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Alcoholism

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Abbreviations:
CuZnSOD: Copper Zinc superoxide dismutase
GSH: glutathione
MnSOD: manganese superoxide dismutase MEOS: microsomal ethanol oxidizing system
POBN: (4-pyridyl-1oxide)-N-t-butylnitrone
ROS: reactive oxygen species

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Abstract

Alcohol consumption can be both beneficial and detrimental to organisms. The small amounts of alcohol can be beneficial in the cardiovascular system. But chronic consumption will result in breakdown of organs and tissue leading to disease. Several enzymes found in the liver perform the metabolism, of alcohol or ethanol. Smaller quantities of alcohol can be metabolized by catalase in peroxisomes and cytochromes P450. Chronic exposure has been shown to increase oxidative stress through superoxide production and free iron leading to lipid peroxidation. The oxidative stress then can lead to damage of several tissue, the liver being the primary, and also the brain and pancreas. This paper will discuss the enzymes and antioxidants that lead to an increase in oxidative stress resulting tissue damage and disease.

Introduction

Most alcoholic drinks consist of ethanol (ethyl alcohol). Ethanol is soluble in water and many organic solvents. The solubility of ethanol enables it to cross the cell membrane and more importantly the blood-brain barrier [1]. A chronic consumption of alcohol can lead to the generation of oxygen free radicals and reactive aldehydic species. The metabolism of ethanol also leads to the generation of hydroxyethyl radicals that can be associated with stimulation of lipid peroxidation. The lipid peroxidation is associated with an increase in liver disease, development of hepatic fibrosis, and cirrhosis. The brain is particularly affected by this increase in lipid peroxidation because of its rich polyunsaturated fatty acid side chains and low amounts of antioxidants. The pancreas is also affected by an increase in ROS leading to the development of pancreatitis. The effects seen by an increase in prooxidants are enhanced by the reduction of many antioxidants like, glutathione (GSH), catalase, and Copper Zinc superoxide dismutase

(CuZnSOD). In experimental animal fed an ethanol diet an increase in lipid peroxidation products such as lipoperoxides and malondialdehyde (MDA) are observed. The decrease in several antioxidant proteins was also noted.

Chronic exposure to alcohol can affect many organs in the body. The liver can develop fibrosis and alcoholic liver disease, which accounts for many of the cirrhosis cases in the country. Cirrhosis is a chronic disease that causes destruction of the liver cells and loss of function. Loss of volume in the brain is also observed followed by memory loss, alter gene expression, induced brain atrophy can contribute to neuronal cell injury, which lead to neurodegenerative disorders.

Metabolism of Alcohol

Metabolism of alcohol is performed in the liver by alcohol dehydrogenase. The first step forms aldehyde ethanal (acetaldehyde) shown in reaction 1 [1]. This oxidation is coupled with the reduction of NAD^+. In this reaction the NAD^+ is then reduced to NADH.

$$\mathbf{CH_3CH_2OH + NAD^+ \rightarrow NADH + CH_3CH{=}O} \qquad \textbf{reaction 1}$$

An excess of reduced NADH can build up if large amounts of ethanol are consumed. The ethanal is rapidly metabolized due to the high toxicity by aldehyde dehydrogenase in the mitochondria as shown in reaction 2.

$$\mathbf{CH_3CHO + NAD^+ \rightarrow NADH + CH_3COOH + H^+} \qquad \textbf{reaction 2}$$

Another source of ethanol removal is by certain cytochromes P450. The oxidation activity is through a microsomal ethanol oxidizing system (MEOS). The ethanol-inducible cytochrome P450 is CYP2E1 and has been found in animals that were chronically exposed to ethanol. The K_m for the MEOS system is higher than that of alcohol dehydrogenase 8-10 mM compared to .2-2 mM respectively. Under normal conditions, alcohol dehydrogenase is probably responsible for the ethanol metabolism but when chronic consumption occurs CYP2E1 levels are seen to increase. The elevated levels of CYP2E1 increase the ethanol that is metabolized by CYP2E1 (reaction 3).

$$\mathbf{CH_3CH_2OH + NADP^+ \rightarrow CH_3CH{=}0 + NADPH} \qquad \textbf{reaction 3}$$

The ethanol metabolism by CYP2E1 is very leaky and is known to release superoxide and hydrogen peroxide increasing in oxidative stress seen in alcoholics. CYP2E1 is probably the main component to the increase in reactive oxygen species (ROS) and other species such as α-hydroxyethyl radical. Hydroxyethyl radicals can go on to form adducts with proteins.

Small quantities are seen in vitro to be oxidized by catalase in peroxisomes in the presence of hydrogen peroxide. This is not the case *in vivo,* because the levels of H_2O_2 are too low to induce the activity of catalase [1].

Ethanol-Induced Free Radical Mechanisms in the Liver

The liver is one of the main targets of toxicity in chronic alcoholism. Several reactive oxygen species are produced which are superoxide, hydrogen peroxide and hydroxyl radicals. In the presence of iron the hydroxyl radical can abstract a hydrogen to yield yet another radical,

1-hydroxyethyl radical [2]. Several studies have determined an iron overload does occur in chronic alcoholics [3]. Several theories are put forth to explain the increase in radical formation in the liver, one is the hepatocytes themselves, activated Kupffer cells and inflammatory cells are able to release reactive oxygen species [4]. The ROS can then go on to produce damage in DNA, lipids and protein through lipid peroxidation or oxidation of the ethanol [4] (Reaction 4 lipid peroxidation). The lipid peroxidation can go through a redox cycling can continue until a termination occurs.

$$\mathbf{LH + X\bullet \rightarrow L\bullet + XH}$$

$$\mathbf{L\bullet + O_2 \rightarrow LOO\bullet} \qquad \textbf{Reaction 4}$$

$$\mathbf{LOO\bullet + LH \rightarrow L\bullet + LOOH}$$

Superoxide can be formed by leakage from the electron transport chain. The decrease in NAD^+/NADH ratios can enhance the leak of superoxide, and this increase could also increase the susceptibility to lipid peroxidation in the mitochondrial liver [2]. The mitochondria is also responsible of the production of hydrogen peroxide and in the presence iron, hydrogen peroxide can generate aggressive radicals could result in the destruction of the mitochondria structure and function [2].

Xanthine oxidase contributes to the production of superoxide and hydrogen peroxide in the liver by oxidizing acetaldehyde. Xanthine oxidase is a superoxide-generating enzyme that is converted from the xanthine dehydrogenase and is a substrate for acetaldehyde. It also contains several iron-sulfur clusters that increase the production of superoxide and hydrogen peroxide. NADH inhibits xanthine oxidase, the decrease of NADH would favor the superoxide generating

activity [2]. The evidence that xanthine oxidase is involved in the increase lipid peroxidation production is determine when an inhibitor of xanthine oxidase is used. Lipid peroxidation was significantly reduced when animals were pretreated with the inhibitor.

Iron can increase the affects of the superoxide and hydrogen peroxide in lipid peroxidation. Iron reactions with superoxide and hydrogen peroxide can accelerate the decomposition of lipid peroxides resulting in peroxyl and alkoxyl radicals the can abstract H^+ and stimulate lipid peroxidation (reaction 5) [1]

$$\mathbf{R\text{-}OOH + Fe^{2+} - chelate \rightarrow Fe\ (III) – chelate + OH^- + R\text{-}O^\bullet} \qquad \textbf{Reaction 5}$$

.

Cytochrome P450 isoform CYP2E1 is also responsible for the generation of reactive oxygen species. It is leaky and therefore releases both superoxide and hydrogen peroxide. CYP2E1 is also involved in the generation of hydroxyethyl free radicals that can go on to form protein adducts found in the blood serum of chronic alcoholics. (4-pyridyl-1oxide)-N-t-butylnitrone (POBN) is utilized in hydroxyethyl radical spin trapping in both liver and pancreas of animals fed large quantities of ethanol [5] (Figure 1).

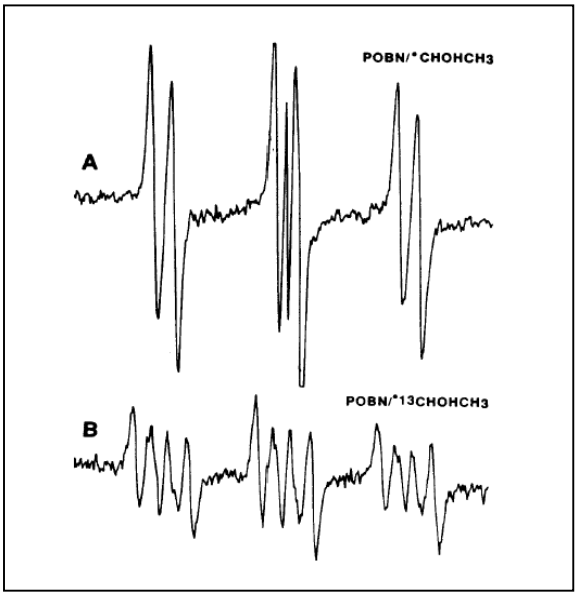

Figure 1. EPR spectra of radical adduct signal of an animal treated with an increased amount of ethanol [5].

The lipid peroxidation was shown to be generated by CYP2E1. Antibodies to CYP2E1 were found to decrease the levels of the lipid peroxidation in rats treated with ethanol [5].

The increases in the prooxidant levels lead to lipid peroxidation and damage to the biomolecules this damage is associated with the liver damage seen in alcoholic liver disease. The increase can also lead to depletion in some of the antioxidants that are needed to terminate the lipid peroxidation and remove the harmful radicals.

Antioxidant Mechanisms in the Liver

Chronic exposure to ethanol will increase the oxidative stress through the production of radicals and lipid peroxidation. The change in prooxidants can and does induce several changes in the antioxidant profile. In studies that placed animals on chronic ethanol feedings an increase in manganese superoxide dismutase (MnSOD) was observed. An increase in superoxide production was observed in the animals fed large amounts of ethanol, which would induce MnSOD [6]. While MnSOD increased a depletion of vitamin E was noted in both the liver and microsomal fractions suggesting an enhancement of lipid peroxidation [6]. Koch et al. proposed the different dietary vitamin E could change the expression in MnSOD levels. Rats on chronic ethanol diets were either deprived of vitamin E or given supplements. Figure 2 shows a significant increase in MnSOD when the rats were supplemented with vitamin E.

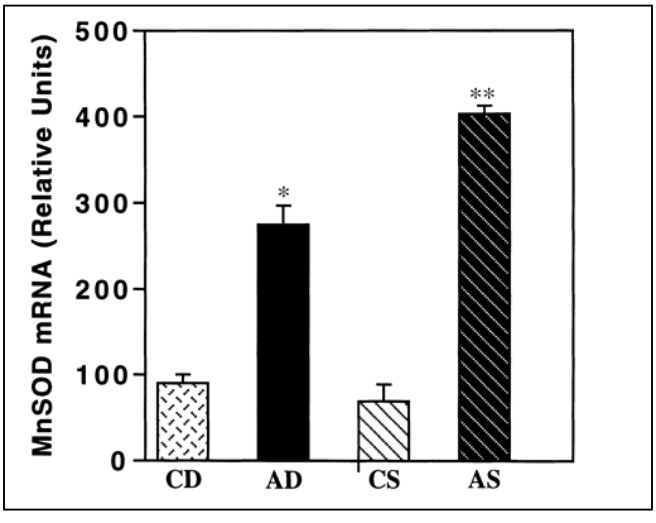

Figure 2. Levels of MnSOD mRNA of the liver with deficient or supplemented vitamin E in ethanol treated rats. CD control vitamin E- deficient, AD vitamin E deficient + Ethanol, CS control vitamin E supplemented , AS vitamin E supplement + ethanol [6].

MnSOD was observed to increase with the consumption of ethanol. Next the levels of both hepatic MnSOD and CuZnSOD were examined in the ethanol treated animal deficient in vitamin E (Figure 3).

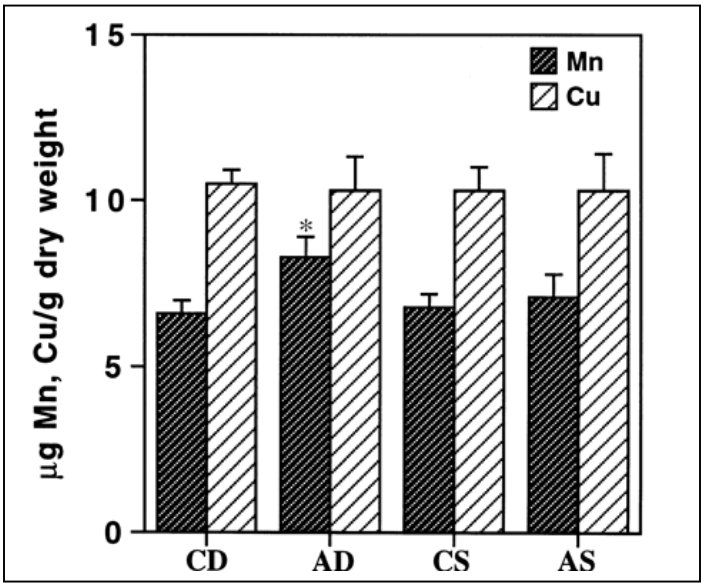

Figure 3. MnSOD and CuZnSOD levels in the liver of the liver with deficient or supplemented vitamin E in ethanol treated rats.
CD control vitamin E- deficient, AD vitamin E deficient + Ethanol, CS control vitamin E supplemented , AS vitamin E supplement + ethanol [6].

The levels of MnSOD vitamin E deficient rats showed elevation, the other levels remained constant. The levels of CuZnSOD and catalase are decreased in the liver of rodents with chronic ethanol administration [2].

α-Tocopherol levels are reported to not be affected when given small amounts of ethanol. However, chronic consumption of ethanol will decrease the levels in the liver mitochondria [2]. Enhanced generation of free radicals is what probably converts α-tocopherol to α-tocopheryl quinone radical. Lipid peroxidation could be reduced when animals were fed with a supplemented diet of vitamin E [2, 6] (Figure 4). Vitamin E deficient diets correlated with increased toxicity to the liver, which can lead to inflammation, fibrosis and alcoholic liver disease.

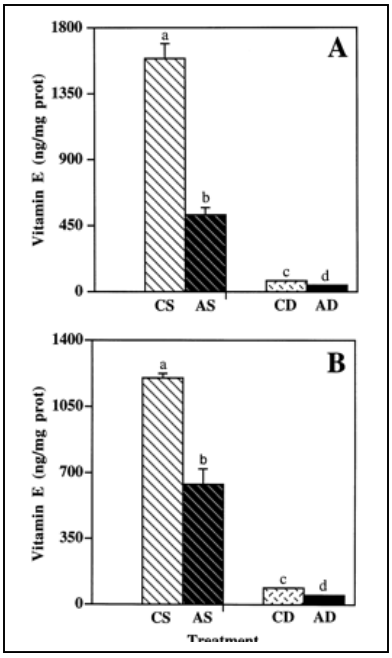

Figure 4. Effects of treatment on vitamin E deficient mice CD control vitamin E- deficient, AD vitamin E deficient + Ethanol, CS control vitamin E supplemented , AS vitamin E supplement + ethanol [6].

Glutathione plays a critical role in the body, it acts to detoxify. Glutathione has been reported to be depleted in animals and people that chronically consumption ethanol and in cirrhosis. Glutathione is thought to be protective in vivo against lipid peroxidation [1]. It is a substrate for glutathione peroxides and can react with several aldehydes formed in lipid peroxidation. The depletion was investigated and although the mechanism is still unclear it was determined that the GSH/GSSG ratios were unchanged [4]. The GSH isn't being oxidized to GSSG, but rather a production failure in the liver. A reduction in the synthesis of cysteine, one of the center amino acids in GSH, is observed. Figure 5 is a schematic of the production of GSH and its redox cycle [2, 4 & 7].

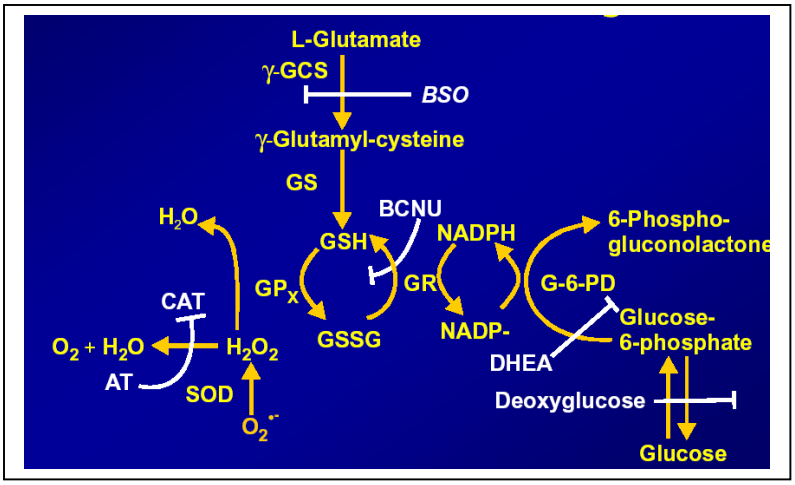

Figure 5. Schematic of the GSH system [7].

Ethanol-Induced Free Radical and Antioxidant Mechanisms in the Brain

The brain is thought to be very susceptible to lipid peroxidation and oxidative injury the free radicals produced can easily change the membrane structure [8]. The brain contains membranes rich in polyunsaturated fatty acid side chains and is antioxidant poor. Alcohol is shown to increase oxidative stress by decreasing the antioxidants that are available such as, α-tocopherol and ascorbate levels.

Rats fed a high ethanol diet were measured for CYP2E1 levels. After consuming the ethanol the rats had an increase in levels of CYP2E1 by 3 fold. The increase in CYP2E1 led to an overall increase in free radical production and lipid peroxidation. Again as seen in the liver the GSH levels were greatly decreased.

The levels of homocysteine in the brain were increased dramatically, which is associated with hyperhomocysteinemia. There is evidence that elevated levels can induce brain atrophy, and contribute to neuronal cell injury, which lead to neurodegenerative disorders [9]. Chronic alcoholism is associated with reduction in hippocampal volume and reduction in brain volume. Homocysteine is also known to autooxidize, which can enhance the levels of reactive oxygen species in an area poor in antioxidants. The generation of homocysteine could also be reason in the reduction GSH. Figure 6 is a schematic of the generation of both homocysteine and glutathione.

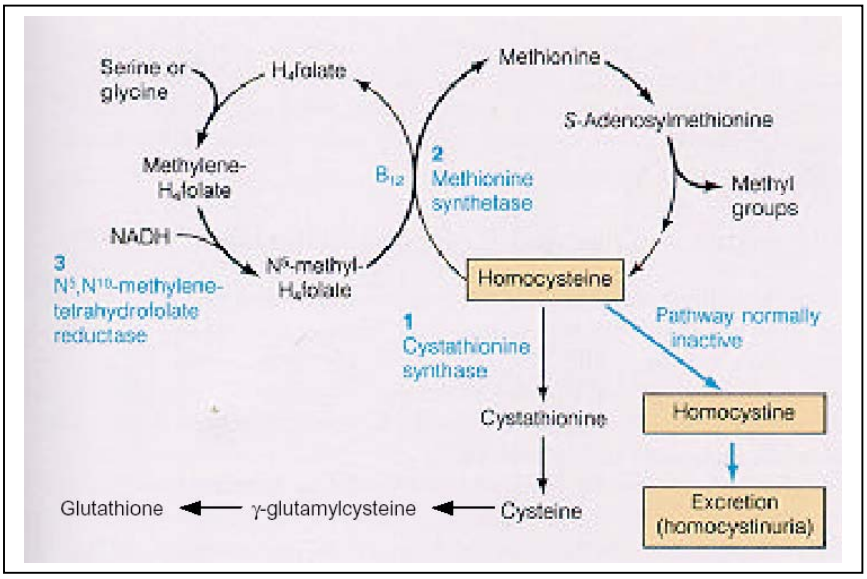

Figure 6 is a schematic of the generation of both homocysteine and glutathione.

The increase in CYP2E1 associated with chronic consumption of alcohol leads to an increase of oxidation in the brain. The brain is rich with unsaturated fatty acids that can easily be involved in lipid peroxidation reactions, which lead to a decrease in brain volume as well as lesions to the cortical and subcortical cerebral atrophy [9].

Future Study

Several studies revealed that many alcoholics have very poor diets consisting of vitally no vitamins. Vitamin E or α-tocopherol function as an antioxidant by breaking redox cycling, like that found in lipid peroxidation. In some animal studies vitamin E supplement was used in an attempt to decrease the lipid peroxidation that was occurring in the tissue. A reduction was noted in some of the experiments, however vitamin E when acting as a change breaking antioxidant requires vitamin C to recycle it back to tocopherol (Figure 7). If a deficiency in vitamin C/ascorbate then vitamin E will no longer act as an antioxidant to protect against lipid peroxidation.

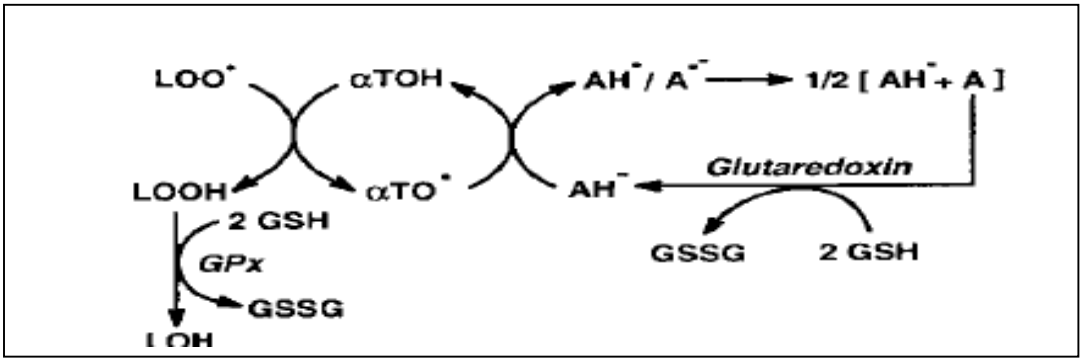

Figure 7. Schematic of the recycling of tocopherol by ascorbate to provide protection against lipid peroxidation.

Hypothesis

We hypothesis that rats placed on chronic ethanol consumption diets can be supplemented with both vitamin E and vitamin C to decrease the lipid peroxidation. Vitamin C is able to recycle vitamin E and repair tocopheroxyl radical. This recycling will allow vitamin E to continue act as a chain breaking antioxidant.

Proposed experimental procedures

In vivo model

- similar model to Koch et. al. Wistar rates
- split rats into five groups: group 1- control vitamin C & E deficient, group 2- vitamin E & C deficient + ethanol, group 3-control vitamin E supplemented,

group 4-vitamin E & C supplemented, group 5- vitamin E & C supplemented + ethanol

- Rats will be fed *ad libitum* with one receiving the vitamin deficient diet and the other supplemented with one or both vitamins. The amount will be basiced on a daily dose per kilogram.
- Approximate time for dietary supplement will be 2 weeks

Goal 1: Detect increased levels of vitamin A and E

We want to determine first if we are increasing the ascorbate and tocopherol levels in the rats compared to the controls. Isolate and examine blood plasma for the different antioxidants using a western blot.

Goal 2: Detection of lipid peroxidation

Examine the amount of lipid peroxidation in each group and determine if the lipid peroxidation is reduced or inhibited when the vitamin C is added compared to vitamin E alone or deficient in both. Again analysis of blood plasma levels looking at the lipid hydroperoxides.

Goal 3: Detect a reduction in tissue damage

Examine the livers and brains of the different groups and look for fibrosis, necrosis of the tissue between the groups. The treatment groups should have less damage to the tissue

Expected Results and possible problems

An increase in antioxidants should reduce the lipid peroxidation. Ascorbate will be able to recycle tocopheroxyl radical back to α-tocopherol to continue the chain breaking activity of vitamin E. Without ascorbate then when the vitamin E was oxidized the peroxidation levels would increase and damage would occur. Since both are now supplemented the lipid peroxidation and the products should be reduced more than with the supplement of vitamin E alone. One potential problem would be that the ratios of vitamin E to C are incorrect and the recycling process is unable to maintain the balance against the increased generation of the reactive oxygen species. Also if the vitamin C should levels should drop then vitamin E would soon decrease because it no longer could be recycled.

Conclusions

Chronic alcoholism can cause many different and unfeasible affects on the body, this can range from nausea to degeneration of tissues. The metabolism of large amounts of ethanol can result in the increase of many ROS and radicals that go to damage lipids, DNA and protein. The antioxidant levels are also affect by the consumption of alcohol some are increased to combat the burst of ROS such as MnSOD but others are depleted and expression is lost like glutathione. The generation of ROS can go and create inflammatory reaction like in the pancreas and if a long enough exposure occur cancer. In the brain the volume can be reduced and there is evidence that elevated homocysteine levels can induce brain atrophy, and contribute to neuronal cell injury, which lead to neurodegenerative disorders [9]. It has been shown with both animal and people that large quantities of ethanol display and increase in oxidative stress and damage suggest the role that radicals and ROS can play in the toxic effects.

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