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Research report

Suppression of neuro-inflammatory signaling cascade by tocotrienol can prevent chronic alcohol-induced cognitive dysfunction in rats

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ABSTRACT

Chronic alcohol intake is known to induce the selective neuronal damage associated with increase oxidative–nitrosative stress and activation of inflammatory cascade finally resulting in neuronal apoptosis and thus dementia. In the present study, we investigated the comparative effect of both the isoforms of vitamin E, α-tocopherol and tocotrienol against chronic alcohol-induced cognitive dysfunction in rats. Male Wistar rats were given ethanol (10 g/kg; oral gavage) for 10 weeks, and treated with α-tocopherol and tocotrienol for the same duration. The learning and memory behavior was assessed using Morris water maze and elevated plus maze test. The rats were sacrificed at the end of 10th week and cytoplasmic fractions of cerebral cortex and hippocampus were prepared for the quantification of acetylcholinesterase activity, oxidative–nitrosative stress parameters, tumor necrosis factor-alpha (TNF-α), and interleukin-1beta (IL-1β). From the 6th week onwards, ethanol-treated rats showed significant increase in transfer latency in both the behavioral paradigms which was coupled with enhanced acetylcholinesterase activity, increased oxidative–nitrosative stress, TNF-α and IL-1β levels in different brain regions of ethanol-treated rats. Co-administration of α-tocopherol as well as tocotrienol significantly and dose-dependently prevented these behavioral, biochemical and molecular changes in the brains of ethanol-treated rats. However, the effects were more pronounced with tocotrienol. The current study thus demonstrates the possible involvement of oxidative–nitrosative stress mediated activation of inflammatory cascade in chronic alcohol-induced cognitive dysfunction and also suggests the effectiveness of vitamin E isoforms, of which tocotrienol being more potent, in preventing the cognitive deficits associated with chronic alcohol consumption.

1. Introduction

Alcohol is the world's most widely used psychoactive drug, but chronic, excessive alcohol consumption leads to permanent organ damage or death. Ethanol consumption is the most common cause of peripheral as well as central nervous system toxicity. Alcohol-induced brain damage produces some of the most insidious effects of alcoholism, including cognitive deficits such as learning and memory impairment [47,61].

Alcohol dependence affects over 14% of the United States population and is the second leading cause of dementia [28], with 50–75% of sober, detoxified former alcoholics having cognitive impairments [16]. An epidemiological study in India by Jha and Patel has demonstrated the occurrence of 10.5% alcohol-related dementia which is more as compared to malnutrition-related dementia (7.2%) and Alzheimer's disease (4.8%) [24].

Both magnetic resonance imaging (MRI) and postmortem studies of alcoholic brains have found both gray and white matter loss in corticolimbic regions including the hippocampus [10]. The mechanism behind ethanol-induced selective neuronal damage is not well understood, but several explanations have been proposed. These include excitotoxicity associated with excessive neurotransmitter release, oxidative stress leading to free radical damage [11] and edema caused by alterations in cellular control of ion transport [9]. Thus, although the occurrence of alcoholic dementia and neurodegeneration are well supported by multiple studies, the mechanisms of neurotoxicity are still poorly understood.

Oxidative stress results from an imbalance between the endogenous antioxidant defense system and free radical generation. Excessive oxidative challenges impair the brain antioxidant defense systems and can activate secondary events leading to apoptosis by affecting DNA integrity, protein function, and membrane lipids [3] and ultimately producing neuronal death [5]. Ethanol enhances oxidative stress directly through generation of oxy free radicals and lipid peroxidation [44] and depletion of endogenous antioxidants such as α-tocopherol, glutathione, ascorbate, and vitamin E. Ethanol is oxidized to acetaldehyde by cytochrome P450, which increases reactive oxygen species, with concomitant changes in redox balance [39,63]. Rats given chronic ethanol show enhanced production of oxidative markers, such as

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thiobarbituric acid-reactive substances, hydrogen peroxide, and OH^- like species [15]. Studies have suggested that chronic ethanol increases oxidative damage to proteins, lipids, and DNA [38]. Reactive oxygen species producing enzymes including NOS, COX2, and NADPH oxidase are all induced by NFkβ activation suggesting that ethanol-induced ROS in brain may be related to NFkβ activation of inflammatory enzymes that produce ROS [12]. Chronic exposure to ethanol results in increased amounts of oxidative damage; translocation of PKC; activation of PKC and NFkβ, which results in DNA fragmentation; and ultimately increased neuronal death through apoptosis or other mechanisms that are responsible for the behavioral deficits including dementia [26].

Oxidative and nitrosative stress has been implicated in a variety of neurodegenerative disorders, including sclerosis, Parkinson's disease, and Alzheimer's disease, and may play an important role in the behavioral deficits (such as dementia) produced by ethanol [5].

There are numerous studies suggesting the protective role of natural antioxidants in variety of neurodegenerative disorders. Ebselen, an organoselenium glutathione peroxidase mimetic, has been found to reverse ethanol-induced inhibition of neurogenesis supporting an antioxidant mechanism [22]. Butylated hydroxyl toluene, a very potent antioxidant, is also known to block NFkβ–DNA binding and reduce neurotoxicity due to a combination of ethanol, TNFα, and glutamate [64]. Further, cannabidiol, is a cannabinoid found in Cannabis, has also been found to protect against binge alcohol-induced brain damage likely due to antioxidant properties [21]. These findings suggest that natural antioxidants may block ethanol-induced neurotoxicity and thus can prevent the behavioral deficits associated with chronic alcohol intake.

Vitamin E is one of the essential, fat-soluble nutrients that functions as an antioxidant in the human body. Burton and Ingold [4] presented the first comprehensive review article discussing that α-tocopherol has near optimal activity as a chain-breaking antioxidant and that both the phenolic head and phytyl tails contributed to the biological properties of the vitamin E molecule. α-Tocopherol gained recognition as the most important lipophilic radical-chain-breaking antioxidant in tissues *in vivo*. Deficiency of α-tocopherol in membranes made them highly permeable and therefore vulnerable to degradation [52]. Tocotrienol, an another isoform of vitamin E possesses numerous functions that are not shared by α-tocopherol. At nanomolar concentrations, α-tocotrienol uniquely prevents inducible neurodegeneration by regulating specific mediators of cell death such as 12-lipoxygenase and c-Src kinase [30,52]. In the presence of nanomolar α-tocotrienol, neurons were resistant to glutamate, homocysteine as well as the L-buthionine sulphoximine-induced toxicity. The study of 12-lipoxygenase activity and metabolism revealed that this key mediator (12-lipoxygenase) of glutamate-induced neurodegeneration is subject to control by the nutrient α-tocotrienol [30]. Micromolar amounts of tocotrienol suppress the activity of HMG-CoA reductase, the hepatic enzyme responsible for cholesterol synthesis [46]. Tocotrienols are thought to have more potent antioxidant properties than α-tocopherol [53,54].

Thus, the aim of the present study was 2 fold, first to investigate the possible involvement of oxidative–nitrosative stress mediated inflammatory cascade in chronic alcohol-induced cognitive dysfunctions and secondly, to evaluate the protective potential of both the isoforms of vitamin E, i.e. α-tocopherol and tocotrienol, on neuroinflammation in rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (150–200 g) bred in Central Animal House facility of Panjab University were used. The animals were housed under standard laboratory conditions, maintained on a 12:12 h light:dark cycle and had free access to food (Ashirwad Industries, Mohali, India) and water. Animals were acclimatized to laboratory conditions before all the behavioral tests. All experiments were carried out between 0900 and 1700 h. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Panjab University and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation.

2.2. Drugs

α-Tocopherol was purchased from Sigma, USA and tocotrienol (mixture of α-, β-, γ-tocotrienol) was received as a gift sample from Golden-Hope Bioganic, Malaysia Palm Oil Board, Malaysia. TNF-α and IL-1β (markers of enhanced neuroinflammation), ELISA kits were purchased from R&D Systems, USA. All other chemicals used for biochemical estimations are of analytical grade.

2.3. Induction of alcoholic dementia and drug treatment schedule

Alcoholic dementia was induced by oral gavage of 10 g/kg of 35% (v/v) ethanol (b.i.d) in double distilled water for 10 weeks. The rats serving as controls were given 10 g/kg p.o. of distilled water. α-Tocopherol and tocotrienol were freshly prepared in double distilled water after triturating with 5% Tween 80 and administered by oral route 1 h before ethanol dosing daily for 10 weeks. The dose of ethanol was decided on the basis of preliminary standardization study conducted in our laboratory (unpublished data). The animals were randomly divided into seven experimental groups with 5–8 animals in each viz. control group (distilled water in place of ethanol), vehicle treated rats with ethanol administration, α-tocopherol (100 mg/kg) and tocotrienol (50, 100 and 200 mg/kg) treated rats with ethanol and tocotrienol (200 mg/kg p.o.) treated rats without ethanol administration.

3. Behavioral tests

3.1. Morris water maze test

Animals were tested in a spatial version of Morris water maze test [42,59]. The apparatus consisted of a circular water tank (180 cm in diameter and 60 cm high). A platform (12.5 cm in diameter and 38 cm high) invisible to the rats was set 2 cm below the water level inside the tank with water maintained at $28.5 \pm 2\,^{\circ}C$ at a height of 40 cm. The tank was located in a large room where there were several brightly colored cues external to the maze; these were visible from the pool and could be used by the rats for spatial orientation. The position of the cues remained unchanged throughout the study. The water maze task was carried out at 6th, 8th and 10th week. The rats received four consecutive daily training trials in the following 5 days, with each trial having a ceiling time of 90 s and a trial interval of approximately 30 s. For each trail, each rat was put into the water at one of four starting positions, the sequence of which being selected randomly. During test trials, rats were placed into the tank at the same starting point, with their heads facing the wall. The rat had to swim until it climbed onto the platform submerged underneath the water. After climbing onto the platform, the animal remained there for 20 s before the commencement of the next trial. The escape platform was kept in the same position relative to the distal cues. If the rat failed to reach the escape platform within the maximally allowed time of 90 s, it was guided with the help of a rod and allowed to remain on the platform for 20 s. The time to reach the platform (escape latency in seconds) was measured.

3.2. Memory consolidation test

A probe trial was performed [59] at the end of 10th week wherein the extent of memory consolidation was assessed. The time spent in the target quadrant indicates the degree of memory consolidation that has taken place after learning. In the probe trial, the rat was placed into the pool as in the training trial, except that the hidden platform was removed from the pool. The total time spent in target quadrant in a time period of 90 s was recorded.

3.3. Elevated plus maze test

Memory acquisition and retention were tested using elevated plus maze test at 6th, 8th and 10th week. The apparatus consisted