Neurochemistry International 61 (2012) 310–320

Contents lists available at SciVerse ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Attenuation of NF-κβ mediated apoptotic signaling by tocotrienol ameliorates cognitive deficits in rats postnatally exposed to ethanol

Vinod Tiwari, Vipin Arora, Kanwaljit Chopra *

Pharmacology Research Laboratory, University Institute of Pharmaceutical Sciences, UGC Center of Advanced Study, Panjab University, Chandigarh 160 014, India

ARTICLE INFO

Article history:
Received 26 January 2012
Received in revised form 2 May 2012
Accepted 9 May 2012
Available online 18 May 2012

Keywords:
Alcohol
Apoptosis
Caspase 3
Cognitive deficits
Fetal alcohol spectrum disorders (FASDs)
Neuroinflammation
Nuclear factor kappa beta (NFκβ)
Oxidative stress
Tocotrienol

ABSTRACT

Ethanol-induced damage in the developing brain may result in cognitive impairment including deficits on neuropsychological tests of learning, memory and executive function, yet the underlying mechanisms remain elusive. In the present study we investigated the protective effect of tocotrienol against cognitive deficit, neuroinflammation and neuronal apoptosis in rat pups postnatally exposed to ethanol. Pups were administered ethanol (5 g/kg, 12% v/v) by intragastric intubation on postnatal days 7, 8 and 9. Ethanol-exposed pups showed significant memory impairment in Morris water maze task as evident from increase in escape latency and total distance travelled to reach the hidden platform. Time spent in target quadrant, % total distance traversed in target quadrant and frequency of appearance in target quadrant was also significantly decreased in ethanol exposed pups in probe trial. Poor memory retention was exhibited by ethanol-exposed pups in elevated plus maze test also. Impaired cognition was associated with significantly enhanced acetylcholinesterase activity, increased neuroinflammation (oxidative-nitrosative stress, TNF-α, IL-1β and TGF-β1) and neuronal apoptosis (NF-κβ and Caspase-3) in different brain regions of ethanol-exposed pups. Co-administration with tocotrienol significantly ameliorated all the behavioral, biochemical and molecular alterations in the different brain regions of ethanol exposed pups. The current study thus demonstrates the possible involvement of NF-κβ mediated apoptotic signaling in cognitive deficits associated with postnatal ethanol exposure in rats and points to the potential of tocotrienol in the prevention of cognitive deficits in children with fetal alcohol spectrum disorders (FASDs).

1. Introduction

Clinical and experimental evidence has demonstrated that ethanol is a teratogen and its consumption during pregnancy induces harmful effects on the developing fetus that leads to mental retardation and long-term cognitive and behavioral deficits in offsprings (Abel, 2004). Central nervous system dysfunctions are the most severe and permanent consequence of maternal alcohol intake and can occur in absence of gross morphological defects (Stratton et al., 1996). Mental retardation and long-term cognitive and behavioral deficits are some of the problems commonly found in children of women who were moderate or heavy drinkers during pregnancy (Stratton et al., 1996). Human prenatal ethanol exposure that occurs during a period of increased synaptogenesis known as the "brain growth spurt" has been associated with significant impairments in attention, learning and memory. Recent studies have shown that administration of ethanol to infant rats during the synaptogenesis period (first 2 weeks after birth) triggers extensive apoptotic neurodegeneration throughout many regions of the developing brain and results in cognitive dysfunctions as the animal matures (Tiwari and Chopra, 2011). The cost of caring for children with fetal alcohol spectrum disorders (FASD) has been estimated at approximately US$ 74.6 million per year, with three quarters of this cost associated with the care of FASD cases with mental retardation (Abel and Sokol, 1991). The mechanisms underlying the deleterious effects of ethanol on the developing brain remain largely unknown and no efficient treatment is currently available. Therefore, understanding how prenatal alcohol exposure produces behavioral and cognitive deficits is of great medical and economic importance.

Experimental evidence demonstrates that alcohol interferes with many molecular, neurochemical and cellular events during the normal development of the brain. Some brain areas are more affected than others and, even within a given region, some cell populations are more vulnerable than others. The neocortex, hippocampus and cerebellum are especially susceptible to alcohol and have been associated with the behavioral deficits (Guerri, 2002). There are several evidences suggesting that deleterious effects of ethanol on neuronal cells have been associated with enhanced oxidative stress (Pirlich et al., 2002) and that oxidative stress in neuronal cells causes both apoptosis and necrosis

* Corresponding author. Tel.: +91 172 2534105; fax: +91 172 2541142.
E-mail address: dr_chopra_k@yahoo.com (K. Chopra).

0197-0186/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuint.2012.05.010

(Oberdoerster et al., 1998; Pirlich et al., 2002). The brain is particularly susceptible to oxidative stress because of its high oxygen consumption, high polyunsaturated fatty acid content and low antioxidant defenses (Lau et al., 2005). The developing brain, which has only a fraction of the antioxidant enzyme activity of the adult brain, is perhaps even more vulnerable to the neurotoxic effects of oxidative stress than the adult brain (Henderson et al., 1999). In addition, certain regions of the central nervous system, such as the hippocampus and cerebellum, may be particularly sensitive to oxidative stress because of their low endogenous levels of Vitamin E, an important biochemical antioxidant, relative to other brain regions (Abel and Hannigan, 1995). Such a depressed defense system may be adequate under normal circumstances. However, in pro-oxidative conditions, such as during alcohol exposure, these low antioxidant defenses can predispose the fetal brain to oxidative damage. Moreover, in vivo studies show that ethanol treatment reduces levels of the endogenous antioxidant glutathione (GSH) (Uysal et al., 1989) and alters activities of the antioxidant enzymes superoxide dismutase and catalase (Heaton et al., 2003). The role of oxidative stress in alcohol-induced neurotoxicity is also supported by studies showing beneficial effects of antioxidant therapy during alcohol exposure. Pretreatment with the peptide NAPVSIPQ, which has potent antioxidant properties *in vitro*, prevents fetal death in a mouse model of FAS (Spong et al., 2001). The treatment also prevents the reduction in glutathione, a common indicator of oxidative stress. Additional studies have demonstrated that the antioxidant vitamins α-tocopherol (Vitamin E) and β-carotene prevent neurotoxicity in cultured hippocampal neurons exposed to alcohol (Mitchell et al., 1999). Another finding from in vivo model of fetal alcohol syndrome showed that inclusion of Vitamin E in the diet prevented alcohol-induced Purkinje cell loss in the cerebellum of neonatal rats (Heaton et al., 2000). These studies suggest that antioxidant therapy either before or during alcohol exposure may protect the developing fetus from alcohol's teratogenic effects.

Tocotrienol, an isoform of vitamin E, is one of the most potent natural anti-oxidants and 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 (Khanna et al., 2003; Sen et al., 2000). Micromolar amounts of tocotrienol suppress the activity of HMG-CoA reductase, the hepatic enzyme responsible for cholesterol synthesis (Pearce et al., 1994). Tocotrienols are thought to have more potent antioxidant properties than α-tocopherol (Serbinova et al., 1991; Serbinova and Packer, 1994). In our previous study, we also found that chronic treatment with tocotrienol ameliorates signs and symptoms of alcoholic neuropathy in rats chronically administered alcohol (Tiwari et al., 2009a).

Thus, the present study was designed with an aim to investigate the effect of tocotrienol on alcohol-induced cognitive dysfunction and NF-κβ mediated apoptotic neurodegeneration in the developing rat brain.

2. Materials and methods

2.1. Animals

Wistar male rat pups (5 day old neonates) housed in Central Animal House facility of Panjab University were used in the study. The neonatal rats were housed under standard laboratory conditions maintained at 22 °C and on a 12:12 h light:dark cycle. 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 carried out in accordance with the guidelines of National Institutes of Health for the care and use of Laboratory animals (NIH Publications No. 80–23, revised 1978) and Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

Tocotrienol (mixture of α-, β-, γ-tocotrienol) was received as a gift sample from Golden-Hope Bioganic, Malaysia Palm Oil Board, Malaysia. TNF-α, IL-1β and TGF-β1 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, California, USA respectively. All other chemicals used for biochemical estimations are of analytical grade.

2.3. Treatment schedule

12–16 pups were housed per cage and both the experimental and control groups were marked with different colors and housed in different cages. The pups were randomly divided into five experimental groups. Group I was the control group in which pups were administered double distilled water in place of ethanol. Group II pups were administered only ethanol (5 g/kg; oral gavage). Group III and IV animals were administered tocotrienol (50 and 100 mg/kg; oral gavage) along with ethanol (5 g/kg; oral gavage). While group V animals (*per se* group) received tocotrienol only (100 mg/kg; oral gavage). *Per se* group means the group received only the drug treatment without ethanol administration. It works as treatment control. The group is included in order to rule out any effect of treatment on the control animals. Tocotrienol (50 and 100 mg/kg) treatment was started on postnatal day (PD) 6 and was continued till the end of study. Ethanol (12% v/v) was administered in the dose of 5 g/kg from PD 7 to PD 9 (Marino et al., 2004) 1 h after the tocotrienol administration. Pups were placed back with the nursing dams right after intubation. From postnatal day 24–28 rat pups were tested for learning and memory task in Morris water maze and elevated plus maze task. At the end of postnatal day 28, the pups 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, TGF-β1, NFκβ and Caspase-3. The samples were stored at −80 °C until processed for biochemical estimations.

2.4. Behavioral tests

2.4.1. Morris water maze (Computer tracking using Ethovision software)

Pups were tested in a spatial version of Morris water maze test (Morris et al., 1982; Tuzcu and Baydas, 2006) from postnatal day 24–28th. The apparatus consisted of a circular water tank