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OXIDANTS, ANTIOXIDANTS AND ALCOHOL: IMPLICATIONS FOR SKELETAL AND CARDIAC MUSCLE

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1. ABSTRACT

The chronic form of alcoholic skeletal myopathy is characterized by selective atrophy of Type II fibers and affects up to two thirds of all alcohol misusers. Plasma selenium and alpha-tocopherol are reduced in myopathic alcoholics compared to alcoholic patients without myopathy. Plasma carnosinase is also reduced in myopathic alcoholics, implicating a mechanism related to reduced intramuscular carnosine, an imidazole dipeptide with putative antioxidant properties. Together with the observation that alcoholic patients have increased indices of lipid peroxidation, there is evidence suggestive of free radical (i.e., unpaired electrons or reactive oxygen species) mediated damage in the pathogenesis of alcohol-induced muscle disease.

Protein synthesis is a multi-step process that encompasses amino acid transport, signal transduction, translation and transcription. Any defect in one or more of the innumerable components of each process will have an impact on protein synthesis, as determined by radiolabelling of constituent proteins. Both acute and chronic alcohol exposure are associated with a reduction in skeletal muscle protein synthesis.

Paradoxically, alcohol-feeding studies in rats have shown that the imidazole dipeptide concentrations are increased in myopathic muscles though alpha-tocopherol contents are not significantly altered. In acutely dosed rats, where protein synthesis is reduced, protein carbonyl concentrations (an index of oxidative damage to muscle) also decline slightly or are unaltered, contrary to the expected increase.

Alcoholic cardiomyopathy can ensue from heavy consumption of alcohol over a long period of time. The clinical features include poor myocardial contractility with reduced left ventricular ejection volume, raised tissue enzymes, dilation of the left ventricle, raised auto-antibodies and defects in mitochondrial function. Whilst oxidant damage occurs in experimental models, however this issues remains to be confirmed in the clinical setting. In the rat, circulating troponin-T release increases in the presence of ethanol, a mechanism ascribed to free radical mediated damage, as it is prevented with the xanthine oxidase inhibitor and beta-blocker, propranolol. However, whist propranolol prevents the release of troponin-T, it does not prevent the fall in whole cardiac protein synthesis, suggestive of localized ischemic damage due to ethanol.

2. INTRODUCTION

Approximately half of chronic alcohol misusers have difficulties in gait, reduced muscle strength, and loss of total lean tissue, giving rise to the entity of alcohol-induced muscle disorders (AIMD). Although AIMD are more common than hereditary conditions of muscle disease (Duchenne muscular dystrophy, for example), the mechanisms for AIMD are poorly understood. Between one and two thirds of chronic alcoholics have skeletal muscle myopathies, making AIMD possibly the most prevalent skeletal muscle disorder (reviewed in (1-4)). Furthermore, excessive alcohol consumption also perturbs the function and metabolism of cardiac muscle: up to one third of chronic alcohol misusers will have some form of cardiovascular impairment (5-7). These figures are quite staggering, considering that very little text is devoted to these problems in standard books on skeletal muscle or cardiology. In this review, we draw critical attention to the features of AIMD and alcoholic cardiomyopathy and raise the possibility that these may be mediated by reactive oxygen species (ROS). With regard to alcoholic cardiomyopathy, we describe *in vivo* studies using a specific plasma marker of cardiac damage, i.e., plasma troponin-T.

3. ALCOHOLIC SKELETAL MUSCLE

In the ensuing paragraphs the nature of the pathogenic lesions in skeletal muscle in response to ethanol are described. Evidence suggests that, currently the only factors to distinguish myopathic alcoholics from non-myopathic alcoholics include differences in plasma alpha-tocopherol, selenium and carnosinase activities. Experiments are subsequently described to test the hypothesis that these variables contribute to the myopathy and the ethanol-induced reductions in protein synthesis that are initial events in the pathogenesis of AIMD.

3.1. The features of alcoholic muscle disease

Quantitative histomorphometry in proximal muscle biopsies from alcoholic subjects have shown that in approx. one half to two thirds of subjects, the diameter of fast-twitch or Type II skeletal muscle fibers (i.e., those with anaerobic glycolytic metabolism) are reduced. The Type IIb fiber subsets (which have few or no mitochondria) are particularly affected. The intermediate Type IIa fiber subsets, and especially the slow-twitch or Type I fibers (aerobic oxidative), are relatively resilient. Inflammation and fibrosis are not commonly found in biopsies and occasionally enhancement in lipid deposition may occur. There is no overt membrane damage, and plasma creatine kinase activities are generally unaltered, though an increase is seen when an acute muscle lesion is superimposed. In general, all muscle groups appear to be affected as indicated by reductions in either mid-arm circumference or urinary creatinine excretion/height ratio. The overall consequences of these changes are difficulties in gait, reduced muscle strength and myalgia, with the concomitant potential for impaired post-operative recovery and compromised immune response due to lost lean body reserves. Rhabdomyolysis is infrequent in alcoholics and occurs in less than 5% of chronic ethanol misusers (1-4).

The myopathy is not directly related to neurological involvement, endocrine imbalance (including defects in cortisol status), nor overt liver disease (reviewed in (1-4)). Assessment of folate, pyridoxine, riboflavin, thiamine, vitamin B12, vitamin D and general food intakes indicates that, tentatively, the myopathy is not nutritional in origin (1-4). However, compared to either non-misusing controls or non-myopathic alcoholics, serum selenium and alpha-tocopherol concentrations are lower in myopathic alcoholics (8). Neither beta-carotene (pro-vitamin A), retinol, copper nor zinc are significantly altered in serum of alcoholics with myopathy (8). Myopathic alcoholics also have reduced carnosinase activities compared to non-myopathic alcoholics or non-misusing controls. Although there may be a neurological basis for the alterations in plasma carnosinase activities (9), the enzyme catalyses the hydrolysis of the substrate carnosine and imidazole dipeptides such as anserine. These imidazole-containing compounds are important anti-oxidants (see below for details). Muscle carnosine contents can be influenced by dietary carnosine concentrations as well as amino acid deficiencies, particularly histidine.

It is not unreasonable to forward a hypothesis that encompasses the concept of free radical mediated damage for the following reasons: (i) selenium and alpha-tocopherol are dietary antioxidants, (ii) carnosine is an important antioxidant in skeletal muscle and (iii) deficiencies of either selenium or alpha-tocopherol can induce myopathic lesions. In the following paragraphs, we deal with each of these points. Other detailed features of alcoholic skeletal muscle disease have been described elsewhere (1-4).

3.2. Antioxidant properties of selenium

Selenium is a cofactor in a number of enzyme systems, especially glutathione peroxidase. This selenoenzyme catalyses the reduction of H_2O_2 and organic hydroperoxides. Glutathione peroxidase is considered the major detoxification enzyme for H_2O_2. In the reaction, reduced glutathione (GSH) is oxidized to the disulphide (GSSG). The enzyme is located in both the cytosol and mitochondria. There is a correlation between reduced plasma selenium and depressed glutathione peroxidase activities (10).

3.3. Antioxidant properties of alpha-tocopherol

There are four different isomers of tocopherol, alpha, beta, gamma and delta: the alpha form is the most potent tocopherol. One of the most characterized features of alpha-tocopherol is its ability to prevent the initiation and propagation of lipid peroxidation by scavenging free radicals. Lipid peroxidation is important in muscle as it modifies the membranes of distinct subcellular organelles, such as the mitochondrion, lysosome or the sarcolemma, with implications for membrane permeability or fragility (11). Plasma levels of alpha-tocopherol are used indirectly to reflect tissue levels, including muscle. With regard to the latter, the most abundant source of alpha-tocopherol is skeletal muscle, by virtue of the fact that it is approx. 40% of body weight. The total content per organ (units per organ) must not be confused with the concentration (units

per g wet weight): the concentration of alpha-tocopherol in hepatic tissue is higher than skeletal muscle, but the liver represents a lower proportion of body mass than muscle.

3.4. Effects of selenium and alpha-tocopherol deficiencies on skeletal muscle

Dietary deficiencies of alpha-tocopherol or selenium cause skeletal muscle myopathy, though in many studies the deficiency of both selenium and alpha-tocopherol has been combined experimentally. Deficiencies of either alpha-tocopherol or selenium also occur naturally. For example, *White muscle disease* (WMD) is seen in farm animals and arises as a consequence of a combination of inadequate soil selenium and enhanced oxidation of alpha-tocopherol in foodstuffs or diets poor in alpha-tocopherol (12-20). In animals with WMD, alpha-tocopherol and selenium contents of affected muscle are reduced with consequential reductions in glutathione peroxidase in plasma (15). Deliberate selenium and alpha-tocopherol deficiencies have been established, for example for 1 year in calves (21). This treatment produces elevations in plasma creatine kinase activity and defined myopathic lesions (21).

Selenium deficiency for 2 months reduces skeletal muscle (gastrocnemius) glutathione peroxidase levels in rats by over 80% (10). In general, however, the effects of selenium deficiency alone are less convincing. Induction of alpha-tocopherol deficiency in rats 20 days after birth induces structural lesions at the electron microscopic levels in muscle after 6, 12, 18 and 24 months (22). However, in the same study, no comparable lesions were observed in selenium deficiency (22). In terms of biochemical indices, a similar lack of effect was recorded by Walsh *et al.,* (21) who demonstrated that selenium deficiency had no effect on muscle TBARS (thiobarbituric acid-reactive substances), though a combination of both alpha-tocopherol and selenium deficiency raised muscle TBARS. However, in interpreting the assay of TBARS, it is important to define the nature of the lesion induced by dietary anti-oxidant deficiencies. In many instances, muscle damage is distinguished in terms of morphological features at the light- or electron-microscopic level, which are less sensitive than biochemical indices of tissue damage.

3.5. Measuring the effects of free-radical damage

From the paragraph above, it is apparent that some consensus should be achieved in identifying criteria for characterizing the nature of the lesions caused by oxidative damage. In many studies, changes in free radical scavenging enzyme activities are measured without consideration of their functional effects on muscle. A good example of how important this approach is relates to a study that induced selenium deficiency for 2 months, reducing muscle glutathione peroxidase activities (10). This had no appreciable effects on endurance capacity (exercise triggers an increase in the generation of ROS) (10). Muscle is unique as it has two functional roles, providing mechanical activity as well as a dominant role in nutritional metabolism. It seems pertinent then, that the synthesis of the muscle proteins should remain optimal (for example, continual renewal of the contractile proteins to maintain adequate mechanical activity). Protein synthesis is therefore a good parameter to assess muscle (skeletal or cardiac) damage in alcohol toxicity, especially as it embraces a number of processes, such as amino acid transport, nucleotide availability, etc.

3.6. A hypothesis to explain the myopathy

To explain the mechanism of the myopathy, two criteria must be met. The myopathy must be explained by (i) defects in protein synthesis and/or protein degradation; (ii) differential changes that emphasize the greater sensitivity of Type II fibers. With respect to both of these factors, this differential sensitivity must be expressed in the final stages of the pathway.

3.7. Animal studies of alcohol misuse and effects on protein synthesis

Biochemical analysis of individual skeletal muscle fibers is technically demanding, and there are no published studies on protein synthesis in individual human muscle fibers. To resolve this problem, one must turn to laboratory animal experiments, as these provide excellent models for alcohol toxicity studies on smooth, cardiac and skeletal muscle (4,23-25). The rat possesses a number of anatomically distinct skeletal muscles containing a predominance of particular fiber types. The plantaris contains a predominance of Type II fibers, whilst the soleus contains mainly Type I fibers. The gastrocnemius is taken to represent the entire musculature, though technically Type II fibers predominate within this muscle. With reference to the gastrocnemius, we have shown that the response of this muscle to alcohol is intermediate between the responses of the plantaris and soleus muscles. In response to chronic ethanol feeding, the changes in weight of the gastrocnemius are exactly the same as either the entire skeletal musculature (assessed in eviscerated carcasses) or entire hind limb muscles (Paice, A G, unpublished observations). Hereafter, for brevity we have referred to the Type I soleus, the Type II plantaris and the mixed gastrocnemius.

Myopathic lesions in skeletal muscle can be reproduced in laboratory rats fed ethanol as 35% of total calories (i.e., Lieber-DeCarli regimen (4)). The weights of the Type II plantaris muscle are reduced, whilst the Type I soleus are relatively unaltered. Detailed analysis shows that the few Type II fibers within the soleus are preferentially affected, indicating that the myopathy selectively targets the fast-twitch, anaerobic fibers (1-4).

Clinical studies with stable isotopes have shown that rates of skeletal muscle protein synthesis in alcohol misusers are lower than the rates seen in non-alcoholic control subjects (26). Animal studies also show that ethanol reduces skeletal muscle protein synthesis in acutely and chronically ethanol-dosed rats, though most studies have focused on the acute model. Certainly, the relevance of this pertains to the fact that the precipitating event in ethanol exposure is a fall in protein synthesis. In the Type II plantaris, the decline in protein synthesis is greater than in the Type I soleus (1-4).

3.8. Oxidant damage and antioxidant status in skeletal muscles

There are three basic approaches to assess the impact of ROS in the etiology of disease processes in muscle. These are:

1. Assessment of ROS directly
2. Assessment of ROS damage, for example lipid or protein oxidation.
3. Assessment of protective mechanisms.

3.8.1. Assessment of ROS per se

There are a few studies examining free radicals directly in muscle disease, using ESR spin-trapping techniques, such as in magnesium deficiency (27,28). However, there are no comparable studies in alcoholic myopathy.

3.8.2. Assessment of ROS damage

A number of indices are used to appraise damage by ROS, particularly TBARS and MDA, reflecting lipid oxidation. There is a particular index of ROS damage to protein, i.e., carbonyl formation, but this has been used less frequently. Carbonyl protein composition has been measured in muscles of rats subjected to exhaustive exercise for 1-1.5 hours (29). These studies showed that protein carbonyl content was increased in rats fed a normal diet then exercised (29). This increase was not seen in exercised and alpha-tocopherol supplemented rats (29).

3.8.3. Assessment of protective mechanisms.

Higher amounts of alpha-tocopherol, and greater activities of catalase, glutathione peroxidase cytosolic and mitochondrial superoxide dismutase are seen in Type I skeletal muscle fibers, reflecting larger free radical formation via the respiratory chain (30-32). Other enzymes, as indicators of muscle pathology, have been examined including glucose-6-phosphate dehydrogenase, phosphogluconate dehydrogenase and glutathione reductase.

Of particular interest is the inverse relationship between the susceptibility to ethanol-induced reductions in protein synthesis and tissue alpha-tocopherol levels. This infers that alpha-tocopherol affords a protective mechanism against alcohol-induced reductions in protein synthesis. Below, we review some studies that have utilized either selenium or alpha-tocopherol as a myoprotective agent.

3.9. Protective effects of alpha-tocopherol and selenium

Administration of alpha-tocopherol appears to limit damage to muscle by ROS species, for example in thyroxine or exercise-induced injury. However, it is important to reiterate that there must be a clear definition of *damage*. Thus the protective effect of alpha-tocopherol is not apparent in exercise-induced injury to skeletal muscle as defined by elevations in the activities of muscle glucose-6-phosphate dehydrogenase (used to assess inflammation due to invasion of phagocytic cells), maximal tetanic force, number of intact fibers and raised plasma creatine kinase activities (11).

The hypothesis that the reductions in protein synthesis in alcohol toxicity are due to changes in lipid peroxidation has been tested in a rat model of ethanol dosage (75 mmol/kg body weight) (33). Despite a daily treatment regimen of alpha-tocopherol supplementation (30 mg/kg per day for 5 days), the acute dose of ethanol still reduces skeletal muscle protein synthesis (33). More recently, studies have shown that in Spanish alcohol misusers with myopathy, both serum and muscle levels of alpha-tocopherol did not influence the presence of skeletal muscle myopathy in chronic alcoholic patients (34). These observations contrast with a UK study, which showed reduced serum alpha-tocopherol and selenium in myopathic alcoholics, which may reflect geographical differences in antioxidant intake (8,34).

We have shown that 24 hours after an acute bolus of ethanol, there is a reduction in muscle protein synthesis (35). Protein carbonyl concentration at the end of this 24 hour period was not elevated compared to controls and may even be reduced (Reilly M, Personal communication). These data do not support a mechanism for ROS in mediating the acute changes in muscle pathology in alcohol toxicity.

3.10. Imidazole dipeptides

The histidine-containing imidazole dipeptides, carnosine and anserine, have a role in intracellular buffering (for example during high lactic acid production in glycolytic skeletal muscle). They occur in high concentrations in muscles, particularly Type II fiber-rich muscles. Additional properties include the ability to

(i) trap peroxyl radicals,
(ii) act as a reducing agent,
(iii) inhibit the oxidative hydroxylation of deoxyguanosine
(iv) chelate metal ions
(v) quench singlet oxygen (see also (36,37)).

We measured imidazole dipeptides in rat muscle after 6 weeks of either ethanol (as 36% of total calories) or isocaloric glucose feeding with the Lieber-DeCarli protocol (38). However, although we were able to show that ethanol feeding preferentially reduced the weight of the Type II fiber-predominant skeletal muscles, carnosine concentrations (micro-mol/g wet weight) increased in the Type II plantaris and total contents (micro-mol per muscle) were not changed (38). In soleus, imidazole dipeptide contents actually decreased after 6 weeks of alcohol feeding (38).

4.0. Alcoholic cardiomyopathy

As with alcoholic skeletal muscle myopathy, we first describe the pathogenic nature of the lesions in the heart due to ethanol misuse. However, it important to remember that there is comparatively little work on the influence of ROS on the heart muscle *per se*, compared to vascular changes. For a review on the effect of ethanol on the cardiovascular system, see (39).

4.1. Clinical features of alcoholic cardiomyopathy

Chronic ethanol consumption can develop into the disease state *alcoholic cardiomyopathy* (ACM), previously

known as *alcoholic heart muscle disease* (AHMD) for which no diagnostic criteria presently exist (40). In ACM there is historical or biochemical evidence of prolonged ethanol consumption for approximately 10 years or more at an average rate of 80 g/day, or a cumulative life time intake of 250 kg (5-7). Although the incidence of alcoholic cardiomyopathy is less than with alcoholic skeletal muscle disease, up to one third of chronic alcohol misusers have defects in contractility.

Features of ACM include striking elevations in tissue enzymes (for example alpha-hydroxybutyric dehydrogenase), marked contractile dysfunction, fibrosis, increased lipid deposits and lipofuscin staining (5-7). Electron microscopy studies on heart muscle from patients with AHMD have shown swollen or ovoid shaped mitochondria, with disruption or fragmentation of the cristae (5-7). Myofibrillary damage include loss of filaments and striations (41).

Various mechanisms have been proposed to account for the changes in the heart muscle in alcoholism, including changes in the synthesis of contractile proteins (25,42-45) and the induction of autoantibodies due to the formation of acetaldehyde-protein adducts (46). The involvement of lipid peroxidation and hence free radical mediated damage has also been hypothesized in the pathogenesis of ACM. Histological features of organelle damage are similar to those resulting from lipid peroxidation (47,48). Certainly, free radical mediated injury occurs in other cardiac pathologies, such as those resulting from selenium deficiency, catecholamine toxicity or adriamycin cardiomyopathy (49-51).

4.2. The involvement of free radical mediated damage

Tissues from patients who had died from acute ethanol consumption, with a previous history of chronic alcohol misuse, show increased myocardial lipopigment (also called "age pigments" as their density is enhanced with age) (52). Animal studies have shown more compelling evidence of ROS-induced damage whereby chronic alcohol consumption causes an increase in cardiac lipid peroxidation, the effects of which are more severe in animals with concomitant zinc deficiency (53,54). Evidence to support some element of a free-radical mediated event in the cause of AHMD has been obtained in studies showing that, in alcohol-treated rats, the shift in fatty acid profile was inhibited by dietary alpha-tocopherol (55). A decrease in cardiac creatine kinase (CK) activity after an acute dose of alcohol has been demonstrated, which was ascribed to damage by ROS (56). The implications of these findings are as follows: xanthine oxidase (XO), which generates superoxide radicals ($O_2^{-\cdot}$) also inactivates CK. CK can also be inactivated by hydrogen peroxide, though the mechanism of CK inactivation in adriamycin toxicity results from the activity of hydroxyl ($OH^{\cdot}$) radical (57). The importance of this relates to the central role of CK in cardiac energetics and contractility. Free radicals generated by XO are capable of reducing calcium uptake velocity in isolated sarcoplasmic reticulum, which contributes to excitation-coupling changes (58). Hydroxyl radicals also change the conformational properties of the contractile proteins themselves, as determined by electrophoretic analysis (59).

In one rat study, chronic alcohol consumption (as 18% of total calories) for 4 weeks was also shown to decrease CK activity (56); however, some clinical studies have shown a significant increase in CK activities in cardiomyopathic alcoholics with a history of heavy (a cumulative intake of greater than 250 kg alcohol) compared to light (a cumulative intake of under 250 kg alcohol) ethanol misuse (60). Clearly, the differences between these studies need to be reconciled. For example, the animal studies used non-alcoholic controls (56) whereas the clinical studies have examined *light* ethanol misusers (60). However, the chronic studies of Hininger *et al.* (56) did not use a pair feeding (i.e., *Lieber-DeCarli*) regimen and ethanol was administered in drinking water making the results difficult to interpret. In contrast, Klein et al., (61), employed careful attention to a pair-feeding regimen where rats were fed liquid diets containing ethanol as 35% of dietary energy for 10 weeks. Controls were pair-fed identical amounts of the same diet, in which ethanol was replaced by isocaloric glucose. However, the activity of cardiac CK was unaltered.

The reductions in cardiac CK activities due to acute ethanol dosage has been employed by to assess three agents, previously proposed to afford protection against free radical-mediated damage: allopurinol, desferrioxamine and propranolol (56). After 2 hours, ethanol alone reduced cardiac CK activities by 15-25%, which was not prevented by either allopurinol or desferrioxamine. Pre-administration of propranolol before acute ethanol caused an increase in CK activity in both control and alcohol treated rats (56). This property conferred by propranolol was thought to be due to the inhibition of XO activities, though propranolol is also a beta-blocker (56,62).

The reason why allopurinol did not prevent the reduction in CK activity requires some explanation as this agent is a well-characterized XO inhibitor. In rats treated with allopurinol alone, a decrease in CK activity was also observed, an event attributed to CK leakage (56). The *pro-oxidant* effects of desferrioxamine *in vivo* (as opposed to its superoxide radical scavenging activities *in vitro*) may explain why this iron-chelator was similarly ineffective in preventing the ethanol-induced reductions in cardiac CK activity (56).

4.3. Alcohol and cardiac ischemic damage

A strong correlation between alcohol intake and sudden cardiac death has been reported (63). During an eight year study, more than half of the patients studied for ischemic heart disease died from sudden cardiac death as a result of heavy alcohol drinking in comparison to light drinkers, implicating a direct effect of alcohol consumption (63). Other studies have also implicated an increase in mortality from ischemic heart disease because of heavy alcohol intake (for example, (64)). This phenomena may be related to rhythm disturbances. In ischemic heart disease, there are increased ectopic beats and arrhythymias

following controlled alcohol ingestion (65). Overall, these data show an increased vulnerability to alcohol in patients with pre-existing cardiac disease.

In vivo studies examining the short term effects of alcohol have reported decreased myocardial blood flow, increased vascular resistance and ECG disturbances (66). Long-term effects of ischemia on the heart include structural alterations to mitochondria, damaged myofibrils and sarcoplasmic reticulum, and an increase in lipofuscin granules. These features are similar to those seen in experimental ischemia in the rat (67). Thus it is not unreasonable to propose mechanisms involving ischemic heart muscle damage in the aetiology of alcoholic heart muscle disease and if this occurs, then a *re-perfusion* type injury will also ensue. It has been suggested that acetaldehyde may be instrumental in inducing myocardial ischemia and coronary vasospasm (assessed by both ECG and thallium 201 scintograms) after alcohol consumption (68). The abnormalities were not perturbed by treadmill exercise alone (68). Studies with troponin-T in the rat (described below) support a mechanism involving ischemia.

4.4. Release of troponin-T and effects of propranolol

The serum marker troponin-T has been successfully used to assess ischemic damage clinically, and we have shown that it increases in acute ethanol exposure (69). However, in the rat, the effects of an acute dose of ethanol can be prevented with propranolol, but not with alpha-tocopherol pre-treatment (69,70). Although propranolol is a beta-blocker, it reverses isoproterenol-induced ischemia by reducing lipid peroxides, lowers xanthine oxidase activity, and increases superoxide dismutase activity. In isolated myocardial preparations, propranolol prevents lipid peroxidation. These effects of propranolol appear to be independent of its beta-blockade activity. The reason why alpha-tocopherol had no effect in preventing the ethanol-induced increases in troponin-T merits comment. It is thought that propranolol acts via a different mechanism of chain-breaking antioxidant activity, compared to alpha-tocopherol (70).

Whilst troponin-T efflux is increased in response to ethanol, whole heart protein synthesis is reduced. Despite the fact that propranolol pre-treatment inhibits the rise in circulating troponin-T, it does not inhibit the fall in protein synthesis, in ethanol dosed rats (70). This suggests that the troponin-T is reflecting a local phenomenon of tissue pathology, a supposition supported by the observation that the proportion of troponin-T released into the circulation is less than 1% of total cardiac free troponin-T (70).

5. CONCLUSIONS

Mechanisms for the involvement of ROS damage in the etiology of alcohol-induced disorders in non-muscle tissues have frequently been proposed. There is also some evidence to indicate that ROS may similarly be involved in the pathogenesis of alcoholic myopathy and cardiomyopathy. We have defined the lesions in skeletal muscle in terms of defects in protein synthesis, though the mechanisms responsible for alcohol-induced heart muscle damage remain esoteric. However, despite the observation that serum levels of alpha-tocopherol are reduced in alcoholics, these observation are not reconciled with the fact that alpha-tocopherol loading studies are ineffective in ameliorating the acute effects of ethanol on protein synthesis. In addition, the protein carbonyl content is not elevated in acutely dosed rats. In chronic alcohol dosed rats, levels of the imidazole dipeptides in skeletal muscle are not reduced (and concentrations actually increase). Although the above do not implicate ROS in skeletal muscle damage, our data also show that the XO inhibitor propranolol reduces alcoholic heart muscle damage as reflected by troponin-T release. However, these alterations reflect local events as more global changes, such as reductions in heart muscle protein synthesis are unaffected by propranolol. Additional studies are clearly warranted as propranolol has other pharmacological properties such as beta-blockade.

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Abbreviations: AIMD: Alcohol-induced muscle disease; CAM: Chronic alcoholic myopathy; CK: Creatine kinase; ROS: Reactive oxygen species; AT: alpha-tocopherol (vitamin E); GSH: Reduced glutathione; GSSG: Oxidized glutathione; WMD: *White muscle disease*; TBARS: Thiobarbituric acid-reactive substances

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