Neurotoxicology and Teratology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

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

Inulin supplementation during gestation mitigates acrylamide-induced maternal and fetal brain oxidative dysfunctions and neurotoxicity in rats ☆

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ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form 5 March 2015
Accepted 11 March 2015
Available online xxxx

Keywords:
Inulin
Prebiotic
Gestation
Acrylamide
Oxidative stress
Neurotoxicity
Fetus
Rat

ABSTRACT

Accumulating evidence suggests that the developing brain is more susceptible to a variety of chemicals. Recent studies have shown a link between the enteric microbiota and brain function. While supplementation of non-digestible oligosaccharides during pregnancy has been demonstrated to positively influence human health mediated through stimulation of beneficial microbiota, our understanding on their neuromodulatory propensity is limited. In the present study, our primary focus was to examine whether supplementation of inulin (a well known fructan) during gestation can abrogate acrylamide (ACR)-induced oxidative impairments and neurotoxicity in maternal and fetal brain of rats. Initially, in a dose-determinative study, we recapitulated the impact of ACR exposure during gestation days (GD 6–19) on gestational parameters, extent of oxidative impairments in brain (maternal/fetal), cholinergic function and neurotoxicity. Subsequently, pregnant rats orally (gavage) administered with inulin (IN, 2 g/kg/day in two equal installments) supplements during gestation days (GD 0–19) were exposed to ACR (200 ppm) in drinking water. IN supplements significantly attenuated ACR-induced changes in exploratory activity (reduced open field exploration) measured on GD 14. Further, IN restored the placental weights among ACR exposed dams. Analysis of biochemical markers revealed that IN supplements effectively offset ACR associated oxidative stress not only in the maternal brain, but in the fetal brain as well. Elevated levels of protein carbonyls in maternal brain regions were completely normalized with IN supplements. More importantly, IN supplements significantly augmented the number of *Bifidobacteria* in the cecum of ACR rats which correlated well with the neurorestorative effect as evidenced by restored dopamine levels in the maternal cortex and fetal brain acetylcholinesterase activity among ACR-exposed dams. Further, IN supplements also conferred significant protection against mitochondrial dysfunction induced by ACR in both milieus. Although the precise mechanism/s by which IN supplements during pregnancy attenuate ACR induced neurotoxic impact merits further investigations, we hypothesize that it may mediate through enhanced enteric microbiota and abrogation of oxidative stress. Further, our study provides an experimental approach to explore the neuroprotective role of prebiotic oligosaccharides during pregnancy in reducing the adverse impact of developmental neurotoxicants.

1. Introduction

Recent evidence has revealed that gut microbiota possesses the propensity to influence brain development and behavior. Epidemiological and animal studies have shown the beneficial effects of the proliferation of bacterial strains on brain chemistry (Messaoudi et al., 2011; Collins and Bercik, 2013). Administration of live bacterial supplements (probiotics) to rodents is shown to alter trophic cytokine (brain-derived neurotrophic factor, BDNF) gene expression (O'Sullivan et al., 2011). Interestingly, probiotic cocktail reduced the process aligned with anxiety in rats and serum cortisol levels associated with psychological distress in humans (Messaoudi et al., 2011). Although the precise mechanisms mediating these effects remain unclear, the involvement of dampening down of the elevated levels of oxidative free radicals and pro-inflammatory cytokines seems likely (Cryan and O'Mahony, 2011). Hence, dietary interventions using prebiotics to stimulate the growth of intrinsic beneficial intestinal microbiota is being explored as a tool to achieve various beneficial effects to the brain.

Fructans, such as inulin are plant derived nondigestible carbohydrates (NDO) are mainly digested by cecal microbiota (Yasuda et al.,

☆ Presented in part at the Joint Meeting of the 20th Biennial Meeting of the International Society for Developmental Neuroscience (ISDN) and the 5th Annual NeuroDevNet Brain Development Conference held on 19–24 July 2014 at Montreal, Canada.

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http://dx.doi.org/10.1016/j.ntt.2015.03.003
0892-0362/

2007) and increase the indigenous bacterial community (Kelly, 2008; van Vlies et al., 2012). Previously, supplementation of non-digestible oligosaccharides during pregnancy, has been reported to ameliorate various metabolic disorders (Qiu et al., 2008). Further, studies have demonstrated a significant diminution in the levels of procarcinogenic biomarkers in rats following prophylactic treatment with inulin (Verma and Shukla, 2013). A recent study reported the potential of inulin in alleviating the LPS-induced oxidative stress in human colon epithelial cell lines (Pasqualetti et al., 2014).

Acrylamide (ACR) is a vinyl, water-soluble alkene used in the production of glue and plastics that have various commercial applications (LoPachin, 2004; Erkekoglu and Baydar, 2014). ACR is demonstrated to be neurotoxic in humans and affects both central and peripheral nervous system. This neurotoxin has gained tremendous importance owing to its formation during frying/baking of commonly consumed foods such as fried potatoes, biscuits and coffee (JECFA, 2011). Studies in rodents have demonstrated that ACR crosses the placenta (Annola et al., 2008; von Stedingk et al., 2011) and causes reproductive/developmental toxicity coupled with adverse effects on the fetus such as lower birth weight, skeletal abnormalities (Manson et al., 2005; El-Sayyad et al., 2011) and neurodevelopmental toxicity (Takahashi et al., 2009; Ferguson et al., 2010; Garey and Paule, 2010; El-Sayyad et al., 2011). The involvement of oxidative stress and inflammatory responses in ACR-induced neurotoxicity are widely accepted (Lopachin and Gavin, 2008; Prasad and Muralidhara, 2012, 2013). Further, ACR is shown to form conjugates with reduced glutathione (GSH), and the resulting complex is metabolized by cytochrome P450 (subtype CYP 2E1) to form glycidamide. Recent evidence suggests that ACR forms adducts within presynaptic proteins resulting in altered neurotransmission and inactivation of enzymes involved in neuronal energy production (LoPachin and Gavin, 2012; Martyniuk et al., 2013).

Compelling evidence exists that chemical agents widely disseminated in the environment are important triggers of neurological anomalies (Grandjean and Landrigan, 2006; Drechsel and Patel, 2008). Developing brain is of particular interest since it is highly susceptible to neurotoxin insult during the critical window of vulnerability. Given the wide industrial applications and the possible neurotoxic effects associated with the chronic human exposure to ACR starting in utero, it is relevant to develop specific therapeutic strategies to abrogate ACR-induced fetal neurotoxic effects. Hence, in the present study our primary objective was to understand whether supplementation of inulin, (a widely consumed NDO) to dams during pregnancy possess the potential to attenuate ACR-induced oxidative stress and neurotoxicity in a rat model. As a prelude, we characterized some of the biochemical perturbations (oxidative impairments and neurotoxicity) in rats associated with gestational exposure to varying doses of ACR. Subsequently we investigated the ameliorative effect of inulin supplements against ACR intoxication in maternal as well as fetal brain.

2. Materials and methods

2.1. Chemicals

Acrylamide (electrophoresis grade; purity > 99%, Product #A8887), inulin from chicory (Product #I2255), 2-thiobarbituric acid, 1,1,3,3-tetramethoxypropane, 2′,7′-dichlorofluorescein (DCF), 2′,7′-dichlorofluorescein diacetate, N,N,N′,N′-tetramethylethylenediamine, L-glutathione reduced, acetylthiocholine iodide, *S*-butyrylthiocholine iodide, nicotinamide adenine dinucleotide, thiazolyl blue tetrazolium bromide, dopamine were procured from Sigma Aldrich, St Louis, MO, USA. 2,4-Dinitrophenylhydrazine, 1-chloro-2,4,-dinitro benzene, nicotinamide adenine dinucleotide phosphate, cytochrome c were procured from Sisco Research Laboratories Pvt. Ltd., India. All other reagents used were of analytical or HPLC grade.

2.2. Experimental animals

Male and female adult Wistar rats were randomly drawn from the stock colony of the institute's animal house facility (200 ± 10 g), and acclimatized for 1 week. Rats were housed in vented polypropylene cages (40 × 28 × 16 cm^3) with dust-free shaved wood bedding. Animals housed in a light- and temperature-controlled room (14 h/10 h light-dark cycle, 21 °C, 50% humidity) were provided with commercial rodent pellet diet (Sai Durga Feeds & Foods, Bengaluru, India) and tap water ad libitum. Following acclimatization, virgin female rats were allowed to mate overnight with males (2:1 ratio). Vaginal smear using 0.9% sodium chloride was performed daily to ascertain pregnancy. The swab was examined on a slide using a bright-field microscope for the presence of spermatozoa. The presence of sperm was designated as gestation day (GD) 0, and the females were separated, weighed and individually housed. All protocols were approved by the institutional animal ethics committee in conformance with the guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, India. Handling, as well as care of animals during sacrifice, was strictly according to the standard guidelines of the Institutional Ethics Committee (Registration #49/1999/CPCSEA).

2.3. Experimental procedure

2.3.1. Study 1: exposure of pregnant rats to acrylamide (ACR): Effect on gestational parameters and oxidative markers in fetal and maternal brain

Sperm-positive female rats were placed in plastic cages and randomly divided into 4 groups. While dams (n = 6) of treatment groups (Groups II, III and IV) received 50, 100 and 200 ppm ACR in drinking water during gestation days (GD) 6 to 19, those of control group (group-I) received deionized water. Water consumption and feed intake were recorded daily while body weight gain was monitored on alternate days. On GD 19, all dams were sacrificed, maternal brains and uterine horns were rapidly exteriorized, immersed in ice-cold phosphate buffered saline (PBS), blotted dry and cryostat cooled as previously described (Shivananjappa and Muralidhara, 2012). Fetuses and placenta were separated from uterine horns after fixing it to a wax base, immersed in ice-cold phosphate buffer saline (PBS). Further, fetal brain were excised from fetuses, rinsed in ice-cold PBS, the meninges were removed and stored at −80 °C until further processing. Similarly, brains were excised from the dams and brain regions viz., cortex and cerebellum were separated on ice and stored until used. Markers of oxidative stress and antioxidant/detoxifying enzymes were determined in the maternal brain regions (cortex and cerebellum) and whole fetal brain.

2.3.2. Study 2: protective effects of inulin supplements in the ACR model

Pregnant rats were assigned four groups and treated follows: Group I — Untreated controls; Group II — Inulin (IN); Group III and Group IV — ACR (200 ppm, GD 6–19) in drinking water. Rats of Groups II and IV were orally administered (gavage) with inulin (IN, 2 g/kg/day) supplements during gestation days (GD 0–19). The total daily dose of IN was given as split dose (twice a day at the rate of 1 g/kg at 09:00 and 15:00 h each day). The IN dose was selected based on our preliminary study, and the doses were adjusted daily to account for the changes in body weight of the dams. Dams of all treatment groups were subjected to open field test on GD 14 and were sacrificed on GD 19. Further procedures were identical as described in Study 1. Biochemical parameters were determined in the maternal brain (cortex and cerebellum) and fetal brain. The cecum from dams of all treatment groups was collected, and subjected to quantification of *Bifidobacteria* and *Lactobacteria* adopting standard micobiological procedures.

Please cite this article as: Gokul K, Muralidhara , Inulin supplementation during gestation mitigates acrylamide-induced maternal and fetal brain oxidative dysfunctions and neurotoxi..., Neurotoxicol Teratol (2015), http://dx.doi.org/10.1016/j.ntt.2015.03.003