Int. J. Devl Neuroscience 37 (2014) 52–57

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

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

Galantamine, an acetylcholinesterase inhibitor, reduces brain damage induced by hypoxia-ischemia in newborn rats

Seishi Furukawa*, Li Yang, Hiroshi Sameshima

Department of Obstetrics & Gynecology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

ARTICLE INFO

Article history:
Received 9 April 2014
Received in revised form 17 June 2014
Accepted 17 June 2014

Keywords:
Acetylcholine
Hypoxia-ischemia
Perinatal
Perinatal brain injury
Microglia
Rat

ABSTRACT

Aim: Our aim is to elucidate whether galantamine, known as an acetylcholinesterase inhibitor, reduces brain damage induced by hypoxia-ischemia (HI).
Study design: 7-day-old Wistar rats were used. Rats were subjected to left carotid artery ligation followed by 2 h of hypoxia (8% oxygen). We injected galantamine intraperitoneally just before hypoxia (5.0 mg/kg, $n = 14$; 2.5 mg/kg, $n = 9$; 1.0 mg/kg, $n = 11$) and after hypoxia (5.0 mg/kg, $n = 7$) to determine its neuroprotective effect. An equivalent volume of saline was administered as a control before ($n = 31$) and after hypoxic load ($n = 7$). We also examined the production of IL-1β in the ligated hemisphere side after injection of galantamine (prior hypoxia; 5.0 mg/kg, $n = 7$) or saline ($n = 8$). Brains were analyzed 7 days after HI.
Results: Two of the 5.0 mg/kg galantamine pre-treated rats and a post-treated rat died during experiments. The remaining survived and 5.0 mg/kg galantamine pre-treated rats showed a marked reduction of brain damage ($p < 0.01$) compared with the control. The other galantamine groups had severe brain damage similar to controls. Microglial accumulation was significantly reduced in rats pre-treated with 5.0 mg/kg of galantamine compared to control rats on both the hippocampus ($p = 0.02$) and cortex ($p < 0.01$). In contrast, the other galantamine groups showed a lower suppressive effect on microglial accumulation compared to the control. Galantamine significantly reduced IL-1β productions when compared to the control ($p < 0.01$).
Conclusion: Pre-treatment of galantamine reduced brain damage with a suppressive effect on microglial accumulation and IL-1β production in a newborn rat model of HI.
© 2014 ISDN. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Perinatal hypoxic-ischemic brain damage is one of the most important medical problems to overcome. The mechanisms involved in the development of brain damage have been extensively studied and the inflammatory response is one contributor to hypoxic-ischemic brain damage (Fleiss and Gressens, 2012). We have also demonstrated recently that the acetylcholine receptor (AChR)-responsive inflammatory pathway in the brain plays an important role in modulating perinatal brain damage, which might provide an opportunity for therapeutic intervention (Furukawa et al., 2011, 2013a,b, 2014).

Activation of the cholinergic response via acetylcholine receptor in macrophage is involved in regulation of the inflammatory pathway (Rosas-Ballina and Tracey, 2009; Wang et al., 2003). Our previous studies have shown that a single dose of AChR agonist (carbachol) prior to hypoxia was effective in reducing hypoxic-ischemic brain damage in the newborn rat (Furukawa et al., 2011). However, it was incapable of suppressing late microglial activation of the cortex, in which tissue damage was in progress after hypoxia-ischemia (HI) (Furukawa et al., 2014). On the other hand, repetitive administration of carbachol rescued progression of hypoxic-ischemic brain damage with a reduction of early and late microglial activation (Furukawa et al., 2013a,b). Thus, an activating cholinergic nervous system during and after HI seems to be important in preventing brain damage caused by HI.

Furthermore, we found that antagonists of acetylcholine receptors (AChRs) that are permeable to the blood–brain barrier significantly enhanced brain damage in 1-h HI. Moreover, 1-h HI does not produce marked brain damage in the Levine-Rice preparation. In particular, the nicotinic AChR antagonist showed a marked

* Corresponding author at: Department of Obstetrics & Gynecology, Faculty of Medicine, University of Miyazaki, 5200 Kihara-Kiyotake, Miyazaki 892-1601, Japan. Tel.: +81 985 85 0988; fax: +81 985 85 6149.
E-mail addresses: shiiba46seishi@gmail.com, seishi_furukawa@med.miyazaki-u.ac.jp (S. Furukawa).

http://dx.doi.org/10.1016/j.ijdevneu.2014.06.011
0736-5748/© 2014 ISDN. Published by Elsevier Ltd. All rights reserved.

enhancement of brain damage (Furukawa et al., 2013a,b). It is therefore desirable to increase the concentration of acetylcholine in the brain or to stimulate nicotinic AChR repetitively and selectively to prevent brain damage.

Several nicotinic AChR agonists are available and have some ameliorating effects on brain disorders. In the model of traumatic brain injury, an acetylcholinesterase inhibitor improves outcome (Holschneider et al., 2011, 2013). Although these drugs are not used clinically except for galantamine, which is known as an acetylcholinesterase inhibitor (Buccafusco, 2004). However, it has yet to be established that galantamine can reduce perinatal brain damage and suppress the activation of microglia induced by HI. We supposed that the prolonged inhibition of microglial activation is crucial in preventing brain damage after HI.

Currently, no therapeutic drug is available for perinatal hypoxic-ischemic brain damage. Therefore, we conducted experiments to determine the effect of galantamine on tissue damage, microglial activation, and cytokine production after HI in the developing brain.

2. Materials and methods

2.1. Animal model

This study was performed in accordance with the Guidelines of the Experimental Animal Center of the University of Miyazaki. Pregnant Wistar rats were housed in the same animal center with free access to water and food under a 12-h on/off lighting schedule. Rat pups were reared with their dams until the time of the experiment. Seven-day-old rats, whose cerebral maturity corresponds to a 23–36 week gestation human fetus (Hagberg et al., 2002), were subjected to the Levine-Rice preparation (Levine, 1960; Vannucci et al., 1999) in an effort to examine hypoxic-ischemic brain damage and local inflammation. Briefly, rats were lightly anesthetized with ether inhalation, and the left common carotid artery was doubly-ligated with 5–0 surgical silk. Following the operation, pups were allowed 2 hours for recovery in the incubator without their dams. Even though a long separation from the mother without feeding may affect the well being of a rat pup, we conducted current experiments according to our previous studies (Ota et al., 1998; Ikeda et al., 2002; Furukawa et al., 2011, 2013a,b). In this experiment, we injected galantamine (5 mg/kg, $n = 14$; 2.5 mg/kg, $n = 9$; 1.0 mg/kg, $n = 11$) intraperitoneally just before 2 h of hypoxia to examine the attenuating effect on brain damage with decreasing accumulation of microglia. These doses were determined in reference to previous experimental doses for adult rats (van Beijsterveldt et al., 2004). Another group of pups received an equivalent volume of saline ($n = 31$) to compare the degree of hypoxic-ischemic brain damage and the accumulation of microglia in different brain regions including the hippocampus and cortex. Pups were then subjected to hypoxia for 2 h. In both groups, hypoxia was induced in a chamber perfused with a mixture of humidified 8% oxygen balanced with nitrogen at 33 °C. This temperature is the usual ambient temperature that rat pups are exposed to when huddling with the mother (Mortola and Dotta, 1992). Following hypoxia, pups were returned to their dam. For comparison of the effect of timing, we also administered galantamine (5 mg/kg; $n = 7$) and an equivalent volume of saline ($n = 7$) after hypoxic load. Another experiment was conducted for measurement of IL-1β using same experimental protocol. We injected galantamine (5 mg/kg, $n = 7$) or saline ($n = 8$) prior to hypoxic load, same as histological studies, to see the production of IL-1β.

2.2. Evaluation of brain damage

Rats were sacrificed on day 14 by a lethal dose of pentobarbital (100 mg/kg) and then used for histological evaluation. The brains were removed and checked for gross brain damage by macroscopic examination. The brains were then fixed overnight in a 19:1 solution of ethanol and acetic acid, dehydrated, and then embedded in paraffin. From each brain, a section 6 μm in thickness was cut that contained the dorsal hippocampus at the A 2.0-mm level in accordance with a stereotactic atlas of a 10-day-old rat brain (Sherwood and Timiras, 1970). Each coronal section was stained with hematoxylin–eosin for the assessment of brain damage, and tomato-lectin stain to evaluate microglial accumulation. In this model, brain damage is usually confined to the ligated side, and the non-ligated side serves as a control. The evaluation of brain damage followed that of our previous study (Furukawa et al., 2011, 2013a,b, 2014). Briefly, once the entire brain in a single section was acquired as an image, we measured the area of both sides using Image J software (http://rsb.info.nih.gov/ij/index.html). The area of the ligated side (left) was divided by the area of the non-ligated side (right) to obtain a ratio as the relative difference of hemisphere area ((ligated side/non-ligated side) × 100%). A value of 100% implies no shrinkage of the ligated side, while 0% implies total loss. Thus, the relative differences of hemisphere were evaluated on day 14. For CA1, CA3 and dentate gyrus (DG) of hippocampus and cortex, we evaluated regional neuronal damage by microscopic observation using a magnification of 150–400×. The neuronal damage was recognized by selective necrosis (the cytoplasm was stained red and the nucleus was shrunken with less basophilic staining) or infarction (all elements were destroyed with or without cystic formation) using hematoxylin–eosin staining. We identified the damaged area in the ligated side and compared it with the non-ligated side. The severity was expressed using a 3-grade scale following our previous study (Furukawa et al., 2011, 2013a,b; Ota et al., 1997): mild, <25% of the surface area on a single section with neuronal damage; moderate, 25–50%; and severe, >50%.

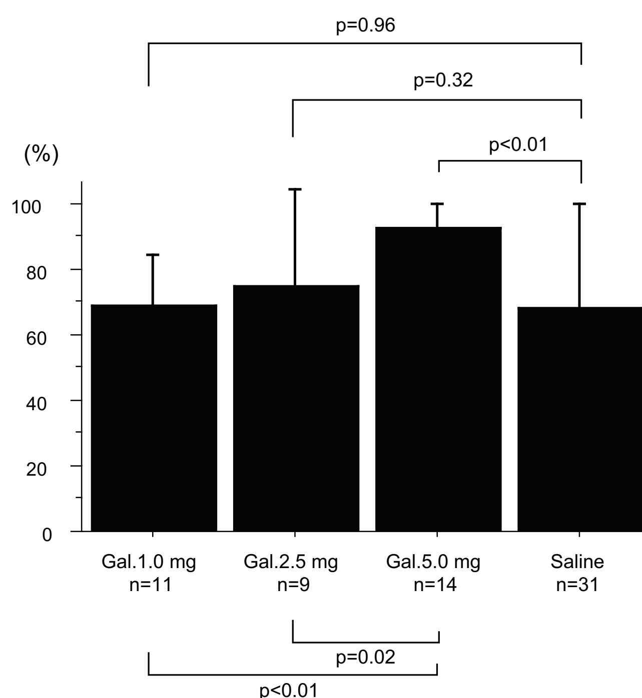

Fig. 1. Comparison of brain damage. The evaluation was made by the relative difference of hemisphere area. It is noted that shrinkage in the 5.0 mg/kg galantamine pre-treated group was significantly less than that observed in the other galantamine pre-treated and saline control groups after hypoxia-ischemia on day 14. The one-way ANOVA with Fisher's PLSD analysis was used to test for group differences. Data are expressed as mean ± SD (%). Gal.: galantamine.

2.3. Immunohistochemical stain and measurement of microglial activation

Microglial activation was evaluated on day 14 by tomato-lectin staining. A thin paraffin-embedded brain section (6 μm) was washed with water and incubated in 3% hydrogen peroxide for 10 min to inactivate the endogenous peroxidase. Each section was then washed with PBS and blocked with 0.5% bovine serum albumin for 30 min before being immunohistochemically stained with biotinylated Lycopersicon esculentum tomato lectin (1:100) (Vector Laboratories) for 1 h. Avidin binding was performed using a Vectastain ABC kit and developed using 3,3′-diaminobenzidine as a peroxidase substrate (Vector Laboratories). Sections were then rinsed in PBS, dehydrated in graded ethanol, cleared in xylene, mounted, and images were then acquired. The procedure used to count activated microglia followed that of our previous study (Furukawa et al., 2013a,b, 2014). Stained microglia are mainly found in white matter, the internal granule cell layer, and it is easy to distinguish the branched (ramified) and amoeboid forms (Furukawa et al., 2014; Acarin et al., 1994). The change from ramified to amoeboid microglia depends on the activation pathway (Czeh et al., 2011). The shape of amoeboid microglia stained with tomato-lectin comprised a rounded body with a dark-brown color. We first enclosed a measurement area, measured the area, and then digitally subtracted the outside of the measurement area. Utilizing the difference in gradation and shape of stained microglia, we then extracted amoeboid microglia in the hippocampal region and cortex of the ligated side. Amoeboid microglia always show stronger binding to tomato-lectin compared with ramified microglia (Acarin et al., 1994). To distinguish stained microglia in regard to shape, we extracted microglia possessing an almost circular shape because amoeboid microglia are more circular in shape compared to ramified microglia. The number of microglia in the hippocampus was expressed as a total count within the ligated side of the hippocampus. The number of microglia in the cortex was expressed as a count per mm^2.

2.4. ELISA for IL-1β

IL-1β production of the ligated hemisphere side was measured on day 14. Rats (galantamine 5.0 mg/kg, $n = 7$; saline, $n = 8$) were sacrificed, and the brain tissues

Download English Version:

https://daneshyari.com/en/article/5893894

Download Persian Version:

https://daneshyari.com/article/5893894

Daneshyari.com