Toxicology Letters 224 (2014) 424–432

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Glycidol induces axonopathy and aberrations of hippocampal neurogenesis affecting late-stage differentiation by exposure to rats in a framework of 28-day toxicity study

Hirotoshi Akane[a], Ayako Shiraki[a,b], Nobuya Imatanaka[c], Yumi Akahori[c], Megu Itahashi[a,b], Hajime Abe[a,b], 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, 1-4-25 Koraku, Bunkyo-ku, Tokyo 112-0004, Japan

HIGHLIGHTS

- Effect of 28-day glycidol exposure on hippocampal neurogenesis was examined in rats.
- Oral doses of glycidol at 200 mg/kg body weight/day induced axonal injury.
- Glycidol at this dose decreased both DCX$^+$ or TUC-4$^+$ cells in the subgranular zone.
- Glycidol at this dose increased reelin$^+$ or calbindin-2$^+$ GABAergic interneurons.
- Glycidol may affect late stage neurogenesis through targeting neurite extension.

ARTICLE INFO

Article history:
Received 26 July 2013
Received in revised form 22 October 2013
Accepted 23 October 2013
Available online 1 November 2013

Keywords:
Glycidol
Neurotoxicity
Axonopathy
Hippocampal dentate gyrus
Neurogenesis

ABSTRACT

Developmental exposure to glycidol induces aberrations of late-stage neurogenesis in the hippocampal dentate gyrus of rat offspring, whereas maternal animals develop axonopathy. To investigate the possibility whether similar effects on adult neurogenesis could be induced by exposure in a framework of 28-day toxicity study, glycidol was orally administered to 5-week-old male Sprague–Dawley rats by gavage at 0, 30 or 200 mg/kg for 28 days. At 200 mg/kg, animals revealed progressively worsening gait abnormalities as well as histopathological and immunohistochemical changes suggestive of axonal injury as evidenced by generation of neurofilament-L$^+$ spheroids in the cerebellar granule layer and dorsal funiculus of the medulla oblongata, central chromatolysis in the trigeminal nerve ganglion cells and axonal degeneration in the sciatic nerves. At the same dose, animals revealed aberrations in neurogenesis at late-stage differentiation as evidenced by decreases of both doublecortin$^+$ and dihydropyrimidinase-like 3$^+$ cells in the subgranular zone (SGZ) and increased reelin$^+$ or calbindin-2$^+$ γ-aminobutyric acid-ergic interneurons and neuron-specific nuclear protein$^+$ mature neurons in the dentate hilus. These effects were essentially similar to that observed in offspring after maternal exposure to glycidol. These results suggest that glycidol causes aberrations in adult neurogenesis in the SGZ at the late stage involving the process of neurite extension similar to the developmental exposure study in a standard 28-day toxicity study.

Abbreviations: Calb1, Calbindin-D-28K; Calb2, Calretinin; C_T, Threshold cycle; DAB, 3,3′-diaminobenzidine; DCX, Doublecortin; Dpysl3, Dihydropyrimidinase-like 3; GABA, Gamma-aminobutyric acid; Gapdh, Glyceraldehyde 3-phosphate dehydrogenase; GFAP, Glial fibrillary acidic protein; Hprt, Hypoxanthine phosphoribosyltransferase 1; MMI, Methimazole; NeuN, Neuron-specific nuclear protein; NF-L, Neurofilament-L; p-NF-H, Phosphorylated neurofilament-H; Pax6, Paired box 6; PBS, Phosphate-buffered saline; PCNA, Proliferating cell nuclear antigen; Pvalb, Parvalbumin; RT-PCR, Reverse-transcription polymerase chain reaction; SGZ, Subgranular zone; T_3, Triiodothyronine; T_4, Thyroxine; Tbr2, T box brain 2; TSH, Thyroid-stimulating hormone; TUNEL, Terminal deoxynucleotidyl transferase dUTP nick end labeling.

* Corresponding author. Tel.: +81 42 367 5771; fax: +81 42 367 5771.
E-mail address: mshibuta@cc.tuat.ac.jp (M. Shibutani).

0378-4274/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2013.10.026

1. Introduction

Glycidol, which is an epoxide alcohol that is a colorless, slightly viscous liquid at room temperature and soluble in both water and organic solvents (IARC, 2000) is used as a chemical intermediate in the pharmaceutical industry, as a stabilizer in the manufacture of vinyl polymers, as a diluent of some epoxy resins, and as an additive for oil (NTP, 1990). Exposure to glycidol through food has recently become a worldwide concern based on the possible release of glycidol in the gastrointestinal tract by hydrolysis of glycidol fatty acid esters. Glycidol fatty acid esters can be found in refined edible oils and fats, including infant formulas, especially in diacylglycerol oil at high concentrations (Bakhiya et al., 2011; BfR, 2009). Glycidol is known to show carcinogenicity and neurotoxicity (IARC, 2000; NTP, 1990). In a repeated-dose toxicity study in rats and mice in which glycidol was administered by oral gavage for 13 weeks, neurotoxicity involving cerebellar necrosis was reported (NTP, 1990).

The hippocampal dentate gyrus of the brain is a unique structure that is known to continue neurogenesis of granule cell lineages in the subgranular zone (SGZ) throughout postnatal life (Supplementary Fig. 1; Eriksson et al., 1998; Kempermann et al., 2004). Postnatal neurogenesis (so called "adult" neurogenesis) in the SGZ occurs from type-1 stem cells, which undergo self-renewal and produce intermediate cell generations in the order of type-2a, type-2b, and type-3 cells. Type-3 progenitor cells differentiate into immature granule cells after final mitosis and then into mature granule cells involving the process of neuritogenesis and synaptogenesis (Hodge et al., 2008; Kempermann et al., 2004; Knoth et al., 2010). The interneuron populations using γ-aminobutyric acid (GABA) as a neurotransmitter in the hilus of the dentate gyrus control neurogenesis in the SGZ, providing projections to growing granule cells to help their differentiation (Duveau et al., 2011; Masiulis et al., 2011). There are several subpopulations in GABAergic interneurons (Houser, 2007), and disruption of neurogenesis can affect interneuron subpopulations (Shiraki et al., 2012). Therefore, toxicants for adult animals targeting axon terminals and synaptosomes as well as cell proliferation may induce both adult and developmental neurotoxicity targeting neurogenesis involving alterations in interneuron subpopulations.

We recently revealed that acrylamide, a well-known neurotoxicant that degenerates distal axons in the central and peripheral nervous systems (Graham, 1999; LoPachin, 2004; Lee et al., 2005), affects the late-stage in the neurogenesis targeting immature granule cells that have dendritic growth cones and recurrent basal dendrites (Ribak et al., 2004) in the SGZ of the hippocampal dentate gyrus of rat offspring after developmental exposure (Ogawa et al., 2011, 2012). In that study, we hypothesized that the effects of acrylamide on neurogenesis could be through a common mechanism targeting mature axon terminals causing distal axonopathy in adult animals.

Recently, we revealed that maternal exposure to glycidol resulted in aberrations of late-stage in the neurogenesis of the hippocampal dentate gyrus of rat offspring, whereas maternal animals developed axonopathy involving both central and peripheral nervous systems (Akane et al., 2013). We hypothesized that the mechanism of toxicity of glycidol has common targets of both mature axon terminals and developing neurites as observed for the pathogenetic mechanism of acrylamide-induced aberrations of neurogenesis (Ogawa et al., 2012). We have also shown a sustained increase in the number of immature interneurons producing reelin, which regulates the maturation and migration of granule cell progenitors (D'Arcangelo et al., 1997; Gong et al., 2007; Lussier et al., 2009; Ramos-Moreno et al., 2006) or expressing calretinin (Calb2), which is a calcium-binding protein, in the hilus of rat offspring after maternal exposure to glycidol. This suggests that developmental exposure to glycidol perturbed neurogenesis and mismigration of granule cell lineages through to the adult stage.

Developmental neurotoxicity testing is a field that is in need of rapid screening systems because testing one chemical with the current guidelines is time-consuming and requires hundreds of animals. It may be reasonable to evaluate both adult-stage and developmental neurotoxic effects in a framework of short-term general toxicity studies. We hypothesize that adult neurogenesis could be a common target of developmental neurotoxicants, and adult neurotoxicants could affect neurogenesis.

The present study was undertaken to confirm whether adult neurotoxicants could affect neurogenesis and to examine the possibility that similar effects suggestive of developmental neurotoxicity could be detected by exposure to glycidol in a standard 28-day toxicity study. To this end, we examined the effects of glycidol on adult neurogenesis in the hippocampal dentate gyrus by analyzing neuronal progenitor cell populations in combination with interneurons using adolescent rats. Because thyroid hormone levels can affect neurogenesis (Saegusa et al., 2010; Shiraki et al., 2012), serum levels of thyroid-related hormones were also examined.

2. Materials and methods

2.1. Chemicals and animals

Glycidol (purity: 97.6%) was purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). Four-week-old male Crl:CD®(SD) rats were purchased from Charles River Japan Inc. (Yokohama, Japan) and maintained in an air-conditioned animal room (temperature: 23 ± 2 °C, relative humidity: 55 ± 15%) with a 12-h light/dark cycle. Animals were housed in plastic cages with wood chip bedding with three or four animals per cage and were provided a pelleted basal diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum* throughout the experimental period.

2.2. Experimental design

After a 1-week acclimatization period, 5-week-old male animals were randomly divided into three groups of 16 animals and were administered 0, 30 or 200 mg/kg glycidol in purified water by gavage for 28 days. The high-dose level was set at 200 mg/kg based on a previous 13-week repeated-dose toxicity study (NTP, 1990) and was expected to cause a slight decrease in body weight. Body weights and food consumption were measured once or twice per week throughout the administration period. To assess neurological abnormalities, animals were scored on the appearance of gait abnormalities into the following four categories: grade 0, normal gait; grade 1, slightly abnormal gait with slight degrees of shuffling; grade 2, moderately abnormal gait with moderate degrees of shuffling; and grade 3, severely affected gait including inability to support the body weight and spreading of all extremities. Assessments were made by placing individual animals in Plexiglas boxes (90 cm × 90 cm × 20 cm) for 3 min in an observer-blinded fashion to the dose group. At the end of the 28-day study, all animals were sacrificed by exsanguination from the abdominal aorta under CO_2/O_2 anesthesia and subjected to necropsy. Body and brain weights were determined for all animals on the necropsy day. Ten animals in each group were subjected to serum hormone analysis and histopathological assessment, and the remaining six animals were subjected to real-time reverse-transcription polymerase chain reaction (RT-PCR) analysis.

All of the study procedures were conducted in compliance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, June 1, 2006) and according to the protocol approved by the Animal Care and Use Committee of Tokyo University of Agriculture and Technology. All efforts were made to minimize animal suffering.

2.3. Hormone analyses

Blood samples collected from 10 animals per group at necropsy were centrifuged at 1600 × *g* for 10 min, and serum was stored at −80 °C. Serum concentrations of thyroid-stimulating hormone (TSH), triiodothyronine (T_3), and thyroxine (T_4) were measured using a rodent ELISA test kit (Endocrine Technologies, Newark, CA, USA) with a microplate reader (FLUOstar Optima, BMG Labtechnologies, Durham, NC, USA).

2.4. Histopathology

Tissues from 10 animals per group were subjected to histopathological evaluation. The brain, trigeminal nerve, sciatic nerve, and spinal cord were removed. The sciatic nerves were exposed at necropsy and subjected to *in situ* fixation by immersion in Bouin's solution for 3 min before sampling for histopathological assessment of axonal changes, according to the method previously described (Takahashi et al.,