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Original article

Oral supplements of inulin during gestation offsets rotenone-induced oxidative impairments and neurotoxicity in maternal and prenatal rat brain

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ABSTRACT

Environmental insults including pesticide exposure and their entry into the immature brain are of increased concern due to their developmental neurotoxicity. Several lines of evidence suggest that maternal gut microbiota influences in utero fetal development via modulation of host's microbial composition with prebiotics. Hence we examined the hypothesis if inulin (IN) supplements during pregnancy in rats possess the potential to alleviate brain oxidative response and mitochondrial deficits employing a developmental model of rotenone (ROT) neurotoxicity. Initially, pregnant Sprague-Dawley rats were gavaged during gestational days (GDs) 6–19 with 0 (control), 10 (low), 30 (mid) or 50 (high) mg/kg bw/day of ROT to recapitulate developmental effects on general fetotoxicity (assessed by the number of fetuses, fetal body and placental weights), markers of oxidative stress and cholinergic activities in maternal brain regions and whole fetal-brain. Secondly, dams orally supplemented with inulin (2×/day, 2 g/kg/bw) on GD 0–21 were administered ROT (50 mg/kg, GD 6–19). IN supplements increased maternal cecal bacterial numbers that significantly corresponded with improved exploratory-related behavior among ROT administered rats. In addition, IN supplements improved fetal and placental weight on GD 19. IN diminished gestational ROT-induced increased reactive oxygen species levels, protein and lipid peroxidation biomarkers, and cholinesterase activity in maternal brain regions (cortex, cerebellum, and striatum) and fetal brain. Moreover, in the maternal cortex, mitochondrial assessment revealed IN protected against ROT-induced reduction in NADH cytochrome *c* oxidoreductase and ATPase activities. These data suggest a potential role for indigestible oligosaccharides in reducing oxidative stress-mediated developmental origins of neurodegenerative disorders.

1. Introduction

Exposure of pregnant women and developing fetus to environmental toxins is a major concern for developmental neurotoxicity [1]. Pesticide exposure during development enhances the susceptibility to neurodegeneration and neuropsychological disturbances since the gestation stage is developmentally unique [2]. Studies have reported a link between rotenone exposure and dopaminergic neurodegeneration involving oxidative and cholinergic factors contributing to neurotoxicity [3,4]. Reports have shown that rotenone induces ganglion cell layer thinning [5], and recently, it has been recognized as a developmental neurotoxicant [6,7]. Environmental insult during gestation elevates fetal brain oxidative stress which could be detrimental to neurodevelopment [8]. Additionally, recent findings indicate the peripheral influence on neurodegenerative disease onset [9] with chemical toxicants disturbing the gut microbiota [10] to promote oxidative stress signalling [11]. Hence, it is important to minimize the adverse events occurring early following developmental insults.

Inulin, a β-1,2-linked fructan oligosaccharide with a terminal 1,2-α-linked glucose has been demonstrated to enhance gut microflora with many beneficial effects [12]. Accruing evidence indicate the influence of gut microbiome on neurodevelopment [13]. Microbiota composition within maternal gut can influence developmental events with microbiota-generated substrates promoting optimal blood-brain barrier functioning [14]. Environmental factors which alter the microbiota composition consequently affect the availability of dietary precursors and reduce mother's beneficial gut microbes exert a profound impact on prenatal brain development [15]. Moreover, emerging findings have indicated the functional role of gut microbes to regulate α-synuclein inclusions suggesting an association between gut microbes and Parkinson's disease (PD) [9].

Gut microbial metabolism contributes to dietary energy transfer

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essential to support neurodevelopment [15]. The oligosaccharide prebiotics to a certain extent promote metabolic benefits and have been recently well-appreciated for their brain benefits [16,17]. Most recent studies show that prebiotics influence developing preterm brain [18] and enhance hippocampal dendritic spine density [19]. Increased brain gene-transcription has been demonstrated in rats supplemented with a blend of galactooligosaccharides-lactoferrin [20]. Our previous findings indicate that the non-digestible ingredients significantly attenuate brain lipid peroxidation and increase neurochemical levels [21]. Interestingly, several prebiotics have also been shown to counteract metabolic syndrome in the brain [22] and reverse psychological phenotype [23].

Reactive oxygen species (ROS) generation and reduced antioxidant system contribute to oxidative-stress-mediated neuronal demise [80]. This is evident during early developmental stages and represents a key process by which dopaminergic toxins promote neurotoxicity [81]. Free radical formation is one of the most potent inducers of lipid peroxidation (LPO) which can introduce carbonyl groups into protein either through malondialdehyde (MDA) or indirectly through oxidation of amino acids to promote the formation of protein carbonyl (PCO). These actions have important implications for loss of function of metabolic enzymatic components and cellular reductases required for redox homeostasis and establish signalling pathways. The affinity of dopaminergic toxins for protein and nonprotein thiols also represents an important phenomenon in neurotoxicity [24,25]. Tripeptide glutathione (GSH) reduction leads to NADH:ubiquinone oxidoreductase (Complex I) inhibition, an early event in mitochondrial functional impairment [26]. The decylubiquinol cytochrome *c* oxidoreductase (Complex III) plays an important role in the respiratory chain allowing oxidation of ubiquinone (respiratory chain component connecting Complex I with III) and cytochrome *c* reduction for proton gradient and ROS production to influence ATPase complex and mitochondrial energy metabolism [27].

With the recent observations of gut microbiota regulating antioxidant metabolism [28], microbiota-mitochondria crosstalk [29] and ability to influence neurodegenerative diseases [9], raises the prospect of prebiotic use to minimize oxidative processes of neurodegeneration following gestational exposure to PD toxin. Accordingly, we investigated the effect of rotenone (ROT) exposure during gestation exposure on oxidative impairments in maternal rat brain and associated implications on fetal brain. Further, we assessed whether gestational inulin (IN) supplements significantly ameliorate maternal behavior, counteract gestational rotenone-induced oxidative stress, mitochondrial dysfunction and neurochemical changes in maternal and fetal rat brain.

2. Materials and methods

2.1. Chemicals

Rotenone (≥ 95% purity), inulin from chicory (Product #I2255), 2-thiobarbituric acid, 1,1,3,3-tetramethoxypropane, 2′,7′-dichlorofluorescein diacetate, N,N,N′,N′-tetramethylethylenediamine, hydrogen peroxide, acetylthiocholine iodide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nicotinamide adenine dinucleotide phosphate, 1-chloro-2,4,-dinitro benzene, cytochrome c, methanol, hydrogen peroxide (all solvents were analytical grade) were purchased from Sisco Research Laboratories Pvt. Ltd., India.

2.2. Animals

Male and virgin female adult Wistar rats were randomly drawn from our institute's animal house facility and housed in a temperature- and humidity-controlled room with *ad lib* food and water. Following one week of acclimatization, 2 females and 1 male were mated and female rats were identified (to determine the gestation day (GD) 0) within a 5-day period by observing the presence of spermatozoids in the vaginal smears. Sperm positive females were individually housed. Treatment protocol began after randomizing to treatment groups within GD 0 body weights.

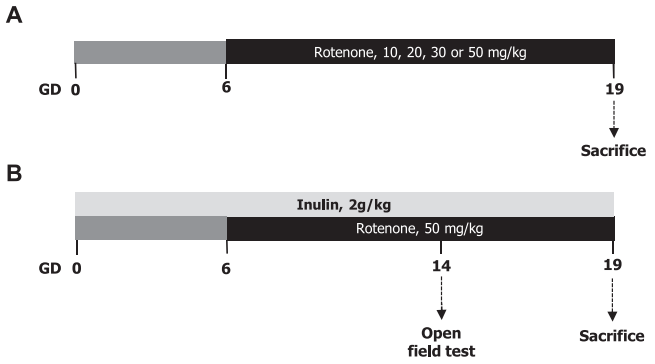

Fig. 1. Schematic representation of the experimental design. The horizontal bars represent maternal treatment regimen. Numerals below the bar indicate gestational day (GD) time point. **A**, Sperm positive rats received one of four doses of ROT (10, 20, 30 and 50 mg/kg bw/day, p.o.; GD 6–19) or vehicle (0.5% CMC) (n = 6 dams/group). **B**, In this study, pregnant rats were administered with inulin (IN, 2 g/kg/day, p.o.; GD 0–19) also received ROT (50 mg/kg bw/day, p.o.; GD 6–19). Exploratory behavior in open field test was assessed on GD 14. (See Materials and Methods for details).

All experiments were approved by the Institutional Animal Ethics Committee and adhered to the guidelines stablished by the Committee for the Purpose of Control and Supervision of Experiments on Animals (49/1999/CPCSEA), Ministry of Environment, Forests and Climate Change, Government of India, India.

2.3. Study design

Experiment 1: Assessment of Rotenone (ROT) Exposure on Gestation Parameters, Fetal Characteristics, and Brain Oxidative Response

A summary of the experimental design is presented in Fig. 1. To assess the effect ROT exposure on gestation, ROT was suspended in 0.5% (by weight) carboxymethyl cellulose sodium salt (CMC) and gavaged to dams with one of four doses, 10 (low), 30 (medium) or 50 (high) mg/ kg body weight/ day [bw/d], beginning on the morning of GD 6 until and including GD 19. Dams used as controls received 0.5% CMC as vehicle once daily for the same duration. The oral ROT doses used here were selected based on the previous report indicating chronic ROT administration to induce nigrostriatal dopaminergic degeneration [82]. Further, the higher doses of ROT used in this study were selected based on the lower oral bioavailability than that for other routes of administration [30]. Since ROT is lipophilic, oils (e.g., corn oil) and dimethylsulfoxide are often used as vehicles. However, reports that oils possess antioxidant effect [31] and estrogenic action [83], and toxicity associated with dimethylsulfoxide, discourages their use. Hence, CMC was chosen here which also offered the advantage of not producing undesirable effects. ROT doses were adjusted for body weights recorded daily prior to dosing. All animals were weighed and sacrificed in the morning of GD 19. The number of implantations, resorptions and placentae weights were recorded. Explanted fetuses were examined to determine body weight and external anomalies. Oxidative stress response in maternal and fetal brains was analysed biochemically.

Experiment 2: Modulatory Effect of Inulin (IN) on Gestational ROT-induced Neurotoxicity

In the present study, we sought to determine if IN administration would counteract gestational ROT induced oxidative impairments and neurotoxicity. Pregnant rats were randomly assigned to one of the four treatment groups (n = 6).

Group I – Vehicle control (CTR, 0.5% CMC, p.o.; GD 0–19)
Group II – Inulin (IN, 2 g/kg bw/d, p.o.; GD 0–19)
Group III – Rotenone (ROT, 50 mg/kg bw/d, p.o.; GD 6–19)
Group IV – Inulin (IN, 2 g/kg bw/d, p.o.; GD 0–19) + Rotenone