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Developmental exposure of citreoviridin transiently affects hippocampal neurogenesis targeting multiple regulatory functions in mice

Kota Nakajima[a,b], Yasunori Masubuchi[a,b], Yuko Ito[a,b], Mari Inohana[a], Masahiko Takino[c], Yukie Saegusa[d], Toshinori Yoshida[a], Yoshiko Sugita-Konishi[e], Makoto Shibutani[a,f,*]

[a] Laboratory of Veterinary Pathology, Division of Animal Life Science, Institute of Agriculture, 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] Agilent Technologies, Japan, Ltd., 9-1 Takakura-cho, Hachioji-shi, Tokyo 192-8510, Japan
[d] Environment Health and Safety Division, Environment Directorate, OECD, 2, rue André Pascal, 75775 Paris Cedex 16, France
[e] Laboratory of Food Safety Sciences, Graduate School of Life and Environmental Sciences, Azabu University, 1-17-71, Fuchinobe, Chuo-ku, Sagamihara-shi, Kanagawa 252-5201, Japan
[f] Institute of Global Innovation Research, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan

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ABSTRACT

To investigate the developmental exposure effect of citreoviridin (CIT) on postnatal hippocampal neurogenesis, pregnant ICR mice were dietary exposed to CIT at 0, 1, 3 and 10 ppm from gestation day 6 to postnatal day (PND) 21 on weaning. Offspring were maintained through PND 77 without CIT exposure. Male offspring were analyzed. At 10 ppm on PND 21, weak changes suggestive of neural stem cell reduction and progenitor cell proliferation were observed. Number of hilar CALB1$^+$ interneurons reduced, suggesting an influence on neurogenesis. In contrast, number of hilar SST$^+$ interneurons increased and *Bdnf* and *Ntrk2* transcripts upregulated in the dentate gyrus, suggesting a facilitation of BDNF-TRKB signaling for progenitor cell proliferation. Transcript expression changes of an outside regulatory system suggested suppressed function of GABAergic interneurons, especially of PVALB$^+$ interneurons for compensation on neural stem cell reduction. At ≥ 3 ppm, number of ARC$^+$ mature granule cells increased, and at 10 ppm, number of hilar GRIA1$^+$ cells increased and *Gria2* and *Gria3* upregulated, suggesting an operation of AMPA receptor membrane trafficking on the increase of ARC-mediated synaptic plasticity. On PND 77, all the transcript expression changes of the neurogenesis regulatory system except for *Grin2d* were inverted, suggesting an operation of a homeostatic mechanism on CIT-induced disruptive neurogenesis. Simultaneous downregulation of *Grin2a* and *Grin2d* suggests suppression of GABAergic interneuron function to adjust neurogenesis at the normal level. The no-observed-adverse-effect level of CIT for offspring neurogenesis was determined to be 1 ppm, translating to 0.13–0.51 mg/kg body weight/day of maternal oral exposure.

1. Introduction

Although mycotoxin contamination in rice has been reported less often than in wheat and corn, there have been examples of serious mycotoxin contamination in stored or imported rice, producing moldy rice, named collectively yellow rice, in the past in Japan (Kushiro, 2015). In the past, moldy rice, named toxic yellowed rice, was shown to cause an acute crisis at the turn of the 20th century, with Japan experiencing a massive epidemic of acute cardiac beriberi, known as “Shoshin-kakke (heart-attacking paralysis)” in relation with the eating of yellow rice (Kushiro, 2015). The causative mycotoxin of this epidemic is now identified, and citreoviridin (CIT) is a toxic secondary metabolite produced by *Penicillium citreonigrum*, *Aspergillus terreus* or *Eupenicillium ochrosalmoneum* in moldy cereals, such as rice and corn (Lima et al., 2010). Acute cardiac beriberi by CIT-contaminated rice has recently been reported in China and Brazil (da Rocha et al., 2015; Sun, 2010). Beriberi is a syndrome caused by thiamine deficiency, characterized by peripheral neuropathy and muscle weakness that is also called “dry” beriberi (López Gastón et al., 2002). CIT interferes with the mitochondrial oxidative phosphorylation in the nerve and muscle

* Corresponding author. Laboratory of Veterinary Pathology, Division of Animal Life Science, Institute of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan.
E-mail address: mshibuta@cc.tuat.ac.jp (M. Shibutani).

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Abbreviations

AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ARC	activity-regulated cytoskeleton-associated protein
BW	body weight
CALB1	calbindin-D-28 K
CALB2	calbindin-D-29 K
CIT	citreoviridin
COX2	cyclooxygenase-2
C_T	threshold cycle
DCX	doublecortin
GABA	γ-aminobutyric acid
Gapdh	glyceraldehyde-3-phosphate dehydrogenase
GCL	granule cell layer
GD	gestational day
GFAP	glial fibrillary acidic protein
GRIA1	glutamate receptor, ionotropic, AMPA1 (alpha 1)
GRIN2D	glutamate receptor, ionotropic, *N*-methyl-D-aspartic acid (NMDA)2D (epsilon 4)
Hprt	hypoxanthine guanine phosphoribosyl transferase
LC-MS/MS	liquid chromatography – tandem mass spectrometry
nAchR	neuronal nicotinic acetylcholine receptor
NeuN	neuronal nuclei
NMDAR	*N*-methyl-D-aspartate receptor
PCNA	proliferating cell nuclear antigen
PFA	paraformaldehyde
PND	postnatal day
PVALB	parvalbumin
RELN	reelin
RT-PCR	reverse-transcription polymerase chain reaction
SGZ	subgranular zone
SOX2	sex determining region Y (SRY)-box 2
SST	somatostatin
TBR2	T box brain 2
TUBB3	tubulin, beta 3 class III
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling
VGLUT	vesicular glutamate transporter

tissues, competing with the absorption of thiamine by the cells of these tissues (Almeida et al., 2012). Thiamine deficiency is also well known to cause central nervous system damage in many mammalian species, such as Wernicke's encephalopathy in humans (Wu et al., 2017). Experimentally, maternal thiamine restriction during lactation induces cognitive impairments and changes in concentrations of glutamate and γ-aminobutyric acid (GABA) in the brain of rat offspring (de Freitas-Silva et al., 2010). Therefore, it could be hypothesized that CIT may affect both peripheral and central nervous systems and the infantile population may be susceptible to CIT-induced toxicity.

The hippocampus located at a temporal lobe brain structure is known to be involved in 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 (Hodge et al., 2008; Sibbe and Kulik, 2017). Adult neurogenesis in the SGZ is a highly regulated process starting from type-1 neural stem cells, which produce proliferative progenitor cells in the order of type-2a, type-2b, and type-3 (Hodge et al., 2008; Sibbe and Kulik, 2017). Type-3 progenitor cells differentiate into post-mitotic immature granule cells and finally into mature granule cells that populate the granule cell layer (GCL; Hodge et al., 2008; Sibbe and Kulik, 2017). In the hilar region of the hippocampal dentate gyrus, GABAergic interneurons are known to connect with adult-born dentate granule cells and play a functional role in adult neurogenesis (Freund and Buzsáki, 1996; Sibbe and Kulik, 2017). In addition to GABAergic neuronal inputs, various types of neurons outside the SGZ also create a synaptic connection with neurons in the dentate gyrus, such as glutamatergic neurons in the entorhinal cortex providing axonal projections to the dentate gyrus (Fonnum et al., 1979) and cholinergic neurons originating from the septal nucleus and nucleus of the diagonal band of Broca innervating neurons in the dentate hilus (Amaral and Kurz, 1985). All of the cell populations and their inherent cellular processes involved in the adult neurogenesis may be sensitive targets of developmental neurotoxicants. Especially, self-renewal of stem cells, proliferation and migration of progenitor cells, neuritogenesis, synaptogenesis and myelinogenesis may be vulnerable developmental processes against chemical toxicity.

We previously reported that a number of chemicals and agents affected proliferation and differentiation of progenitor cells in the SGZ (Shibutani, 2015). In addition to changes in granule cell lineage subpopulations, we also reported aberrant numbers of GABAergic interneuron subpopulations, as well as cholinergic or glutamatergic inputs, as part of the regulatory system of neurogenesis (Shibutani, 2015). Therefore, monitoring granule cell lineage in the SGZ, and GABAergic interneurons in the dentate hilus, as well as cholinergic or glutamatergic inputs, is important for the detection of target cell populations in the study of developmental neurotoxicity in adult neurogenesis. We recently reported that developmental exposure to representative mycotoxins, such as aflatoxin B_1, T-2 toxin and ochratoxin A, affects proliferation and differentiation of granule cell lineages in the hippocampal adult neurogenesis at the end of exposure in mice or rats (Tanaka et al., 2015, 2016a; b).

The present study was performed to elucidate whether CIT causes developmental neurotoxicity by oral administration to maternal animals. For this purpose, we examined the effect of maternal exposure to CIT on developmental neurotoxicity from gestational day (GD) 6 to postnatal day (PND) 21 in mouse offspring in accordance with the exposure scheme of OECD Test Guideline 426 (Test No. 426: Developmental Neurotoxicity Study; OECD, 2007). For risk assessment purposes, we examined the effects on adult neurogenesis of granule cell lineages in the SGZ, as well as on the regulatory systems of neurogenesis, such as GABAergic, cholinergic and glutamatergic neuronal systems in the dentate gyrus after developmental exposure and at the adult stage in mice.

2. Materials and methods

2.1. Chemicals and animals

Citreoviridin (CAS No. 25425-12-1; purity: 82.4%) was provided by Hayashi Pure Chemical Ind., Ltd., (Osaka, Japan). Mated female Slc:ICR mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan) at GD 1 (the appearance of vaginal plugs was designated as GD 0). Mated female mice were housed individually with their offspring in plastic cages with paper chip bedding until 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 mice were kept under free access to a pelleted basal diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) using lid-type mouse feeder until the start of exposure to CIT and tap water using water bottle throughout the experimental period. Offspring were housed three or four animals per cage and provided *ad libitum* with the pelleted basal diet and tap water from PND 21 onwards. Because no measurable degradation of CIT in powdered diet at 10 ppm for 1 week at room temperature was confirmed by means of liquid chromatography – tandem mass spectrometry (LC-MS/MS; analyzed by Japan Food Research Laboratories , Tokyo, Japan), powdered basal diet (MF) was used for dietary exposure of CIT.