

Effects of ethanol on the pathogenesis of Alzheimer's disease

Kaixuan Yi, Yue Shi and Yan Zhao*

Department of Bioengineering, Harbin Institute of Technology, Weihai, Shandong, P. R. 264209 China.

*Corresponding author e-mail: zhaoyan@hitwh.edu.cn

Abstract. Alzheimer's disease (AD) is an age-related neurodegenerative disease associated with cognitive decline and memory loss. It has been shown that alcohol drinking affects the mechanisms of neurodegeneration in patients with AD. This paper reviews the effects of alcohol on major factors involved in AD pathogenesis, including the formation and aggregation of beta-amyloid, posttranslational modulation of microtubule-associated proteins, induction of oxidative stress and degeneration of acetylcholine system.

1. Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders. Patients with AD suffer progressive brain tissue damage and cognitive dysfunction [1]. Epidemiological studies have shown that alcohol drinking is associated with cognitive impairment and dementia [2]. Heavy drinking and alcohol abuse cause brain shrinkage and cognitive decline and damage to the nervous system [2]. In contrast, light or moderate drinking (<1 drinks/day) does not increase the risk of cognitive impairment [3]. In AD patients, heavy drinking (8 or more alcoholic drinks/week) causes a faster cognitive decline compared to the abstainers, while light to moderate alcohol drinking (1-7 alcoholic drinks/week) leads to a slower cognitive decline [4]. It is also found that moderate drinking protects cognitive function in individuals carrying *Apo lipoprotein E epsilon4 (APOE epsilon4)*, which is a widely accepted risk factor for AD [5]. Therefore, the effects of alcohol drinking on AD development depend on the quantity and the frequency of drinking, although the detailed mechanisms are not totally understood. This article discusses how ethanol affects major factors involved in AD pathogenesis, including the formation and aggregation of beta-amyloid, the phosphorylation of microtubule-associated proteins, oxidative stress and the degeneration of acetylcholine system.

2. Effect of ethanol on Abeta toxicity

Beta-amyloid (Abeta) protein, the main component of the senile plaques seen in AD brain, is produced by sequential proteolytic cleavages of Abeta precursor protein (APP) by beta-secretase (BACE1) and gamma-secretase [6]. Abeta can form toxic oligomers that trigger synapse loss and dysfunction, causing cognitive impairment [7] [8]. In rat model of chronic alcohol consumption, the protein levels of APP and BACE1 were increased in the hippocampus and cerebellum compared to that in the rats fed with control diet, suggesting that chronic alcohol consumption might increase the risk of AD by elevating the expression of APP and Abeta-producing enzymes [9]. On the other hand, it has been shown that ethanol treatment inhibits the aggregation of Abeta *in vitro* and decreased the toxicity of Abeta in HEK and PC-12 cell lines [10]. It is suggested that ethanol weakens the formation of amyloid dimers



and more complex aggregates by destroying the salt bridge composed by residues Asp 23 and Lys 28 [10] which is important for the formation of the amyloid dimer. Electron microscopy (EM) study showed that ethanol treatment (10 mM) changed the ultrastructure of Abeta from fibrillar form to a smaller amorphous and/or disordered appearance. Furthermore, ethanol treatment reduced the association between Abeta oligomers and neuronal membranes and decreased the synaptic toxicity induced by Abeta oligomers [11]. Treatment of ethanol with moderate dose also inhibits Abeta-induced synaptotoxicity by reducing the loss of key presynaptic proteins such as synaptic vesicle 2 and synaptophysin in mouse hippocampal cultures [11]. In mouse cortical neurons exposed to ethanol (0.02-0.08 % v/v), Abeta42 oligomers-induced reduction of synaptophysin was also prevented. Abeta-induced activation for cytoplasmic phospholipase A2 (cPLA₂), which can lead to synaptic damage, was attenuated by ethanol treatment [12].

3. Effect of ethanol on microtubule-associated proteins

Tau is a major microtubule-associated protein (MAP) in neurons, which plays a key role in maintaining the microtubule (MT) assembly and stability [13]. Phosphorylation and dephosphorylation of MAPs can modulate the function of MT [14]. The abnormal hyperphosphorylated Tau can aggregate into neurofibrillary tangles (NFTs), which is a pathological characteristic presented in AD brain [15]. In human neuroblastoma cells overexpressing Tau, it is found that ethanol (1.25-5 mg/ml) treatments increase the level of Tau protein and decrease the viability of cells by reducing the expression of calpain 1, which participates in tau degradation, thus causing the accumulation of Tau [16]. It has been shown that ethanol treatment increases tau phosphorylation during neurodegeneration in the forebrains of ethanol-treated postnatal day 7 mice. And the phosphorylation of tau induced by the ethanol treatment is mediated by glycogen synthase kinase-3beta [17]. Microtubule-associated proteins like MAP2 also plays an important role in the assembly and stability of microtubule [18]. Using microtubules extracted from the brains of rats, it is shown that low levels of ethanol (6, 12, 24, and 48 mM) stimulate, whereas high levels of ethanol (96, 384, and 768 mM) suppress the phosphorylation of MAP2 *in vitro* [19]. These studies suggest that alcohol drinking may affect both the accumulation and phosphorylation of microtubule-associated proteins.

4. Effect of ethanol on oxidative stress in brain

Studies have indicated that oxidative stress is associated with the pathogenesis of neurodegenerative diseases such as AD [20] [21]. The oxidation of ethanol by cytochrome P450-2E1 (CYP2E1) generates H₂O₂, which can lead to the production of reactive oxygen species (ROS) when interacting with copper or iron [22]. After exposure to 17.5 mM ethanol, CYP2E1 activity is induced 3-folds together with the paralleled increase of ROS production in human neurons [22]. Long-term alcohol use can increase the expression of *CYP2E1* in rat brain, cerebral cortex and human brain [23,24], accompanied by the decrease of glutathione and superoxide dismutase activity [25,26]. 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 [22], which are the main source of superoxide production [27][28], thus contributing to the oxidative stress [22]. Animal studies have shown that alcohol treatment increases malondialdehyde (MDA) and protein carbonyls in the synaptosomal membranes of rats [29]. It has also been shown that the activities of catalase and superoxide dismutase and the content of glutathione are decreased in synaptosomes in rats treated with 20 % (v/v) ethanol at 5 g/kg body weight every day for sixty days, the resulted oxidative stress changes the lipid composition and damages the function of synaptosomal membranes [29].

5. Effect of ethanol on acetylcholine system

The cholinergic system is an important neurotransmitter system in animal brains. The neurotransmitters released such as acetylcholine (Ach) play critical roles in memory and learning [30]. The degeneration seen in brains of AD patients is often associated with the deficits of cholinergic

systems [31]. Cerebral cholinergic neurons are susceptible to ethanol. Alcohol abuse can lead to the loss of cortical muscarinic cholinergic receptors [32] and cholinergic neurons [33]. Low concentration of alcohol consumption (0.8 g/kg) in rats promotes the release of Ach in hippocampus, while high concentration of alcohol consumption (2.4 g/kg) has an opposite effect, suggesting that the cognitive impairment caused by alcohol abuse may be due to the inhibitory effect of ethanol on Ach release [34]. Similarly, in adult rats given ethanol (20 % v/v) as their sole source of drinking water for three to six weeks, choline acetyltransferase activity is decreased in the cerebral cortex, hippocampus and cerebellum, and Ach release is reduced [35]. These studies suggest that high concentration of ethanol can suppress the release of Ach by reducing its synthesis, thus may aggravate the symptoms of dementia in AD patients. However, low concentration of ethanol can promote the Ach release, thus may protect nervous system and ameliorate the symptoms of AD patients [34].

6. Conclusion

AD is a complicated neurodegenerative disease. Depending on the quantity and the frequency of drinking, alcohol may have detrimental or protective effects on brain function. Heavy alcohol use causes a faster cognitive decline in AD patients, while moderate alcohol drinking may protect against the development of AD. This may reflect the differential effects of different concentrations of ethanol on Abeta production and aggregation, function of microtubule-associated proteins, ROS production and Ach release.

Acknowledgments

This work was financially supported by National Natural Science Foundation of China (31371082), research fund from Harbin Institute of Technology at Weihai [HIT(WH)Y200902] and Weihai Science and Technology Development Program (2009-3-93 and 2011DXGJ14).

References

- [1] Kar S, Slowikowski SP. Interactions between beta-amyloid and central cholinergic neurons: implications for Alzheimer's disease. *J Psychiatry Neurosci*. 2004; 29: 427–441.
- [2] Tyas SL. Alcohol use and the risk of developing Alzheimer's disease. *Alcohol Res Health*. 2001; 25: 299-306.
- [3] Neafsey EJ, Collins MA. Moderate alcohol consumption and cognitive risk. *Neuropsychiatric Disease and Treatment*. 2011; 7:465-484.
- [4] Devorah H, Yaakov S. The association between alcohol use and the progression of Alzheimer's disease, *Curr Alzheimer Res*. 2016; 13 (12): 1356-1362.
- [5] Carmelli D, Swan GE. The effect of apolipoprotein E epsilon4 in the relationships of smoking and drinking to cognitive function. *Neuroepidemiology* 1999; 18: 125-33.
- [6] Roberts HL, Schneider BL. α -Synuclein increases β -amyloid secretion by promoting β - γ -secretase processing of APP, *Plos one*, 2017, 12 (2): e0171925.
- [7] Shankar GM, Bloodgood BL. Natural oligomers of the Alzheimer amyloid-protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway, *Journal of Neuroscience*, 2007, 27 (11): 2866-2875.
- [8] Scheff SW, Price DA. Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. *Neurology*, 2007, 68 (18): 1501-1508.
- [9] Kim SR, Jeong HY. Effects of chronic alcohol consumption on expression levels of APP and A β -producing enzymes, *Bmb Reports*, 2011, 8 (4) :135-139.
- [10] Ormeno D, Romero F. Ethanol reduces amyloid aggregation in vitro and prevents toxicity in cell lines, *Archives of Medical Research*, 2013, 44 (1): 1-7.
- [11] Muñoz G, Urrutia JC. Low concentrations of ethanol protect against synaptotoxicity induced by A β in hippocampal neurons, *Neurobiology of Aging*, 2015, 36 (2): 845-856.
- [12] Bate C, Williams A. Ethanol protects cultured neurons against amyloid- β and α -synuclein-induced synapse damage, *Neuropharmacology*, 2011, 61 (8): 1406-1412.

- [13] Feinstein SC, Wilson L. Inability of tau to properly regulate neuronal microtubule dynamics: a loss of function mechanism by which tau might mediate neuronal cell death. *Biochim Biophys Acta* 2005; 1739: 268-279.
- [14] Hashiguchi M, Hashiguchi T. Kinase-kinase interaction and modulation of tau phosphorylation, *International Review of Cell & Molecular Biology*, 2013, 300:121.
- [15] Nakano H, Kobayashi K. Regional analysis of differently phosphorylated tau proteins in brains from patients with alzheimer's disease, *Dementia & Geriatric Cognitive Disorders*, 2004, 17 (3): 122-131.
- [16] Gendron TF, McCartney S. Ethanol enhances tau accumulation in neuroblastoma cells that inducibly express tau. *Neuroscience Letters*, 2008,443 (2): 67-71.
- [17] Saito M, Chakraborty G. Tau phosphorylation and cleavage in ethanol-induced neurodegeneration in the developing mouse brain, *Neurochemical Research*, 2010, 35 (4): 651-659.
- [18] Dehmelt L, Halpain S, The MAP2/Tau family of microtubule-associated proteins, *Genome Biology*, 2005,6 (1): 1-10.
- [19] Ahluwalia B, Ahmad S. Low levels of ethanol stimulate and high levels decrease phosphorylation in microtubule-associated proteins in rat brain: an in vitro study. *Alcohol & Alcoholism* 2000;35:452-457.
- [20] Markesbery WR . oxidative stress hypothesis in Alzheimer's disease, *Free Radic Biol Med*, 1997, 23 (1): 134-147.
- [21] Barnham KJ, Masters CL. Neurodegenerative diseases and oxidative stress, *Biomedicine & Pharmacotherapy*, 2004, 58 (1): 39-46.
- [22] Haorah J, Ramirez SH. Mechanism of alcohol-induced oxidative stress and neuronal injury, *Free Radical Biology & Medicine*, 2008, 45 (11): 1542-1550.
- [23] Anandatheerthavarada HK, Shankar SK. Induction of brain cytochrome P-450IIE1 by chronic ethanol treatment. *Brain Res.* 601: 279-285; 1993.
- [24] Upadhyya SC, Tirumalai PS. Cytochrome P4502E (CYP2E) in brain: constitutive expression, induction by ethanol and localization by fluorescence in situ hybridization. *Arch. Biochem. Biophys.* 373: 23-34; 2000.
- [25] Bai J, Cederbaum AI. Overexpression of CYP2E1 in mitochondria sensitizes HepG2 cells to the toxicity caused by depletion of glutathione. *J. Biol. Chem.* 281: 5128–5136; 2006.
- [26] Kessova IG, Cederbaum AI. Mitochondrial alterations in livers of Sod1^{-/-} mice fed alcohol. *Free Radic. Biol. Med.* 42: 1470–1480; 2007.
- [27] Vaziri ND, Dicus M. Oxidative stress and dysregulation of superoxide dismutase and NADPH oxidase in renal insufficiency, *Kidney International*, 2003, 63 (1): 179-185.
- [28] George J, Struthers AD. Role of urate, xanthine oxidase and the effects of allopurinol in vascular oxidative stress. *Vasc Health Risk Manag* 2009; 5: 265-272.
- [29] Reddy VD, Padmavathi P. Association between alcohol-induced oxidative stress and membrane properties in synaptosomes: A protective role of vitamin E, *Neurotoxicology & Teratology*, 2017, 63: 60-65.
- [30] Zhen J, Qian Y. Deep brain magnetic stimulation promotes neurogenesis and restores cholinergic activity in a transgenic mouse model of alzheimer's disease, *Front Neural Circuits*, 2017, 11: 48.
- [31] Perry E, Walker M. Acetylcholine in mind: a neurotransmitter correlate of consciousness *Trends Neurosci* 1999; 22: 273-80.
- [32] Freund G, Ballinger W. E. Loss of cholinergic muscarinic receptors in the frontal cortex of alcohol abusers. *Alcohol. clin. exp. Res.* 1988; 12, 630-638.
- [33] Arendt T, Bigl V. Loss of neurons in the nucleus basalis of Meynert in Alzheimer's disease, paralysis agitans and Korsakoff's disease. *Acta neuropath.* 1983, 61 (2): 101.
- [34] Henn C, Loffelholz K. Stimulatory and inhibitory effects of ethanol on hippocampal acetylcholine release. *Arch Pharmacol* 1998; 357: 640-47.

- [35] Casamenti F, Scali C. Long-term ethanol consumption by rats: effect on acetylcholine release in vivo, choline acetyltransferase activity, and behavior. *Neuroscience*. 1993; 56: 465-471.