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Minireview

Ethanol toxicity and oxidative stress

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SUMMARY

The mechanisms underlying the toxicity of ethanol have been the subject of much study, but are not well understood. Unlike many selective pharmacological agents, ethanol clearly has several major loci of action. One deleterious factor in ethanol metabolism is the potential for generation of excess amounts of free radicals. The extent to which this activity accounts for the overall toxicity of ethanol is unknown. This review outlines the enzymic steps that have the capacity to generate reactive oxygen species. These steps are likely to differ in acute and extended exposures to ethanol. Acetaldehyde catabolism also has the likelihood of contributing to ethanol-related oxidative stress. The review focuses on the ethanol-induced production of excess amounts of pro-oxidant reactive species in both the liver and the central nervous system. The potential of various stages of ethanol catabolism to involve generation of free radicals is described.

INTRODUCTION

Ethanol (ethyl alcohol) is a widely consumed organic solvent with toxic properties both systemically and to the central nervous system. Its toxic properties can be expressed following an acute overdose or after extended chronic consumption. The rather high LD₅₀ of ethanol [1], suggests that this agent acts relatively non-specifically

on a wide range of cellular targets rather than at a single critical site. Thus, the toxicity of ethanol probably represents a summation of a series of adverse metabolic modulations.

There is a significant body of evidence which suggests that the cytosolic sites of generation of reactive oxygen species (ROS) constitute some of the targets accounting for the toxicity of ethanol. This review is intended to summarize both direct and circumstantial evidence for the generation of excess oxidative species participating in the overall toxicity of ethanol. In addition, the relative contributions of various steps in ethanol catabolism to this process in brain and liver are evaluated, and the intracellular loci potentially involved are discussed. Finally, a comparison with the ROS-inducing potential of other solvents, both aliphatic and aromatic, is made.

EVIDENCE FOR ETHANOL-RELATED OXIDATIVE STRESS

Levels of glutathione and related enzymes

Dramatic reductions in hepatic glutathione levels following acute or extended exposure to ethanol have been reported for liver [2]. Glutathione peroxidase is also depressed in the liver following chronic ethanol dosing [3]. Lesser reductions, and also increases, in glutathione levels have been found in brain [4–7]. Since compensatory processes can be rapid after induction of excessive ROS, both reductions and increases in cerebral glutathione are regarded as indices of oxidative stress [8]. The turnover of glutathione is elevated after chronic ethanol treatment. However, this may be due to increased degradation via the γ -glutamyl cycle rather than increased oxidation of glutathione [9].

Lipid peroxidation and evidence of damage to other macromolecules

Lipid peroxidation is an indirect measure which reflects the consequences of ROS activity upon lipid-rich cell membranes. Results are often reported in terms of thiobarbituric acid reactive materials such as malondialdehyde. This procedure is subject to various artifacts [10] but is widely used and has some validity. Elevations in lipid peroxidation following ethanol treatment have been frequently reported for both brain and liver [11–15]. Acetaldehyde treatment also elevates hepatic lipid peroxidation [10]. Evidence of widespread systemic elevations in lipid peroxidation have also been detected in vivo by assay of exhalation of metabolically produced ethane [16,17]. The attenuation of some of these ethanol-induced changes by the lipid-soluble antioxidant vitamins A or E, and the depletion of antioxidant vitamins in ethanol-treated rats [6,13,18,19] are consistent with the presence of induced oxidative stress.

The metabolism of ethanol has been shown to lead to the formation of oxidative products capable of cleaving DNA [20]. The capacity of free radicals to facilitate expression of proto-oncogenes [21] may underlie this finding. This may account for the weakly carcinogenic properties of ethanol, and also its teratogenic actions [22]. However, there is no evidence for ethanol causing DNA damage in intact animals.

Direct detection of active oxidant species

Free radicals can be detected after conversion to a derivative with a long half-life by electron spin resonance. By this means excess microsomal ROS production has been found during ethanol metabolism [23–25]. Using spin-trapping techniques, an active, relatively short-lived oxidant species, the hydroxyethyl radical, has been suggested to be the dominant deleterious species [26]. This radical is not as reactive as the hydroxyl radical and thus has the potential to diffuse toward, and damage more distant target molecules [27]. ROS activity can also be determined using an intracellular fluorescent probe generated by the oxidation of a non-fluorescent precursor [28]. Using this probe (2',7'-dichlorodihydrofluorescein) we have found evidence of ethanol-induced hepatic and cerebral ROS generation (Bondy, S.C., unpublished data).

Iron mobilization

Iron is well-recognized as an initiator of intracellular ROS production. This may be by way of catalyzing the generation of the hydroxyl radical from peroxides by the Haber-Weiss reaction, or by direct formation of the perferryl radical. Free ionic, or incompletely sequestered iron may be essential for the appearance of ethanol-induced ROS. Several investigators have found ethanol to effect the liberation of low molecular weight iron from bound intracellular reserves [12,20,29,30]. The superoxide anion can release iron from ferritin [31] and this may underlie such observations. That the presence of small amounts of iron in complexes of low molecular weight may lead to the formation of ROS is illustrated by the protective effects of iron chelators such as deferoxamine on ethanol-related changes in oxidative events [12,32]. It has even been suggested that iron-sequestering chemicals can reduce physical dependence on ethanol [33].

Induction of enzymes relating to oxidant status

Since oxidases utilize molecular oxygen directly, their induction has the potential for enhancing ROS production. Ethanol is known to induce microsomal mixed function oxidase enzymes in the liver [34,35], and this has been found to specifically involve P-450 11E1 [36]. More recently, a parallel induction of P-450 11E1 has been found in the CNS where it is present in much lower concentrations [37,38].

Increased levels of superoxide dismutase (SOD), an inducible enzyme, are generally taken as indirect evidence of an increased oxidant milieu. However, since this is a sulfhydryl enzyme, depression of its level in a tissue can also reflect oxidative denaturation. Following ethanol treatment, decreases in SOD have been found in liver [39,40] and brain [41]. Ethanol-effected increases in SOD have also been reported [42]. As with glutathione, biphasic fluxes of SOD levels are common and a change in either direction may relate to the presence of excess ROS.

SOURCE OF REACTIVE OXYGEN SPECIES GENERATED BY ETHANOL

The catabolic steps involved in the degradation of ethanol are candidates for the origin of excess ROS. Enzymes with ROS-producing potential are shown in Figure 1. Two major classes of enzyme need to be considered; those oxidizing ethanol to acetaldehyde, and those completing the oxidation to acetate.

Alcohol dehydrogenase

This relatively non-specific soluble enzyme is considered the primary initial step in the catabolism of ethanol. There is evidence that the activity of several dehydrogenases, including alcohol dehydrogenase, can bring about ROS formation, despite the fact that oxygen is not directly involved [43]. The mechanism underlying this may involve the formation of hydroperoxyl radical by the zinc moiety found in this enzyme (Adams, J., personal communication). The inhibition of ethanol-effected free radical production by an inhibitor of alcohol dehydrogenase, 4-methylpyrazole [25], suggests that ROS are generated by this enzyme. However, 4-methylpyrazole is also capable of inhibition of mixed function oxidases [44], so use of this agent does not allow a clear distinction to be made between the two major routes of ethanol breakdown.

Catalase

The evidence that this soluble enzyme is involved in ethanol degradation is that pretreatment of rats with ethanol can block the inhibition of cerebral catalase normally brought about by 3-amino-1,2,4-triazole [45]. This inhibitor can also attenuate some behavioral effects of ethanol, further suggesting the relevance of this route of oxidation to the brain [46]. Such a pathway would be more likely to absorb, rather than to generate free radicals, since hydrogen peroxide is a co-substrate in this oxidation.

Mixed function oxidases

After chronic consumption of ethanol, or in its presence at a high concentration, its metabolism by hepatic microsomal P-450 enzymes becomes more pronounced [47]. Several of these enzyme species are able to oxidize ethanol but cytochrome P-450 11E1 (P-450j) is the most selective [48]. As mentioned above, this enzyme is inducible by ethanol and can generate oxidizing species in the absence of substrates as long as NADPH is present [49]. Under such conditions, cytochrome P-450 11E1 has been reported to exhibit an unusually high rate of oxidase, and H₂O₂-generating activity [36]. The enzyme has also been detected in the CNS, albeit at a lower level. In the cerebellum, this enzyme is confined to glia, while it is also present in cortical pyramidal cells, and especially prominent in hippocampus and striatum [37].

P-450-independent microsomal oxidation of ethanol

The powerful influence of iron on ethanol-related ROS formation has led to the

Fig. 1. Enzymic pathways of ethanol catabolism.

suggestion that there is an $OH^{\cdot}$ -producing pathway of ethanol oxidation that is independent of cytochrome (P-450). This reaction appears to involve NADPH-P-450 reductase acting in the presence of iron [29]. This pathway is generally not of major quantitative significance, but may be inducible and may make a disproportionate contribution to total ethanol-related oxidant processes. Electron spin resonance studies also support the concept of this alternate microsomal pathway [23].

Another potential route of ethanol oxidation involves the elevation of NADH levels that can occur after alcohol consumption. The consequent inhibition of fatty acid oxidation allows increased triglyceride formation and fat deposition in the liver, which may predispose this tissue to peroxidative events [50]. The increased and prolonged availability of NADH may also promote the microsomal generation of ROS [51].

Aldehyde dehydrogenase

This mitochondrial NAD^+ -dependent enzyme constitutes the major means of oxidation of acetaldehyde by the liver. It is also present in brain [52]. As discussed previously, this dehydrogenase is capable of producing hydroxyl ions. Inhibition of this enzyme by sodium cyanamide, prevents the onset of ethanol-stimulated hepatic lipid peroxidation [53]. This result also shows that in addition to causing formation of proteinaceous adducts, the oxidation of acetaldehyde may be a key element in ethanol toxicity.

Cysteine and ascorbic acid are protective against the acute behavioral toxicity of acetaldehyde, supporting the concept of the significance of acetaldehyde-inducible oxidative stress to the brain [54]. However, such agents may also have non-antioxidant ameliorative effects such as preventing the formation of adducts of acetaldehyde with protein [55].

Aldehyde oxidase and xanthine oxidase

Both xanthine oxidase and aldehyde oxidase are molybdenum- and flavin-containing enzymes. The presence of the latter enzyme in the brain is equivocal but xanthine oxidase, which can also oxidize acetaldehyde, is present in all tissues. This enzyme is derived by the proteolytic and oxidative modification of xanthine dehydrogenase. Ethanol treatment may also bring about this enzyme conversion [33], and acetaldehyde appears to be the agent directly responsible for this effect [56]. The affinity of aldehyde oxidase toward acetaldehyde, is much greater than that of xanthine oxidase [32]. By use of various selective inhibitors of aldehyde oxidase and xanthine oxidase, it was concluded that, in the liver of the ethanol-treated animal, both enzymes were roughly equal in their ability to promote free radical generation [57].

Non-enzymic reaction of acetaldehyde with proteins

It has been postulated that acetaldehyde generated during the metabolism of ethanol can initiate oxidative stress by reaction with, and depletion of, protective thiols such as cysteine and glutathione. Microsomal mixed function oxidases may also be a direct target of acetaldehyde. Binding of this ethanol metabolite to these enzymes, forming a stable adduct, can impair their properties [35]. Thus, it is possible that the P-450 11E1 enzyme induced by ethanol is malfunctioning [58]. The extent to which acetaldehyde found within the brain after ethanol dosing, is generated intrinsically rather than systemically transported from the liver, is unclear. The reactivity of acetaldehyde with many biological constituents suggests that it is likely to be synthesized close to the site where it is detected. However, following ethanol treatment, acetaldehyde levels of brain interstitial fluid are above those present in the CNS [52]. This suggests that acetaldehyde can cross the blood brain barrier.

Mitochondrial impairment by ethanol

Most cellular superoxide is produced by mitochondria. This can represent a significant proportion of the oxygen utilized by the respiratory chain [59]. This proportion is elevated after acute, but not after prolonged treatment with ethanol, despite the fact that mitochondrial morphology is affected by such extended exposure [32]. This handicaps the concept that free radical generation by chronic exposure to ethanol is mediated by impairment of mitochondrial efficiency.

Ethanol-stimulated lipid mobilization

Ethanol has been shown to activate phospholipases A1 and A2, in an isolated cardiac preparation [60]. This effect is blocked in the presence of α -tocopherol. The liberation of arachidonic acid by phospholipase A2 sets in motion a range of oxidative catabolic processes; the 'arachidonic acid cascade'. This polyunsaturated fatty acid contains four ethylenic bonds and is readily oxidizable. The enzymic conversion of this compound to prostaglandins, leukotrienes and thromboxanes by cyclooxygenases and lipoxygenases leads to considerable ROS generation [61].

SYNERGISM AND COMPARISON WITH OTHER TOXICANTS

Several other organic solvents are known to increase levels of free radical generation in the liver. These include aliphatic compounds, such as carbon tetrachloride, and aromatic solvents, such as benzene, toluene, styrene and xylenes [43,62–64]. Several of these compounds also stimulate CNS production of ROS [43,64]. In the case of toluene catabolism, the oxidation of benzaldehyde by aldehyde dehydrogenase appears to be the major source of free radicals [65]. Synergistic interactions have been reported between ethanol and other solvents including carbon tetrachloride, toluene, and carbon disulfide [66–68]. These synergisms may be attributable to the induction of a mixed function oxidase complex by a specific solvent. This elevated mixed function oxidase may then act on a range of solvents which, alone, are not powerful inducers. However, there will also be competition for oxidation between different chemicals. Obviously, complex agonistic and antagonistic events can result from such competition. For example, ethanol may retard the rate of toluene catabolism [69], but whether this would increase or decrease the toxicity of the latter solvent is not clear. Nevertheless, such interactions have obvious relevance to the circumstances under which ethanol is generally consumed.

Further suggestion of the ability of ethanol to enhance induced neural oxidative stress comes from results of studies combining ethanol with other toxicants. The toxicity of manganese, 6-hydroxydopamine and 1-methyl-4-phenyl pyridinium ion (MPP⁺), three agents suspected to owe part of their neurotoxicity to oxidative events, interact with ethanol or acetaldehyde in a synergistic manner [42,70,71]. The dopaminergic system seems peculiarly susceptible to oxidative damage and also declines with normal aging. The maintenance of adequate dopaminergic function throughout life depends on several interacting environmental and occupational exposure factors. The degree of ethanol consumed may constitute one of these significant variables.

CONCLUSIONS

With our current information, it is not yet possible to apportion the extent of participation of several key enzymes in ethanol degradation. While there are a plethora of candidates for ethanol-stimulated ROS production, the relative importance of each is not clear. This issue needs to be resolved before one can begin to assess the degree to which each of these steps contributes to incurred oxidative damage. The relative quantitative significance of the various potential pathways of ethanol breakdown described is defined by at least two major factors.

The tissue in question

Alcohol dehydrogenase is only present in brain in low amounts [72], and thus may play a lesser role there than in the liver. Catalase may be the major primary oxidant of alcohol in the CNS. The low levels of most cerebral oxidative enzymes may mean that acetaldehyde within the CNS largely originates from a hepatic source. Evidence

for hepatic oxidative stress following ethanol treatment is more unequivocal than for a parallel cerebral insult. This may be a reflection of the free radical generation occurring at the initial steps of ethanol oxidation. Liver mitochondria seem much more sensitive to disruption by ethanol than are brain mitochondria [73], and hepatic glutathione levels can be strongly depleted by ethanol. In addition, the liver is susceptible to other sources and ethanol-effected ROS, such as chemotactic attraction of phagocytes [74].

The duration of exposure to ethanol

While alcohol dehydrogenase may be of primary importance in the acutely ethanol-exposed liver, more chronic exposures can lead to induction of mono-oxygenases and the P-450 system may increase in relevance in both brain and liver. The further oxidation of ethanol is primarily by way of aldehyde dehydrogenase. More extended dosing is also likely to effect conversion of xanthine dehydrogenase to the oxidase thereby increasing the contribution of xanthine oxidase to ethanol catabolism.

Other features that define the extent of oxidative damage incurred by a tissue relate to the intrinsic susceptibility of the tissue to pro-oxidant conditions and the status of antioxidant processes in that tissue. For example, dopamine-containing neurons are especially sensitive to oxidant damage. The distinct susceptibility of the cerebellum and the hippocampus to ethanol-induced morphological damage may relate to their relatively high content of cytochrome P-450 11E1 mono-oxygenase [37].

A final unsolved issue is the extent to which overall ethanol toxicity is related to oxidative stress. The hepatic events consequent to prolonged and high levels of ethanol ingestion such as lipid mobilization are very likely to involve harmful pro-oxidant events. Such dramatic changes are generally not seen in nervous tissue in the absence of thiamine deficiency and whether excess free radical production is involved in the neurotoxicity of ethanol is an unresolved question. However, the brain is an organ with very limited potential for cell replacement and can be vulnerable to gradual incremental deficits. Such slowly accumulating lesions, although difficult to quantitate, can be irreversible. These subtle changes may accelerate deficits resulting from normal physiological aging and enhance susceptibility to additional neurological stressors.

The potential clinical utility of antioxidants in the therapy of ethanol toxicity is unknown. However, the evaluation of this possibility in alcoholics does not require delay until the mechanisms subserving ethanol-induced oxidative stress are fully understood.

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