Published in final edited form as:

Alcohol Clin Exp Res. 2013 August ; 37(8): 1253–1260. doi:10.1111/acer.12109.

The good, the bad and the ugly with alcohol use and abuse on the heart

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Abstract

Since its advent, alcohol has been utilized throughout history socially, for rituals, worship, for its therapeutic, antibacterial, and analgesic properties. In moderation, alcohol consumption and its use are generally viewed as clinically beneficial. Excessive alcohol consumption on the other hand has been recognized as having several adverse implications. Excessive use increases the risk of liver and heart disease, metabolic disturbances, nutritional deficiencies, certain cancers, brain damage, dementia, neuropathy, as well as other facets of morbidity and mortality. This review targets the sequelae of alcohol consumption on the heart, specifically on myocardial contractility, calcium channel signaling, and intracellular signaling pathways. With the incidence of alcohol-induced cardiac abnormalities being higher than previously thought, it is of increasing importance to elucidate the mechanisms behind them. Here the cardiac effects of alcohol were not discussed in isolation but in conjunction with other important factors, such as HDL and LDL levels and vascular dilatory influences. We explore these mechanisms, in particular, the oxidative stress as the major contributor, as well as pathways that may prove to be cardioprotective. As such, we demonstrate the involvement of Nuclear factor (erythroid-derived 2)-like 2 (NFE2L2/ NRF2) as well as Akt, that act as regulators of oxidative balance during oxidative stress responses. Thus, alcohol consumption may confer a cardioprotective effect when used in moderation through an Akt/NRF2-dependent mechanism.

Introduction

Excessive alcohol consumption continues to be one of the major causes of mortality and morbidity in the U.S., thereby necessitating the scrutiny of several studies assessing the dose-dependent effects of alcohol on the myocardium. Alcohol can stimulate or exacerbate a number of pathogenic disorders including but not limited to cardiomyopathy, liver injury, (breast, oral and GI) cancers, neuronal toxicity (Krenz and Korthuis, 2012), bone disease, Alzheimer's disease, and diabetes mellitus (Guo et al., 1998). This is generally a consequence of chronic alcohol use. On the other hand, other studies suggest that light to moderate alcohol use, defined as 1–2 drinks/day (Guo et al., 1998; Krenz and Korthuis, 2012), can have beneficial effects on an individual's overall health. This dichotomy lends itself to a discussion regarding the amount of alcohol consumed with the varying effects on health, and specifically cardiac function. This review highlights the molecular markers and mechanisms of alcohol metabolism that contribute to its toxic effects on oxidative homeostasis, and physiological function of cardiac tissue. It has been well noted that alcohol-induced oxidative stress mediates cellular and tissue damage and dysfunction (Guo et al., 1998).

Pathogenesis of Alcohol-Induced Cardiomyopathy

Research has shown an association between heavy alcohol intake and cardiomyopathy (Zakhari, 1999). In individuals with hypertension, the elevated afterload leads to hypertrophic cardiomyopathy. As cardiomyopathy worsens, the heart muscle weakens and becomes less efficient at distributing blood throughout the body, thereby increasing the likelihood of other sequelae such as cardiac arrhythmias and heart failure. Alcohol exacerbates this process by inducing hypertension through stimulation of the sympathetic nervous system, which is responsible for vascular constriction and increased contraction of the heart. Alcohol has also been shown to reduce the sensitivity of baroreceptors. Under normal conditions, baroreceptors respond to stretch caused by high blood pressure, triggering signaling to the central nervous system (CNS). In turn, efferent signals inhibit vascular contraction which leads to a reduction in blood pressure. In the presence of alcohol, baroreceptors tend to have reduced sensitivity, resulting in increased blood pressure (El-Mas et al., 2009) and possibly worsening hypertrophic cardiomyopathy.

The biochemical mechanisms of alcohol-induced cardiomyopathy also involve the disruption in cardiovascular metabolism. Specifically, high amounts of alcohol in the blood decrease oxygen supply to the heart, decreasing aerobic metabolism and increasing anaerobic metabolism (Ginter and Simko, 2008). As a result, there would be a reduction in the levels of adenosine triphosphate (ATP) (Ginter and Simko, 2008). Metabolites of alcohol, such as acetaldehyde, and reactive oxygen species also contribute to cardiomyopathy by reducing protein synthesis and expression (Guo and Ren, 2010). Alcohol consumption also increases the expression of the *c-myc* gene, which promotes apoptosis (Paice et al., 1996), and causes cardiomyocyte loss and further heart damage. In that regard, microarray studies performed on cardiac tissue from alcoholic human-males showed a down regulation in key extracellular matrix genes like titin, collagen type III, and calponin. These data allude to alcohol-induced etiology of heart failure (Haddad et al., 2008).

Although acetaldehyde has a short life-span, it can orchestrate significant cellular and tissue damage. It has been previously reported that acetaldehyde impairs endoplasmic reticulum (ER) calcium release and cardiac contractility. It has also been noted that acetaldehyde can activate a stress signal, c-Jun, via phosphorylation which triggers the onset of apoptosis. On the other hand, a beneficial aspect of acetaldehyde has been recently linked to its binding to the precursors of advanced glycation end products (AGEs) and forming stable complexes that are unable to activate AGEs. AGEs are known to induce extensive oxidative stress and inflammation in many organs. As such, acetaldehyde can prevent the harmful effects of AGEs to human health (Guo and Ren, 2010).

In cardiomyocytes, the mitogen activated protein kinase (MAPK) is activated in response to stressful stimuli. Components of this pathway such as the extracellular signal regulated protein kinases (ERK), and p38 have been heavily studied for their role in the MAPK signaling matrix as well as modulating gene expression. ERK1/2 working as a pro-proliferative mechanistic pathway is upregulated in immature cardiomyocytes and activated during the onset of cardiomyopathy. P38 has a dual role (1) acting in vivo to prevent hypertrophy and (2) acting to promote hypertrophy in other cases. We have shown that p38 and ERK1/2 work together to orchestrate a negative auto-regulation which diminishes with the induction of cardiac hypertrophy (Zhao et al., 2010). MAPKs overall regulate apoptosis. Alcohol-induced injury causes an onset of apoptosis which also contributes to other physiological and biochemical changes in the cardiovascular system. Global studies on rat pair-fed alcohol liquid diet as compared to control ones showed an elevation in MAPK and apoptotic genes in liver tissue (Bardag-Gorce et al., 2010). Unpublished data from our lab confirms a similar trend in MAPK signaling in chronic alcohol-treated heart tissue.

Cardioprotection Mechanisms of Alcohol

Benefits of moderate alcohol consumption include a reduction in the risk of developing coronary heart disease (CHD), congestive heart failure (CHF), intermittent claudication (IC), and myocardial infarction (MI) (Stockley, 2012). There is a 20–45% reduction in the risk of developing CHD which is causally related to moderate alcohol consumption compared with abstinence (Rimm et al., 1999). According to Krenz et al. (2012), the beneficial effects of light to moderate alcohol consumption prevented 12–14% of the observed CHD deaths each year among men aged 30–69 years. This emphasizes how vital it is to distinguish which levels of alcohol intake play a protective role rather than a harmful one as well as the mechanisms underlying these effects. Alcohol has been found to exert its actions by increasing high density lipoprotein levels (Ginter and Simko, 2008), fibrinolytic activity (Collins et al., 2009), interleukin 10, and protein kinase C ϵ (PKC ϵ). There is also an increase in myocardial blood flow, endothelial nitric oxide synthase (eNOS) and expression of heat shock protein 70, heme oxygenase-1 and manganese superoxide dismutase (MnSOD) associated with moderate alcohol consumption. It also decreases LDL oxidation, platelet aggregation (Collins et al., 2009; El-Mas et al., 2011; Krenz and Korthuis, 2012).

Moderate alcohol intake has been found to adjust cholesterol levels of HDL and LDL in several ways. Plasma HDL levels are increased due to alcohol which not only increases HDL-3 as once thought but also HDL-2. Both of these contribute equally and cooperatively to the overall efficiency of reverse transport of cholesterol (Schafer et al., 2007). Therefore alcohol intake may raise plasma HDL levels either by altering the synthesis or clearance of HDL or by effects on enzymes and proteins influencing HDL metabolism (Agarwal, 2002). There was a 16.8% reduction in CHD that is directly attributable to increased HDL from an intake of 30 g of alcohol per day (Rimm et al., 1999). Alcohol intake also alters HDL by increasing the plasma concentrations of apolipoprotein AI and apolipoprotein AII, which are the main components of HDL. Alcohol intake appears to increase HDL-C concentrations by increasing the transportation rate of these 2 major HDL apolipoproteins apoA-I and -II (De Oliveira E Silva ER et al., 2000). Low to moderate alcohol intake raises HDL in an approximately dose dependent manner and to a greater degree than currently available pharmacological options (Rimm et al., 1999). Alcohol has also been shown to reduce LDL oxidation through its antioxidative effects by hindering atherosclerotic plaque formation (Serafini et al., 2000). Human serum HDL-linked paraoxanase enzyme limits LDL peroxidation by preventing transformation of LDL into biologically active atherogenic particles. Fasting paraoxanase activity was higher after intake of wine, beer and spirits (Vasdev et al., 2006). Thus, moderate alcohol consumption increases HDL but decreases LDL, lowering the risk of CHD due to cholesterol. In addition to the benefits of moderate alcohol consumption, epidemiological and clinical evidence suggests that low alcohol consumption is also associated with reduced morbidity and mortality from CHD (Agarwal, 2002). People that completely abstain from alcohol consumption also have a higher mortality than those that consume light/moderate amounts of alcohol on a daily basis. Furthermore, clinical findings also suggest that low alcohol consumption also protects against peripheral artery disease and ischemic stroke. These outcomes were specifically found among people that drink light/moderate amounts of alcohol every day during meals, as opposed to binge drinking on a weekly basis (Di Minno et al., 2011).

Social drinking is correlated with low Lp(a) lipoprotein in middle-aged men and concluded that low Lp(a) lipoprotein concentration may be one factor explaining low mortality and retarded progression of coronary artery disease in social drinkers. Reduction of Lp(a) concentrations by light to moderate alcohol consumption may have a favorable effect on the atherosclerotic risk profile hypertensive patients thereby decreasing cardiovascular morbidity and mortality (Catena et al., 2003). Hemostatic factors are also affected by

moderate alcohol consumption. Plasma fibrinogen concentrations are decreased by moderate alcohol intake (Lacoste et al., 2001), as well as blood platelet aggregability (Ruf, 1999). Wine and wine phenolics antioxidants also affect platelet aggregation by inhibiting it (Agarwal, 2002). Moderate drinking also decreases plasma levels of C-reactive protein and fibrinogen. In support of that, there is also a decrease in blood homocysteine levels, blood pressure but an increase in fibrinolysis and coronary blood flow with moderate alcohol consumption (Collins et al., 2009). Accordingly, It was shown that a reduction of one drink per day in moderate and heavy drinkers led to approximately a millimeter of mercury reduction in blood pressure (Xin et al., 2001).

Gender Differences

It is important to note that epidemiological evidence suggests that the cardioprotective benefits by alcohol exclude men under the age of 40 and women that have not undergone menopause. There is also literature that suggests that the cardioprotective effects can only be obtained if the individual does not wait for middle age to begin drinking (Wannamethee and Shaper, 2002). In addition, the putative advantages could be exaggerated based on different health accounts and lifestyles (Wannamethee and Shaper, 2002). Research does however suggest that moderate alcohol consumption among men and women reduces the risk of heart disease (Mukamal et al., 2005), where moderate alcohol consumption in men was considered two drinks or less, and moderate in women as one drink or less each day. Of note, it has been shown that women are more likely to develop alcohol-induced cardiomyopathy. Similar research also suggests that alcohol consumption for both men and women is related to a decreased risk of myocardial infarction, when alcohol intake occurs 3–4 days a week (Mukamal et al., 2005).

Calcium Signaling and Inotropics in Cardiac Myocytes

Several mechanisms have been identified to explain the detrimental effects that alcohol has on the heart muscle. Excessive alcohol reduces the functionality of actin and myosin, contributing to a decrease in sarcomeric stroke power, and thus, stroke volume and cardiac output (Preedy et al., 1997; Vary and Deiter, 2005). The reduction of cardiac protein synthesis is especially prominent in alcoholics with hypertension (Preedy et al., 2003). In addition, calcium is involved in both the cellular shortening as intracellular calcium concentrations increase, but also relaxation as calcium is pumped out of the cell and back into the SR mainly through SERCA2a. Alcohol reduces the permeability of the sarcoplasmic tubules, causing a reduction in calcium release, and a subsequent decrease in cardiac muscle contraction (Thomas et al., 1996). Clinically relevant concentrations of ethanol induced elevation of intracellular calcium (Brown et al., 1996; Solem et al., 2000). It was shown that the density of dihydropyridine binding sites and the $I_{Ca,L}$ flux were reduced in the heart of ethanol-consuming (chronic) compared to control rats (Brown et al., 1996; Solem et al., 2000). Alcohol effects on $I_{Ca,L}$ have been shown to be regulated by protein kinase C (PKC) isozymes (Hajnoczky et al., 2005). On the other hand, acute exposure to ethanol has been shown to elicit a concentration-dependent depression in cell shortening and intracellular calcium (Brown et al., 1999). The same group also reported that short-term exposure to acetaldehyde (the first metabolic product of ethanol) depressed cell shortening amplitude, maximal velocity of shortening/re-lengthening and intracellular calcium clearing (Brown et al., 1999).

Moderate alcohol consumption also increases several signaling molecules such as PKC ϵ

Acute (Chen et al., 2000) and chronic (Zhou et al., 2002) cardioprotection stimulated by physiological concentrations of alcohol were associated with selective activation of PKC ϵ . This is recognized as a powerful cyto-protectant in myocytes through interactions with the substrates and anchoring molecules in numerous compartments. Localization of PKC ϵ to mitochondrial fractions is 3-fold greater after ischemia reperfusion in the hearts of alcohol-fed mice compared to controls. These data suggest that alcohol pretreatment promotes rapid recruitment of PKC ϵ during stress to heart mitochondria. PKC ϵ aids the myocardium in resisting ischemic damage. One way PKC ϵ resists ischemic damage is through the mitochondrial ATP-sensitive potassium channel, which has been identified as the putative effector of classic cardioprotection (Zhu et al., 2000). PKC ϵ antagonists prevent the opening of mitochondrial K_{ATP} channels and abolish cardioprotection. PKC ϵ knockout mice are resistant to ischemic preconditioning and unresponsive to the cardioprotective effect of alcohol (Lucas et al., 2005).

Alcohol Induced Oxidative Stress

Oxidative stress is a pathological condition in which normal protective antioxidant mechanisms are unable to prevent a build-up of reactive oxygen species (ROS), free radicals which can be produced in a number of physiological as well as pathological processes (Lucas et al., 2005; Wu et al., 2006). Chronic as well as acute alcohol exposures increase the generation of ROS. Studies have shown roles for cytochrome P450, cytochrome C, xanthine oxidase, and NADPH oxidase in generating the ethanol-induced increases in ROS (Lucas et al., 2005; Wu et al., 2006). Once present, ROS may lead to a number of downstream effects including structural changes in DNA, activation or inhibition of genes related to growth and cell death, increased cell motility, vasodilation, and angiogenesis. These changes are mediated by several key regulators that interact with the effector molecules phosphatidylinositol-3 kinases/protein kinase B (PI3K/AKT). In the PI3K/AKT pathway there are downstream mediating transcription factors, Forkhead box O (FoxO), that modulate cellular homeostasis including cell cycle arrest through p21, p27, apoptosis through FasL and Bim, and oxidative stress resistance through MnSOD (Ma and Wang, 2012). FoxO 1 and 3 are expressed in the heart defending against oxidative stress through the upregulation of antioxidant proteins and activation of survival pathways (Akhtar et al., 2012). A new potential key player, Nuclear factor (erythroid-derived 2)-like 2 (NFE2L2/NRF2), the central regulator of antioxidant machinery, may also act in alcohol-induced cardiomyopathy. Through other parallel studies we have discovered that NRF2 (as discussed later) can serve as a metabolic switch in regulating the downstream targets of the antioxidant response elements (ARE), energy (mTOR) and cell survival pathways (PI3K/AKT). The details of the interplay between NRF2 and mTOR/PI3K as well as AKT during alcohol-induce cardiomyopathy remain unanswered.

Alcohol metabolism requires two steps. First, ethanol is converted to acetaldehyde by alcohol dehydrogenase (ADH). The ADH enzyme is not produced in cardiac tissue; however, aldehyde dehydrogenase (ALDH2), which completes the second step converting acetaldehyde to acetate, is expressed in the heart (Krenz and Korthuis, 2012; Wu et al., 2006). During these enzymatic steps NAD is employed as a required cofactor, a hydrogen acceptor, which plays a role in alcohol-induced organ damage. Increased levels of NADH can induce an increase in super oxide (O₂⁻) free radicals leading to lipid peroxidation and damage to mitochondrial DNA. Once the oxidative balance is disturbed this will lead to tissue damage. As a side note, ethanol exposure leads to a decrease in the NAD⁺/NADH

ratio in mitochondria, which inactivates sirtuin-3 (SIRT3). It is the inactivation of SIRT3 that leads to ALDH2 activation in the heart (Cahill and Redmond, 2012).

Acetaldehyde is toxic in cardiomyocytes, causing uncoupling of excitation and contraction, so ALDH2 is critical for normal cardiac function, but it also helps to reduce the accumulation of ROS (Cahill and Redmond, 2012; Ma et al., 2010). This reduction of ROS by ALDH2 is also involved in mediating the vasorelaxation benefit of chronic alcohol exposure. This vasodilation is mostly due to increases in circulating nitric oxide (NO) produced by endothelial cells and induced forms of nitric oxide synthase (eNOS and iNOS respectively), which are activated by decreased ROS accumulation that can be attributed to ALDH2 (Cahill and Redmond, 2012). This interplay contributes to the regulation of oxidative stress. The acetaldehyde-independent mechanisms partly involve the fatty acid ethyl esters (FAEEs) which disrupt the physiological function of the heart. After excessive alcohol consumption, the concentration of FAEEs increases by 115,000-fold over normal levels in the myocardium. FAEEs interrupt the respiratory exchange of oxygen, impair mitochondrial function, and enables inefficient energy production (Bora et al., 1996).

Nitric oxide (NO) has a significant role in ischemic injury. Alcohol-mediated cardioprotection is partly achieved through up-regulation of eNOS expression. Moderate alcohol consumption increased eNOS protein expression in the vascular endothelium. Total nitrates and nitrites were increased in the blood of rats after 8 weeks consumption of moderate alcohol, suggesting a functional eNOS protein increase (Abou-Agag et al., 2005). NO may also function in developing an anti-inflammatory phenotype. Plasma levels of nitrite/nitrate, a marker for NO production, are increased during the period of alcohol exposure. Ethanol also has direct effects on the coronary vasculature to increase myocardial blood flow. Ethanol-induced vasodilation occurs as a result of NO generation secondary to increased NOS expression and activity and via activation of transient receptor potential vanilloid 1 (TRPV1) channels on perivascular sensory nerve terminals, which subsequently release the potent vasodilator calcitonin gene related peptide (CGRP), thereby increasing coronary blood flow (Abou-Agag et al., 2005).

Alcohol-Induced Apoptosis

A mutant allele of ALDH2 is known to occur in humans (ALDH2*2); it confers a significant reduction in ALDH2 enzyme activity (Chen et al., 2010). Given the known cardioprotective benefits of ALDH2, alcohol exposure would be expected to have more detrimental effects in carriers of the ALDH2*2 mutation. Indeed, ALDH2 knock-out mouse models showed increased sensitivity to ethanol, not only was the expected acetaldehyde toxicity seen in the form of contractile dysfunction and mitochondrial damage, but another study also showed that loss of function at that locus lead to an up-regulation of protein phosphatase (PP2A), possibly leading to decreased phosphorylation of Akt, a key anti-apoptotic protein (Chen et al., 2000; Ma et al., 2010). Insofar as reduced expression of ALDH2 proved to be detrimental, overexpression in mouse models had a positive effect on Akt and other anti-apoptotic signaling molecules (Chen et al., 2000) and rescuing chronic alcohol intake-induced cardiomyopathy and contractile dysfunction (Doser et al., 2009). Alcohol decreases both the Akt and mitogen activated protein kinase (MAPK) anti-apoptotic pathways in cardiomyocytes (Li et al., 2006). Meanwhile, lower doses of ethanol have been shown to activate the PI3-Kinase/Akt pathway in H9C2 cardiomyocyte cells in culture (Ma et al., 2010; Zhou et al., 2009). In these cases, the p44/42 MAPK seems to be activated by alcohol and acts synergistically with Akt (Markou et al., 2011). Acute alcohol exposure has been shown to cause early activation of the Akt pathway, but as the exposure becomes chronic there is a shift to deactivation (Ma et al., 2010). However, high-doses of alcohol induce apoptosis in a dose-dependent manner even if the exposure is acute. Reduced PI3-Kinase

signaling has been implicated as a mechanism of alcoholic injury, while activation of the same pathway has been shown by our lab and others to mediate survival benefits (Chen et al., 2000). However, a clinical study has shown that Akt activation is also increased in patients with alcohol-induced chronic heart failure, indicating a maladaptive impact of long term Akt activation (Markou et al., 2011). The mitochondrion has been demonstrated to be a vital part of the various pathways to apoptosis, and some studies have shown that ethanol may be able to affect apoptosis at the mitochondrial level. The opening of the mitochondrial permeability transition pore (mPTP) is an important step in induction of mitochondrial dysfunction that leads to the activation of apoptotic signaling cascades. Glycogen synthase kinase 3 beta (GSK-3 β) activation is a key step required to open the mPTP (Ma et al., 2010; Zhou et al., 2009). Low-doses of ethanol-induced activation of the PI3-Kinase/Akt pathway leads to inactivation of GSK-3 β and consequently reduces opening of the mPTP in H9C2 cells (Zhou et al., 2009). Interestingly, this interaction seems to be involved in one of the most widely described myocardial benefits of chronic moderate alcohol, cardioprotection from ischemia reperfusion (I/R) injury (Zhou et al., 2009).

Alcohol-Induced Ischemic Preconditioning

It was recognized that brief ischemia induced shortly before a larger ischemic event reduced the extent and severity of I/R injury, a phenomenon of ischemic preconditioning (IPC) (Veighey and Macallister, 2012). Several mechanisms have been implicated in IPC including MAPK signaling, ROS generation and NO response, and desensitization of the mPTP. As previously discussed, these are also players in mediating the action of alcohol in the heart. Indeed, studies have shown that chronic moderate alcohol consumption protects from I/R and also suggest that it prevents or improves outcomes following myocardial infarction (MI) (Chen et al., 2010; Collins et al., 2009; Krenz and Korthuis, 2012). Further investigation indicated that alcohol-induced IPC may be characterized by early and late phases which involve different mechanisms. While the early phase involves natively expressed cellular response pathways such as those that deal with ROS, the late phase is characterized by de novo expression of proteins as a downstream result of ethanol's influence on signaling pathways and transcription factors (Krenz and Korthuis, 2012). Protein kinase C epsilon (PKC ϵ) is involved in both early and late phases of ethanol induced IPC; PKC ϵ antagonists could negate the IPC effects and PKC ϵ agonists could induce IPC even in the absence of alcohol (Chen et al., 2010; Collins et al., 2009). Mitochondrial potassium ATPase channels are stimulated by PKC ϵ which seems to be a key factor in ethanol induced IPC possibly by preventing the opening of the mPTP (Lucas et al., 2005). Other factors (NOS, calcitonin gene related peptide, and heme oxygenase) seem to be involved in mediating the ethanol-induced microvasculature effects that contribute to IPC (Collins et al., 2009). Long-term moderate alcohol exposure has been shown to increase NO production via increased expression of NOS (Cahill and Redmond, 2012; Collins et al., 2009; El-Mas et al., 2011; Krenz and Korthuis, 2012). Most studies implicate endothelial NOS (eNOS), but there is some controversy suggesting inducible NOS (iNOS) and neuronal NOS (nNOS) (El-Mas et al., 2011). In any event, the NO produced causes decreased blood pressure and, early in ischemia, induces reactive hyperemia, both of which have been shown to reduce I/R injury (Cahill and Redmond, 2012; Collins et al., 2009; El-Mas et al., 2011). Caspase 3, which is involved in carrying out apoptosis, has also been implicated in mediating tissue damage following I/R (Hajnóczky et al., 2005). As discussed earlier, alcohol exposure has negative impacts on the function of the mitochondria and leads to the accumulation of ROS. Following these events, release of cytochrome c activates caspases leading to DNA fragmentation and apoptosis. Only a small percentage of alcohol treated cells in culture were apoptotic at any given time, however, even a small amount of apoptosis can have a very detrimental effect since cardiomyocytes are terminally differentiated and do

not replicate. Thus, anti-apoptotic processes may indeed be critically beneficial to the long-term cardiac function, as in low alcohol exposure.

Discussion and Future Directions

Although excessive alcohol consumption has been shown to exhibit deleterious effects on cardiac muscle, light to moderate alcohol consumption has been found to have numerous cardioprotective actions. Many studies have examined the relationship between a persons' alcohol consumption and alcohol related morbidity and mortality. Long term alcohol abuse on cardiac muscle can cause many problems including decreased contractility and stroke volume, with the development of cardiomyopathy, cardiomegaly, and heart failure (Preedy et al., 2003). Interestingly, low alcohol consumption, 1–2 drinks, seems to have beneficial effects. However, the type of drink, age, gender, and weight, are all important factors when discussing levels of alcohol consumption. Several hypotheses have been proposed for the low-dose beneficial cardiovascular effects. Understanding the mechanisms involved in shifting the alcohol paradigm from its advantageous to its hazardous outcomes on the heart will help prevent the harmful and fatal progression in alcoholics.

Therefore, there are also gender differences that should be considered in assessing consumption parameters. Mortality rates among men that are moderate drinkers are much lower compared to men who do not drink at all. Low and moderate drinking was associated with a reduced risk of cardiomyopathy, stroke, and heart disease with epidemiological studies suggesting that heavy drinking worsens all of the previously stated conditions (Zakhari, 1999). There is a dose-response curve that is depicted as a J or U shaped model, relating the amount of alcohol consumption with coronary heart disease (CHD). The risk of developing CHD is lower in individuals that consume low and moderate amount of alcohol, and higher when amount of alcohol consumption is increased (Agarwal, 2002).

Recent studies from our laboratory connect the NRF2 antioxidant molecular switch with interaction from alcohol-induced oxidative stress. In Figure 1, we depict a proposed mechanistic interaction between NRF2 signaling in concert with AKT/PI3K through mTOR. During low alcohol exposure, an oxidative burst occurs activating the AKT/PI3K pathway and simultaneously the complex between NRF2 and Keap1. They are cleaved by mTOR allowing NRF2 to enter the nucleus and interact with antioxidant response elements (ARE) that will induce expression of antioxidant machinery (including phase II antioxidant enzymes). Preliminary data shown in Figure 2, demonstrates that during chronic high alcohol consumption there is a decrease in NRF2 expression and its homolog NRF1. However, low alcohol exposure increases their expression. This data suggests that chronic low alcohol exposure has beneficial effects on oxidative balance and is dose-dependent on its effects on NRF2/NRF1 expression. These data also correlate with AKT expression which is upregulated with chronic low alcohol and downregulated with high alcohol exposure (Figure 2). These results (and other unpublished data) suggest that NRF2 regulate the downstream targets of the ARE, energy (mTOR) and cell survival pathways (PI3K/AKT). We see these interactions occur during dose-dependent chronic alcohol-induced oxidative stress responses. However, further examination of key players in this paradigm must continue to be evaluated.

Acknowledgments

This work was supported in part by grants 1 R15 AA019816-01A1, GM08016-38 NIGMS/NIH, and 2G12 RR003048 RCMI, Division of Research Infrastructure to GEH.

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Figure 1. The proposed mechanistic pathways mediated by Nrf2 system on alcohol-induced cardiomyopathy

Keap1 sequesters Nrf2 in the cytoplasm by binding to its amino-terminal regulatory domain allowing Nrf2 to translocate to the nucleus. In the nucleus, Nrf2 binds to the ARE. As protective machinery, Nrf2 activation promotes the expression of a variety of key anti-oxidative enzymes that scavenge reactive oxidative species and attenuate oxidative stress. Binding of Nrf2 to the ARE activates the Nrf2-targeted phase II detoxification enzymes, HO-1, NQO1, SOD and GCL, which protect from oxidative stress-induced abnormalities. Nrf2 also activates the PI3K/AKT/mTOR signaling which are the key regulators in cell protein synthesis, cell growth, and cytoskeletal modeling. The protein kinase, AMPK also phosphorylates and enhances the activity of mTOR which in turn signals the Nrf2. Subsequently, Nrf2 enhances insulin signaling components, such as GSK-3 or mTOR, which in turn can promote Nrf2 function by regulating its content and nuclear location by a feedback loop mechanism. Keap1: Kelch-like ECH-associated protein 1; Nrf2: Nuclear factor (erythroid-derived 2)-like 2; ARE: Antioxidant response element; HO-1: Heme oxygenase-1; NQO1: NAD(P)H dehydrogenase, quinone 1; SOD: superoxide dismutase; GCL: Glutamate cysteine Ligase; PI3K: phosphatidylinositol 3-kinase; Akt, also known as

protein kinase B; mTOR: Mammalian target of rapamycin; S6K1, ribosomal S6 kinase 1; AMPK:AMP-activated protein kinase.

Figure 2. Effects of dose-dependent chronic alcohol exposure on oxidative stress biomarkers
 Real-time PCR was performed on RNA extracts from rat cardiac tissue. The $\Delta\Delta\text{CT}$ values were derived from the equation ($\Delta\Delta\text{CT} = \Delta\text{CT}_{\text{reference}} - \Delta\text{CT}_{\text{Target}}$) to retrieve relative expression for each condition Low Alcohol (5mM ethanol) and High Alcohol (100mM ethanol) as compared to control values. The biomarkers examined in this figure were NrF2: Nuclear factor (erythroid-derived 2)-like 2, NrF1: Nuclear factor (erythroid-derived 2)-like 1, and Akt, also known as protein kinase B. A T-test was performed to evaluate significance of the study. The “*” indicates a p value of 0.05.