The Neurotoxicity of Ethanol and The Underlining Mechanisms

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Abstract. After ingestion, ethanol is mainly metabolized in the liver, eventually producing ATP and end products such as carbon dioxide and water. Because it is both water- and fat-soluble, it is easy to pass through the blood-brain barrier and affect the function of the nervous system. Overconsumption of alcohol or alcohol abuse is associated with various diseases including neurocognitive disorders. This article reviews the neurotoxicity of ethanol and discusses its possible molecular mechanisms.

1. Introduction
Ethanol (C2H5OH) is a major component of alcoholic beverages. After absorption through digestive tract, ethanol is mainly metabolized in the liver. First, alcohol dehydrogenase (ADH) catalyzes the oxidation of ethanol, forming acetaldehyde. Acetaldehyde is then oxidized to acetic acid by aldehyde dehydrogenase 2 (ALDH2). Acetic acid enters the tricarboxylic acid cycle, generating ATP, carbon dioxide and water [1]. In addition to the alcohol dehydrogenase system, two additional pathways participate in the metabolism of ethanol. One is the microsomal ethanol oxidizing system (MEOS), in which the enzyme closely related to ethanol catabolism is the cytochrome P450 second family E subtype polypeptide 1 (cytochrome P450 2E1, CYP2E1). The other pathway metabolizing ethanol involves catalase.

The amount of alcohol that human liver can metabolize is about 1 gram per kilogram of body weight per day. It has been suggested that the amount of alcohol allowed per day for a 60 kg-person should be limited to 60 grams. For people whose body weight below 60 kg, the daily intake of alcohol should be reduced accordingly, preferably less than 45 grams [2]. Overconsumption of alcohol or alcohol abuse can induce a variety of diseases, such as alcoholic liver disease [3], chronic alcoholic encephalopathy [4] and neurocognitive disorders [5]. It is also well known that maternal alcohol use during gestation results in fetal alcohol syndrome (FAS), which causes developmental and learning problems.

Studies have shown that ethanol is a neurotoxic substance that can severely damage the normal function of the nervous system. Heavy drinking or alcohol abuse causes damages of the nervous system, leading to brain shrinkage and cognitive decline [6]. Compared with non-alcoholic individuals, the alcoholic patients have impaired executive functions and below normal performance on both free and delayed recall [7]. About 50-70% of alcohol-dependent adults exhibit permanent cognitive impairment due to damages of central nervous system caused by excessive drinking [8]. Studies of young adults with prenatal alcohol exposure show that the total volume of brain as well as the volume
of hippocampus is significantly smaller in these individuals than in the controls. The brain structure changes seen in these individuals with prenatal alcohol exposure are associated with their poor performance in learning and recall [9], suggesting that alcohol exposure causes severe damages to the developing brain. The neurotoxic mechanism of ethanol may be related to its induction of glutamate excitotoxicity, promotion of intracellular oxidative stress and inhibition of cell survival signals [10]. Here we review the neurotoxic effects of ethanol and discuss its possible molecular mechanisms.

2. Ethanol induces glutamate excitotoxicity

In the nervous system, glutamate, aspartate, and their structural analogues bind to receptors that regulate ion channels or activate phosphoinositide hydrolysis. Among them, glutamate is a major excitatory transmitter of the central nervous system, which is essential for learning and memory processes. Glutamate is released by exocytosis of the presynaptic membrane or directly from the cytosol. It binds to specific glutamate receptors on the postsynaptic membrane, causing depolarization of the posterior membrane and neuronal excitation [11]. Glutamate receptors can be divided into four kinds according to the characteristics of their agonists. The N-methyl-D-aspartate (NMDA) receptor is a glutamate receptor coupled with calcium ion channel found in nerve cells, which plays an important role in synaptic plasticity and synapse formation [12]. In brain cells isolated from new-born rats, ethanol (25, 50, 100 mM) treatments reduce the glutamate receptor agonist NMDA-induced rise of intracellular calcium [13]. Similarly, in primary cerebellar granule cell culture, ethanol as low as 10 mM inhibits NMDA-stimulated calcium uptake [14]. Thus, ethanol may have direct actions on NMDA receptor-induced calcium flux and its associated function [13,14]. In hippocampal neurons, ethanol (5-50 mM) inhibits the ion current induced by NMDA in a concentration-dependent manner. The inhibition of NMDA receptors by ethanol can lead to neurological and cognitive impairment associated with alcoholism [15,16]. In alcoholics, the density of glutamate binding sites in synaptic membranes prepared from the hippocampus is significantly higher than that in the control individuals [17]. Both acute and chronic ethanol exposure in rats increases glutamate binding in the brains [18]. These results suggest an association of glutamate and ethanol-induced changes in neuronal function. Thus, ethanol may affect the central nervous system by altering glutamate-mediated synaptic transmission.

3. Ethanol increases the levels of reactive oxygen species

The excessive generation of reactive oxygen species (ROS) and oxidative stress has been associated with many neurodegenerative disorders including Alzheimer’s disease and Parkinson’s disease [19]. It has been shown that oxidative stress is also an important factor in ethanol-caused brain damages [20]. Incubation of hippocampal astrocytes with 50 mM ethanol results in an increase in ROS and impedes the formation of normal nerve contact [21]. Several pathways may be involved in the ROS production induced by ethanol. The oxidation of ethanol by cytochrome P450-2E1 (CYP2E1) generates hydrogen peroxide [22]. Long-term alcohol use in both animals and human can increase the expression of CYP2E1 in the brains, which may contribute to the increased production of ROS in chronic alcohol treatment [23,24]. Leakage of electrons from mitochondria induced by ethanol causes the generation of large amounts of ROS by NADPH oxidase complex (NOX) [25]. In human primary neuron cells, ethanol treatment increases the expression of NOX and xanthine oxidase (XOX), which are the main source of superoxide production. Acetaldehyde, a metabolite of ethanol by alcohol dehydrogenase, can activate NADPH oxidase (NOX) and xanthine oxidase (XOX) and increase the expression of NOX and XOX [26].

4. Ethanol promotes neuronal apoptosis

Studies have shown that hippocampal volume is significantly smaller in alcoholic patients [27,28]. The loss of brain volume by overconsumption of alcohol may cause by increased neuronal apoptosis. It has been found that apoptosis is increased in the brains of animal models after acute alcoholism [29,30]. Both mitochondrial-mediated apoptotic pathway and extrinsic apoptotic pathway mediated by
Fas death receptor and the activation of caspase 8 are involved in the neuronal apoptosis induced by acute alcohol treatment [29,30,31].

Several cell signal pathways have been shown to be associated with alcohol-induced neuronal apoptosis. Mitogen-activated protein kinases (MAPKs) are a family of serine/threonine-specific protein kinases including extracellular signal-regulated kinases 1 and 2 (ERK1/2), ERK3/4, ERK5, p38 MAPK, C-Jun N-terminal kinase (JNK) 1, 2 and 3, which are the core pathways in many receptors signaling pathways and play important roles in regulating cell proliferation and apoptosis [32]. After treatment with 100 mmol/L ethanol for 24 hours, SK-N-SH neuroblastoma cells showed cell cycle G1 arrest, activation of caspase-3 and increased apoptosis. The activation of JNK, p38 MAPK and p53 were increased [33]. p53 is a downstream target of JNK and p38 MAPK. Activated p53 is involved in the induction of cell cycle arrest and apoptosis [34]. Therefore, ethanol may block cell cycle and induce apoptosis by activating MAPK-p53-related pathway.

Glycogen synthase kinase 3 (GSK3) is a serine protein kinase that phosphorylates key enzymes of a variety of signaling pathways. In mammals, GSK3 is encoded by two genes, GSK3α and GSK3β, which share 85% homology. The relative molecular masses of GSK3α and GSK3β are 51 KD and 47 KD, respectively [35]. GSK3β mainly participates in the regulation of cellular metabolism, proliferation, senescence, apoptosis and its abnormal expression level will trigger a series of diseases such as Alzheimer's disease [35], cervix cancer [36] and inflammation [35]. Phosphorylation of Ser9 of GSK3β inhibits GSK3 activity, while phosphorylation of Tyr216 of GSK3β increases its activity [36]. It has been shown that ethanol treatment induces dephosphorylation of GSK3β at Ser9 and the activation of Bax as well as caspase-3 in the brains of C57BL/6 mice, while pretreatment with GSK3β inhibitor ameliorates these ethanol-induced alterations. In human neuronal cell line SK-N-MC, overexpressing wildtype GSK3β or active form of GSK3β increases the sensitivity of cells to ethanol-induced apoptosis. In contrast, overexpression of dominant-negative GSK3β confers resistance to ethanol toxicity [37]. Therefore, GSK3β may be a mediator of ethanol neurotoxicity and blocking the activity of GSK3β may inhibit ethanol-induced neurotoxicity. As ROS can induce overexpression of GSK-3β [38], ethanol-induced oxidative stress could be an upstream signal for GSK3β activation that eventually leads to neuronal apoptosis.

5. Conclusion

After ingestion, ethanol enters the blood within 2-5 minutes, and the blood concentration of ethanol peaks 30-90 minutes after ingestion. Because of its water- and fat-soluble characteristics, ethanol passes the blood-brain barrier easily, and affects the function of the nervous system [39]. Ethanol induced neurotoxicity may be associated with ethanol-induced glutamate excitotoxicity and oxidative stress-promoting apoptosis signals, ultimately impairing neuronal synapses and inducing apoptosis, thereby causing nervous system dysfunction and triggering multiple diseases.

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