Published in final edited form as:

Curr Eye Res. 2009 September ; 34(9): 785–790.

Toxic Effect of Methamphetamine on the Retina of CD1 Mice

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Abstract

Purpose—To investigate whether systemic administration of methamphetamine (METH) induces retinal damage in CD1 mice.

Materials and Methods—Eighteen male CD1 mice were randomly assigned to three groups, six mice per group: Group 1 receiving a single dose of 40 mg/kg METH, Group 2 receiving four doses of 10 mg/kg METH, and Group 3 (control) receiving 40 mg/kg 0.9% NaCl solution. METH and NaCl were administered by intraperitoneal injection. Immunostaining of glial fibrillary acidic protein (GFAP), S-100 for astrocytes and Muller cells, CD11b for microglia, and tyrosine hydroxylase (TH) and TUNEL labeling for apoptotic cell death were performed on the retinal sections on day 1 and day 7 post-exposure.

Results—GFAP and S-100 immunoreactivity was observed in Group 1 mice. CD11b+ cells in Group 1 mice showed more intensely stained shorter and thicker cellular processes than Groups 2 and 3, indicating activated microglia in the mice exposed to large dose METH. No significant difference in TH level was seen among the three groups. TUNEL labeling did not reveal positive cells in the retinas of any of the 18 CD1 mice.

Conclusions—A single large dose of METH induces an increase in short-term protein expression of GFAP and S-100 and in microglial activation. The results suggest that METH has a neurotoxic effect on CD1 mouse retina.

INTRODUCTION

Methamphetamine (METH) is a highly addictive psychostimulant. In the United States, an estimated 600,000 people are weekly users of the drug.¹ METH exerts pharmacological effects on the central nervous system (CNS) through a non-exocytotoxic mechanism that releases monoamine neurotransmitters including dopamine, norepinephrine, and serotonin.^{2,3} In the brain, however, METH affects both monoaminergic and non-monoaminergic systems.⁴ It is

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The authors have no commercial, proprietary, or financial interest in any of the products or companies described in this article.

generally believed that METH-induced toxicity involves an excessive release of dopamine which initiates the production of oxygen-based free radicals, causing neuronal death through apoptosis.^{5,6}

Though much progress has been made in understanding the neurological consequences of METH use, we know far less about the effects of METH on the retina, which is part of the CNS with many METH-targeted dopaminergic cells. It was shown in an *in vivo* study that even a low concentration of METH (1.2 mM) could alter retinal plasma membrane integrity, esterase activity, and/or pH in rat retina homogenates.⁷ It was also found that although intravitreal injection of METH had no detectable detrimental effect on the retina of normal adult rat, it exacerbated the damaging effects of kainic acid.⁸ Some studies suggest that prenatal-induced METH toxicity in rat retina may be related to lipid peroxidation processes and oxidative stress,⁹ and that any substance altering dopaminergic system of the retina influences the visual system.^{10,11} However, little is known about how systemic administration of METH affects adult mouse retina. The purpose of the present study was to determine whether systemic administration of METH by intraperitoneal injection would have a toxic effect on retina of CD1 mice. CD1 mice have been shown to be susceptible to METH and to sustain extensive damage to neurons in the brain with METH exposure.¹² CD-1 mice have been extensively used in methamphetamine studies and are considered the most sensitive model for investigating METH toxicity. We thus chose CD1 mice retina as the animal model for this investigation.

MATERIALS AND METHODS

Animals and Drug Treatment

Eighteen male CD1 mice (Charles River, Raleigh, NC, USA), weighing 23–25g (8 weeks old), were used. Eyeball weight, though not measured, was controlled through body weight. The animals were housed in cages, three mice per cage, with free access to food and water. Temperature and humidity in the animal facility were controlled at $23 \pm 1^\circ\text{C}$ and $53 \pm 15\%$, respectively. Each mouse was randomly assigned to one of three groups, six mice per group. Group 1 received a single intraperitoneal dose of 40.0 mg/kg METH. The animals showed no evidence of seizures. Three mice in Group 1 died on day 1 post-injection, and their eyeballs were taken for study. The other three mice in Group 1 survived through day 7. Group 2 received four intraperitoneal doses of 10.0 mg/kg METH at 2-hour intervals. Three mice in Group 2 were sacrificed on day 1 post-injection and the other three on day 7. Group 3 (control) received one injection of 40.0 mg/kg 0.9% NaCl solution and were sacrificed on day 7 post-injection. Euthanasia was performed by placing the animal in a CO₂ chamber. Methamphetamine hydrochloride (M8750, Sigma), a D isomer of methamphetamine, was used in the present study. Details are described elsewhere.¹² The use of 40.0 mg/kg METH constituted an attempt to mimic the large doses taken by human METH abusers. Because of significant species differences in METH elimination half-life and metabolism, the frequently used bingeing patterns introduced in 1988 by Sonsalla et al.¹¹ are only approximations of what is actually encountered in clinical situations.

Tissue Preparation

After the mice were sacrificed, their eyeballs were immediately enucleated, fixed in 4% paraformaldehyde in PBS for 12–18 hours at 4°C , and then cryopreserved in 25% sucrose in 0.1 M PBS overnight at 4°C . The eyeballs were frozen in Tissue-Tek O.C.T. compound (Sakura, Tokyo, Japan) in liquid nitrogen and stored at -80°C until sectioning. The tissue blocks were cut vertically at $7\ \mu\text{m}$ with a cryostat.

Immunohistochemistry and TUNEL Assay

Sections of each eyeball were processed for immunohistochemistry using an anti-rabbit polyclonal antibody directed against glial fibrillary acidic protein (GFAP; 1:5000; Chemicon, Temecula, CA) and S-100 (1:400; Sigma, St. Louis, MO) to assess glial changes, using the rat anti-mouse CD11b monoclonal antibody to assess microglia (1:300; Serotec, Oxford, UK), or using an anti-rabbit polyclonal antibody against tyrosine hydroxylase (TH; 1:2000; Chemicon) to examine the neuronal terminal damage. The immunohistochemistry analysis was performed using the rabbit or rat *Elite* ABC kit (Vector Corporation). The sections were incubated with primary antibodies overnight at 4 °C and were finally developed in DAB chromogen. Following progressive dehydration in a series of ethyl alcohol rinses (70%, 90%, 95%, 100%) and xylene, the sections were cover slipped with permanent mounting reagent, and were observed under a light microscope. For S-100 fluorescent immunolabeling, the donkey anti-rabbit Cy-3 secondary antibody (1:100; Jackson ImmunoResearch, West Grove, PA) was employed. The images were captured by a computer-assisted digital camera, Nikon DXM 1200 (Nikon USA, NY), connected to the microscope. Retinal cell apoptosis was determined by terminal deoxynucleotide transferase nick end labeling (TUNEL) (Roche Applied Science, Indianapolis, IN), according to the manufacturer's instructions. Diaminobenzidine (DAB) was used as color substrate.

All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The research protocol was approved by the Johns Hopkins Animal Care and Use Committee.

RESULTS

GFAP and S-100 Immunostaining

In the control retina, the GFAP was primarily expressed in the astrocytes and Muller cell foot plates in the retinal ganglion cell (RGC) layer and internal limiting membrane (Figure 1, arrows), with weak immunolabeling in the processes of Muller cells in the inner plexiform layer (IPL). In mice treated with single-dose 40.0 mg/kg METH, a marked increase of GFAP expression in the processes of Muller cells was noted in the retinas 1 day and 7 days after METH exposure. The increase of GFAP expression in Muller cells was not remarkable in the retinas 1 day and 7 days after treatment of four doses of 10.0 mg/kg METH, as compared with control (not shown). S-100 immunolabeling (Figure 2) showed a similar increased expression in the IPL of the retinas of mice receiving single-dose 40.0 mg/kg METH, as well as in the cell bodies of Muller cells in the inner nuclear layer (INL). No discernable changes were found for the 10×4 mg/kg METH group in terms of GFAP and S-100.

Tyrosine Hydroxylase Immunostaining

Tyrosine hydroxylase (TH) immunostaining showed intense labeling in the junction of the INL and IPL with scattered intensely positive cells in the innermost layer of the INL in the control retinas. The staining patterns were similar in all three groups (Figure 3). No significant difference was noted between retinas with and those without METH treatment.

Microglial Staining

CD11b immunostaining showed many microglial cells with delicate cellular processes in the control retinas, mostly in the IPL and outer plexiform layer (Figure 4, A). After the single-dose 40.0 mg/kg METH treatment, the CD11b+ cells were slightly increased in number, became more hypertrophic, and were activated with shorter and thicker cellular processes (Figure 4, B and C). The mice receiving four doses of 10.0 mg/kg METH did not reveal notable differences in microglial cell immunoreactivity, as compared with the control.

TUNEL for Retinal Cell Death

TUNEL did not reveal any positive cells in the retinas of any group.

DISCUSSION

Our *in vivo* study demonstrates that a single large dose (40 mg/kg) of METH causes an increase in GFAP and S-100 immunoreactivity in the adult mouse retina and alters microglial response. Our results also show no significant reduction of TH-like immunoreactivity and no evidence of apoptotic cell death. The lack of apoptotic cell death suggests mild neuronal toxicity to the retinal tissues.

In the normal retinas of humans and rodents, Muller cells express minimal GFAP activity. However, up-regulated GFAP expression is found in virtually all retinas in pathological conditions.^{13,14} Our study shows a marked increase in GFAP expression in the retina 1 day and 7 days after treatment with a single large dose (40 mg/kg) of METH as compared with the controls, but not as compared with GFAP expression after treatment with multiple small doses (4×10 mg/kg) of METH. Miller et al., using the immunoassay method, showed a large increase in striatal GFAP protein 72 hours after METH injection.¹⁵ Hess et al. demonstrated that the lightly stained GFAP-positive cellular processes found in normal conditions is transformed to more heavily stained thicker cellular processes after METH exposure.¹⁶ Fukumura et al.¹⁷ and Cappon et al.¹⁸ reported that a single-dose METH injection induces pronounced GFAP overexpression in the neostriatal and caudate-putamen, and the effect is larger in a single-dose (40 mg/kg) group than in a multiple-small-dose (10 mg/kg $\times$ 4 times/2hrs) group. Our results demonstrate that in the retina a similarly increased GFAP expression occurs, indicating mild retinal damage after METH exposure. To better quantify GFAP expression, Elisa immunoassay or Western blot for GFAP protein of whole retina is called for in future studies.

We observed that TH immunoreactivity is localized in the junction of the INL and IPL. Our finding is consistent with Witkovsky's.¹⁹ In mammalian retinas the dopaminergic cell bodies are in the layers of amacrine cells, and the density of dopamine neurons in vertebrate retinas is low.¹⁹ In general, the efficacy of dopamine synthesis depends mostly on the amount and activity of TH as this is the rate-limiting enzyme.²⁰ In our study, we did not observe significant changes of TH immunoreactivity in the retinas of CD1 mice after METH treatment with either a single large dose or multiple small doses as compared with the controls. In the CNS, METH has been shown to induce decreased activity of TH.^{17,18,21} For example, in the study by Fukumura et al.,¹⁷ decrease in METH-induced TH was not significant with a single dose of 10 mg/kg METH, but reaches maximum decrease at the 40 mg/kg dosage level. In the study by Kita et al.,²² TH-positive terminal depletion was not present in young rats less than 60 days old with a 10 or 20 mg/kg single dose METH exposure. TH depletion in the striatum could occur even as early as 48 hours after METH exposure.²³ We speculate that the tissue response in the retina may be different from that in the brain.

Microglia respond to any disturbance in CNS that poses a threat to physiological homeostasis.²⁴ The microglia, which normally lie dormant in adult retina, become rapidly activated in response to injury, infection, inflammation, ischemia, or neurodegeneration.²⁵ Microglial activation has been found in the brain of METH-treated mice and contributed to neuronal cell death.^{26,27} Microglial activation represents an early phase in METH-induced neurotoxicity.²⁷ Other studies using MK-801²⁸ or ketoprofen²⁹ also demonstrate that inhibition of microglial activation protects against METH-induced neurotoxicity. In our study, an increased population of activated CD11 b-labeled microglia is noted in the mice treated with a single large dose (40 mg/kg) of METH. Their microglia appear heavily stained with more cellular processes. The astrocytic and microglial cell responses after METH exposure may contribute to the pathogenesis of the METH-related retinal or other CNS injury.

Although the literature is limited, it shows that METH affects human visual functions in a broader context of cognitive impairment.^{30,31} We believe that it is important to develop an animal model of cognitive deficiency to better understand the neurological impact of METH on humans.

ACKNOWLEDGMENTS

This work was supported by a grant from NIH/NIDA DA-15020 (SL).

Funding Support:

NIH/NIDA Grant DA-15020.

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Figure 1.

GFAP immunolabeling. *A*: In the control mouse retina, GFAP showed intense staining in the foot processes of Muller cells and the astrocytes in the retinal ganglion cell (RGC) layer and internal limiting membrane (arrows) and weak staining in the Muller cell processes in the IPL. *B and C*: Retina 1 day (*B*) and 7 days (*C*) after single-dose 40 mg/kg METH injection, with GFAP staining showing marked increase in the Muller cell processes in the IPL. (ONL: outer nuclear layer; INL: inner nuclear layer; IPL: inner plexiform layer) Bar = 100 μ m.

Figure 2.

S-100 immunostaining. *A*: S-100 immunolabeling showed a similar pattern of staining as GFAP labeling in the control mouse retina, with intense staining in the astrocytes and Muller cell processes in the RGC layer and internal limiting membrane but weak staining in the Muller cell processes in the IPL. *B and C*: After a single-dose 40 mg/kg METH injection, marked expression of S-100 was seen in the cell bodies of Muller cells in the INL and Muller cell processes in the IPL at 1 day (*B*) and 7 days (*C*) after exposure. Bar = 50 um.

Figure 3.

TH immunostaining. In the control mouse retina (A), the TH immunostaining was seen in the junction between INL and IPL (arrows). Scattered darkly stained cells were seen in the innermost layer of the INL (short arrows), possibly representing amacrine cells. There were no significant changes in the immunoreactivity between the control (A) and the single-dose 40 mg/kg METH-treated group 1 day (B) and 7 days (C) after exposure. Bar = 100 μ m.

Figure 4.

CD 11b immunolabeling. *A*: In the control mouse retina, CD11b-immunoreactive microglial cells in the inner retina showed delicate cellular processes in the IPL and outer plexiform (arrows). *B and C*: The number of microglial cells was moderately increased 1 day (*B*) and 7 days (*C*) after treatment with a single-dose 40 mg/kg METH. The microglial cells in the IPL appeared hypertrophic or activated with shorter and thicker cellular processes (arrows).