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Ethanol and acetaldehyde in alcoholic cardiomyopathy: from bad to ugly en route to oxidative stress

Xiaochun Zhang^a, Shi-Yan Li^a, Ricardo A. Brown^b, Jun Ren^{a,*}^a*Division of Pharmaceutical Sciences, Graduate Neuroscience Program, and Center for Cardiovascular Research and Alternative Medicine, University of Wyoming College of Health Sciences, Laramie, WY 82071-3375, USA*^b*Biomedical Research Branch, Division of Basic Research, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892-7003, USA*

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Abstract

Alcoholic cardiomyopathy is characterized by cardiomegaly, disruptions of myofibrillary architecture, reduced myocardial contractility, decreased ejection fraction, and enhanced risk of stroke and hypertension. Although several mechanisms have been postulated for alcoholic cardiomyopathy, including oxidative damage, accumulation of triglycerides, altered fatty acid extraction, decreased myofilament Ca²⁺ sensitivity, and impaired protein synthesis, neither the mechanism nor the ultimate toxin has been unveiled. Primary candidates acting as specific toxins of myocardial tissue are ethanol; its first and major metabolic product, acetaldehyde; and fatty acid ethyl esters. Acetaldehyde has been demonstrated to impair directly cardiac contractile function, disrupt cardiac excitation–contractile coupling, and contribute to oxidative damage and lipid peroxidation. Acetaldehyde-elicited cardiac dysfunction may be mediated through cytochrome P450 oxidase, xanthine oxidase, and the stress-signaling cascade. Unfortunately, the most direct approach that can be used to examine toxicity is hampered by the fact that direct intake of acetaldehyde is highly toxic and unsuitable for long-term study. To overcome this obstacle, transgenic mice have been used to alter artificially ethanol/acetaldehyde metabolism, resulting in elevated acetaldehyde concentrations after ethanol ingestion. In this review, we summarize results obtained with the use of transgenic animal models to elucidate the role of acetaldehyde in the mechanism of action in alcoholic cardiomyopathy. © 2004 Elsevier Inc. All rights reserved.

Keywords: Ethanol; Acetaldehyde; Alcoholic cardiomyopathy; Oxidative stress

1. Alcohol and heart disease

Alcoholism is a major health problem throughout the world, with approximately 10% of the adult population in the United States suffering from alcoholism or its complications, regardless of racial, ethnic, and socioeconomic factors (Schoppet & Maisch, 2001). Although light-to-moderate alcohol consumption protects against cardiovascular diseases largely by reducing coronary artery-related events, alcohol consumption from either long-term misuse or binge drinking usually leads to cardiac dysfunction, mitochondrial dysfunction, and ventricular arrhythmias (Preedy et al., 2001; Richardson et al., 1998; Spies et al., 2001). Almost one of every three individuals who are chronically dependent on alcohol exhibit cardiac dysfunction, characterized as a unique type of dilated cardiomyopathy termed *alcoholic cardiomyopathy* (Liang et al., 1999; Spies et al., 2001). Cardiac damage because of alcohol use is usually evident

within several years if alcohol consumption exceeds 90 to 100 g per day. Alcoholic cardiomyopathy is also known as alcoholic heart muscle disease and is discernible by cardiomegaly, disruptions in myofibrillary architecture, reduced myocardial contractility, decreased ejection fraction and stroke volume, as well as enhanced risk of stroke and hypertension (Schoppet & Maisch, 2001). These cardiac morphologic and functional alterations may ultimately lead to cardiac failure and therefore are considered the end points of alcoholic cardiac toxicity in a manner similar to that observed for several other toxins, such as doxorubicin, cocaine, acetaldehyde, monocrotaline, and azide. These cardiac toxins may contribute directly to cardiac dysfunction and hypertrophy by enhancing levels of prooxidants, such as catecholamines and reactive oxygen species (ROS), as well as by inducing hemodynamic load, such as hypoxia and hypertension. Cardiac failure associated with alcoholic cardiomyopathy may be the result of a direct toxic effect of ethanol or its metabolites, or of an indirect action owing to neurohumoral, hormonal, or nutritional factors (Badger et al., 2003; Schoppet & Maisch, 2001). Other scenarios have also been hypothesized for the pathogenesis of alcoholic

* Corresponding author. Tel.: +1-307-766-6131; fax: +1-307-766-2953.

E-mail address: jren@uwyo.edu (J. Ren).

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cardiomyopathy, such as oxidative stress (Hannuksela et al., 2002; Zima et al., 2001), protein–aldehyde adducts (Niemelä, 2001), accumulation of fatty acid ethyl esters (Patel et al., 1997), and modifications of lipoprotein and apolipoprotein particles (Hannuksela et al., 2002). In addition, it may be possible that the cardiac toxicity of ethanol is secondary to inflammatory response in cardiac tissue or damage done to other organs such as the liver. However, none of these hypotheses has received convincing clinical and experimental support to be validated fully. For example, direct ethanol toxicity may require development of oxidative stress as a permissive factor, whereas the ethanol-induced oxidative stress may require metabolism of ethanol into more reactive compounds such as acetaldehyde first. Furthermore, little correlation is found between the onset of alcoholic cardiomyopathy and damage to other organs such as the liver, making the theory of cardiac toxicity secondary to other organ damage rather weak. It is important to mention that some of or all these pathways may share certain common cellular mechanisms such as enhanced oxidative stress en route to the development of alcoholic cardiomyopathy (Preedy et al., 2001, 2002; Spies et al., 2001), although convincing evidence is lacking. Advances in transgenic, genomic, and proteomic techniques are in high demand to reveal the mechanism of chronic cardiac toxicity of ethanol at cellular and molecular levels.

2. Acetaldehyde and cardiovascular function

Acetaldehyde (CH_3CHO) is a chemically reactive organic compound with low molecular weight (44.05) and low boiling point (21°C). It is formed by the oxidation of ethanol primarily through the action of alcohol dehydrogenase (ADH). Liver is often considered the primary site of oxidation, although other organs, including the heart, may also participate in the metabolism of ethanol. Simultaneous to acetaldehyde production, ADH reduces nicotinamide adenine dinucleotide (NAD^+) to nicotinamide adenine dinucleotide (reduced form: NADH). Concomitant alterations in NADH and $\text{NADH}:\text{NAD}^+$ ratio may, in turn, modulate the activity of certain free radical–generating enzymes, including xanthine oxidase, and cause oxidation of NADH (Mantle & Preedy, 1999). These mechanisms may play a role in ethanol-induced generation of ROS. In addition to ethanol metabolism, acetaldehyde, also known as reactive aldehyde, can be generated endogenously during the degradation process of biologic molecules (e.g., lipid peroxidation) (Uchida, 2000). Acetaldehyde is oxidized to acetic acid mainly through aldehyde dehydrogenase (ALDH). It may also react with amino, hydroxyl, and sulfhydryl groups to interfere with or modify the structure and function of macromolecules in the body, such as proteins and enzymes. Acetaldehyde is about 10 times more toxic than ethanol on the basis of its 50% lethal dose (LD_{50}) value (Brien & Loomis, 1983). Through the years, evidence has accumulated to support the suggestion

that acetaldehyde, the first metabolite of ethanol, may play an important role in the pathogenesis of alcoholic cardiomyopathy (Aberle & Ren, 2003a; Duan et al., 2002, 2003; Eriksson, 2001; Hintz et al., 2003; Liang et al., 1999).

Elevated concentrations of circulating acetaldehyde are detected in human beings who consume, as well as in animals that experimentally consume, excessive alcohol (Hintz et al., 2003; Jänkälä et al., 2000; Nishimura et al., 2002; Watanabe et al., 1985), and increased acetaldehyde concentrations have been found within the hearts (Espinet & Argiles, 1984). The physiologic concentrations of circulating acetaldehyde that are achievable are usually undetectable ($<0.5 \mu\text{M}$) in non–alcohol-dependent control subjects after moderate alcohol intake. To the contrary, blood acetaldehyde concentrations are much higher in alcohol-dependent individuals after alcohol intake (Nuutinen et al., 1983). With the blood alcohol level decreasing from 54 to 33 mM after intravenous alcohol administration, the blood acetaldehyde plateau concentration was significantly higher in alcohol-dependent individuals ($42.7 \mu\text{M}$) than in non–alcohol-dependent individuals ($26.5 \mu\text{M}$) (Korsten et al., 1975). The main reason for the elevated blood acetaldehyde concentrations in individuals who are chronically dependent on alcohol is believed to be the impaired ALDH enzymatic capacity to metabolize acetaldehyde (Nuutinen et al., 1983). Blood acetaldehyde concentrations were approximately tenfold higher in human beings with defective mitochondrial class 2 aldehyde dehydrogenase (ALDH2) than in healthy individuals (Nishimura et al., 2002). Results from clinical studies have revealed blood acetaldehyde concentrations of $\sim 5 \mu\text{M}$ in healthy subjects versus 30–125 μM in Asians with defective ALDH2 after heavy alcohol consumption (Chen et al., 1999; Nishimura et al., 2002; Watanabe et al., 1985). A blood acetaldehyde concentration up to 500 μM has been documented after severe ethanol intoxication in human beings (Watanabe et al., 1985). In animal studies, the blood acetaldehyde concentrations have been found to be in the range of 2 to 164 μM after alcohol intake (Hintz et al., 2003; Jänkälä et al., 2000). The apparent discrepancy in blood acetaldehyde concentrations among alcohol-dependent subjects (i.e., either in human subjects or in animals in which this state occurred experimentally) may be explained by assay technique, amount and duration of drinking, time of blood sampling after last drink, and difference in race or species. It has been suggested that elevated blood acetaldehyde concentrations occur regularly after interrupted drinking in alcohol-dependent individuals classified as heavy drinkers (alcohol consumption $>80 \text{ g/day}$), with fast ethanol elimination, possibly combined with reduced liver ALDH activity. However, this elevation in blood acetaldehyde concentration may quickly disappear on abstinence and hospitalization, which improves hepatic malfunctions and reduces ethanol elimination rate (Lindros et al., 1980). Nevertheless, increasing evidence supports the suggestion that elevated blood acetaldehyde concentrations may not be just a parallel phenomenon in alcohol-dependent individuals with compromised

ventricular function. Rather, they actually participate in the cause of alcoholic cardiomyopathy. Direct actions of acute (5–10 min) acetaldehyde exposure on cardiac and vascular contractile function have been studied extensively (Brown et al., 1999, 2001; Brown & Savage, 1996; Ren et al., 1997; Savage et al., 1995). Acetaldehyde produces vasoconstriction, as well as positive inotropic and chronotropic responses, in vitro at concentrations of less than 3 mM, which are mediated indirectly by norepinephrine release from intramural sympathetic nerve terminals and blocked by appropriate adrenoceptor antagonists. Higher concentrations of acetaldehyde (>3 mM) produce cardiac depression, vasodilation, and hypotension in vitro, which cannot be abolished by reserpine pretreatment or adrenoceptor antagonists (Brown & Carpentier, 1989, 1990). Findings from studies of papillary muscle have shown that acetaldehyde (>1 mM) exerts a concentration-dependent negative inotropic effect in vitro, and the underlying mechanisms may be intimately related to reduced intracellular Ca^{2+} release from sarcoplasmic reticulum (Ren et al., 1997, 2001; Savage et al., 1995). The acetaldehyde-induced negative inotropic effect in cardiac and vascular preparations may also be related to its inhibitory effect on voltage-dependent Ca^{2+} channels, although such effect needs to be confirmed in cardiac myocytes (Morales et al., 1997). However, most of the earlier studies conducted in our laboratories failed to reveal any discernible cardiac contractile effect at physiologically achievable acetaldehyde concentrations (i.e., in the micromolar concentration range). This may be attributed to the highly volatile property of acetaldehyde. To reconcile this volatile property of acetaldehyde, Aberle and Ren (2003b) incubated ventricular myocytes in sealed vials with silicone septa for 4 to 6 h and showed that acetaldehyde may depress myocyte contraction amplitude, depress maximal velocity of contraction/relaxation, and prolong duration of relaxation and intracellular Ca^{2+} clearing at concentrations between 10 and 100 μM .

In addition to its action on the functional components involved in cardiac excitation–contraction coupling, acetaldehyde may also interfere with gene expression and protein synthesis in the heart. In isolated heart preparation, addition of acetaldehyde to the perfusate reduced rates of protein synthesis, and a more pronounced reduction in translation rates occurred when cardiac muscles were treated with ethanol and the ALDH inhibitor calcium carbimide concurrently, indicating the role of acetaldehyde as a potent inhibitor for protein synthesis (Siddiq et al., 1993). Elevated mRNA expression of atrial natriuretic peptide (ANP), often used as a marker for cardiac hypertrophy, stress, and apoptosis (Wu et al., 1997), was found in rat left ventricle after a 2- or 8-day combined ethanol and calcium carbimide treatment. Interestingly, there was no significant change of ANP mRNA levels in left ventricles from rats treated with ethanol alone, supporting the suggestion of a possible role of acetaldehyde in the up-regulation of gene encoding for ANP (Jänkälä et al., 2000). In a different study (Jänkälä et al., 2002), investigators

from the same laboratory found increased p21 gene expression and Bax:Bcl-2 mRNA ratio after a 2-day treatment with ethanol and calcium carbimide. However, after an 8-day treatment with ethanol and calcium carbimide, mRNA concentration of p21 was depressed, whereas that of p53 and Bcl-2 was elevated. These results indicate that acetaldehyde may regulate the expression of apoptosis-related genes in a time-dependent manner, which may play a role in the development of alcoholic cardiomyopathy. Interestingly, there have been disparate reports regarding contributions of ethanol and acetaldehyde to cardiac gene expression. Short-term infusion of ethanol alone has been shown to induce significantly mRNA expression of Bax, ANP, and p21 in ventricular myocardium. However, acetaldehyde alone did not exert any significant effect on either ANP or p21 mRNA expression (Jänkälä et al., 2001; Patel et al., 2001). These findings seem to indicate that ethanol may induce certain genes associated with cardiac hypertrophy and dysfunction, independent of its metabolism into acetaldehyde and acetaldehyde-induced cardiac apoptotic injury. Although the mechanisms behind the disparate contribution to cardiac gene expression between ethanol and acetaldehyde are largely unknown, differences in experimental conditions, such as duration and dose of ethanol treatment, should be considered.

3. Transgenic modification of acetaldehyde metabolism

The focus of this section is on the role of altered acetaldehyde metabolism in alcoholic cardiomyopathy. Advancement of the acetaldehyde toxicity theory has been trivial to date, mostly because of the lack of a suitable method of altering acetaldehyde concentrations chronically in vivo. Although blood acetaldehyde concentrations may reach 500 μM after alcohol intake in Asians and African-Americans owing to an ALDH polymorphism (Tsukamoto et al., 1989; Watanabe et al., 1985; Yoshida, 1992), intolerance to alcohol ingestion makes it practically impossible for these individuals to be considered as subjects for clinical study. Earlier work with metabolic inhibitors to alter acetaldehyde concentrations revealed that the metabolic inhibitors for acetaldehyde are nonspecific, ineffective, toxic, and difficult to manage chronically (Aberle & Ren, 2003a; Preedy et al., 2002). To overcome such obstacles in the assessment of acetaldehyde, we have artificially changed the tissue exposure of acetaldehyde by altering ethanol metabolism with the use of two highly specific transgenes, cytosolic class 1 alcohol dehydrogenase (ADH1) and ALDH2, for acetaldehyde synthesis and breakdown, respectively. A transgenic mouse line was developed to overexpress ADH1 specifically in the heart, which elicited higher cardiac acetaldehyde concentrations after chronic (8 weeks) ethanol intake (Liang et al., 1999). In contrast, nonspecific overexpression of the ALDH2 transgene with the use of chicken β -actin promoter should facilitate removal of acetaldehyde and alleviate the cardiac

burden of acetaldehyde, thus reducing its exposure without altering ethanol metabolism. If acetaldehyde toxicity is permissive to the development of alcoholic cardiomyopathy, alcoholic cardiomyopathy should develop at a faster rate after alcohol consumption in mice with cardiac overexpression of the ADH1 gene, and mice with the ALDH2 transgene overexpression should be protected from development of alcoholic cardiomyopathy after alcohol consumption. Allelic variation of the ADH and ALDH genes, especially deficiency in ALDH2 owing to point mutation in the active *ALDH2*1* gene, has been shown to affect significantly the blood acetaldehyde concentrations and vulnerability for the onset of alcoholism (Chen et al., 1999; Peng et al., 1999, 2002; Yoshida, 1992). However, the relation between such allelic variation (e.g., *ALDH2*2*) and development of alcoholic cardiomyopathy has not been elucidated, largely because of the intolerance of individuals with this point mutation to alcohol drinking.

3.1. Alcohol dehydrogenase transgene

A transgenic mouse model of acetaldehyde overproduction specifically in the heart was designed to increase significantly cardiac exposure to acetaldehyde and determine its impact on alcoholic cardiomyopathy after alcohol ingestion (Liang et al., 1999). The cDNA for the murine ADH1 gene was driven by the mouse α -myosin heavy chain (MHC) promoter to be specifically expressed in the heart. This cDNA was chosen because ADH1 is the most efficient enzyme in the oxidation of ethanol. A second gene containing cDNA for the enzyme tyrosinase was co-injected with ADH1, the product of which generates a grey coat pigmentation in albino mice to be used for identification purposes (Liang et al., 1999). The ADH activity was increased by ~40-fold in the heart of the ADH transgenic mice, which results in a fourfold to sixfold increase in cardiac acetaldehyde production after alcohol ingestion (Hintz et al., 2003; Liang et al., 1999). Both acute (5 min) (Duan et al., 2002, 2003) and chronic (8 weeks) (Hintz et al., 2003; Liang et al., 1999) administration of alcohol enhanced local production of acetaldehyde and promoted ethanol-induced cardiac contractile depression, development of alcoholic cardiomyopathy, or both. Acute exposure (5–10 min) to ethanol (80–640 mg/dl) depressed cardiac contractile function and intracellular Ca^{2+} transients, indicative of intracellular Ca^{2+} release, in ventricular myocytes from wild-type FVB mice. The maximal inhibitions were 23.3% and 23.4%, respectively, for cardiac contractile function and intracellular Ca^{2+} transients (Duan et al., 2002). Interestingly, the ethanol-induced depressant effects on cardiac contraction and intracellular Ca^{2+} transients were significantly augmented in myocytes from the ADH transgenic mice, with maximal inhibitions of 43.7% and 40.6%, respectively. The ADH inhibitor 4-methylpyrazole prevented, whereas the ALDH inhibitor cyanamide exacerbated, the ADH transgene-induced augmentation in cardiac depression in response to

ethanol exposure (Duan et al., 2002). In a chronic study after 10 weeks of alcohol ingestion, mRNA expression of ANP and α -skeletal actin, both characteristic of cardiomyopathy (Colbert et al., 1997), was increased significantly in alcohol-consuming ADH mice compared with findings for the wild-type FVB mice consuming alcohol (Liang et al., 1999). After 18 weeks of alcohol diet treatment, enlarged hearts, disrupted cardiac ultrastructure, and reduced contractility were observed, with the morphologic and functional damage being much more severe in ADH mice than in FVB mice consuming alcohol (Liang et al., 1999). Ventricular myocytes obtained from ethanol-fed (8 weeks) ADH and FVB mice displayed significantly depressed cell contraction, velocity of contraction/relaxation, Ca^{2+} -induced intracellular Ca^{2+} release, and sarcoplasmic reticulum Ca^{2+} load associated with similar duration of contraction/relaxation compared with findings for myocytes obtained from non-drinking ADH or FVB mice. Strikingly, the ethanol-induced mechanical and intracellular Ca^{2+} defects were exacerbated in ADH myocytes compared with findings for the FVB group. Formation of the lipid peroxidation end-product malondialdehyde, which directly depresses cardiac contractile function (Folden et al., 2003), and protein carbonyl, indicative of protein damage, was significantly elevated in both livers and hearts after chronic (8 weeks) alcohol consumption, with the cardiac lipid and protein damage being exaggerated by ADH transgene (Hintz et al., 2003). Collectively, these results support the notion that acetaldehyde may play a significant role in lipid peroxidation, cardiac injury, and, ultimately, development of alcoholic cardiomyopathy. The ADH transgene did not affect morphologic, mechanical, and intracellular Ca^{2+} properties, with the exception of a reduction in the resting intracellular Ca^{2+} concentrations and Ca^{2+} resequestration at low pacing frequency (Hintz et al., 2003; Liang et al., 1999), supporting the suggestion that the ADH transgene is not innately harmful. Although ADH reduces NAD^+ to NADH at the same time it converts ethanol to acetaldehyde, the $\text{NAD}^+:\text{NADH}$ ratio was similar in ADH transgenic and FVB wild-type mice consuming alcohol (Liang et al., 1999), indicating that depletion of NAD^+ was not likely an adequate factor to interpret the enhanced cardiac damage in ADH transgenic mice after alcohol intake.

3.2. Aldehyde dehydrogenase transgene

Although the ADH transgene causes elevation in acetaldehyde accumulation after alcohol intake, ALDH facilitates acetaldehyde removal by converting it to acetate. This finding is supported by the evidence that blood acetaldehyde concentrations were tenfold higher in human beings with defective mitochondrial ALDH (i.e., ALDH2) than in healthy individuals (Nishimura et al., 2002). To examine the impact of ALDH gene expression on development of alcoholic cardiomyopathy and other tissue damage, transgene encoding of the low Km mitochondrial isozyme of

ALDH2–ALDH2 was constructed by using chicken β -actin promoter. The cardiac-specific α -MHC promoter was not used for cardiac local expression because diffusion of acetaldehyde from peripheral regions would easily offset the facilitated cardiac metabolism of the toxin. High efficacy of ALDH2 transduction was demonstrated in human umbilical vein endothelial cells [(HUVECs); ATCC, Manassas, VA] and human cardiac myocytes (ScienCell Research Laboratories, San Diego, CA; Product #6200) by using green fluorescent protein (GFP) and confirmed with ALDH2 activity assay (Li et al., 2004). Results indicated that overexpression of ALDH2 significantly attenuates acetaldehyde exposure-induced oxidative stress and apoptosis in HUVECs and human cardiac myocytes (Li et al., 2004). Examination of Fig. 1 (A–F) reveals that 24 h of incubation of acetaldehyde with myocytes ($\sim 10^5$ cells per milliliter) significantly enhanced intracellular ROS generation, measured by 5-(6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H₂DCFDA), which enters the cells and produces a fluorescent signal after intracellular oxidation by ROS, at a concentration greater than 50 μ M in human left ventricular cardiac myocytes. Interestingly, myocytes transfected with ALDH2 transgene (Fig. 2D), but not those transfected with pBsCAG-2 vector only, were significantly protected from acetaldehyde (100 μ M)-induced ROS accumulation (Fig. 2), supporting the suggestion that facilitation of acetaldehyde breakdown may lessen or detoxify its cellular toxicity. These results confirm the notion that acetaldehyde may directly elicit cell injury because facilitation of its metabolism by ALDH2 alleviates the cellular toxicity.

The ALDH2 transgene should be a useful tool to modify artificially acetaldehyde metabolism. The availability of both ADH and ALDH2 transgenes has made it possible to examine the precise role and mechanism of acetaldehyde in the development of alcoholic cardiomyopathy. Although several hypotheses have been postulated for alcoholic tissue injury, including oxidative damage, lipid peroxidation, and altered membrane integrity attributed to the hydrophobic interaction between ethanol and membrane phospholipids or protein components (Bailey et al. 1999; Cederbaum et al., 2001; Mantle & Preedy, 1999), the ultimate culprit for alcoholic cardiomyopathy remains unidentified. The notion that acetaldehyde may directly enhance free radical generation by its oxidation by aldehyde oxidase, xanthine oxidase, or both with concurrent accumulation of superoxide anion (Guerra et al., 1994; Lieber, 1999; McDonough, 1999; Oei et al., 1986; Zima et al., 2001) is consistent with the observation of alleviated oxidative stress and apoptosis in HUVECs (Li et al., 2004) and cardiac myocytes (S.-Y. Li & J. Ren, unpublished observations, 2004). It is thus reasonable to postulate that the toxic effects of ethanol may be mediated, at least in part, through acetaldehyde.

3.3. Studies in human beings

The rate of acetaldehyde metabolism differs between individuals and races, especially in Asians possessing deficient phenotypes of ALDH (*ALDH2*2/1* and *ALDH2*2/2* genotypes). These mutant alleles usually result from a single point mutation of the active *ALDH2*1* gene and are confined

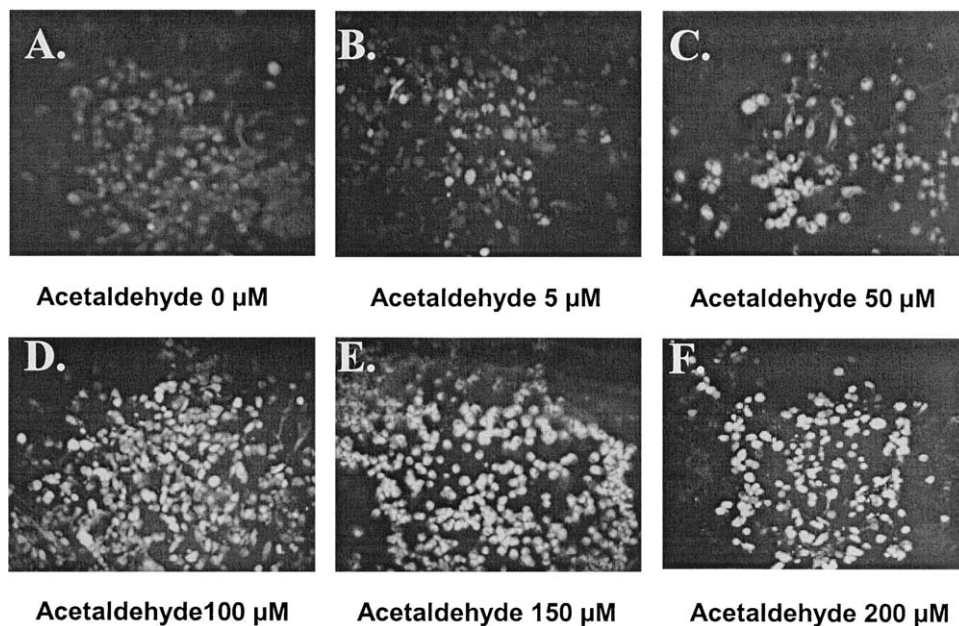


Fig. 1. Effect of 24-h incubation of acetaldehyde on intracellular reactive oxygen species production in human cardiac myocytes. Cells were loaded with the membrane-permeable probe 5-(6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate [(CM-H₂DCFDA); 1 μ M] for 30 min at 37°C before being evaluated with the use of an Olympus IX-70 microscope and ImagePro image analysis software (Media Cybernetics, Silver Spring, MD). Panels A–F display fluorescence intensity under six concentrations of acetaldehyde, respectively: 0, 5, 50, 100, 150, and 200 μ M.

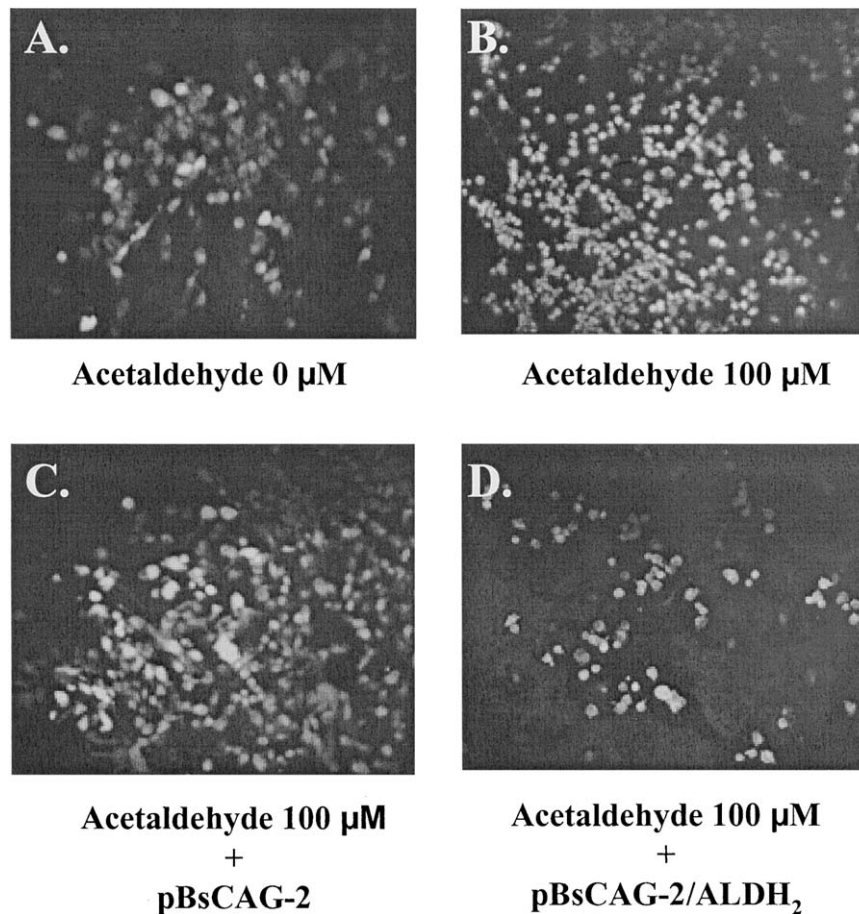


Fig. 2. Effect of mitochondrial class 2 aldehyde dehydrogenase (ALDH2) transgene overexpression on acetaldehyde (100 μM)-induced intracellular reactive oxygen species production in human cardiac myocytes. The amplified ALDH2 fragment was digested with EcoRI and cloned into the EcoRI site of vector pBsCAG-2 under the CAG cassette to be pBsCAG-2-ALDH2 [see Li et al. (2004) for details]. Cells were loaded with 5-(6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate [(CM-H₂DCFDA); 1 μM] for 30 min at 37°C before being evaluated with the use of an Olympus IX-70 microscope and ImagePro image analysis software. Panels A–D display, respectively, control human cardiac myocytes without acetaldehyde treatment and DNA transfection; human cardiac myocytes treated with acetaldehyde (100 μM); human cardiac myocytes with pBsCAG-2 vector transfection grown under acetaldehyde treatment (100 μM); and human cardiac myocytes transfected with constructed pBsCAG-2–ALDH2 grown under acetaldehyde treatment (100 μM).

to up to 50% of Asian populations (Nishimura et al., 2002; Yoshida, 1992). The effect of ALDH2 genotype on cardiovascular responses to alcohol ingestion was evaluated in young healthy Japanese subjects (Nishimura et al., 2002). The blood acetaldehyde concentrations were found to be approximately tenfold higher in subjects with the deficient ALDH2 genotype than in subjects with normal ALDH2 genotype (Nishimura et al., 2002). Alcohol ingestion significantly increased sympathetic activity and heart rate, whereas it inhibited cardiac parasympathetic activity in subjects without the mutant ALDH2 genotype. These effects are likely mediated by an acetaldehyde-induced increase in blood norepinephrine concentrations and changes in the autonomic nervous system (Nishimura et al., 2002). With the use of blood pressure readings, serum lipid concentrations, and uric acid levels as indices to evaluate the risk factors for coronary heart disease among alcohol drinkers with genetic polymorphism of the alcohol-metabolizing enzymes ADH and ALDH, it was found that individuals with *ADH2**1/2*1 genotype might suffer fewer negative effects

of drinking (Hashimoto et al., 2002). The gene status of *ALDH2**2/2 can greatly protect against development of alcohol dependence and alcohol-related disease (Chen et al., 1999). To test genetic susceptibility loci for alcohol-related heart muscle disease, 700 middle-aged male victims of sudden death were examined, and no conclusive genetic susceptibility factors (including ADH and ALDH2) were identified for alcoholic cardiomyopathy (Kajander et al., 2001). It is worth mentioning that these surveys are unlikely to provide direct information regarding the relation between acetaldehyde and cardiac function, largely owing to intolerance to alcohol among these individuals with genetic polymorphisms of ADH/ALDH2. The role of acetaldehyde in alcoholic cardiomyopathy was substantiated by the observation that the ALDH inhibitor cyanamide potentiates the alcohol intake-induced rise of plasma cardiac troponin-T concentrations within 6 h, a key index for myocardial cell death (Patel et al., 2001). These observations have convincingly indicated the critical role of acetaldehyde in cardiac myopathic change and alcoholic cardiomyopathy.

A functional polymorphism of ALDH2 is speculated to play a role in the sensitivity to alcohol-elicited injury, with the variant allele (*ALDH2*2*) encoding a protein subunit conferring low activity to the tetrameric enzyme. Homozygosity for the allele *ALDH2*2* is believed to inhibit sufficiently the development of alcoholism in Asians. After administration of a small dose of alcohol (0.2 g/kg), the cardiac and extracranial/intracranial arterial hemodynamic parameters, as well as self-rated sensations, were strikingly responsive in individuals homozygous for *ALDH2*2*, as evidenced by the pronounced cardiovascular hemodynamic effects as well as subjective perception of general discomfort for as long as 2 h after alcohol ingestion. This low-dose alcohol hypersensitivity is accompanied by a prolonged and large accumulation of acetaldehyde in the blood. The accumulated blood acetaldehyde and its toxicity has provided an apparent explanation for the protection against heavy drinking and alcoholism in individuals homozygous for the *ALDH2*2* gene allele (Chen et al., 1999; Peng et al., 1999, 2002). However, it is unrealistic and perhaps unethical to use individuals as “guinea pigs” to assess the precise role of acetaldehyde in the cardiac toxicity or injury characteristic of alcoholic cardiomyopathy.

4. Influence of sex on acetaldehyde-induced cardiac effect

In comparison with men, women often drink less and experience less alcohol-related medical problems. Only one third of all alcohol-dependent individuals in the United States are women. However, results from recent studies revealed that an equivalent amount of alcohol is more unpleasant and causes tissue damage more rapidly in women than in men, supporting the suggestion that being female may be a risk factor for the development of alcoholic cardiomyopathy. Women in whom alcoholic cardiomyopathy develops are reported to have a lower lifetime alcohol consumption compared with that in men with alcoholic cardiomyopathy (Piano, 2002). Women have a higher vulnerability to the development of alcohol-related diseases, possibly because of their higher blood alcohol levels after drinking and relatively smaller total blood volume, although Lucey et al. (1999) found no difference in blood alcohol levels between age-matched men and women. These investigators suggested that age, rather than sex, may influence peak blood alcohol levels. In a recent study, healthy men and women drank ethanol at 0.3 g/kg, and, subsequently, the activities of ADH isozymes were assessed by evaluation of specimens obtained through gastric biopsy. Women, in comparison with men, were found to have less first-pass metabolism of alcohol at high levels of alcohol intake (Baraona et al., 2001). This was associated with a lower gastric ADH activity in women, which may explain the greater sex difference in first-pass metabolism, with high, rather than low, levels of alcohol intake. In comparison with findings for men, alcohol gastric

emptying was 42% slower, hepatic oxidation was 10% higher, and the blood volume of alcohol distribution was 7.3% smaller in women. It is believed that the sex difference in alcohol levels is attributed mainly to a smaller gastric metabolism in women (lower ADH activity), rather than to the differences in gastric emptying or in hepatic oxidation of ethanol. However, Lai et al. (2000) failed to observe any difference in gastric ADH activity between age-matched men and women, supporting the suggestion that combined pharmacokinetic differences (e.g., emptying and oxidation) may increase the vulnerability of women to the effects of ethanol (Baraona et al., 2001). This finding is consistent with the occurrence of elevated blood acetaldehyde concentrations formed during alcohol oxidation in women, but not in men, after alcohol intake. An association between elevated acetaldehyde concentrations and high estrogen phases was established in normal cycling women or women taking oral contraceptives (Eriksson et al., 1996). The likely correlation between estrogen and acetaldehyde concentrations after alcohol intake may play a role in the sex-related differences in the cardiac effects of alcohol. This notion is supported by results from recent studies with the use of cardiac-specific ADH overexpression transgenic mice, which demonstrated that the ventricular myocytes obtained from both sexes of ADH mice exhibited similar mechanical properties but a higher efficacy to produce acetaldehyde compared with findings for the wild-type FVB group (Duan et al., 2002, 2003; Hintz et al., 2003). Exposure to ethanol (80–640 mg/dl) for 60 min elicited a concentration-dependent reduction in cell shortening in both FVB and ADH groups of mice. Interestingly, the ethanol-induced depression of myocyte contraction was augmented significantly in the female, but not in the male, ADH group of mice. The ADH transgene did not exacerbate the ethanol-induced slowing of contraction/relaxation velocity in either sex. These findings confirmed that, in comparison with male animals, female animals may be more sensitive to the acetaldehyde-induced cardiac contractile depression, which may explain sex-related differences in alcoholic cardiomyopathy (Duan et al., 2003).

5. Stress signaling behind acetaldehyde-induced cardiac injury

5.1. Oxidative stress theory

Although several hypotheses have been postulated, enhanced oxidative stress after alcohol ingestion seems to be central in explaining the toxicity of ethanol and acetaldehyde. A role for free radicals in the development of alcohol tissue damage has been speculated since the early 1960s. Ethanol-induced oxidative stress is linked directly to its metabolic pathways. Each metabolic pathway of ethanol [e.g., ADH, microsomal ethanol oxidizing system (MEOS), and catalase] is able to produce free radicals, including superoxide anion (Nakano et al., 1995) and 1-hydroxyethyl radical (Knecht et al., 1995; Rao et al., 1996), and to diminish the

enzymatic/nonenzymatic antioxidant defense systems (Zima et al., 2001). Acetaldehyde is considered to be a “second cytotoxic messenger,” which participates in the pathogenesis of several cardiovascular diseases (Uchida, 2000). However, exactly how acetaldehyde produces cytotoxicity and mediates cardiomyopathy remains unclear. As a potent electrophile, acetaldehyde can readily react with nucleophiles in proteins, phospholipids, and nucleic acids to produce adducts (Conduah Birt et al., 1998; Fang & Vaca, 1997). Oxidation of acetaldehyde to acetate was accompanied by free radical generation, including superoxide anion, hydroxyl, acetyl, and methyl radicals, in a manner similar to that in metabolism of ethanol (Guerra et al., 1994; Nakao et al., 2000). Formation of acetyl radicals is considered to occur from reaction of acetaldehyde with hydroxyl radical ($\text{CH}_3\text{CHO} + \cdot\text{OH} \rightarrow \text{CH}_3\text{C}\cdot\text{O} + \text{H}_2\text{O}$), which results in parallel formation of methyl radical from acetyl radical decarbonylation ($\text{CH}_3\text{C}\cdot\text{O} \rightarrow \cdot\text{CH}_3 + \text{CO}$). Several enzymes or cellular fractions, such as xanthine oxidase, aldehyde oxidase, mitochondria, and microsomes, may oxidize acetaldehyde into free radical intermediates (Albano et al., 1994; Boh et al., 1982; Gonthier et al., 1991; Guerra et al., 1994; Oei et al., 1986; Rajasinghe et al., 1990). Oxidation of acetaldehyde by xanthine oxidase has been shown to produce superoxide anion, hydrogen peroxide, and acetyl radicals, possibly through the above-mentioned Fenton mechanism, escape of acetyl radicals from enzymatic oxidation of acetaldehyde, and nucleophilic addition of peroxide to acetaldehyde (Nakao et al., 2000). Both consumption of ethanol and direct administration of acetaldehyde have been demonstrated to elicit enhanced oxidative stress (Cunningham & Bailey, 2001; Gómez-Quiroz et al., 2003; Vendemiale et al., 2001). An elevated ROS generation was observed in mitochondria obtained from individuals with a history of chronic ethanol consumption, and it was suspected that this was a result of mitochondrial-associated reoxidation of NADH produced during ethanol and acetaldehyde metabolism. It is likely due to decreases in mitochondrial-derived electron transport components, which, in turn, results in higher concentrations of the semiquinone forms of flavin mononucleotide and ubiquinone. Both these semiquinones readily donate electrons to molecular oxygen to form superoxide (Cunningham & Bailey, 2001). It was demonstrated that catalase activity was decreased in HepG2 cells by both ethanol and acetaldehyde treatment. However, acetaldehyde, but not ethanol, can significantly increase lipid peroxidation, supporting the suggestion of a differential role of ethanol and acetaldehyde in the induction of oxidative stress (Gómez-Quiroz et al., 2003). Nevertheless, significantly elevated activity of catalase, but unchanged superoxide dismutase (SOD), glutathione peroxidase, or glutathione reductase, were found in brain after a single intraperitoneal injection of acetaldehyde (5 mmol/kg) (Heap et al., 1995). Taken together, although it is well established that free radicals are produced when acetaldehyde is metabolized to acetate, how prooxidant/antioxidant

balance is affected by acetaldehyde in vivo remains highly controversial.

Oxygen free radicals or ROS have been established to induce lipid peroxidation, protein modification, enzyme inactivation, and DNA strand break and base modifications (Camougrand & Rigoulet, 2001), a major upstream component in the signaling cascade leading to several cellular functions, such as cell proliferation, apoptosis, inflammatory responses, and accumulation of adhesion molecules (Napoli et al., 2001; Yoon et al., 2002). They are thus implicated in the pathogenesis of a variety of cardiac diseases, such as hypoxia-reperfusion injury, heart failure, and diabetic and hypertrophic cardiomyopathies (Tanaka et al., 2001).

One potential target for oxidative stress may be the mitogen-activated protein (MAP) kinase family. One or more of the three distinct but parallel MAP kinase cascades—extracellular signal-regulated kinase (ERK), c-Jun NH₂-terminal kinase (JNK), and p38 MAP kinase—may be activated and subsequently trigger downstream intracellular responses such as gene expression. Activation of the three branches of MAP kinases (i.e., ERK, p38, and JNK) is important in the development of a hypertrophic phenotype, as well as in the initiation of mechanical dysfunction in cardiomyocytes. Characteristic changes in gene expression include the elevated transcription of atrial natriuretic factor (ANF), β -MHC, α -skeletal actin, and certain variants of integrins and perhaps tubulin genes, as well as reduced expression of the sarcoplasmic reticulum proteins phospholamban and sarco(endo)plasmic reticulum Ca^{2+} -ATPase2a (SERCA2a). It has become apparent that acetaldehyde and other reactive aldehydes are potential inducers of this oxidative stress-signaling cascade (Che et al., 1997; Uchida et al., 1999), and they participate in the development of alcoholic cardiomyopathy (Parola et al., 1998; Ruef et al., 1998). A finding from a recent study from our laboratory (Li et al., 2004) indicates that short-term treatment (24–48 h) with a low micromolar range of acetaldehyde induces a significant increase in the phosphorylation of p38 MAP kinase, ERK, and JNK in human cardiac myocytes without affecting total p38 MAP kinase, ERK, or JNK levels. This finding is consistent with results of earlier reports of the involvement of ERK1/2, JNK, and p38 MAP kinase in acetaldehyde-induced cellular toxicity (Cao et al., 2002; Svegliati-Baroni et al., 2001), supporting the suggestion that stress-signaling pathways may be involved in the cardiac toxicity of acetaldehyde. It has been indicated that activation of MAP kinase family may serve as a significant signaling cascade for the chronic alcohol ingestion-related oxidative injury (Masamune et al., 2002; Weng & Shukla, 2000). Acetaldehyde, as a highly reactive product from the oxidative metabolism of ethanol, was shown to be a critical mediator of ethanol-induced apoptosis by means of activation of the MAP kinase signaling pathway (Chen & Davis, 2000; Lee et al., 2002; Svegliati-Baroni et al., 2001). It is thus possible that acetaldehyde may selectively activate a certain member of the MAP

kinase family, although further study is warranted to elucidate the stress-signaling mechanisms involved in the acetaldehyde-induced cellular toxicity. The oxidative stress mechanism in ethanol- or acetaldehyde-induced cardiac damage may be further buttressed by up-regulated antioxidant defense after alcohol exposure. Edes et al. (1987) reported elevated concentrations of the antioxidant enzymes SOD, catalase, and glutathione peroxidase after 15 weeks of alcohol ingestion, and results from several other studies revealed increased myocardial catalase levels after chronic administration of alcohol. These investigators claimed that oxygen free radicals may not play a significant role in alcoholic cardiomyopathy, at least in some species that may have greater antioxidative reserves. Although the exact toxicants that induce molecular changes remain elusive, increasing lines of evidence support the notion that prooxidants play a central role in cardiac hypertrophy. Recent evidence from our laboratory demonstrated that transgenic mice with cardiac overexpression of antioxidant catalase displayed preserved cardiac mechanical function and intracellular Ca^{2+} -regulating proteins against ethanol-induced damage (Zhang et al., 2003), supporting the notion of oxidative stress in alcoholic cardiomyopathy.

5.2. Aldehyde adducts

Generation of protein–aldehyde adducts as a result of excessive ethanol intake has been well established (Badger et al., 2003; Niemelä, 2001). Acetaldehyde can bind to reactive lysine residues, some aromatic amino acids, cysteine, or free alpha-amino groups (Niemelä, 1999). Although virtually all reactive aldehydes can bind to proteins in vitro, there seems to be some preferential targets in vivo (Niemelä, 2001). These include erythrocyte membrane proteins, albumin, lipoproteins, hemoglobin, collagens, tubulin, and cytochrome enzymes (Niemelä, 1999, 2001). Although most protein–aldehyde adducts are located in liver (Jeong et al., 2000; Worrall et al., 2001), some are distributed in muscle, brain, and blood cells. As a consequence of adduct formation, the physicochemical properties of proteins, nucleic acids, and lipids may be altered (Niemelä, 1999). Protein function is disturbed particularly when a lysine residue is in a functionally critical location, such as tubulin and in lysine-dependent enzymes. Acetaldehyde–DNA binding has been considered to promote carcinogenesis in alcohol-dependent individuals (Niemelä, 2001). It has been reported that acetaldehyde–protein adducts and lipid peroxidation products increase collagen mRNA levels and the expression of connective tissue proteins.

Both humoral and cell-mediated immunologic responses against various types of protein adducts are stimulated by ethanol intake. Circulating immunoglobulins anti-acetaldehyde and anti-malondialdehyde have been detected in ethanol-fed rats (Niemelä, 1999). However, the actions of these antibodies are not clear. The demonstration of specific protein adducts in alcohol-dependent individuals has stimulated

new research initiatives by using these adducts as biologic markers of alcohol-induced disease. Harcombe et al. (1995) reported that 33% of patients with alcoholic cardiomyopathy possessed circulating antibody, especially immunoglobulin G. However, so far results of adduct assays have shown insufficient sensitivities for such purposes. Further studies are needed before autoimmune responses become a useful diagnostic tool.

6. Non-acetaldehyde-related mechanisms in alcoholic cardiomyopathy

Although acetaldehyde may be considered as the possible ultimate toxin in ethanol-induced cardiac damage, other mechanisms may influence cardiac metabolism of ethanol and thus contribute to the pathogenesis of alcoholic cardiomyopathy. Ethanol may elicit a direct toxic effect on the cardiovascular system or alter the neurohumoral and hormonal regulation of cardiac function (Schoppet & Maisch, 2001). Other metabolites of ethanol metabolism may also affect cardiac function independent of acetaldehyde. For example, fatty acid ethyl esters were found to be the myocardial metabolites of ethanol. Incubation of myocardial mitochondria with fatty acid ethyl esters may result in reduced respiratory control ratio index of coupling of oxidative phosphorylation and maximal rate of oxygen consumption, leading to mitochondrial dysfunction and, subsequently, oxidative stress. Because uncoupling of mitochondrial oxidative phosphorylation associated with cleavage of fatty acid ethyl ester may induce fatty acid generation near the mitochondrial membrane, fatty acid ethyl esters are likely to serve as a toxic shuttle to transport fatty acid from the physiologic intracellular binding sites to the mitochondrial membrane. Accumulation of fatty acid ethyl esters after alcohol intake may account for the impaired cardiac mitochondrial function and inefficient cardiac energy production (Lange & Sobel, 1983). Although it has been speculated that nutritional status may play a role in the heart function in alcohol-dependent individuals, few patients with alcoholic cardiomyopathy showed either clinical or laboratory evidence of malnutrition (Estruch et al., 1993). It is possible that alcoholic complications in the cardiovascular system may be due largely to an accumulative toxic effect of ethanol or its metabolites, rather than to nutritional status. Nevertheless, recent observations from our laboratory support the suggestion that acetaldehyde-induced cardiac mechanical dysfunction may be alleviated by administration of folate or thiamine (Aberle et al., 2003; N. S. Aberle & J. Ren, unpublished observations, 2004) and, therefore, that there is possibly an interaction between acetaldehyde-induced cardiac toxicity and nutritional status. This finding is somewhat consistent with the favorable response of patients with alcoholic cardiomyopathy to treatment with thiamine and other dietary measures (Constant, 1999). Responsiveness to treatment with thiamine and other dietary measures was recommended as a criterion to differentiate genuine alcoholic

cardiomyopathy from alcoholic dilated cardiomyopathy with viral cause (Constant, 1999).

7. Conclusions

In this article, we have reviewed some aspects of the role of acetaldehyde in the development of alcoholic cardiomyopathy. It can be concluded that elevated acetaldehyde concentration during acute and chronic alcohol ingestion participates in the development of alcoholic cardiomyopathy through alterations in protein synthesis, excitation–contractility coupling, myocardial function, responsiveness to inotropic agents, and oxidative stress, and it may be an essential culprit for alcoholic cardiomyopathy. In previous studies, however, investigators have focused on the effects of acetaldehyde on cardiac function, and its direct cytotoxicity has not been well addressed. Moreover, convincing results from human case studies are still lacking. There is no clear demonstration that acetaldehyde shifts the antioxidative/oxidative balance in the heart. Further studies are needed to illuminate how far and how acetaldehyde is involved in alcoholic cardiomyopathy.

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References

- Aberle, N. S. II, Privratsky, J. R., Burd, L., & Ren, J. (2003). Combined acetaldehyde and nicotine exposure depresses cardiac contraction in ventricular myocytes: prevention by folic acid. *Neurotoxicol Teratol* 25, 731–736.
- Aberle, N. S. II, & Ren, J. (2003a). Experimental assessment of the role of acetaldehyde in alcoholic cardiomyopathy. *Biol Proceed Online* 5, 1–12.
- Aberle, N. S. II, & Ren, J. (2003b). Short-term acetaldehyde exposure depresses ventricular myocyte contraction: role of cytochrome P450 oxidase, xanthine oxidase, and lipid peroxidation. *Alcohol Clin Exp Res* 27, 577–583.
- Albano, E., Clot, P., Comoglio, A., Dianzani, M. U., & Tomasi, A. (1994). Free radical activation of acetaldehyde and its role in protein alkylation. *FEBS Lett* 348, 65–69.
- Badger, T. M., Ronis, M. J. J., Seitz, H. K., Albano, E., Ingelman-Sundberg, M., & Lieber, C. S. (2003). Alcohol metabolism: role in toxicity and carcinogenesis. *Alcohol Clin Exp Res* 27, 336–347.
- Bailey, S. M., Pietsch, E. C., & Cunningham, C. C. (1999). Ethanol stimulates the production of reactive oxygen species at mitochondrial complexes I and III. *Free Radic Biol Med* 27, 891–900.
- Baraona, E., Abittan, C. S., Dohmen, K., Moretti, M., Pozzato, G., Chayes, Z. W., Schaefer, C., & Lieber, C. S. (2001). Gender differences in pharmacokinetics of alcohol. *Alcohol Clin Exp Res* 25, 502–507.
- Boh, E. E., Baricos, W. H., Bernofsky, C., & Steele, R. H. (1982). Mitochondrial chemiluminescence elicited by acetaldehyde. *J Bioenerg Biomembr* 14, 115–133.
- Brien, J. F., & Loomis, C. W. (1983). Pharmacology of acetaldehyde. *Can J Physiol Pharmacol* 61, 1–22.
- Brown, R. A., Anthony, M. J., Petrovski, P., & Ren, J. (2001). The influence of gender, diabetes, and acetaldehyde on the intrinsic contractile properties of isolated rat myocardium. *Cardiovasc Toxicol* 1, 35–42.
- Brown, R. A., & Carpentier, R. G. (1989). Effects of acetaldehyde on the automaticity of the guinea pig sinus node. *Alcohol* 6, 103–107.
- Brown, R. A., & Carpentier, R. G. (1990). Effects of acetaldehyde on membrane potentials of sinus node pacemaker fibers. *Alcohol* 7, 33–36.
- Brown, R. A., Jefferson, L., Sudan, N., Lloyd, T. C., & Ren, J. (1999). Acetaldehyde depresses myocardial contraction and cardiac myocyte shortening in spontaneously hypertensive rats: role of intracellular Ca^{2+} . *Cell Mol Biol (Noisy-le-grand)* 45, 453–465.
- Brown, R. A., & Savage, A. O. (1996). Effects of acute acetaldehyde, chronic ethanol, and pargyline treatment on agonist responses of the rat aorta. *Toxicol Appl Pharmacol* 136, 170–178.
- Camougrand, N., & Rigoulet, M. (2001). Aging and oxidative stress: studies of some genes involved both in aging and in response to oxidative stress. *Respir Physiol* 128, 393–401.
- Cao, Q., Mak, K. M., & Lieber, C. S. (2002). Dilinoleoylphosphatidylcholine decreases acetaldehyde-induced TNF- α generation in Kupffer cells of ethanol-fed rats. *Biochem Biophys Res Commun* 299, 459–464.
- Cederbaum, A. I., Wu, D., Mari, M., & Bai, J. (2001). CYP2E1-dependent toxicity and oxidative stress in HepG2 cells. *Free Radic Biol Med* 31, 1539–1543.
- Che, W., Asahi, M., Takahashi, M., Kaneto, H., Okado, A., Higashiyama, S., & Taniguchi, N. (1997). Selective induction of heparin-binding epidermal growth factor-like growth factor by methylglyoxal and 3-deoxyglucosone in rat aortic smooth muscle cells. The involvement of reactive oxygen species formation and a possible implication for atherogenesis in diabetes. *J Biol Chem* 272, 18453–18459.
- Chen, A., & Davis, B. H. (2000). The DNA binding protein BTEB mediates acetaldehyde-induced, Jun N-terminal kinase-dependent α (I) collagen gene expression in rat hepatic stellate cells. *Mol Cell Biol* 20, 2818–2826.
- Chen, Y.-C., Lu, R.-B., Peng, G.-S., Wang, M.-F., Wang, H.-K., Ko, H.-C., Chang, Y.-C., Lu, J.-J., Li, T.-K., & Yin, S.-J. (1999). Alcohol metabolism and cardiovascular response in an alcoholic patient homozygous for the *ALDH2*2* variant gene allele. *Alcohol Clin Exp Res* 23, 1853–1860.
- Colbert, M. C., Hall, D. G., Kimball, T. R., Witt, S. A., Lorenz, J. N., Kirby, M. L., Hewett, T. E., Klevitsky, R., & Robbins, J. (1997). Cardiac compartment-specific overexpression of a modified retinoic acid receptor produces dilated cardiomyopathy and congestive heart failure in transgenic mice. *J Clin Invest* 100, 1958–1968.
- Conduah Birt, J. E., Shuker, D. E., & Farmer, P. B. (1998). Stable acetaldehyde–protein adducts as biomarkers of alcohol exposure. *Chem Res Toxicol* 11, 136–142.
- Constant, J. (1999). The alcoholic cardiomyopathies—genuine and pseudo. *Cardiology* 91, 92–95.
- Cunningham, C. C., & Bailey, S. M. (2001). Ethanol consumption and liver mitochondria function. *Biol Signals Recept* 10, 271–282.
- Duan, J., Esberg, L. B., Ye, G., Borgerding, A. J., Ren, B. H., Aberle, N. S. II, Epstein, P. N., & Ren, J. (2003). Influence of gender on ethanol-induced ventricular myocyte contractile depression in transgenic mice with cardiac overexpression of alcohol dehydrogenase. *Comp Biochem Physiol A Mol Integr Physiol* 134, 607–614.
- Duan, J., McFadden, G. E., Borgerding, A. J., Norby, F. L., Ren, B. H., Ye, G., Epstein, P. N., & Ren, J. (2002). Overexpression of alcohol dehydrogenase exacerbates ethanol-induced contractile defect in cardiac myocytes. *Am J Physiol Heart Circ Physiol* 282, H1216–H1222.
- Edes, I., Piros, G., Forster, T., & Csanady, M. (1987). Alcohol-induced congestive cardiomyopathy in adult turkeys: effects on myocardial antioxidant defence systems. *Basic Res Cardiol* 82, 551–556.
- Eriksson, C. J. P. (2001). The role of acetaldehyde in the actions of alcohol (update 2000). *Alcohol Clin Exp Res* 25, 15S–32S.
- Eriksson, C. J. P., Fukunaga, T., Sarkola, T., Lindholm, H., & Ahola, L. (1996). Estrogen-related acetaldehyde elevation in women during alcohol intoxication. *Alcohol Clin Exp Res* 20, 1192–1195.

- Espinet, C., & Argiles, J. M. (1984). Ethanol and acetaldehyde concentrations in the rat foeto-maternal system after an acute ethanol administration given to the mother. *Arch Int Physiol Biochim* 92, 339–344.
- Estruch, R., Nicolas, J. M., Villegas, E., Junque, A., & Urbano-Marquez, A. (1993). Relationship between ethanol-related diseases and nutritional status in chronically alcoholic men. *Alcohol Alcohol* 28, 543–550.
- Fang, J. L., & Vaca, C. E. (1997). Detection of DNA adducts of acetaldehyde in peripheral white blood cells of alcohol abusers. *Carcinogenesis* 18, 627–632.
- Folden, D. V., Gupta, A., Sharma, A. C., Li, S.-Y., Saari, J. T., & Ren, J. (2003). Malondialdehyde inhibits cardiac contractile function in ventricular myocytes via a p38 mitogen-activated protein kinase-dependent mechanism. *Br J Pharmacol* 139, 1310–1316.
- Gómez-Quiroz, L., Bucio, L., Souza, V., Escobar, C., Farfán, B., Hernández, E., Konigsberg, M., Vargas-Vorackova, F., Kershenevich, D., & Gutiérrez-Ruiz, M. C. (2003). Interleukin 8 response and oxidative stress in HepG2 cells treated with ethanol, acetaldehyde or lipopolysaccharide. *Hepatology* 37, 134–141.
- Gonthier, B., Jeunet, A., & Barret, L. (1991). Electron spin resonance study of free radicals produced from ethanol and acetaldehyde after exposure to a Fenton system or to brain and liver microsomes. *Alcohol* 8, 369–375.
- Guerri, C., Montoliu, C., & Renau-Piqueras, J. (1994). Involvement of free radical mechanism in the toxic effects of alcohol: implications for fetal alcohol syndrome. *Adv Exp Med Biol* 366, 291–305.
- Hannuksela, M. L., Liisanant, M. K., & Savolainen, M. J. (2002). Effect of alcohol on lipids and lipoproteins in relation to atherosclerosis. *Crit Rev Clin Lab Sci* 39, 225–283.
- Harcombe, A. A., Ramsay, L., Kenna, J. G., Koskinas, J., Why, H. J., Richardson, P. J., Weissberg, P. L., & Alexander, G. J. (1995). Circulating antibodies to cardiac protein–acetaldehyde adducts in alcoholic heart muscle disease. *Clin Sci (Lond)* 88, 263–268.
- Hashimoto, Y., Nakayama, T., Futamura, A., Omura, M., Nakarai, H., & Nakahara, K. (2002). Relationship between genetic polymorphisms of alcohol-metabolizing enzymes and changes in risk factors for coronary heart disease associated with alcohol consumption. *Clin Chem* 48, 1043–1048.
- Heap, L., Ward, R. J., Abiaka, C., Dexter, D., Lawlor, M., Pratt, O., Thomson, A., Shaw, K., & Peters, T. J. (1995). The influence of brain acetaldehyde on oxidative status, dopamine metabolism and visual discrimination task. *Biochem Pharmacol* 50, 263–270.
- Hintz, K. K., Relling, D. P., Saari, J. T., Borgerding, A. J., Duan, J., Ren, B. H., Kato, K., Epstein, P. N., & Ren, J. (2003). Cardiac overexpression of alcohol dehydrogenase exacerbates cardiac contractile dysfunction, lipid peroxidation, and protein damage after chronic ethanol ingestion. *Alcohol Clin Exp Res* 27, 1090–1098.
- Jänkkälä, H., Eklund, K. K., Kokkonen, J. O., Kovanen, P. T., Linstedt, K. A., Härkönen, M., & Mäki, T. (2001). Ethanol infusion increases ANP and p21 gene expression in isolated perfused rat heart. *Biochem Biophys Res Commun* 281, 328–333.
- Jänkkälä, H., Eriksson, C. J. P., Eklund, K. K., Härkönen, M., & Mäki, T. (2002). Combined calcium carbimide and ethanol treatment induces high blood acetaldehyde levels, myocardial apoptosis and altered expression of apoptosis-regulating genes in rat. *Alcohol Alcohol* 37, 222–228.
- Jänkkälä, H., Eriksson, C. J. P., Petersen, N. E., Härkönen, M., & Mäki, T. (2000). Role of acetaldehyde in the induction of heart left ventricular atrial natriuretic peptide gene expression in rats. *Alcohol Alcohol* 35, 331–335.
- Jeong, K.-S., Soh, Y., Jeng, J., Felder, M. R., Hardwick, J. P., & Song, B. J. (2000). Cytochrome P450 2E1 (CYP2E1)-dependent production of a 37-kDa acetaldehyde–protein adduct in the rat liver. *Arch Biochem Biophys* 384, 81–87.
- Kajander, O. A., Kupari, M., Perola, M., Pajarinen, J., Savolainen, V., Penttilä, A., & Karhunen, P. J. (2001). Testing genetic susceptibility loci for alcoholic heart muscle disease. *Alcohol Clin Exp Res* 25, 1409–1413.
- Knecht, K. T., Adachi, Y., Bradford, B. U., Iimuro, Y., Kadiiska, M., Xuang, Q. H., & Thurman, R. G. (1995). Free radical adducts in the bile of rats treated chronically with intragastric alcohol: inhibition by destruction of Kupffer cells. *Mol Pharmacol* 47, 1028–1034.
- Korsten, M. A., Matsuzaki, S., Feinman, L., & Lieber, C. S. (1975). High blood acetaldehyde levels after ethanol administration. Difference between alcoholic and nonalcoholic subjects. *N Engl J Med* 292, 386–389.
- Lai, C.-L., Chao, Y.-C., Chen, Y.-C., Liao, C.-S., Chen, M.-C., Liu, Y.-C., & Yin, S.-J. (2000). No sex and age influence on the expression pattern and activities of human gastric alcohol and aldehyde dehydrogenases. *Alcohol Clin Exp Res* 24, 1625–1632.
- Lange, L. G., & Sobel, B. E. (1983). Mitochondrial dysfunction induced by fatty acid ethyl esters, myocardial metabolites of ethanol. *J Clin Invest* 72, 724–731.
- Lee, Y. J., Aroor, A. R., & Shukla, S. D. (2002). Temporal activation of p42/44 mitogen-activated protein kinase and c-Jun N-terminal kinase by acetaldehyde in rat hepatocytes and its loss after chronic ethanol exposure. *J Pharmacol Exp Ther* 301, 908–914.
- Li, S.-Y., Gomelsky, M., Duan, J., Zhang, Z., Gomelsky, L., Zhang, X., Epstein, P. N., & Ren, J. (2004). Overexpression of aldehyde dehydrogenase-2 (ALDH2) transgene prevents acetaldehyde-induced cell injury in human umbilical vein endothelial cells: role of ERK and p38 mitogen-activated protein kinase. *J Biol Chem* 279, 11244–11252.
- Liang, Q., Carlson, E. C., Borgerding, A. J., & Epstein, P. N. (1999). A transgenic model of acetaldehyde overproduction accelerates alcohol cardiomyopathy. *J Pharmacol Exp Ther* 291, 766–772.
- Lieber, C. S. (1999). Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968–1998)—a review. *Alcohol Clin Exp Res* 23, 991–1007.
- Lindros, K. O., Stowell, A., Pikkarainen, P., & Salaspuro, M. (1980). Elevated blood acetaldehyde in alcoholics with accelerated ethanol elimination. *Pharmacol Biochem Behav* 13 Suppl 1, 119–124.
- Lucey, M. R., Hill, E. M., Young, J. P., Demo-Dananberg, L., & Beresford, T. P. (1999). The influences of age and gender on blood ethanol concentrations in healthy humans. *J Stud Alcohol* 60, 103–110.
- Mantle, D., & Preedy, V. R. (1999). Free radicals as mediators of alcohol toxicity. *Adverse Drug React Toxicol Rev* 18, 235–252.
- Masamune, A., Kikuta, K., Satoh, M., Satoh, A., & Shimosegawa, T. (2002). Alcohol activates activator protein-1 and mitogen-activated protein kinases in rat pancreatic stellate cells. *J Pharmacol Exp Ther* 302, 36–42.
- McDonough, K. H. (1999). The role of alcohol in the oxidant antioxidant balance in heart. *Front Biosci* 4, d601–d606.
- Morales, J. A., Ram, J. L., Song, J., & Brown, R. A. (1997). Acetaldehyde inhibits current through voltage-dependent calcium channels. *Toxicol Appl Pharmacol* 143, 70–74.
- Nakano, M., Kikuyama, M., Hasegawa, T., Ito, T., Sakurai, K., Hiraiishi, K., Hashimura, E., & Adachi, M. (1995). The first observation of O₂⁻ generation at real time in vivo from non-Kupffer sinusoidal cells in perfused rat liver during acute ethanol intoxication. *FEBS Lett* 372, 140–143.
- Nakao, L. S., Kadiiska, M. B., Mason, R. P., Grijalba, M. T., & Augusto, O. (2000). Metabolism of acetaldehyde to methyl and acetyl radicals: in vitro and in vivo electron paramagnetic resonance spin-trapping studies. *Free Radic Biol Med* 29, 721–729.
- Napoli, C., de Nigris, F., & Palinski, W. (2001). Multiple role of reactive oxygen species in the arterial wall. *J Cell Biochem* 82, 674–682.
- Niemelä, O. (1999). Aldehyde–protein adducts in the liver as a result of ethanol-induced oxidative stress. *Front Biosci* 4, d506–d513.
- Niemelä, O. (2001). Distribution of ethanol-induced protein adducts in vivo: relationship to tissue injury. *Free Radic Biol Med* 31, 1533–1538.
- Nishimura, F. T., Fukunaga, T., Kajiura, H., Umeno, K., Takakura, H., Ono, T., & Nishijo, H. (2002). Effects of aldehyde dehydrogenase-2 genotype on cardiovascular and endocrine responses to alcohol in young Japanese subjects. *Auton Neurosci* 102, 60–70.
- Nuutinen, H., Lindros, K. O., & Salaspuro, M. (1983). Determinants of blood acetaldehyde level during ethanol oxidation in chronic alcoholics. *Alcohol Clin Exp Res* 7, 163–168.

- Oei, H. H., Zoganas, H. C., McCord, J. M., & Schaffer, S. W. (1986). Role of acetaldehyde and xanthine oxidase in ethanol-induced oxidative stress. *Res Commun Chem Pathol Pharmacol* 51, 195–203.
- Parola, M., Robino, G., Marra, F., Pinzani, M., Bellomo, G., Leonarduzzi, G., Chiarugi, P., Camandola, S., Poli, G., Waeg, G., Gentilini, P., & Dianzani, M. U. (1998). HNE interacts directly with JNK isoforms in human hepatic stellate cells. *J Clin Invest* 102, 1942–1950.
- Patel, V. B., Ajmal, R., Sherwood, R. A., Sullivan, A., Richardson, P. J., & Preedy, V. R. (2001). Cardioprotective effect of propranolol from alcohol-induced heart muscle damage as assessed by plasma cardiac troponin-T. *Alcohol Clin Exp Res* 25, 882–889.
- Patel, V. B., Why, H. J., Richardson, P. J., & Preedy, V. R. (1997). The effects of alcohol on the heart. *Adverse Drug React Toxicol Rev* 16, 15–43.
- Peng, G. S., Wang, M. F., Chen, C. Y., Luu, S. U., Chou, H. C., Li, T.-K., & Yin, S. J. (1999). Involvement of acetaldehyde for full protection against alcoholism by homozygosity of the variant allele of mitochondrial aldehyde dehydrogenase gene in Asians. *Pharmacogenetics* 9, 463–476.
- Peng, G. S., Yin, J. H., Wang, M. F., Lee, J. T., Hsu, Y. D., & Yin, S. J. (2002). Alcohol sensitivity in Taiwanese men with different alcohol and aldehyde dehydrogenase genotypes. *J Formos Med Assoc* 101, 769–774.
- Piano, M. R. (2002). Effects of gender on alcohol's heart dysfunction. In R. R. Watson, & A. K. Myers (Eds.), *Alcohol and Heart Disease* (pp. 28–47). New York: Taylor & Francis.
- Preedy, V. R., Adachi, J., Asano, M., Koll, M., Mantle, D., Niemelä, O., Parkkila, S., Paice, A. G., Peters, T., Rajendram, R., Seitz, H., Ueno, Y., & Worrall, S. (2002). Free radicals in alcoholic myopathy: indices of damage and preventive studies. *Free Radic Biol Med* 32, 683–687.
- Preedy, V. R., Adachi, J., Peters, T. J., Worrall, S., Parkkila, S., Niemelä, O., Asamo, M., Ueno, Y., Takeda, K., Yamauchi, M., Sakamoto, K., Takagi, M., Nakajima, H., & Toda, G. (2001). Recent advances in the pathology of alcoholic myopathy. *Alcohol Clin Exp Res* 25, 54S–59S.
- Rajasinghe, H., Jayatilake, E., & Shaw, S. (1990). DNA cleavage during ethanol metabolism: role of superoxide radicals and catalytic iron. *Life Sci* 47, 807–814.
- Rao, D. N., Yang, M. X., Lasker, J. M., & Cederbaum, A. I. (1996). 1-Hydroxyethyl radical formation during NADPH- and NADH-dependent oxidation of ethanol by human liver microsomes. *Mol Pharmacol* 49, 814–821.
- Ren, J., Davidoff, A. J., & Brown, R. A. (1997). Acetaldehyde depresses shortening and intracellular Ca^{2+} transients in adult rat ventricular myocytes. *Cell Mol Biol (Noisy-le-grand)* 43, 825–834.
- Ren, J., Natavio, M., Jefferson, L., Pavlik, M. L., & Brown, R. A. (2001). Loss of cardiac contractile response to tetrahydropapaveroline with advanced age and hypertension. *Cell Mol Biol (Noisy-le-grand)* 47 Online Pub, OL15–OL22.
- Richardson, P. J., Patel, V. B., & Preedy, V. R. (1998). Alcohol and the myocardium. *Novartis Found Symp* 216, 35–45.
- Ruef, J., Rao, G. N., Li, F., Bode, C., Patterson, C., Bhatnagar, A., & Runge, M. S. (1998). Induction of rat aortic smooth muscle cell growth by the lipid peroxidation product 4-hydroxy-2-nonenal. *Circulation* 97, 1071–1078.
- Savage, A. O., Dunbar, J. C., & Brown, R. A. (1995). Effects of acetaldehyde on the isolated papillary muscle of diabetic rats. *J Cardiovasc Pharmacol* 26, 251–258.
- Schoppet, M., & Maisch, B. (2001). Alcohol and the heart. *Herz* 26, 345–352.
- Siddiq, T., Richardson, P. J., Mitchell, W. D., Teare, J., & Preedy, V. R. (1993). Ethanol-induced inhibition of ventricular protein synthesis in vivo and the possible role of acetaldehyde. *Cell Biochem Funct* 11, 45–54.
- Spies, C. D., Sander, M., Stangl, K., Fernandez-Sola, J., Preedy, V. R., Rubin, E., Andreasson, S., Hanna, E. Z., & Kox, W. J. (2001). Effects of alcohol on the heart. *Curr Opin Crit Care* 7, 337–343.
- Svegliati-Baroni, G., Ridolfi, F., Di Sario, A., Saccomanno, S., Bendia, E., Benedetti, A., & Greenwel, P. (2001). Intracellular signaling pathways involved in acetaldehyde-induced collagen and fibronectin gene expression in human hepatic stellate cells. *Hepatology* 33, 1130–1140.
- Tanaka, K., Honda, M., & Takabatake, T. (2001). Redox regulation of MAPK pathways and cardiac hypertrophy in adult rat cardiac myocyte. *J Am Coll Cardiol* 37, 676–685.
- Tsakamoto, S., Muto, T., Nagoya, T., Shimamura, M., Saito, M., & Tainaka, H. (1989). Determinations of ethanol, acetaldehyde and acetate in blood and urine during alcohol oxidation in man. *Alcohol Alcohol* 24, 101–108.
- Uchida, K. (2000). Role of reactive aldehyde in cardiovascular diseases. *Free Radic Biol Med* 28, 1685–1696.
- Uchida, K., Shiraishi, M., Naito, Y., Torii, Y., Nakamura, Y., & Osawa, T. (1999). Activation of stress signaling pathways by the end product of lipid peroxidation. 4-hydroxy-2-nonenal is a potential inducer of intracellular peroxide production. *J Biol Chem* 274, 2234–2242.
- Vendemiale, G., Grattagliano, I., Altomare, E., Serviddio, G., Portincasa, P., Prigigallo, F., & Palasciano, G. (2001). Mitochondrial oxidative damage and myocardial fibrosis in rats chronically intoxicated with moderate doses of ethanol. *Toxicol Lett* 123, 209–216.
- Watanabe, A., Kobayashi, M., Hobara, N., Nakatsukasa, H., Nagashima, H., & Fujimoto, A. (1985). A report of unusually high blood ethanol and acetaldehyde levels in two surviving patients. *Alcohol Clin Exp Res* 9, 14–16.
- Weng, Y.-I., & Shukla, S. D. (2000). Ethanol alters angiotensin II stimulated mitogen activated protein kinase in hepatocytes: agonist selectivity and ethanol metabolic independence. *Eur J Pharmacol* 398, 323–331.
- Worrall, S., Niemelä, O., Parkkila, S., Peters, T. J., & Preedy, V. R. (2001). Protein adducts in type I and type II fibre predominant muscles of the ethanol-fed rat: preferential localisation in the sarcolemmal and subsarcolemmal region. *Eur J Clin Invest* 31, 723–730.
- Wu, C.-F., Bishopric, N. H., & Pratt, R. E. (1997). Atrial natriuretic peptide induces apoptosis in neonatal rat cardiac myocytes. *J Biol Chem* 272, 14860–14866.
- Yoon, S.-O., Yun, C.-H., & Chung, A.-S. (2002). Dose effect of oxidative stress on signal transduction in aging. *Mech Ageing Dev* 123, 1597–1604.
- Yoshida, A. (1992). Molecular genetics of human aldehyde dehydrogenase. *Pharmacogenetics* 2, 139–147.
- Zhang, X., Klein, A. L., Alberle, N. S. II, Norby, F. L., Ren, B. H., Duan, J., & Ren, J. (2003). Cardiac-specific overexpression of catalase rescues ventricular myocytes from ethanol-induced cardiac contractile defect. *J Mol Cell Cardiol* 35, 645–652.
- Zima, T., Fialova, L., Mestek, O., Janebova, M., Crkovska, J., Malbohan, I., Stipek, S., Mikulikova, L., & Popov, P. (2001). Oxidative stress, metabolism of ethanol and alcohol-related diseases. *J Biomed Sci* 8, 59–70.