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Maternal carbamazepine alters fetal neuroendocrine-cytokines axis

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ABSTRACT

This study detected the impact of maternal carbamazepine (CBZ) on the fetal neuroendocrine-cytokines axis. 25 or 50 mg/kg of CBZ was intraperitoneally administrated to pregnant albino rats from the gestation day (GD) 1 to 20. Both administrations of CBZ caused a hypothyroidism in dams and fetuses whereas the decreases in serum thyroxine (T4) and triiodothyronine (T3) and increases in serum thyrotropin (TSH) levels were highly significant (LSD; $P < 0.01$) at GD 20 compared to untreated control dams. Also, both administrations had undesirable impacts on the maternofetal body weight, litter weight, survival of dams and fetuses, and their food consumption in comparison to the corresponding control. These administrations also elicited a reduction in fetal serum growth hormone (GH), interferon-γ (IFNγ), interleukins (IL-2 & 4) and prostaglandin E2 (PGE2) levels. Also, the elevation in fetal serum tumor necrosis factor-alpha (TNFα), transforming growth factor-beta (TGFβ), and interleukins (IL-1β & 17) levels was observed at embryonic day (ED) 20. Moreover, there were a cellular fragmentation, distortion, hyperemia, oedema and vacuolation in the fetal cerebellar cortex due to both maternal administrations. These developmental changes were dose-dependent. These novel results suggest that CBZ may act as a developmental immunoneuroendocrine disruptor.

1. Introduction

In the last 40 years, the administration of antiepileptic drugs (AEDs) during pregnancy has adverse actions on the fetuses (Liguori and Cianfarani, 2009; Cassina et al., 2013; Thomas et al., 2017; Wen et al., 2017). Indeed, AEDs therapy has been disclosed to induce the teratogenicity (Veiby et al., 2009; British National Formulary (BNF), 2011; Kaushik et al., 2016) and endocrine disorders in both children and adults (Svalheim et al., 2015). In this context, there is intrauterine growth restriction (IUGR), and impairment in the neurocognitive behaviors (Luef, 2009). Carbamazepine (CBZ), dibenzoazepine derivative, is recognized as antiepileptic and tricyclic anticonvulsant drug (Kaushik et al., 2016; Juhel et al., 2017; Lu and Wang, 2017; Wijnen et al., 2017). However, it is considered a human teratogen and transferred the placenta to accumulate in the fetal tissues (Bath and Scharfman, 2013; Nie et al., 2016). Also, it has a moderate effect on the thyroid, hepatic and metabolic markers of children (Yılmaz et al., 2014). Importantly, CBZ initiates a central hypothyroidism (Sigurjonsdottir et al., 2014), subclinical hypothyroidism (Hamed, 2015) and growth retardation (Rättyä et al., 1999). On the contrary, one study has reported that CBZ does not initiate a hypothyroidism in patients (Post et al., 1983). Another study has postulated that CBZ can reduce the free thyroxine (FT4) level and disrupt the thyrotropin (TSH) level (Vainionpää et al., 2004). Otherwise, maternal administration of CBZ induces a neural tube defect (Morrow et al., 2006), memory loss (Uddin et al., 2016), and neuronal injury (Barkovich and Raybaud, 2004; Leventer et al., 2008; Åberg et al., 2013). Additionally, any cortical abnormalities during the first or second trimester of gestation can cause epileptic

Abbreviations: GABA, γ-aminobutyric acid; ANOVA, analysis of one way of variance; AEDs, antiepileptic drugs; BNF, British National Formulary; CBZ, carbamazepine; DC, degenerative changes; Ds, deiodinases; ED, embryonic day; EGL, external granular layer; ERK1/2, extracellular signal regulated kinase; FT4, free thyroxine; GD, gestation day; GHRH, GH-releasing hormone; GH, growth hormone; H&E, Haematoxylin and Eosin; HBV, hyperemic blood vessel; HPTA, hypothalamic-pituitary-thyroid-axis; IgA, IgG and IgM, immunoglobulins; IGF1, insulin growth factor 1; IFNγ, interferon-γ; IL-1β, 2, 4 & 17, interleukins; IGL, internal granular layer; IUGR, intrauterine growth restriction; I^-, iodide; LSD, least significant degree; ML, molecular layer; NIC, National Cancer Institute; NGF, nerve growth factor; NF-κB, nuclear factor kappa of B cells; O, oedema; PGE2, prostaglandin E2; AKT, protein kinase B; PC, Purkinje cell; PL, Purkinje layer; rT3, reverse triiodothyronine; SE, standard error; TEGL, thickened external granular layer; TRs, TH receptors; THTs, TH-transporters; TBG, thyroid binding globulin; THs, thyroid hormones; TSH, thyrotropin; T4, thyroxine; TT4, total thyroxine; TGFβ, transforming growth factor-beta; T3, triiodothyronine; TNFα, tumor necrosis factor-alpha; UDP-GTs, uridine diphosphate glucuronyl transferases; V, vacuoles; WM, white matter.

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seizures (Leventer et al., 2008; Tamijani et al., 2015). Alternatively, the levels of cytokines are also impacted by CBZ treatment. Some findings have reported that the CBZ changes the levels of interleukins (IL-1β, 2, 4–6 & 10) (Basta-Kaim et al., 2008; Himmerich et al., 2013), transforming growth factor-beta (TGFβ) (Basta-Kaim et al., 2008), and tumor necrosis factor-alpha (TNFα) (Himmerich et al., 2013). Also, it causes inflammation (Björnsson, 2008), hypereosinophilia and hypersensitivity (skin involvement) (Mathieu et al., 2011). Conversely, the variation in immune markers can cause epilepsy or bipolar disorder (Himmerich et al., 2013). However, there are conflicting results regarding the impact of maternal CBZ on the fetal thyroid-brain and immune axis.

As the fetal thyroid hormones (THs) (Ahmed et al., 2008) and cytokines (Dean et al., 2012) showed a vital role in the developing brain, the goal of this study was to assess the impact of maternal administrations of CBZ (25 or 50 mg/kg) on the fetal neuroendocrine-cytokines axis. Thus, the current experiment was performed on pregnant albino rats to evaluate the following: **(1)** the alterations in the materno-fetal thyroid markers, body weight, food consumption, and survival of the dams and their fetuses; **(2)** the variations in the serum concentrations of fetal growth hormone (GH), pro-fibrotic marker (TGFβ), pro-inflammatory cytokines (TNFα, INFγ, IL-1β & 17), anti-inflammatory cytokines (IL-2 & 4) and acute-inflammatory cytokine (PGE2); and **(3)** the abnormalities in the histogenesis of the fetal cerebellum at the embryonic day (ED) 20. Indeed, this brain region is highly sensitive to any stress throughout the development (Ahmed, 2011; Ahmed et al., 2014).

2. Materials and methods

2.1. Experimental animals

Twenty-four mature virgins female Wistar rats (Rattus norvegicus) weighing 160–170 g and twelve adult males for mating only were purchased from the animal house of VACSERA in Helwan (Egypt). The rats were kept in stainless steel cages with constant light/dark cycle, temperature, and humidity throughout the experimental period. Tap water and food were provided ad libitum (Ahmed et al., 2015a,b). Before the beginning of the experiment, all animals were kept for 14 days to eliminate any intercurrent contaminations. Then, one male was coupled with two proestrous females for one or two days in a separate cage (Marcondes et al., 2002). The sperms in the vaginal smears confirmed the beginning of pregnancy and our experiment. The treatments and housing of pregnant rats were followed the overall rules of animal care in Egyptian (Zoology Department, Faculty of Science, Beni-Suef University) and Canadian Committees (Olfert et al., 1993). Notably, we did our best to decrease the animal suffering.

2.2. Experimental strategy

CBZ (Sigma Chemical Co.; dissolved in dimethylsulfoxide) was administered (i.p. injection) to pregnant rats at a dose of 25 or 50 mg/kg body weight/day during the whole pregnancy. These doses were selected according to Sitges et al. (2012) and Gómez et al. (2014), respectively. The high dose of CBZ (50 mg/kg) was equivalent to a comparable human dose of 486 mg and was given to epileptic patients per day (Reagan-Shaw et al., 2008). The control pregnant rats were injected the solvent vehicle only (i.p.). In contrast to human patients, we avoided the oral administration to prevent the stress, which could interfere with the gestation period and delay the developing brain (Manent et al., 2007).

At the end of the experiment, the dams and fetuses of all groups were sacrificed after anesthesia and tested at the gestation day (GD) 20. We followed the maternal body weight gain, the maternal mortality, the number of aborted dams, the food consumption, the litter weight, the live fetuses/litter, the number of dead fetuses/total fetuses, and the fetal body weight. We collected the blood samples for each dam and their fetuses from the jugular vein and umbilical cord, respectively. These samples were left to coagulate and centrifuged for 20 min at 3000 rpm (1006.2*g*). Their clear supernatants were directly separated into 3 Eppendorf tubes/each animal and reserved at −70 °C till utilized for various analysis. Also, the histopathological changes in the fetal cerebellum were examined at ED 20.

2.3. ELISA examination of the maternal and fetal markers

The serum concentrations of maternal and fetal TSH, T3 and T4, and fetal TNFα, GH, TGFβ, IFNγ, interleukins (IL-1β, 2, 4 & 17) and PGE2 were determined by ELISA (Spectra Max 190-Molecular Devices, USA) in Cairo University (Dep. of Biochemistry, Fac. of Medicine), Egypt. The practical kits were applied for estimation the serum concentrations of TSH, T3, T4, GH, TGFβ, and PGE2 (Millipore ELISA Kit, USA). Serum TNFα and IFNγ concentrations were examined using kits obtained from Invitrogen Corporation, USA. The IL-1β, IL-2, and IL-4 kits were purchased from R and A systems (USA) while the IL-17 kit was purchased from Cusabio (USA) according to manufacturer's instructions.

2.4. Histological examination of the fetal cerebellum

Cerebellar tissue samples were directly fixed in 10% neutral buffered formalin for twenty-four hours and ordered through the ethanol solutions (50%, 70%, 95% and 100%; 2 h for each change). These samples were sent to the National Cancer Institute (NIC) (Cairo, Egypt) for additional processing, clearing in xylene, embedding in paraffin wax, blocking, and serially sectioning at 6 μm and staining with Haematoxylin and Eosin (H&E) (Bancroft and Gamble, 2008).

2.5. Statistical analysis

The software of PC-STAT program was used for the statistical examination (Roa et al., 1985). Analysis of one way of variance (ANOVA) and the least significant degree (LSD) was used to distinguish the effects between the experimental groups. The results were expressed as a mean ± standard error (SE). The overall changes among the groups were established by F-probability. The means were highly significant ($P < 0.01$) and very highly significant ($P < 0.001$) different. Also, the mean values with similar superscript symbols were negligible alterations. Particularly, the number of examined samples/parameter/group was six.

3. Results

3.1. Maternal CBZ-disrupted the materno-fetal thyroid functions

Both administrations of 25 mg and 50 mg CBZ during pregnancy induced a maternofetal hypothyroidism as confirmed by an increase in the serum TSH and a decrease in the serum T4 and T3 levels at GD 20 (Table, 1). Notably, the fetal hypothyroid state was more potently in the 50 mg CBZ-treated group (−44.67% for T4, −92.44% for T3 & +228.69% for TSH) than in the 25 mg CBZ-treated group (-29.45% for T4 & −49.28% for T3 & +109.46% for TSH) (Table 1).