Cytokine 71 (2015) 173–180

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Interplay between pro-inflammatory cytokines and brain oxidative stress biomarkers: Evidence of parallels between butyl paraben intoxication and the valproic acid brain physiopathology in autism rat model

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ARTICLE INFO

Article history:
Received 30 April 2014
Received in revised form 28 September 2014
Accepted 28 October 2014

Keywords:
Butyl paraben
Autism animal model
Valproic acid
Brain cytokine
Brain oxidative stress

ABSTRACT

Butyl paraben is a preservative used in food, drugs and cosmetics. Neurotoxic effect was reported recently beside the potential estrogenic activity of parabens. There is controversy as to the potential harmful effects of butyl parabens, which are suspected to contribute to autism and learning disabilities. The purpose of this study was to examine the similarities between paraben intoxication signs in the rat brain and brain markers in an autistic like rat model. This study provides evidence of many parallels between the two, including (1) oxidative stress, (2) decreased reduced glutathione levels and elevated oxidised glutathione, (3) mitochondrial dysfunction, and (4) neuroinflammation and increased pro-inflammatory cytokine levels in the brain (tumour necrosis factor-alpha, interleukin-1-beta, and interleukin-6). (5) Increased protein oxidation reported by a significant increase in 3-nitrotyrosine (3-NT)/tyrosine ratio. (6) A marked disturbance was found in the production of energy carriers (AMP, ATP and AMP/ATP ratio) in comparison with the control. The evidence suggests that paraben may, to some extent, either cause or contribute to the brain physiopathology in ASDs or pathogens that produce the brain pathology observed in the diagnosed rat model of ASD.

1. Introduction

Autism is a heterogeneous behaviourally defined severe neurodevelopment disorder that is characterised by an impairment in verbal and nonverbal communication, imagination, and reciprocal social interaction [1] Autism belongs to a group of neurodevelopmental disorders known as autism spectrum disorders (ASDs). Although the cause of autism remains elusive, autism is considered a multi-factorial disorder that is influenced by genetic, epigenetic, environmental, and immunological factors [2,3].

Accumulating evidence has suggested that oxidative stress may be a common feature in autism through which environmental factors exert their deleterious effects, which may be further exacerbated by the interaction of genetically susceptible alleles [3,4]. Antioxidants, specifically glutathione (GSH), are essential for neural survival during the early critical period [5,6]. In addition to serving as an antioxidant, GSH plays an important role in cell differentiation, proliferation, and apoptosis [7–9]. There is also ample evidence on the role of glutathione in both innate and adaptive immune functions, as well as an anti-inflammatory role [8,10,11]. Recently, evidence has suggested that increased exposure to oxidative stress, together with mitochondrial dysfunction [12,13] may contribute to the aetiology of multifactorial autism disorder, inflammation, and immune abnormalities [13]. Additionally, overactivation and inflammation of autism can result in an overproduction of NO. The levels of NO were found to be increased in autism, and cytokines were implicated in the production of higher levels of NO compared with control ones [14].

The activity of the immune system can elicit profound effects on behaviour. Several immune protein functions within the nervous system function as mediators of normal neural-development [15]. Cytokines such as TNF-α and IL-1β mediate direct effects on neural activity; for example, TNF-α is produced in a wide variety of cells during an inflammatory event [16]. In addition, it can modulate neural cell proliferation or cell death and play an important role

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http://dx.doi.org/10.1016/j.cyto.2014.10.027
1043-4666/

in synaptic pruning [17–19]. Other neuropoeitic cytokines, such as IL-6 with IL-1β, also exert varied effects on neural survival, proliferation synapse formation, migration, and differentiation [20].

Foetal exposure to parabens via the use of paraben-containing personal care products (PCPs) by pregnant women is of particular relevance for three reasons. First, the foetus is particularly susceptible to endocrine disrupting agents, such as parabens, that can pass the blood-placenta barrier [21]. Moreover, the slightest exposure of such agents to a pregnant woman can result in severe deleterious effects in her offspring. Second, free forms of parabens are expected to circulate in the maternal bloodstream after their dermal absorption following the application of PCPs. Furthermore, it is expected that, in oral administration of parabens, conjugation forms of the gluconide with sulphate serves as a detoxification process during first-pass metabolism in the liver. Third, women are, in general, more cautious about establishing harmful habits, such as smoking and drinking alcohol during pregnancy; however, they may not be cognizant of the potential harm of using PCPs, and the exposure may not be contracted during pregnancy [22].

In this study, exposure to butyl paraben (oral or subcutaneous administration) of pregnant female rats was assessed by measuring pro-inflammatory cytokines and oxidative stress biomarkers of exposure in the pups of these dams as a sign of paraben intoxication compared with markers in the autistic rat pup model.

2. Materials and methods

2.1. Animals and chemicals

Male and female Wister rats were obtained from the National Organization for Drug Control and Research (NODCAR) laboratory (Cairo, Egypt). The rats were housed in a room in which the temperature and relative humidity were controlled with a 12-h light–dark cycle. The rats were fed a standard commercial pellet diet, and tap water was provided *ad libitum*. Animal experiments began after an acclimation period for two weeks after arrival. To obtain pregnant animals, virgin females at the age of 16–18 weeks and weighing 200 g were placed with males weighing 220–240 g (1–2 females/male) overnight. The next morning, plugged females with sperm in their vagina were regarded as pregnant and the day was assigned as the first gestational day [23]. The experimental protocols and procedures were approved by Ain Shams University authorities and followed Egyptian rules for animal protection, which was performed according to the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimise animal suffering, to reduce the number of animals used, and to utilise alternatives to in vivo techniques, if available.

Sodium valproate (VA, Sigma, St. Louis, MO, USA) was dissolved in 1% Tween 80 (polyethylene sorbitol ester) immediately prior to use. VA (800 mg/kg body weight) was orally administered via gavage to pregnant rats on gestational day 12.5. Pups of VA-injected dams represented the autistic-like group [24]. Butyl paraben (BP, Sigma, St. Louis, MO, USA) was dissolved in Tween 80 and orally administered at 200 mg BP/kg body weight/day [25] or subcutaneously at 200 mg/kg body weight [26]. The pups of these females represented the two paraben groups.

As a vehicle control, oral and subcutaneous (0.25 ml/100 g body weight of 1% Tween 80/day) administration of Tween 80 was performed on each day of the experiments. The pups of these dams represented the control groups.

Females of all groups were maintained individually and were born healthy. The number of offspring was normal. The young rats were weaned at 21 days old and were housed separately by gender.

2.2. Experimental design

Control groups: male pups of dams that received oral (COi) or subcutaneous (CSc) 0.25 ml/100 g body weight 1% Tween 80 for 6 consecutive weeks from the first day of gestation until day 21 of lactation.

Autistic-like group: male pups of dams orally administered 800 mg/kg body weight sodium valproate as a single dose on day 12.5 of gestation (VA) and treated as the oral control (COi) from the first day of gestation to 21 days of lactation.

Paraben groups: males pups of dams injected orally (BPOi) or subcutaneously (BPSc) with butyl paraben (200 mg/kg body weight/day) [25,26] from the first day of gestation until day 21 of lactation. The selected dose dissolved in 1% Tween 80 and each animal received 0.25 ml/100 g b.w. from the solution.

2.3. Behaviour tests

Three days prior to decapitation, the pups from all groups were subjected to the three-chamber sociability test and the Morris water maze as previously described [27].

2.4. Biochemical analyses

At the end of the experimental period, the male pups at 21 day age were sacrificed by cervical decapitation. Brain tissues were immediately excised and dissected into ice-cold physiological saline. Six whole brain tissues were homoginzed in cooled phosphate buffer saline (PH, 7.4) and centrifuged to obtain the supernatant. The brain supernatant was used to assess the total nitric oxide (NO) levels using an ELISA Kit specific for rat assays (Assay Designs, Inc-Germany). The levels of brain rat interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor alpha (TNF-α) were determined using enzyme immunoassay (EIA) techniques (IBL Gesellschaft, Hamburg, Germany). The levels of reduced per oxidised glutathione (GSH/GSSG) were determined using an ELISA Kit (Oxford Biomedical Research, Germany). The activity of cytochrome P450 reductase (CYP 450) was determined using an ELISA Kit (Life Science, Inc-Germany). The amount of tri-nitrotyrosine (3-NT), tyrosine (Tyr), adenosine triphosphate (ATP), and adenosine monophosphate (AMP) concentrations were measured in different brain regions (cortex, hippocampus, and midbrain) using high-performance liquid chromatography (HPLC) according to [28,29]. All the used ELISA kits are specific for rats.

Preparation of samples for HPLC:

The weighted brain tissues (cortex, hippocampus, and midbrain) were hemogenized in 10% (w/v) 75% methanol HPLC grade. The homogenized tissue was centrifuged and the supernatant was used for HPLC–UV analysis as follows.

2.4.1. AMP and ATP assay

A 20 μl samples were injected into the system. The separation was carried out in room temperature on Agilent 1100 Model system with an analytical column Nucleosil C-18 (15 × 0.4 cm). The mobile phase for the adenine nucleotides was 50 mM potassium phosphate 1% (v/v) methanol at PH 5.5 and flow rate 1 ml/min. The UV detector was set at 210 nm. The nucleotides were quantified using standard samples areas and the concentrations were expressed as μmole/g wet tissue.

2.4.2. 3-Nitrotyrosine assay

A part of the supernatant was incubated in 110 °C for 24 h with 6 N HCl and 0.1% phenol which is added to rule out the artifactual formation of NO2-Tyr during acid hydrolysis in the presence of nitrite or nitrate [30].