholic hepatitis [27]. On the other hand, it is conceivable that inhibition of TNFα activity could improve "NASH" because anti-TNFα antibodies improved "NASH" in ob/ob mice [19]. Potential sources of TNFα are macrophages and activated lymphocytes infiltrating the liver tissue of NASH patients, whereas adipose tissue is a significant source of endogenous TNFα production [28]. The expression of TNFα is elevated in most rodent models of obesity and implicated in human obesity, and TNFα has been shown to induce insulin resistance [29]. Insulin resistance represents the most reproducible predisposing factor for NASH, and NAFLD has been appropriately considered as one of the components of the insulin resistance syndrome. Insulin resistance favors accumulation of free fatty acids in the liver and predisposes to oxidative stress by stimulating microsomal lipid peroxidases, and by the direct effect of high insulin levels in decreasing mitochondrial β-oxidation of fatty acids [30]. Recent reports have shown that overexpression of TNFα and TNF receptor1 mRNA is increased in the liver tissue of NASH patients [31]. Valenti et al. reported that TNF-α polymorphisms could represent a susceptibility genotype for NAFLD [32]. These findings support that TNFα is necessary for steatohepatitis in both alcoholic and nonalcoholic conditions.

The common pathogenic mechanisms in ASH and NASH might include other conditions such as hypoxia and impaired methionine metabolism [8,33]. As mentioned above, mechanisms other than CYP2E1 play roles in the pathogenesis of NASH. We agree that increased CYP2E1 per se is not sufficient to cause NASH; however, some reports on the strong causal relationship between CYP2E1 induction and occurrence of NASH have enabled the establishment of the important role of CYP2E1 in the pathogenesis of NASH.

The review by Lieber in this issue suggests the beneficial effects of CYP2E1 inhibitors in the treatment of ASH and NASH. They have recently reported that lycopene, the most abundant carotenoid in tomatoes, has a high anti-oxidant capacity and protects HepG2 cells expressing CYP2E1 against arachidonic acid toxicity [34]. Lieber and his group have also elucidated that lycopene opposes the ethanol-induced oxidative stress and apoptosis in HepG2 cells overexpressing CYP2E1 [35]. These results support that this carotenoid may be useful as a therapeutic agent for ASH and NASH and merit further evaluation.

In summary, the review by Lieber has taken us a step further in our understanding of the association of CYP2E1 and steatohepatitis. The forthcoming results of clinical trials evaluating new medications that specifically inhibit CYP2E1 activity will provide further insights into the relationship of CYP2E1 and steatohepatitis.

References

[1] Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55:434–8.

[2] Falck-Ytter Y, Younossi ZM, Marchisini G, McCullough AJ. Clinical features and natural history of nonalcoholic steatosis syndromes. Semin Liver Dis 2001;21:17–26.

[3] Lieber CS. Cytochrome P4502E1: its physiological and pathological role. Physiol Rev 1997;77:517–44.
[4] Lieber CS, DeCarli LM. Hepatic microsomal ethanol oxidizing system. In vitro characteristics and adaptive properties in vivo. J Biol Chem 1970;245:2505–12.
[5] Lieber CS, Garro A, Leo MA, Mak KM, Worner T. Alcohol and cancer. Hepatology 1986;6:1005–19.
[6] Weltman MD, Farrell GC, Liddle C. Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. Gastroenterology 1996;111:1645–53.
[7] Weltman MD, Farrell GC, Hall P, Ingelman-Sundberg M, Liddle C. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. Hepatology 1998;27:113–28.
[8] Chalasani N, Gorski JC, Asghar MS, Asghar A, Foresman B, Hall SD, et al. Hepatic cytochrome P450 2E1 activity in nondiabetic patients with nonalcoholic steatohepatitis. Hepatology 2003;37:544–50.
[9] Emery MG, Fisher JM, Chien JY, Kharasch ED, Dellinger EP, Kowdley KV, et al. CYP2E1 activity before and after weight loss in morbidly obese subjects with nonalcoholic fatty liver disease. Hepatology 2003;38:428–35.
[10] Robertson G, Leclercq I, Farrell GC. Nonalcoholic steatosis and steatohepatitis. Part II. Cytochrome P-450 enzymes and oxidative stress. Am J Physiol Gastrointest Liver Physiol 2001;281:G1135–9.
[11] Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. Hepatology 2003;37:1202–19.
[12] Leclercq I, Farrell G, Field J, Bell DR, Gonzalez FJ, Robertson GR. CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis. J Clin Invest 2000;105:1067–75.
[13] Seki S, Kitada T, Yamada T, Sakaguchi H, Nakatani K, Wakasa K. In situ detection of lipid peroxidation and oxidative DNA damage in nonalcoholic fatty liver disease. J Hepatol 2002;37:56–62.
[14] Chitturi S, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. Semin Liver Dis 2001;21:27–41.
[15] Tamai H, Horie Y, Kato S, Yokoyama H, Ishii H. Long-term ethanol feeding enhances susceptibility of the liver to orally administered lipopolysaccharides in rats. Alcohol Clin Exp Res 2002;26:75S–80S.
[16] Su GL. Lipopolysaccharides in liver injury: molecular mechanisms of Kupffer cell activation. Am J Physiol Gastrointest Liver Physiol 2002;283:G256–65.
[17] Adachi Y, Moore LE, Bradford BU, Gao W, Thurman RG. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. Gastroenterology 1995;108:218–24.
[18] Cope K, Risby T, Diehl AM. Increased gastrointestinal ethanol production in obese mice: implications for fatty liver disease pathogenesis. Gastroenterology 2000;119:1340–7.
[19] Li Z, Yang S, Lin H, Huang J, Watkins PA, Moser AB, et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatology 2003;37:343–50.
[20] Nair S, Cope K, Risby TH, Diehl AM, Terence RH. Obesity and female gender increases breath ethanol concentration: potential implications for the pathogenesis of nonalcoholic steatohepatitis. Am J Gastroenterol 2001;96:1200–4.
[21] Allard JP. Other disease associations with non-alcoholic fatty liver disease (NAFLD). Best Pract Res Clin Gastroenterol 2002;16:783–95.
[22] Jarvelainen HA, Orpana A, Perola M, Savolainen VT, Karhumen PJ, Lindros KO. Promoter polymorphism of the CD14 endotoxin receptor gene as a risk factor for alcoholic liver disease. Hepatology 2001;33:1148–53.
[23] Day C. CD14 promoter polymorphism associated with risk of NASH. J Hepatol 2002;36:21.
[24] Solga SF, Diehl AM. Non-alcoholic fatty liver disease: lumen-liver interactions and possible role for probiotics. J Hepatol 2003;38:681–7.
[25] Yin M, Wheeler MD, Kono H, Bradford BU, Gallucci RM, Luster MI, et al. Essential role of tumor necrosis factor α in alcohol-induced liver injury in mice. Gastroenterology 1999;117:942–52.
[26] Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. N Engl J Med 2003;343:1467–76.
[27] Tilg H, Jalan R, Kaser A, Davies NA, Offner FA, Hodges SJ, et al. Anti-tumor necrosis factor-alpha monoclonal antibody therapy in severe alcoholic hepatitis. J Hepatol 2003;38:419–25.
[28] Hotamisligil GS, Shargill NS, Spiegelmen BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91.
[29] Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-α production. Nature 1997;389:610–4.
[30] Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120:1183.
[31] Crespo J, Cayon A, Fernandez-Gil P, Hernandez-Guerra M, Mayorga M, Dominguez-Diez A, et al. Gene expression of tumor necrosis factor α and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis. Hepatology 2001;34:1158–63.
[32] Valenti L, Fracanzani AL, Dongiovannni P, Santorelli G, Branchi A, Taioli E, et al. Tumor necrosis factor α polymorphisms and insulin resistance in nonalcoholic fatty liver disease. Gastroenterology 2002;122:274–80.
[33] Santamaria E, Avila MA, Latasa MU, Rubio A, Martin-Duce A, Lu SC, et al. Functional proteomics of nonalcoholic steatohepatitis: mitochondrial proteins as targets of S-adenosylmethionine. Proc Natl Acad Sci USA 2003;100:3065–70.
[34] Xu Y, Leo MA, Lieber CS. Lycopene attenuates arachidonic acid toxicity in HepG2 cells overexpressing CYP2E1. Biochem Biophys Res Commun 2003;303:745–50.
[35] Xu Y, Leo MA, Lieber CS. Lycopene attenuates alcoholic apoptosis in HepG2 cells expressing CYP2E1. Biochem Biophys Res Commun 2003;308:614–8.
[36] Lieber CS, CYP2E1: from ASH to NASH, Hepatol Res, in press.

Hiromasa Ishii
Division of Gastroenterology
Department of Internal Medicine
Keio University School of Medicine
Tokyo 1608582 Japan
Tel.: +81-333531211
fax: +81-333569654
E-mail address: hishii@med.keio.ac.jp
(H. Ishii)

J Gastroenterol 2004; 39:140–147
DOI 10.1007/s00535-003-1278-5

Journal of Gastroenterology

Acetaldehyde accumulation suppresses Kupffer cell release of TNF-α and modifies acute hepatic inflammation in rats

Yuji Nakamura[1,2], Hirokazu Yokoyama[1], Susumu Higuchi[2], Sachiko Hara[2], Shinzo Kato[1], and Hiromasa Ishii[1]

[1]Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan
[2]National Institute on Alcoholism, Kurihama National Hospital, 5-3-1 Nobi, Yokosuka 239-0841, Japan

Background. Alcohol-related diseases have multiple and varied associations with acetaldehyde, a highly toxic product of ethanol oxidation that accumulates in the absence of active aldehyde dehydrogenase (ALDH). This study was designed to clarify the role of acetaldehyde in liver injury, specifically in vivo and in vitro effects on Kupffer cell release of the inflammatory cytokine tumor necrosis factor-alpha (TNF-α). ***Methods.*** Rats pretreated overnight with the ALDH inhibitor disulfiram (or saline control) were ethanol loaded and challenged with lipopolysaccharide (LPS), and their blood and histological parameters were examined 3 h later. Similarly, isolated rat Kupffer cells were pretreated with disulfiram or cyanamide incubated in ethanol (1 h), then challenged with LPS and evaluated 2 h later for TNF-α and acetaldehyde levels in the culture medium. TNF-α release from Kupffer cells after LPS challenge was also evaluated following incubation in acetaldehyde and acetate for comparison with ethanol loading. ***Results.*** Higher blood acetaldehyde concentration following disulfiram pretreatment significantly attenuated acute hepatic inflammation in the ethanol-loaded, LPS-challenged rat (18 ± 2.9 vs 30 ± 3.7 polymorphonuclear cells/portal area; $P = 0.01$). After LPS challenge, ALDH inhibitor pretreatment attenuated Kupffer cell release of TNF-α in the presence of disulfiram at 5063 ± 151 pg/ml and cyanamide at 4390 ± 934 pg/ml, versus no inhibitor, 5869 ± 265 pg/ml ($P < 0.01$), but not in the absence of ethanol. Acetaldehyde significantly suppressed Kupffer cell TNF-α release ($P < 0.05$), but acetate treatment did not. ***Conclusions.*** Acetaldehyde accumulation suppresses macrophage function, at least suppressing TNF-α release, which plays a role in modifying acute hepatic inflammation in rats.

Received: May 26, 2003 / Accepted: November 21, 2003
Reprint requests to: Y. Nakamura

Key words: acetaldehyde, TNF-α, Kupffer cells, lipopolysaccharide, inflammation

Introduction

Alcohol is one of the most important factors that can modify the course of human diseases. Despite recent evidence that drinking an appropriate quantity of alcohol can decrease the incidence of ischemic heart disease and stroke,[1] alcohol abuse injures many organs, most commonly the liver, where it can result in acute or chronic liver injury and eventually lead to cirrhosis. Although the pathogenesis of alcoholic liver injury remains obscure, both hepatic macrophages known as Kupffer cells and endotoxin (lipopolysaccharide [LPS]) are known to play major roles. Adachi and colleagues showed that the inactivation of Kupffer cells using gadolinium chloride ($GdCl_3$) prevents early alcohol-induced liver injury.[2] They also showed that intestinal sterilization with antibiotics (polymixin B and neomycin) can prevent alcohol-induced liver injury by reducing intestinal bacterial counts and lowering the risk for endotoxinemia.[3]

Several reports have suggested that alcoholic hepatitis is milder in Japan than in the United States and most Western countries.[4] Although some investigators attribute this circumstance to the difference in the eating and drinking habits of these countries, e.g., the quantity of alcohol consumed and/or the amount of fat in the diet,[5] the underlying reason for these differences is not clear. Another possible reason for these differences may be associated with an excessive accumulation of acetaldehyde in the blood after drinking. The difference between acetaldehyde accumulation in Asians and others is readily apparent: because of their low aldehyde dehydrogenase (ALDH) activity, about half of Asians have high acetaldehyde levels after drinking.[6] Such

Table 1. In vivo experiments

Pretreat (overnight)	ALDH inhibitor (n = 20)			Saline (n = 20)		
Treat (rapid injection to 4-h infusion)	Ethanol (n = 10)	Saline (n = 10)		Ethanol (n = 10)	Saline (n = 10)	
Challenge (1 h after start of treatment)	LPS (n = 10)	LPS (n = 5)	Saline (n = 5)	LPS (n = 10)	LPS (n = 5)	Saline (n = 5)

Rats (n = 40)
Control (pretreatment, treatment, challenge) = saline
Aldehyde dehydrogenase (ALDH) inhibitor pretreatment = disulfiram, 75 mg/kg body weight, by gastric tube
Treatment = ethanol injection, 1.75 g/kg i.v., followed by ethanol infusion, 250 mg/kg/h
Challenge = lipopolysaccharide (LPS), 1 mg/kg i.v.

genetic differences in alcohol metabolism may affect the progression of alcohol-related illnesses.

Acetaldehyde, a highly reactive product of alcohol metabolism, forms adducts with cytochrome P450 2E1, collagen fibrils, albumin, hemoglobin, microtubules, and DNA.[7-12] The accumulation of acetaldehyde may influence various genomic and protein functions as well as cellular function. Our previous research has shown that Kupffer cells possess the alcohol-metabolizing enzymes cytochrome P450 2E1 and ALDH2; consequently, these cells are capable of oxidizing ethanol to acetaldehyde and acetate.[13] Kupffer cells produce various mediators (e.g., cytokines, eicosanoids, proteases, and oxygen radicals) that participate in inflammation, usually during the acute phase, and contribute toward later immune responses.[14]

Acute inflammation in many tissues is associated with the activation of macrophages and monocytes through the production of various mediators during the acute phase.[15,16] One important monocytic mediator of acute inflammation is tumor necrosis factor-alpha (TNF-α), a cytokine produced by numerous cells, including monocytes, natural killer cells, B cells, T cells, basophils, eosinophils, neutrophils, and nonimmune cells (e.g., mast cells, Kupffer cells, astrocytes, granulosa cells, epithelial cells, keratinocytes, and glial cells). In the liver, the major source of TNF-α is the resident macrophage or Kupffer cell.[17]

The pathogenesis of liver injury with alcohol consumption remains obscure, and few studies have addressed the role of acetaldehyde in that regard.[18] In this study to clarify the effect of acetaldehyde on Kupffer cell activity during the acute phase of liver injury, we used ALDH inhibitors and ethanol to evaluate the in vitro and in vivo effects of acetaldehyde accumulation on liver injury caused by LPS, as demonstrated by the release of TNF-α from Kupffer cells.

Materials and methods

Animals

Male Wistar rats weighing about 250 g (Orientalkoubo, Tokyo, Japan) were used for the experiments. All animal care protocols were approved by the Laboratory Animal Users Committee at Keio University.

In vivo experiment

Forty rats were divided into two groups of 20 each for overnight pretreatment with or without an ALDH inhibitor, disulfiram (75 mg/kg; Sigma, St. Louis, MO, USA) by gastric tube, and treatment with or without ethanol and/or LPS (Table 1). Each rat was anesthetized with pentobarbital (30 mg/kg i.p.; Abbott, North Chicago, IL, USA), and a 24-gauge Teflon catheter (Terumo, Tokyo, Japan) was inserted into the left femoral vein for the rapid injection of 1.75 g/kg ethanol, followed immediately by ethanol infusion (250 mg/kg/h) or saline, respectively, for 4 h. One hour after the initial ethanol injection, to create endotoxinemia, we injected 1 mg/kg LPS in saline into the femoral vein of half of each group of rats; those not treated with LPS received sham injections of saline.

Three hours after LPS injection, the rat livers and serum were examined to determine the degree of liver injury. Each liver was evaluated by light microscopy, using hematoxylin and eosin (HE) staining to facilitate the counting of infiltrating polymorphonuclear inflammatory (PMN) cells (10 portal area per liver for each of 10 rats per group). The serum samples were used to measure the levels of two liver enzymes that indicate organ damage, alanine aminotransferase (ALT) and lactate dehydrogenase (LDH), as well as TNF-α and blood acetaldehyde.

In vitro experiment

Ten rats were killed by pentobarbital injection and Kupffer cells were isolated from their excised livers by a

densitometric procedure[19] using metrizamide (Sigma). The isolated cells were seeded in six-well plastic plates (5×10^6 cells/well) and divided into three groups for incubation in RPMI 1640 medium (Gibco, Grand Island, NY, USA) fortified with 10% heat-inactivated fetal calf serum (Gibco), 50 μM kanamycin (Sigma), and one of two ALDH inhibitors (2 μM disulfiram or 200 μM cyanamide; Sigma) or neither (control). After 1-h incubation with or without an ALDH inhibitor, ethanol was added to each well to achieve a concentration of 50 mM. One hour later, 100 ng/ml LPS (Sigma) in saline was added to each well to stimulate the Kupffer cells. In another set of experiments, we added 50 mM ethanol and one of its oxidation products, acetaldehyde (50 μM–20 mM) or acetate (20 mM), to media containing Kupffer cells for 1 h, and then incubated the media with LPS (100 ng/ml) for 2 h to estimate TNF-α release (Table 2).

Two hours after LPS challenge, the Kupffer cells were collected for Northern blot analysis to examine TNF-α mRNA expression, using a TRIzol reagent (Gibco) according to the manufacturer's instructions to obtain the total cellular mRNA hybridized with rat TNF-α cDNA probes generated by polymerase chain reaction (PCR). Two hours after the LPS challenge we also collected the culture media and analyzed the levels of acetaldehyde and TNF-α. To measure the acetaldehyde concentration in medium by headspace gas chromatography, we mixed perchloric acid (Sigma) with the culture solution as quickly as possible in a procedure described by Eriksson et al.[20] The amount of TNF-α released by Kupffer cells into the culture medium was measured using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (R & D Systems, Minneapolis, MN, USA). We observed no difference between the ELISA results for control TNF-α levels in the absence and presence of acetaldehyde (20 mM).

Table 2. In vitro experiments

	ALDH inhibitor ($n = 50$)				No ALDH inhibitor ($n = 102$)					
Pretreatment (1 h before treatment)	Disulfiram ($n = 25$)		Cyanamide ($n = 25$)							
Treatment	Ethanol ($n = 15$)	Saline ($n = 10$)	Ethanol ($n = 10$)	Saline ($n = 15$)	Ethanol ($n = 25$)		Saline ($n = 25$)		Acetate ($n = 12$)	Acetaldehyde ($n = 40$)
Challenge (1 h after treatment)	LPS ($n = 15$)	LPS ($n = 10$)	LPS ($n = 10$)	LPS ($n = 15$)	LPS ($n = 15$)	Saline ($n = 10$)	LPS ($n = 15$)	Saline ($n = 10$)	LPS ($n = 12$)	LPS ($n = 40$)

Cultures in 152 wells
Control = saline for both inhibitor pretreatment and ethanol treatments, and for LPS challenge
Pretreatment = disulfiram, 2 μM; cyanamide, 200 μM
Treatment = ethanol, 50 mM; acetate, 20 mM; acetaldehyde (four treatments, $n = 10$ each), 50 μM, 500 μM, 5 mM, 20 mM
Challenge = lipopolysaccharide (LPS), 100 ng/ml

Statistical analysis

We expressed all data as the mean ± standard deviation (SD). We analyzed differences between groups using Student's unpaired t test. A value of $P < 0.05$ was considered to be statistically significant.

Results

In vivo study

After 3-h LPS challenge, PMN cells infiltrated the livers, predominantly in the portal areas (Fig. 1A), of the ethanol-loaded rats that had not received disulfiram. In

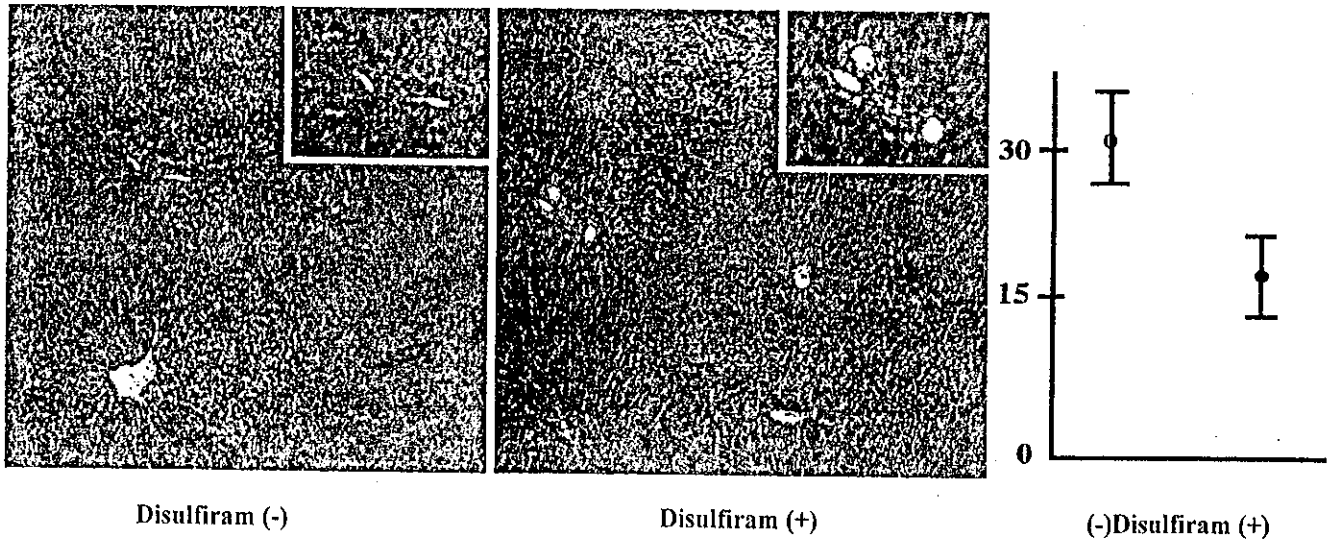

Fig. 1. Polymorphonuclear (PMN) cell infiltration following ethanol treatment and 3-h lipopolysaccharide (LPS) challenge, as shown by hematoxylin and eosin (HE) stain: **A** Without disulfiram pretreatment (×40); **B** with disulfiram pretreatment (×40); **C** PMN cells/portal area of individual liver specimens (n = 10 rat livers ×10 portal areas each; P = 0.01)

Table 3. Effects of ALDH inhibitor pretreatment, ethanol, and LPS challenge on the liver enzymes ALT and LDH and on TNF-α in rat serum

Inhibitor Pretreatment/ethanol Treatment/LPS challenge	No. rats	ALT (IU/l)	LDH (IU/l)	TNF-α (pg/ml)
None/none/none*	5	22 ± 5	372 ± 15	1.7 ± 1.78
Disulfiram/none/none*	5	29 ± 4	372 ± 84	1.8 ± 1.74
None/none/LPS**	5	115 ± 52	961 ± 184	495 ± 138
Disulfiram/none/LPS**	5	103 ± 67	1000 ± 308	506 ± 161
None/ethanol/LPS***	10	127 ± 102	1080 ± 334	481 ± 198
Disulfiram/ethanol/LPS***	10	42 ± 21	680 ± 279	255 ± 150

ALT, alanine aminotransferase; LDH, lactate dehydrogenase; TNF-α, tumor necrosis factor-alpha
*Disulfiram vs. no ALDH inhibitor: no significant difference in effect on any of the three parameters
**LPS with and without disulfiram: no significant difference in effect on any of the three parameters
***Ethanol and LPS, with and without disulfiram: statistically significant difference in effect for all three parameters ($P < 0.05$)

contrast, the livers of the disulfiram-pretreated rats had significantly fewer infiltrating cells (Fig. 1B,C) (with disulfiram 18 ± 2.9 vs without disulfiram 30 ± 3.7 cells/portal area, P = 0.01). The serum levels of ALT, LDH, and TNF-α in disulfiram-treated ethanol-loaded rats were also attenuated, but in the absence of ethanol, disulfiram pretreatment did not alter the levels of ALT, LDH, or TNF-α (Table 3). In ethanol-loaded rats, the serum ethanol concentration was 72 ± 14.8 mM. As expected, the acetaldehyde concentration in the serum after 4 h ethanol infusion was significantly higher in the ethanol-infused rats pretreated with disulfiram than in the rats receiving no disulfiram (Table 4). Thus, the livers of rats in which acetaldehyde accumulation was evoked by ethanol plus an ALDH inhibitor had reduced acute hepatic inflammation after LPS challenge.

In vitro study

As demonstrated in vivo, 3 h after the addition of 50 mM ethanol to the culture media, the acetaldehyde concentrations were significantly higher in the disulfiram-pretreated cultures than in the cultures receiving no ALDH inhibitor (see Table 4). The release of TNF-α from Kupffer cells after ethanol plus LPS challenge was significantly reduced in cultures pretreated with an ALDH inhibitor (disulfiram or cyanamide), but the inhibitors alone (without ethanol) did not suppress TNF-α release (Table 5). The addition of acetaldehyde alone, in lieu of ethanol, decreased the release of TNF-α from LPS-challenged Kupffer cells in dose-dependent fashion, whereas substituting acetate for ethanol did not produce this effect (Table 5). Northern blot analysis showed TNF-α mRNA expression in Kupffer cells after

Table 4. Effects of ALDH inhibitor (Disulfiram) pretreatment and ethanol treatment on acetaldehyde levels in vivo and in vitro

Source	*n*	Acetaldehyde concentration		*P* value
		Without disulfiram[a] (μM)	With disulfiram[a] (μM)	
Blood	6	6.2 ± 2.6	42.3 ± 5.2	<0.01
Kupffer cell culture medium	10	3.2 ± 1.2	14.1 ± 2.5	<0.01

[a] Disulfiram, 75 mg/kg body weight in vivo, 2 μM in vitro; no-disulfiram control = saline

Table 5. In vitro effect of LPS challenge on TNF-α concentration in Kupffer cell cultures with and without ALDH inhibitor pretreatment and ethanol or ethanol metabolic product treatment

Pretreatment/treatment/LPS challenge	*n*	TNF-α (pg/ml)	*P* value*
None/ethanol/LPS	15	5869 ± 265	—
None/saline/LPS	15	5761 ± 668	N.S.
None/saline/none	10	32 ± 3.05	<0.001
None/ethanol/none	10	34 ± 3.20	<0.001
Disulfiram/ethanol/LPS	15	5063 ± 151	<0.01
Disulfiram/saline/LPS	10	6033 ± 1171	N.S.
Cyanamide/ethanol/LPS	10	4390 ± 934	<0.01
Cyanamide/saline/LPS	15	5653 ± 1036	N.S.
None/acetate (20 mM)/LPS	12	5641 ± 1095	N.S.
None/acetaldehyde (50 μM)/LPS	10	5197 ± 397	<0.05
None/acetaldehyde (500 μM)/LPS	10	4536 ± 614	<0.01
None/acetaldehyde (5 mM)/LPS	10	2429 ± 205	<0.001
None/acetaldehyde (20 mM)/LPS	10	31 ± 12.0	<0.001

*Reference value represents no ALDH inhibitor, ethanol loading, LPS challenge; N.S., not significant

ethanol plus LPS challenge demonstrated no significant differences between cells with and without disulfiram pretreatment (Fig. 2A). TNF-α mRNA expression in LPS-challenged Kupffer cells was not significantly changed by adding 500 μM acetaldehyde (Fig. 2B), in contrast to decreased TNF-α release associated with the addition of only 50 μM acetaldehyde (see Table 5).

Discussion

Despite several reports indicating that the inflammation is suppressed by ethanol[21,22] or acetaldehyde,[18,23] the precise progression of hepatic inflammation and the function of hepatic macrophages, which are closely associated with liver diseases including alcoholic hepatitis,[2,3] have not yet been elucidated with ethanol and acetaldehyde loading. The results of our experiments, demonstrating that ALDH inhibitor disulfiram attenuates acute hepatic inflammation induced by LPS with ethanol loading, suggest one reason for the suppression of TNF-α release in the blood: the accumulation of acetaldehyde. This response, observed in vivo, also held for the in vitro experiments, in which acetaldehyde accumulated in response to treatment with ethanol and ALDH inhibitors suppressed Kupffer cell release of TNF-α. Additional experiments proved that acetaldehyde, but neither ethanol nor acetate alone, significantly attenuates TNF-α release from LPS-challenged Kupffer cells. The degree to which the disulfiram plus ethanol treatment suppressed both in vivo and in vitro TNF-α levels after LPS challenge was similar to the extent of the suppression of hepatic inflammation in vivo.

In our in vivo experiments, polymorphonuclear cells infiltrated the liver within 3 h of initiating the LPS challenge. Disulfiram in the presence of ethanol, but not disulfiram alone, attenuated this infiltration. Moreover, the serum levels of the liver enzymes ALT and LDH showed that disulfiram suppresses liver injury caused by LPS and ethanol loading. Because the serum acetaldehyde concentrations in our study were markedly elevated by disulfiram plus ethanol treatment, we speculated that acetaldehyde accumulation could be linked to hepatic protection. In vitro, administration of ethanol following either disulfiram or cyanamide had suppressed TNF-α release by Kupffer cells, an effect not produced by either ALDH inhibitor alone. Moreover, the acetaldehyde concentration in the Kupffer cell culture medium was elevated by disulfiram plus ethanol. In fact, additional experiments showed that acetaldehyde, but neither ethanol nor acetate alone, signifi-

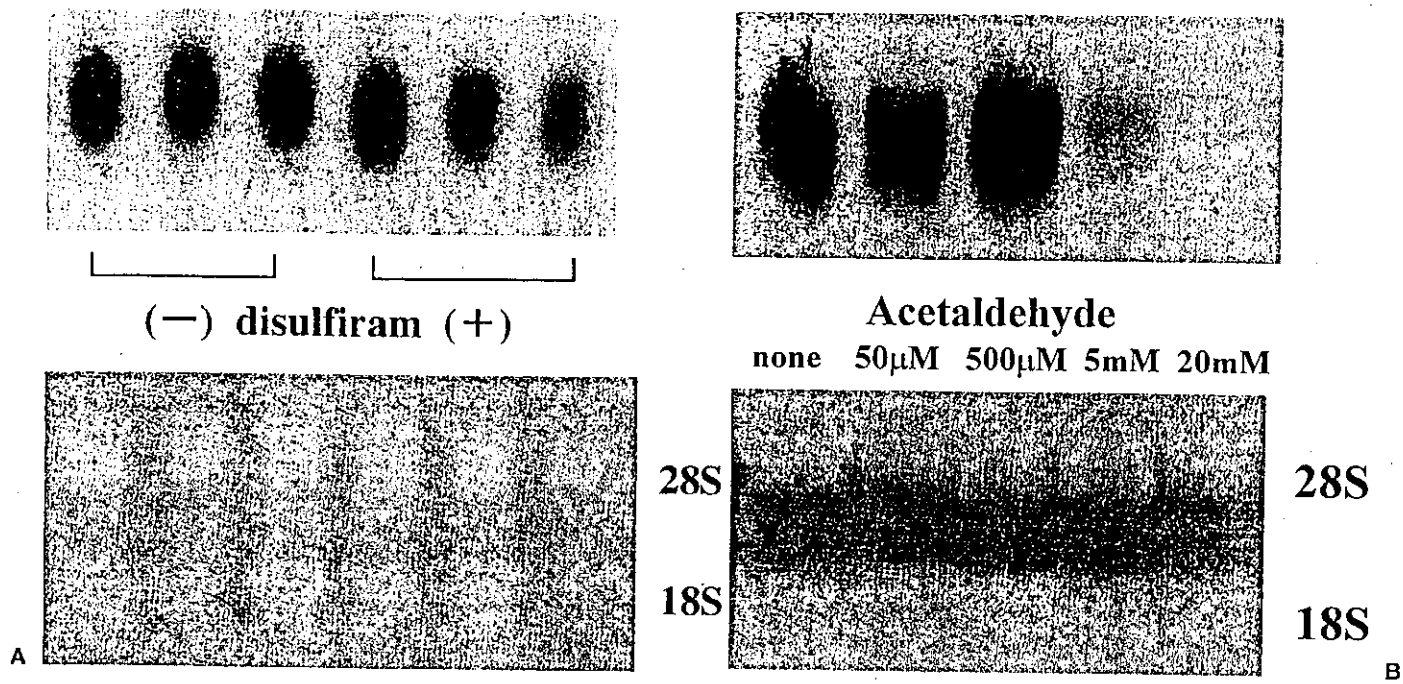

Fig. 2. Northern blot analysis showing Kupffer cell expression of tumor necrosis factor-alpha (TNF-α) mRNA as the effects of (**A**) ethanol treatment plus LPS challenge following pretreatment with or without disulfiram and (**B**) LPS challenge in the presence of various concentrations of acetaldehyde

cantly attenuates TNF-α release from LPS-challenged Kupffer cells.

Two clinical situations in which acetaldehyde accumulation could affect the internal organs, including the liver, are (1) medical treatment that inhibits ALDH activity and (2) the presence of a genotype in which the inactive ALDH2*2 allele readily incurs the accumulation of high levels of acetaldehyde (a condition especially prevalent in Asians). In addition to disulfiram and cyanamide, drugs that have an ALDH inhibitory effect include cephalosporins,[24] chlorpropamide,[25] and metronidazol.[26] These drugs might have the ability to affect macrophage function and hepatic injury after drinking alcohol. The proposed protective role for acetaldehyde could help to explain why Asians have milder alcoholic hepatitis than people in the United States and most Western countries.[4] Observations concerning acetaldehyde accumulation may help to answer some of the molecular and biochemical questions about acute hepatic inflammation with alcohol consumption.

The degree to which the disulfiram/ethanol treatment suppressed both in vivo and in vitro TNF-α levels after LPS challenge in this investigation was similar to the extent of suppression of the hepatic inflammation. Although treatment with an ALDH inhibitor before ethanol loading induced a greater accumulation of acetaldehyde and suppressed TNF-α release from Kupffer cells, our Northern blot experiments did not demonstrate significant suppression in TNF-α mRNA levels in Kupffer cells, with or without disulfiram pretreatment. Moreover, TNF-α mRNA expression from LPS-challenged Kupffer cells was not significantly affected by the addition of 500μM acetaldehyde, in contrast to the decrease in TNF-α release when we added only 50μM acetaldehyde. This finding suggests the likelihood that posttranscriptional inhibition is involved in this process.

Recent experiments by Lindros et al.[18] and Jokelainen et al.[23] indicated that acetaldehyde inhibits and suppresses hepatic inflammation in ethanol-fed rats by preventing nuclear factor kappa-B (NFκ-B) activation through the suppression of TNF-α mRNA and its release. However, the Lindros group incubated Kupffer cells with acetaldehyde for 4h in vitro, and examined the in vivo effects of treatment with an ALDH inhibitor for 1 month, whereas in our study we examined the effects of ethanol loading for only 1h (i.e., after 1h of acetaldehyde accumulation) in vitro, and overnight in vivo. The differences in duration of exposure to accumulated acetaldehyde (1 month versus overnight in vivo, 4h versus 1h in vitro) may explain the differences between our results and theirs. Some additional parameters, the concentrations of ALDH inhibitor and ethanol, are differed between the Lindros study and ours.

Acetaldehyde decreases the activity of enzymes such as sucrase, maltase, and chymotrypsin.[27,28] There is considerable evidence of the influence with acetaldehyde on numerous proteins, microtubules, hemoglobin, globulin, collagen, and DNA.[7–12] Thus, it is not surprising that no difference in the expression of TNF-α mRNA was found in our study, although the differences in mature TNF-α protein levels were significant. Among the many processes that must influence the reduction of TNF-α release to be considered are the translation by ribosomes within the endoplasmic reticulum, the intracellular movements within microtubules, the conversion of pro-TNF-α to mature TNF-α, and the surrounding conditions after TNF-α release. Our data appear to support those of Zhang and colleagues,[29] who demonstrated that ethanol inhibits the processing of pro-TNF-α to TNF-α by TNF-α-converting enzyme (TACE). Posttranscriptional inhibition such as TACE activity could offer a clue concerning acute acetaldehyde effects, but this possibility requires further investigation.

Although 500 μM acetaldehyde significantly reduced actual Kupffer cell release of TNF-α, whereas 50 μM acetaldehyde decreased the response by up to 10%, disulfiram plus ethanol suppressed TNF-α secretion much more effectively when the level of acetaldehyde detected was less than 50 μM. These differences in effect were achieved following different periods of exposure to acetaldehyde. Because the boiling point of acetaldehyde is 20.1 °C, most of the acetaldehyde added to media and incubated at 37 °C would evaporate immediately. Further, in exposing Kupffer cells to 50 μM acetaldehyde, without an ALDH inhibitor, we showed a more rapid breakdown of acetaldehyde than we observed following disulfiram pretreatment and 50 mM ethanol loading. The accumulation of acetaldehyde during 3 h incubation in a 14-μM concentration of acetaldehyde would likely have had a much greater effect on Kupffer cell function than a bolus 50-μM dose of acetaldehyde, which evaporated and metabolized readily in the presence of active ALDH during a 3-h incubation.

In conclusion, this study has provided evidence that acetaldehyde accumulation resulting from simultaneous ALDH inhibition and ethanol loading suppresses LPS-induced acute hepatic inflammation in rats. One mechanism of this effect might involve the reduction in TNF-α release from hepatic macrophages. The person who has a low-activity ALDH subtype or whose ALDH activity is impaired (e.g., due to medication) would thus experience the protective effect of acute alcoholic hepatitis because of accumulated acetaldehyde. Further studies are needed to clarify the role of acetaldehyde accumulation in acute hepatic inflammation and to address the obscure role of acetaldehyde accumulation in chronic liver injury with alcohol consumption.

References

1. Muntwyler J, Hennekens CH, Buring JE, Gaziano JM. Mortality and light to moderate alcohol consumption after myocardial infarction. Lancet 1998;352:1882–5.
2. Adachi Y, Bradford BU, Gao W, Bojes HK, Thurman RG. Inactivation of Kupffer cells prevents early alcohol-induced liver injury. Hepatology 1994;20:453–60.
3. Adachi Y, Moore LE, Bradford BU, Gao W, Thurman RG. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. Gastroenterology 1995;108:218–24.
4. Takada A, Takase S, Tsutsumi M. Characteristic features of alcoholic liver disease in Japan: a review. Gastroenterol Jpn 1993;28: 137–48.
5. Weisburger JH. Worldwide prevention of cancer and other chronic diseases based on knowledge of mechanisms. Mutat Res 1998;402:331–7.
6. Harada S, Misawa S, Agarwal DP, Goedde HW. Liver alcohol dehydrogenase and aldehyde dehydrogenase in the Japanese: isozyme variation and its possible role in alcohol intoxication. Am J Hum Genet 1980;32:8–15.
7. Hecht SS, McIntee EJ, Wang M. New DNA adducts of crotonaldehyde and acetaldehyde. Toxicology 2001;166:31–6.
8. Jeong KS, Soh Y, Jeng J, Felder MR, Hardwick JP, Song BJ. Cytochrome P450 2E1 (CYP2E1)-dependent production of a 37-kDa acetaldehyde-protein adduct in the rat liver. Arch Biochem Biophys 2000;384:81–7.
9. Svegliati-Baroni G, Baraona E, Rosman AS, Lieber CS. Collagen-acetaldehyde adducts in alcoholic and nonalcoholic liver diseases. Hepatology 1994;20:111–8.
10. Niemela O, Israel Y, Mizoi Y, Fukunaga T, Eriksson CJ. Hemoglobin-acetaldehyde adducts in human volunteers following acute ethanol ingestion. Alcohol Clin Exp Res 1990;14: 838–41.
11. Fleisher JH, Lung CC, Meinke GC, Pinnas JL. Acetaldehyde-albumin adduct formation: possible relevance to an immunologic mechanism in alcoholism. Alcohol Alcohol 1988;23:133–41.
12. Smith SL, Jennett RB, Sorrell MF, Tuma DJ. Substoichiometric inhibition of microtubule formation by acetaldehyde-tubulin adducts. Biochem Pharmacol 1992;44:65–72.
13. Nakamura Y, Yokoyama H, Okamura Y, Ohgo H, Fukuda M, Horie Y, et al. Evidence for ethanol oxidation by Kupffer cells. Alcohol Clin Exp Res 1999;23(suppl 4):92S–5S.
14. Maher JJ, Friedman SL. Parenchymal and nonparenchymal cell interactions in the liver. Semin Liver Dis 1993;13:13–20.
15. Iversen OH. The cell kinetics of the inflammatory reaction. Introduction and overview. Curr Top Pathol 1989;79:1–5.
16. van Furth R. Macrophage activity and clinical immunology. Origin and kinetics of mononuclear phagocytes. Ann N Y Acad Sci 1976;278:161–75.
17. Sheila S, Dooley J. Diseases of the liver and biliary system. 10th ed. Oxford: Blackwell Science, 1997.
18. Lindros KO, Jokelainen K, Nanji AA. Acetaldehyde prevents nuclear factor-κB activation and hepatic inflammation in ethanol-fed rats. Lab Investig 1999;79:799–806.
19. Nakamura Y, Yokoyama H, Kato S, Ishii H. Major role of cytochrome P450 2E1 in ethanol oxidation by Kupffer cells. Res Commun Alcohol Subst Abuse 1999;20:15–25.
20. Eriksson CJ, Atkinson N, Petersen DR, Deitrich RA. Blood and liver acetaldehyde concentrations during ethanol oxidation in C57 and DBA mice. Biochem Pharmacol 1984;33:2213–6.
21. Fox ES, Cantrell CH, Leingang KA. Inhibition of Kupffer cell inflammatory response by acute ethanol: NF-kappa B activation and subsequent cytokine production. Biochem Biophys Res Commun 1996;225:134–40.
22. Szabo G. Consequences of alcohol consumption on host defence. Alcohol Alcohol 1999;34:830–41.
23. Jokelainen K, Thomas P, Lindros K, Nanji AA. Acetaldehyde inhibits NF-κB activation through IκBα preservation in rat

Kupffer cells. Biochem Biophys Res Commun 1998;253:834–6.
24. Drummer S, Hauser WE Jr, Remington JS. Antabuse-like effect of beta-lactam antibiotics. N Engl J Med 1980;303: 1417–8.
25. Little RG 2nd, Petersen DR. Effect of tolbutamide and chlorpropamide on acetaldehyde metabolism in two inbred strains of mice. Toxicol Appl Pharmacol 1985;80:206–14.
26. Winter D, Stanescu C, Sauvard S. The effect of metronidazol on the toxicity of ethanol. Biochem Pharmacol 1969;18:1246–8.
27. Koivisto T, Salaspuro M. Effects of acetaldehyde on brush border enzyme activities in human colon adenocarcinoma cell line Caco-2. Alcohol Clin Exp Res 1997;21:1599–605.
28. Brecher AS, Yang MP. Acetaldehyde inhibits chymotrypsin and serum anti-chymotrypsin activity. J Investig Med 1998;46:146–52.
29. Zhang Z, Cork J, Ye P, Lei D, Schwarzenberger PO, Summer WR, et al. Inhibition of TNF-α processing and TACE-mediated ectodomain shedding by ethanol. J Leukoc Biol 2000;67:856–62.

GASTROENTEROLOGY 2004;126:873–885

Pioglitazone Prevents Alcohol-Induced Fatty Liver in Rats Through Up-regulation of c-Met

KENGO TOMITA,* TOSHIFUMI AZUMA,* NAOTO KITAMURA,* JIRO NISHIDA,† GEN TAMIYA,§ AKIRA OKA,§ SAYAKA INOKUCHI,* TAKESHI NISHIMURA,* MAKOTO SUEMATSU,‖ and HIROMASA ISHII*

Departments of *Internal Medicine and ‖Biochemistry, Keio University School of Medicine, Tokyo, Japan; †Department of Gastroenterology, Ichikawa General Hospital, Tokyo Dental College, Chiba, Japan; and §Department of Molecular Life Science, School of Medicine, Tokai University, Kanagawa, Japan

Background & Aims: Treatment of steatosis is important in preventing development of fibrosis in alcoholic liver diseases. This study aimed to examine if pioglitazone, an antidiabetic reagent serving as a ligand of peroxisome proliferator-activated receptor gamma (PPARγ), could prevent alcoholic fatty liver. *Methods:* Rats fed with an ethanol-containing liquid diet were given the reagent at 10 mg/kg per day intragastrically for 6 weeks. Hepatic genes involved in actions of the reagent were mined by transcriptome analyses, and their changes were confirmed by real-time polymerase chain reaction and Western blotting analyses. The direct effects of pioglitazone on primary-cultured hepatocytes were also assessed in vitro. *Results:* Pioglitazone significantly attenuated steatosis and lipid peroxidation elicited by chronic ethanol exposure without altering insulin resistance. Mechanisms for improving effects of the reagent appeared to involve restoration of the ethanol-induced down-regulation of c-Met and up-regulation of stearoyl-CoA desaturase (SCD). Such effects of pioglitazone on the c-Met signaling pathway resulted from its tyrosine phosphorylation and resultant up-regulation of the apolipoprotein B (apoB)-mediated lipid mobilization from hepatocytes through very low–density lipoprotein (VLDL) as well as down-regulation of sterol regulatory element binding protein (SREBP)-1c and SCD levels and a decrease in triglyceride synthesis in the liver. *Conclusions:* Pioglitazone activates c-Met and VLDL-dependent lipid retrieval and suppresses triglyceride synthesis and thereby serves as a potentially useful stratagem to attenuate ethanol-induced hepatic steatosis.

Chronic consumption of excess alcohol is hepatotoxic in humans and produces an accumulation of hepatic triglycerides to cause steatosis. These changes are pathologically characterized by macrovesicular fatty degeneration and occur in pericentral regions. Recent clinical studies provided evidence that such an accumulation of triglycerides in the liver is not benign but could lead to fibrosis and cirrhosis with effective treatment remaining to be established.[1,2] Because the hepatic steatosis often coincides with hyperinsulinemia and insulin resistance, treatment that renders patients sensitive to the hormone could be beneficial. Such a possibility was well supported by previous studies showing that metformin, an agent improving insulin resistance of the liver, improved steatosis, hepatomegaly, and the release of transaminases in insulin-resistant ob/ob mice with nonalcoholic fatty liver; mechanisms for such beneficial effects of metformin appear to involve down-regulation of tumor necrosis factor-α (TNF-α) and TNF-inducible genes such as SREBP-1 and uncoupling protein-2[3] as well as increased phosphorylation and activation of AMP-activated protein kinase (AMPK).[4] The fact that the histological features and natural history of alcoholic fatty liver are similar to those of nonalcoholic fatty liver suggests that unidentified common mechanisms for pathogenesis of hepatic steatosis could be involved in these 2 disease conditions. However, utilization of metformin is unlikely to be applicable to treat alcoholic steatosis because of its side effect of lactic acidosis, an important complication of clinical alcoholic liver injury.

Such circumstances led us to examine if another class of antidiabetic agents such as thiazolidinediones are effective to treat hepatic steatosis caused by chronic ethanol administration. These reagents are ligands of perox-

Abbreviations used in this paper: ALT, alanine aminotransferase; AMPK, AMP-activated protein kinase; apoB, apolipoprotein B; AST, aspartate aminotransferase; BADGE, bisphenol A diglycidyl ether; HGF, hepatocyte growth factor; 4-HNE, 4-hydroxynonenal; HOX, heme oxygenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PPAR, peroxisome proliferator-activated receptor; PCR, reverse-transcriptase polymerase chain reaction; TG, triglyceride; TNF-α, tumor necrosis factor-α; VLDL, very low-density lipoprotein; ZDF fa/fa, Zucker diabetic fatty.

0016-5085/04/$30.00
doi:10.1053/j.gastro.2003.12.008