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Developmental exposure to cuprizone reduces intermediate-stage progenitor cells and cholinergic signals in the hippocampal neurogenesis in rat offspring

Hajime Abe [a,b], Takeshi Tanaka [a,b], Masayuki Kimura [a,b], Sayaka Mizukami [a,b], Nobuya Imatanaka [c], Yumi Akahori [c], Toshinori Yoshida [a], Makoto Shibutani [a,*]

[a] Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan
[b] Pathogenetic Veterinary Science, United Graduate School of Veterinary Sciences, Gifu University, 1-1 Yanagido, Gifu-shi, Gifu 501-1193, Japan
[c] Chemicals Evaluation and Research Institute, Japan, 1-4-25 Koraku, Bunkyo-ku, Tokyo 112-0004, Japan

HIGHLIGHTS

- Hippocampal neurogenesis following maternal exposure to CPZ in rats was investigated.
- ER-stress mediated apoptosis reduces the numbers of Tbr2$^+$ type-2b cells in the SGZ.
- CPZ suppresses myelination and the BDNF signaling cascade in the dentate gyrus.
- CPZ suppresses cholinergic signals to intermediate-stage progenitor cell populations.
- CPZ increases reelin$^+$ interneurons in response to decreased neuronal plasticity.

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ABSTRACT

The exposure to cuprizone (CPZ) leads to demyelination in the central nervous system in rodents. To examine the developmental effects of CPZ exposure on hippocampal neurogenesis, pregnant rats were treated with 0, 0.1 or 0.4% CPZ in the diet from gestational day 6 to day 21 after delivery. On postnatal day 21, male offspring had a decreased density of new glue2$^+$ oligodendrocyte progenitor cells in the dentate hilus and in the area of the cerebellar medulla in the presence of 0.4% CPZ. With regard to neurogenesis-related parameters, offspring had decreased T box brain 2$^+$ progenitor cells and increased apoptotic cells, as detected by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling, which was accompanied by the up-regulation of *Casp12* and *Bcl2l11* in the subgranular zone, and increased reelin$^+$ interneurons in the dentate hilus. In addition, the density of phosphorylated TrkB$^+$ interneurons decreased in the dentate hilus, which was accompanied by transcript down-regulation of *Bdnf* and *Chrna7* in the dentate gyrus. Moreover, granule cells expressing gene products of immediate-early genes, i.e., *Arc* and *Fos*, decreased. These results suggest that maternal exposure to 0.4% CPZ decreases proliferative type-2 progenitor cells via endoplasmic reticulum stress-mediated apoptosis and inhibition of cholinergic signals to intermediate-stage progenitor cells following reduced oligodendrocyte production and suppression of the brain-derived neurotrophic factor signaling cascade. Increases in reelin-expressing interneurons may compensate for impaired granule cell migration and/or correct positioning due to decreased immediate-early gene-mediated neuronal plasticity. However, all observed

Abbreviations: Arc, activity-regulated cytoskeleton-associated protein; Bcl2, B-cell CLL/lymphoma 2; BDNF, brain-derived neurotrophic factor; Bim, Bcl2-like 11; BLBP, brain lipid binding protein; Calb1, calbindin-D-28K; Calb2, calbindin-D-29K; C_T, threshold cycle; CPZ, cuprizone; DAB, 3,3-diaminobenzidine; Dcx, doublecortin; ER, endoplasmic reticulum; GABA, γ-aminobutyric acid; Gapdh, glyceraldehyde 3-phosphate dehydrogenase; GCL, granule cell layer; GD, gestational day; Fos, FBJ osteosarcoma oncogene; Hprt1, hypoxanthine phosphoribosyltransferase 1; Iba1, ionized calcium-binding adaptor molecule 1; IEG, immediate-early gene; MBP, myelin basic protein; NeuN, neuron-specific nuclear protein; NG2, new glue2; Olig2, oligodendrocyte lineage transcription factor 2; PCNA, proliferating cell nuclear antigen; PFA, paraformaldehyde; PND, postnatal day; RT-PCR, reverse-transcription polymerase chain reaction; Pvalb, parvalbumin; SGZ, subgranular zone; Sox2, SRY (sex determining region Y)-box 2; Tbr2, T box brain protein 2; TrkB, Tropomyosin receptor kinase B; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling.

* Corresponding author. Tel.: +81 42 367 5771; fax: +81 42 367 5771.
E-mail addresses: abeichi@cc.tuat.ac.jp (H. Abe), tatanaka@cc.tuat.ac.jp (T. Tanaka), mskimura@cc.tuat.ac.jp (M. Kimura), smizuka@cc.tuat.ac.jp (S. Mizukami), imatanaka-nobuya@ceri.jp (N. Imatanaka), akahori-yumi@ceri.jp (Y. Akahori), yoshida7@cc.tuat.ac.jp (T. Yoshida), mshibuta@cc.tuat.ac.jp (M. Shibutani).

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fluctuations disappeared at the adult stage, suggesting that CPZ-induced developmental neurotoxicity was reversible.

1. Introduction

Cognitive function is dependent on the effective communication between various brain regions. Myelination influences processing speed and strength, and plays a vital role in signal transduction. In humans, the majority of myelination in the central nervous system occurs postnatally and continues into adulthood. Thus, myelination in brain regions during development is thought to be important for the development of cognitive function (Nagy et al., 2004).

The hippocampus is a temporal lobe brain structure involved in various types of learning and memory. The subgranular zone (SGZ) of the dentate gyrus, a subregion of the hippocampus, uniquely continues to generate new neurons during postnatal life (McDonald and Wojtowicz, 2005). This process is called adult neurogenesis and consists of multistep processes, including a proliferative phase, such as the self-renewal of type-1 stem cells, facilitation of cell division of intermediate type-2 and type-3 progenitor cells to generate newborn granule cells (immature granule cells), and the subsequent differentiation and migration of new cells within the granule cell layer (McDonald and Wojtowicz, 2005). In the hilar region of the hippocampal dentate gyrus, γ-aminobutyric acid (GABA)-ergic interneurons are known to connect with adult-born dentate granule cells and play a functional role in adult neurogenesis (Toni et al., 2008). In particular, GABAergic interneurons secrete reelin, an extracellular matrix glycoprotein that modulates progenitor cell migration to maintain normal integration in the neonatal and adult mammalian dentate gyrus (Gong et al., 2007). Furthermore, there are subpopulations of GABAergic interneurons expressing calcium-binding proteins, such as calbindin-D-28K (Calb1), parvalbumin (Pvalb), and calbindin-D-29K (Calb2), which are a family of proteins containing the EF-hand Ca-binding motif, and potentially serve important roles in the development and function of the brain (Freund and Buzsáki, 1996). Various neurons outside the hippocampus also make synaptic connections with neurons in the dentate gyrus. For example, cholinergic neurons originating from the septal nucleus and nucleus of the diagonal band of Broca innervate neurons in the dentate hilus (Amaral and Kurz, 1985).

In the hippocampal dentate gyrus, all of the cell populations and their inherent phenomena involved in the process of adult neurogenesis may be a sensitive target of developmental neurotoxicity. Especially, self-renewal of stem cells, proliferation and migration of progenitor cells, neuritogenesis, synaptogenesis and myelinogenesis may be the vulnerable developmental processes against chemical toxicity. We have recently shown that developmental exposure to acrylamide and glycidol, both of which target axon terminals, impair late-stage differentiation of the neurogenic process in rat offspring (Akane et al., 2013a; Ogawa et al., 2012). In contrast, developmental hypothyroidism, which targets a broad range of neuronal stem/progenitor cells, decreases these cell populations in rat offspring (Shiraki et al., 2012). In addition, maternal transient exposure to methylnitrosourea, an anti-proliferating alkylating agent, mainly targets transient populations of highly proliferative progenitor cells without affecting the ability of stem cells to undergo self-renewal in both rat and mouse offspring (Itahashi et al., 2014; Takimoto et al., 2014). These results suggest that neurogenesis can be affected by neurotoxicants through targeting different cellular populations of neuronal cell lineage. Because the molecular mechanisms to control neuronal development processes have many similarities with those described for mechanisms for neuronal maintenance after maturation, neurogenesis process may be vulnerable to "adult-type neurotoxicants" to show affection of mature nervous tissue, suggesting that adult-type neurotoxicants may cause developmental neurotoxicity by affecting neurogenesis.

Cuprizone (CPZ) is a copper chelator and had been used in blood biochemistry for quantitative analysis of copper levels in sera (Peterson and Bollier, 1955). In the 1960s, it was reported that CPZ selectively injures oligodendrocytes and induces subsequent demyelination in the central nervous system in mice (Carlton, 1967). Thus, CPZ has been used as a model for multiple sclerosis (Matsushima and Morell, 2001). Cortical demyelination can be observed after 3 weeks of CPZ exposure in mice (Skripuletz et al., 2008). Demyelination is also observed in other brain regions such as the hippocampus and cerebellum (Koutsoudaki et al., 2009; Skripuletz et al., 2010). In addition, CPZ-induced demyelinating mice show behavioral deficits, loss of social interaction, and spatial working memory impairment (Xu et al., 2009). The mechanism of oligodendrocyte damage by CPZ is still not completely understood; however, mitochondrial disturbances seem to be the key factor for oligodendroglial apoptosis because enlarged giant-mitochondria were observed in the brains of CPZ-treated mice (Komoly et al., 1987). In rats, oral CPZ exposure also induces demyelination in the brain and decreases mRNA and protein levels of oligodendrocyte-specific genes in the prefrontal cortex (Adamo et al., 2006; Gregg et al., 2009; Kanno et al., 2012).

Among adult-type neurotoxicants, myelin toxicants may affect myelination of interneuron populations and neuronal inputs from outside the SGZ, and granule cells may not directly be affected because they are consisted of non-myelinated fibers. Thus, we hypothesized that developmental CPZ exposure to myelin toxicant can affect hippocampal neurogenesis through inhibition of GABAergic interneurons and/or neuronal inputs from outside the SGZ. There are chemicals that exert myelin toxicity. However, there is limited information with regard to the developmental exposure effect of myelin toxicants. The present study was performed to examine the effect of maternal exposure to CPZ as a representative myelin toxicant on hippocampal neurogenesis in rat offspring later in life. For this purpose, we administered CPZ to pregnant rats during periods of gestation and lactation, and examined the dose-effect relationship on the distribution, proliferation, and apoptosis of granule cell lineages in the SGZ and on the distribution of interneuron subpopulations in the dentate hilus on weaning and also at the adult stage.

2. Materials and methods

2.1. Chemicals and animals

CPZ (purity: >99.0%; CAS No. 370-81-0) was purchased from Sigma–Aldrich Japan Co. (Tokyo, Japan). Pregnant Crl:CD (SD) rats were purchased from Charles River Japan Inc. (Yokohama, Japan) at gestational day (GD) 2 (the appearance of vaginal plugs was designated as GD 0). Pregnant rats were housed individually with their offspring in plastic cages with paper chip bedding until postnatal day (PND) 21 (where PND 0 is the day of delivery). Animals were maintained in an air-conditioned animal room (temperature: 23 ± 2 °C, relative humidity: 55 ± 15%) with a 12-h light/dark cycle. Pregnant rats were provided ad libitum with a pelleted basal diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan)