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Alcohol metabolism and generation of free radicals: A deep insight

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Abstract

Introduction

Alcohol is readily distributed throughout the body in the aqueous blood stream after consumption as it is miscible in water.

This paper critically evaluates and highlights the various aspects of alcohol metabolism and elucidates the role of Reactive oxygen species (ROS).

Alcohol is rapidly absorbed in the bloodstream and metabolized primarily in the liver by the enzyme alcohol dehydrogenase (ADH). ADH plays a crucial role in the conversion of alcohol to acetaldehyde, which is then converted to acetate by the actions of acetaldehyde dehydrogenase (ALDH). The impact of alcohol metabolism on generation of ROS in the cell is linked to its metabolism via oxidative processes. It has also been observed that alcohol deplete GSH levels, particularly in the mitochondria, which normally are characterized by high levels of GSH needed to eliminate the reactive oxygen species generated during the various activities of the respiratory chain.

Conclusion

This paper reports the mechanism of alcohol metabolism, the role of reactive oxygen species from some of our studies and highlights the exact role of alcohol metabolism and its byproducts in the generation of ROS.

Introduction

Because of the complete miscibility with water, alcohol is readily distributed throughout the body in the aqueous blood stream after consumption. The distribution follows the water content in the particular tissue[1]. It readily crosses important biological membranes, such as the blood brain barrier, by simple passive diffusion along concentration gradients and affects a large number of organs and biological processes in the body. The diffusion takes place through aqueous channels, including vascular endothelium. Hence, despite the high water content in blood, depending on the ratio between the tissue water content and the volume of blood flow, some tissues can attain equally high alcohol concentrations. Tissues with lower blood flow rates such as resting muscle, adipose (fat) tissue, and skin require longer time to reach equilibrium with the blood. In contrast tissues with higher blood flow

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such as the brain, kidney, and lung attain equilibrium more rapidly.

For most people alcohol use is enjoyable, unassociated with problems, and socially accepted. However, for about 10% of people, alcohol use progresses to alcohol abuse or alcohol dependency (alcoholism). This latter group suffers from a primary illness (a brain disease) with terrible morbidity and underreported mortality.

Absorption of alcohol into the blood can occur through the skin and via the lungs, though the major route is via drinking alcoholic beverages. Inhalation of alcohol vapor allows its absorption across the pulmonary epithelium. Gastric emptying seems to be the most important determinant of the rate of absorption of alcohol, taken orally. In general, faster the gastric emptying, more rapid is the absorption. Since food delays gastric emptying, it also delays alcohol absorption, irrespective the type of food. Physiological factors like strenuous physical exercise, and drugs (e. g. nicotine and marijuana), that modify physiological factors regulate gastric emptying also modify alcohol absorption[2].

Discussion

Alcohol metabolism in humans

More than 90% of body alcohol is completely oxidized to acetic acid; while the remainder is excreted either in the sweat, urine, or given off through breath. The oxidation occurs primarily in the liver via several routes, the major pathway being by the cytosolic enzyme, alcohol dehydrogenase (ADH). At higher alcohol concentrations, the oxidation is catalyzed by the microsomal cytochrome P450 system (MEOS) system. In addition, minor routes via catalase-dependent oxidation and oxidations by the stomach also provide its metabolism.

The ADH-catalyzed oxidation of alcohol (shown below) produces the highly toxic acetaldehyde.

$$CH3CH2OH + NAD+ \longrightarrow CH3CHO + NADH + H+$$

ADH has broad specificity, is not a solitary enzyme and comprises of five different ADH genes, two of which ADH2 and ADH3 show polymorphism. Of importance is the fact that the ability of people to oxidize alcohol is dependent upon the genetic makeup of the individual. People with alleles (types) of ADH2 and ADH3 may protect those having these genes from developing alcoholism. These genes are common in the Asian population and convert alcohol to acetaldehyde more rapidly than normal. The increased production of acetaldehyde leads to uneasiness and sickness. The second step of ethanol metabolism, which is catalyzed by acetaldehyde dehydrogenase leads to

conversion of acetaldehyde to acetic acid, a normal, non-toxic metabolite in humans.

$$CH3CHO \xrightarrow{NADH} CH3COOH$$
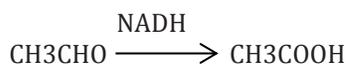
Certain individuals, again common in Asians, have a defective aldehyde dehydrogenase gene, ALDH2, which retards the metabolism of acetaldehyde causing sickness.
The MEOS system of liver, which comprises of the enzyme cytochrome P450IIE1 (CYP2E1), catalyzes alcohol oxidation as follows.

$$CH3CH2OH + NADPH + O2 \longrightarrow CH3CHO + NADP^+ + H2O$$

It is not surprising that there are variations in the P450E1 enzyme, which lead to differences in the rate of ethanol metabolism. This may have implications for tissue damage from ethanol, particular in the liver. Against the above backdrop, it is easy to conceive that alcohol is metabolized primarily in liver by oxidative pathway. The oxidative stress, resulting from this is responsible for its poor manifestation in health[3].

Consequences of alcohol consumption
Despite many decades of research, the reasons why only a relatively small proportion of individuals who consume excessive quantities of alcohol develop clinically significant alcohol related diseases remain unknown[4]. The precise mechanisms leading to alcohol related diseases are still imprecisely known. It depends not only on the total amount of alcohol consumed, but also on drinking patterns. The metabolic effects of alcohol are due both to its direct action and to that of its first metabolite acetaldehyde, and can also be connected with the changes in redox state. Cells are protected against oxidation by the action of certain enzymes, vitamins, and other substances, known collectively as antioxidants. Ethanol increases the rate of generation of free radicals, decreases the antioxidant levels, and potentiates oxidative stress[5].

Alcoholism and lipid metabolism
Levels of free fatty acids and fatty acid ethyl esters are elevated in ethanol – treated rats' liver, kidney, brain, and heart. These are probably the possible mediators in the production of alcohol dependent syndromes[6]. These esters were found to be toxic to the human cells [7]. Fatty acid ethyl esters are non-oxidative products of ethanol metabolism and have been implicated as mediators of cholesterol induced organ damage[8]. Fatty acid ethyl esters bind with lipoproteins and albumin in human plasma and are carried to the different parts of the body and induce organ damage. Ethanol administration to pregnant rats during gestation period leads to significant accumulation of saturated fatty acid and ethyl esters of long chain fatty acids in both maternal and foetal organs. Maternal ethanol consumption during pregnancy leads to decrease in decosahexaenoic acid content in foetal brain and liver phosphatidyl choline

and phosphatidyl ethanolamine due to reduction of polyunsaturated fatty acid levels[9].
Ethanol consumption is also associated with increased level of triglycerides and HDL cholesterol[10]. Ethanol ingestion leads to severe alteration of cholesterol metabolism resulting in both elevated serum cholesterol and hepatic cholesterol ester levels. It also causes alteration of the plasma membrane cholesterol domains that alter transbilayer fluidity gradients in plasma membrane, and are associated with decreased Ca^{2+}, Na^+, K^+- ATPase activities[11]. Probably there is decreased expression of LDL receptor gene and HMG-CoA reductase gene. Alcohol induces defective lipoprotein metabolism. The accumulation of VLDL and LDL in the blood of ethanol-ingesting individuals is due to reduced expression of LDL-receptors [12].Ethanol provokes a change in apolipoprotein b conformation[13]. Chronic ethanol exposure affected the deacylation and reacylation of membrane phospholipids [14]. The increase of LDL level is probably the main risk factor in the formation of atherosclerosis, and oxidation of LDL increased atherogenicity [15]. This oxidized LDL, produced due to ethanol oxidation in the cells, induced smooth muscle proliferation through the activation of phospholipase D[16, 17].

Immunomodulatory activity of alcohol
Ethanol acts as an immunomodulator. The ingestion of ethanol was shown to be associated with immunodeficiency[18]. Researchers have shown that alcohol administration is directly related to immunosuppression. Alcohol intake increases the susceptibility of the individual to different types of infections. Both humoral and cell mediated immunity have been shown to be suppressed in chronic alcoholics. Ethanol reduces the number of lymphocytes and phagocytosis by macrophages[19]. It can suppress the host defence mechanisms to bacterial infections and inhibit neutrophil function. Ethanol modifies the specificity of antibody functions against a defined epitope, probably due to changes of the conformation of the antibodies and this effect is concentration dependent [20]. Ethanol administration impaired cell-mediated immune response, probably by inhibiting early events in T-lymphocyte activation. Ethanol is involved in impairment of IgM synthesis and secretion by plasma cells especially in the mesenteric lymph node. It also suppresses the synthesis of IL-1, 2 & 4 in spleen, probably by inhibiting translation of mRNA for the cytokines[21].

Absorption of alcohol in humans
Although alcohol has high calorific value, it reduces nutrient intake from other foods by decreasing appetite. Alcohol consumption decreases the absorption of a number of nutrients and can alter their storage, metabolism and excretion. It affects the metabolism and storage of fat-soluble vitamins and also causes deficiency of many water-soluble vitamins[22].
Ethanol being soluble both in water and lipids and having a small molecular size can diffuse rapidly through the

mucous membranes of the oesophagous and stomach. After absorption ethanol appears in both expired air and in urine. Ethanol diffuses out from the lungs and kidneys. It is not stored in the body, as whatever is ingested is oxidized. It is metabolized entirely in the liver[22].

**Role of reactive oxygen species in alcohol metabolism**

The impact of ethanol on generation of ROS in the cell is linked to its metabolism via oxidative processes. The major mechanisms for ethanol oxidation are designated by the following reactions. In the individual consuming moderate amounts of ethanol most of it is metabolized by alcohol dehydrogenase.

$$CH_3CH_2OH + NAD^+ \longrightarrow CH_3CHO + NADH + H^+$$

In this reaction a hydride ion is being transferred from ethanol to NAD^+. This mechanism for ethanol metabolism is predominant. When a person is drinking in moderation, the alcohol dehydrogenase in the liver would metabolize most of the ethanol consumed [23].
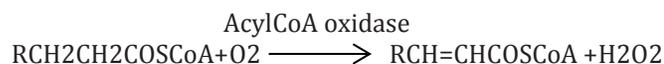
Alcohol acts through numerous pathways to affect the liver and other organs and to lead to the development of alcoholic liver disease (ALD). No single process or underlying mechanism can account for all the effects of alcohol on an organism or even on one specific organ; instead, many mechanisms act in concert, reflecting the spectrum of the organism's response to a myriad of direct and indirect actions of alcohol [24].

The microsomal electron transport system also participates in ethanol oxidation via catalysis by the cytochrome P450 isoenzymes [25]. The enzymes in this family vary in their capacity to oxidize ethanol, including the 2E1, 1A2 and 3A4 isoforms [26] and catalyze the following reaction sequence.

$$CH_3CH_2OH + NADPH + H^+ + O_2 \longrightarrow CH_3CHO + NADP^+ + 2H_2O$$
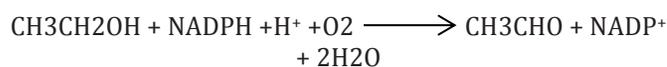
One H_2O is from the reducing equivalents of $NADPH + H^+$ and the other from the reducing equivalents transferred from ethanol. Ethanol at hepatic levels in low concentrations is metabolized mostly by alcohol dehydrogenase. An important consideration with the microsomal electron transport system is that the cytochrome P450 2E1 isoform is induced to higher tissue concentrations as a result of chronic ethanol consumption and becomes more important quantitatively in ethanol oxidation in alcohol abusers. In addition to the mitochondrion, the 2E1 isoenzyme may also be a significant catalyst for formation of ROS in the alcohol consumer, as it has been demonstrated to generate higher amounts of H_2O_2 in the presence or absence of oxidizable cosubstrate. Its elevation in the livers of ethanol consumers has also been linked to increased generation of hydroxyl radicals [27]. There are many processes and factors involved in causing alcohol induced free radical generation and oxidative stress (Table 1).

Peroxisomal activity also contributes to ethanol oxidation in the liver, as is seen in the following reactions:

$$\text{AcylCoA oxidase}$$
$$RCH_2CH_2COSCoA + O_2 \longrightarrow RCH=CHCOSCoA + H_2O_2$$

$$\text{Catalase}$$
$$CH_3CH_2OH + H_2O_2 \longrightarrow CH_3CHO + 2H_2O$$

In heavy ethanol consumers where there is usually an elevation in fatty acids in the liver. It is possible that this mechanism might be more prominent due to increased peroxisomal oxidation of fatty acids. Ethanol oxidation gives rise to acetaldehyde, which is further oxidized by hepatic aldehyde dehydrogenases that are quite efficient in keeping acetaldehyde levels low.

$$CH_3CHO + NAD^+ \longrightarrow CH_3COOH + NADH + H^+$$
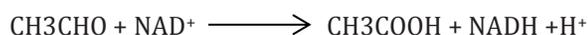
The mitochondrial form of aldehyde dehydrogenase plays a prominent role in maintaining low concentration of acetaldehyde. The acetate is then activated by acetyl CoA synthase to acetyl CoA [23].

As a result of oxidation of ethanol by alcohol dehydrogenase and subsequent oxidation of acetaldehyde there is a significant increase in the hepatic $NADH+H^+$/NAD^+ ratio. This shift occurs both in the cytoplasm and the mitochondria, as measured by the lactate/ pyruvate and b-hydroxybutyrate/ acetoacetate ratios, respectively. The mitochondrial, low Km aldehyde dehydrogenase generates much of the NADH within the mitochondria and the reducing equivalents of the cytoplasmic NADH are transported into the mitochondria, primarily via the malate-aspartate shuttle, which is predominant in liver. In addition to GSH and NADPH, numerous other nonenzymatic antioxidants are present in the cells, most prominently vitamin E (α–tocopherol) and vitamin C (ascorbate). Vitamin E is a major antioxidant found in the lipid phase of membranes and, like other chemically related molecules, acts as a powerful terminator of lipid peroxidation. During the reaction between vitamin E and a lipid radical, the vitamin E radical is formed, from which vitamin E can be regenerated in a reaction involving GSH and ascorbate. Alcohol also appears to interfere with the body's normal vitamin E content because patients with ALD commonly exhibit reduced vitamin E levels [23].

Conclusion

Alcohol consumption and alcoholism is one important cause of an acute illness and chronic disease worldwide. The metabolic aspect of alcohol have immense role in controlling the alcohol induced toxicity and its consequences. It is also observed that excess levels of ROS resulting oxidative stress and have been implicated in a variety of human diseases. Many studies have demonstrated that alcohol increases lipid per oxidation as well as the modification of proteins; however, it is not

Table 1: FACTORS INVOLVED IN CAUSING ALCOHOL INDUCED FREE RADICAL GENERATION AND OXIDATIVE STRESS IN HUMANS FACTORS INVOLVED IN CAUSING ALCOHOL INDUCED FREE RADICAL GENERATION AND OXIDATIVE STRESS IN HUMANS.

Changes in the NAD^+/NADH ratio in the cell as a result of alcohol metabolism. Each of these reactions leads to formation of one molecule of NADH, thereby providing more starting material and thus enhanced activity of the respiratory chain, including heightened O_2 use and ROS formation.

Production of acetaldehyde during alcohol metabolism, which through its interactions with proteins and lipids also can lead to radical formation and cause cellular damage in individuals.

Damage to the mitochondria resulting in decreased ATP production.

Effects on cell structure (e.g., the membranes) and function caused by alcohol's interactions with either membrane components (i.e., phosphate–containing lipids [phospholipids]) or enzymes and other protein components of the cells.

Alcohol–induced oxygen deficiency (i.e., hypoxia) in tissues, especially in certain areas of the liver lobules (i.e., the pericentral region), where extra oxygen is required to metabolize the alcohol and an important aspect for Angiogenesis.

Alcohol's effects on the immune system, which lead to altered production of certain signaling molecules called cytokines, which in turn lead to the activation of an array of biochemical processes.

Alcohol–induced increase in the ability of the bacterial molecule endotoxin to enter the bloodstream and liver, where it can activate certain immune cells causing cell damage.

Alcohol–induced increases in the activity of the enzyme cytochrome P450 2E1 (CYP2E1), which metabolizes alcohol and other molecules and generates ROS in the process.

Alcohol–induced increases in the levels of free iron in the cell (i.e., iron that is not bound to various proteins), which can promote ROS generation.

Effects on antioxidant enzymes and chemicals, particularly a molecule called glutathione (GSH) which has an important role in ROS activity.

Biochemical reactions generating an alcohol–derived radical (i.e., the 1hydroxyethyl radical and also in the conversion of the enzyme xanthine dehydrogenase into a form called xanthine oxidase, which can generate ROS.

always clear if these changes are the causes rather than consequences of alcohol–induced tissue injury. Although researchers already have gained substantial insight into the mechanisms and consequences of alcohol–induced oxidative stress, additional studies are required to further clarify how alcohol produces oxidative stress in various tissues. Additional analyses need to determine the role of alcohol metabolism and its byproducts (e.g., acetaldehyde) in the production of ROS.

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