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Resveratrol abrogates alcohol-induced cognitive deficits by attenuating oxidative–nitrosative stress and inflammatory cascade in the adult rat brain

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ABSTRACT

Chronic alcohol intake is known to induce permanent cognitive deficits along with enhanced oxidative–nitrosative stress and activation of neuroinflammatory cascade. In the present study, we investigated the protective effect of resveratrol, a natural polyphenolic phytoalexin against chronic alcohol-induced cognitive dysfunction and neuroiflammatory cascade in the brain of adult rats chronically administered ethanol. Male Wistar rats were adminstered ethanol (10 g/kg; oral gavage) for ten weeks and treated with resveratrol (5, 10 and 20 mg/kg) for the same duration. Ethanol-exposed rats showed impaired spatial navigation in the Morris water maze test and poor retention in the elevated plus maze task which was coupled with enhanced acetylcholinesterase activity, increased oxidative–nitrosative stress, cytokines (TNF-alpha and IL-1beta), NF-kappa β and caspase-3 levels in different brain regions (cerebral cortex and hippocampus) of ethanol-treated rats. Co-administration with resveratrol significantly and dose-dependently prevented all the behavioral, biochemical and molecular deficits. Correlatively, the results of the present study revealed that treatment with resveratrol significantly prevented cognitive deficits induced by chronic ethanol exposure not only by modulating oxido–nitrosative stress but also by attenuating the enhanced levels of pro-inflammatory cytokines (TNF-α and IL-1β), NF-kβ and caspase-3 in different brain regions of ethanol treated rats. Therefore, mechanism underlying the neuroprotective effects of resveratrol observed in our study may be due to its antioxidant, anti-inflammatory and neuromodulating activities.

1. Introduction

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. Alcohol-induced brain damage produces some of the most insidious effects of alcoholism, including cognitive deficits such as learning and memory impairment (White, 2003). 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 (Crews et al., 2005). Early observations using computerized tomography identified "shrinkage" in the brains of chronic alcoholics (Cala et al., 1981) that were later verified with higher resolution imaging based on magnetic resonance techniques. Specific brain regions affected by chronic alcohol exposure and described by structural MRI include cortical gray and white matter particularly prefrontal areas in older alcoholic individuals, anterior hippocampus, thalamus and cerebellum (De Bellis et al., 2005; Cardenas et al., 2007).

Although the occurrence of alcoholic dementia and neurodegeneration are well supported by multiple studies, the mechanisms of neurotoxicity are still poorly understood. Among possible causative factors is neurotoxicity of the ethanol molecule itself or its metabolic products (acetaldehyde). Greater expression of GluT5 in several brain regions of alcoholics relative to controls supports the concept that neuroinflammation occurs in response to chronic and excessive alcohol consumption (He and Crews, 2008). Neuroinflammation is a feed-forward/feed-back process and may lead to neurodegeneration which is supported by several converging lines of evidence. Any immune reaction primes microglia to activate proinflammatory cytokines (IL-1β and TNFα), which in turn stimulate microglia to produce MCP-1, which in turn leads to excessive production of proinflammatory cytokines and potentially neurodegeneration (Qin et al., 2007). Systemic cytokines particularly TNFα may enter the brain to initiate the inflammatory process (Qin et al., 2007) and lead to neuronal loss by increasing brain glutamate levels (Zou and Crews, 2005).

Rats given chronic ethanol showed enhanced production of oxidative markers, such as thiobarbituric acid-reactive substances, hydrogen peroxide, and OH^- like species (Dicker and Cederbaum, 1992). Studies have suggested that chronic ethanol increases oxidative damage to proteins, lipids and DNA (Mansouri et al.,

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2001). Reactive oxygen species producing enzymes including NOS, COX-2, and NADPH oxidase are all induced by NF-κβ activation suggesting that ethanol-induced ROS in brain may be related to NF-κβ activation of inflammatory enzymes that produce ROS (Crews et al., 2006). Chronic exposure to ethanol results in increased amounts of oxidative damage, translocation of PKC, activation of PKC and NF-kβ which results in DNA fragmentation and ultimately increased neuronal death through apoptosis or other mechanisms that are responsible for the behavioral deficits including dementia (Jung et al., 2005). Oxidative and nitrosative stress has been implicated in a variety of neurodegenerative disorders, including multiple sclerosis, Parkinson's disease and Alzheimer's disease and may play an important role in the behavioral deficits (such as dementia) produced by ethanol (Butterfield et al., 2002).

There are numerous studies suggesting the protective role of natural antioxidants in variety of neurodegenerative disorders. Butylated hydroxy toluene, a very potent antioxidant, is also known to block NF-kβ–DNA binding and reduced neurotoxicity due to a combination of ethanol, TNF-α and glutamate (Zou and Crews, 2005). 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 (Hamelink et al., 2005). These findings suggest that natural antioxidants may block ethanol-induced neurotoxicity before or during alcohol exposure. Thus, the present study was designed with an aim to investigate the effect of resveratrol, a potent antioxidant and anti-inflammatory molecule against chronic alcohol-induced cognitive deficits and associated neuroinflammatory cascade.

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 natural 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 and reagents

Resveratrol was purchased from Sigma (St. Louis, MO, USA) and prepared freshly by suspending in 0.5% carboxymethylcellulose just before administration. TNF-α and IL-1β ELISA kits were purchased from R&D Systems, USA. While NF-κβ and Caspase-3 ELISA kits were procured from Imagenex, San Diego, USA and Biovision, USA respectively. All other chemicals and reagents used for biochemical estimations were of analytical grade.

2.3. Drug treatment schedule

The animals were randomly divided into six experimental groups with 5–8 animals in each group. Group I comprised of control animals given double distilled water in place of ethanol by oral gavage; Group II animals were administered ethanol (10 g/kg oral gavage); Group III–V consisted of resveratrol (5, 10 and 20 mg/kg; oral gavage) treated rats along with administration of ethanol (10 g/kg) and Group VI animals received resveratrol (20 mg/kg; oral gavage) alone. The dose of ethanol was decided on the basis of standardization study (unpublished data) and previous study conducted in our laboratory (Tiwari et al., 2009). Resveratrol was administered by oral route 1 h before ethanol dosing daily for ten weeks starting from day 1. All the behavioral assays were done by an observer blind to the drug treatment on 6th, 8th and 10th week. After 10 weeks, blood was collected from tail vein and rats were sacrificed under deep anesthesia and brains were rapidly removed and placed on dry ice for isolation of cerebral cortex and hippocampus. Brain tissues were incubated with 1 ml of ice cold 1X hypotonic buffer supplemented with 1 mM DTT (Dithiothreitol) and 1% detergent solution for 30 min on ice. After incubation, the samples were centrifuged for 10 min at 10,000 rpm at 4 °C. The supernatant (Cytoplasmic Fraction) was transferred into a separate tube and stored at 4 °C. The nuclear pellet was resuspended in 100 μl nuclear lysis buffer by pipetting up and down. The samples were vortexed vigorously and suspension was incubated at 4 °C for 30 min. The suspension was vortexed again for 30 s and centrifuged at 14,000 rpm for 10 min at 4 °C in a microcentrifuge. The supernatant was transfered (Nuclear Fraction) into a pre-chilled microcentrifuge tube. Cytoplasmic and nuclear fractions were separated from the brain homogenate for the biochemical estimations and for quantification of TNF-α, IL-1β, NFκβ and Caspase-3. The samples were stored at −80 °C until processed for biochemical estimations.

3. Behavioral tests

3.1. Morris water maze test

Animals were tested in a spatial version of Morris water maze test (Morris et al., 1982; Tuzcu and Baydas, 2006). 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 ± 2 °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 trial, 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 (Tuzcu and Baydas, 2006) 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