Metabolic Brain Disease (2019) 34:951–956
https://doi.org/10.1007/s11011-019-00404-5

SHORT COMMUNICATION

Fluoro-Jade B histofluorescence staining detects dentate granule cell death after repeated five-minute transient global cerebral ischemia

Ji Hyeon Ahn[1] · Taek Geun Ohk[2] · Dae Won Kim[3] · Hyunjung Kim[4] · Minah Song[4] · Tae-Kyeong Lee[4] · Jae-Chul Lee[4] · Go Eun Yang[5] · Myoung Cheol Shin[2] · Jun Hwi Cho[2] · Soo Young Choi[1] · Moo-Ho Won[4] · Joon Ha Park[1]

Received: 13 December 2018 / Accepted: 25 February 2019 / Published online: 4 March 2019

Abstract

Transient global cerebral ischemia (tGCI)-induced neuronal damage is variable according to its duration and degree. There are many studies on the damage or death of pyramidal cells of the hippocampus proper (CA1–3) in rodent models of tGCI. However, studies on the death of granule cells in the hippocampal dentate gyrus (DG) following tGCI have not yet been addressed. In this study, we examined the damage/death of granule cells in the gerbil DG at 5 days after various durations (5, 10, and 15 min) of single tGCI and repeated tGCI (two 5-min tGCI with 1-h interval) using cresyl violet staining, NeuN immunohistochemistry and Fluoro-Jade B (F-J B) histofluorescence staining. Neuronal death was observed only in the polymorphic layer in all single tGCI-operated groups. However, in the repeated tGCI-operated group, massive neuronal death was observed in the granule cell layer as well as in the polymorphic layer by using F-J B histofluorescence staining. In addition, microgliosis in the DG was significantly increased in the repeated tGCI-operated group compared to the 15-min tGCI-operated group. Taken together, these findings indicate that repeated brief tGCI causes granule cell death in the DG which could not occur by a longer duration of single tGCI.

Keywords Transient global cerebral ischemia · Repeated transient ischemia · Dentate gyrus · Granule cells · Microgliosis

Introduction

Cardiac arrest and cardiopulmonary resuscitation can lead to transient global cerebral ischemia (tGCI), which is a major cause of serious and long-term disabilities (Benjamin et al. 2018; Soler and Ruiz 2010). During tGCI, blood supply to the brain is severely reduced or interrupted, showing that it initiates a cascade of pathological events which result in irreversible neuronal damage in certain brain areas (Dietrich et al. 1991; Olsson et al. 2003). The hippocampus, which is considered as one of brain areas very sensitive to tGCI, has two main interlocking parts: the hippocampus proper (also called Cornu Ammonis [CA]) and the dentate gyrus (DG) (Wible 2013). Neuronal populations in the CA, which consists of CA1, CA2 and CA3, and DG show different vulnerability to tGCI. For example, pyramidal neurons of the CA1 and polymorphic cells in the DG are highly vulnerable to tGCI, whereas pyramidal neurons of the CA3 and granule cells in the DG are relatively resistant to tGCI: especially, DG granule cells are known to have the highest resistance to tGCI (Kirino and Sano 1984; Schmidt-Kastner and Freund 1991; Yu et al. 2012).

Ji Hyeon Ahn and Taek Geun Ohk contributed equally to this work.

✉ Moo-Ho Won
mhwon@kangwon.ac.kr

✉ Joon Ha Park
jh-park@hallym.ac.kr

[1] Department of Biomedical Science and Research Institute for Bioscience and Biotechnology, Hallym University, 1 Hallimdaehak-gil, Chuncheon, Gangwon 24252, Republic of Korea

[2] Department of Emergency Medicine, School of Medicine, Kangwon National University, Chuncheon, Gangwon 24341, Republic of Korea

[3] Department of Biochemistry and Molecular Biology, and Research Institute of Oral Sciences, College of Dentistry, Gangnung-Wonju National University, Gangneung, Gangwon 25457, Republic of Korea

[4] Department of Neurobiology, School of Medicine, Kangwon National University, 1 Kangwondaehak-gil, Chuncheon, Gangwon 24341, Republic of Korea

[5] Department of Radiology, Kangwon National University Hospital, Chuncheon, Gangwon 24289, Republic of Korea

Many animal models of tGCI have been developed to study neuronal injury in certain brain areas (Ginsberg and Busto 1989; Traystman 2003). Gerbils have been proposed as a good animal model to investigate pathomechanisms of tGCI-induced neuronal death and characterization of repetitive ischemic insults, because they lack the communicating arteries which connect the carotid and the vertebrobasilar arterial systems: for that reason, tGCI is easily developed in the gerbils by bilateral common carotid artery occlusion (Araki et al. 1990; Lee et al. 2017; Martinez et al. 2012).

There is accumulating information on the death or loss of particular neuronal populations in certain brain areas: especially, pyramidal cells in the CA1 of the hippocampus are easily lost following tGCI (Schmidt-Kastner and Freund 1991; Yu et al. 2012). However, to the best of our knowledge, studies on the death or loss of granule cells in the DG of the hippocampus after tGCI have not yet been addressed. The aim of this study, therefore, was to investigate whether granule cells in the DG following various durations of single tGCI and repeated tGCI in the gerbil are lost or not. For this purpose, we employed Fluoro-Jade B (F-J B) histofluorescence staining which is a very useful technique to detect the death of neurons in damaged brain tissues (Lim et al. 2015; Schmued and Hopkins 2000).

Materials and methods

Experimental animals

We used male Mongolian gerbils *(Meriones unguiculatus)* when they were about 6 months of age (body weight, about 75 g). They were housed in a conventional facility (temperature, 23 °C; relative humidity, 60%) with a 12-h light/12-h dark cycle. The protocol of this study was approved (No., KW-180124-1) by the Institutional Animal Care and Use Committee of Kangwon University (Chuncheon, Kangwon, Republic of Korea).

These animals (total $n = 35$) were grouped as follows: 1) sham group ($n = 7$), 2)-4) 5-min, 10-min and 15-min tGCI groups (subtotal $n = 21$), which received single 5-min, 10-min and 15-min tGCI, respectively ($n = 7$), 5) repeated tGCI group ($n = 7$), which received 2 cycles of 5-min tGCI with 1-h interval.

Induction of single and repeated tGCI

tGCI was induced according to our published method (Song et al. 2018). In brief, the experimental animals were anesthetized with a mixture of 2.5% isoflurane in 33% oxygen and 67% nitrous oxide. Both common carotid arteries were occluded for single episode of 5-min, 10-min and 15-min tGCI. To induce repeated tGCI (2 cycles of tGCI), 5-min tGCI was repeated with 1-h interval. The reperfusion of blood was directly observed under an ophthalmoscope (HEINE K180, Heine Optotechnik, Herrsching, Germany). Sham animals received the same procedure without the occlusion of both common carotid arteries. Body (rectal) temperature was maintained under normothermic (37 ± 0.5 °C) condition until the animals completely recovered from anesthesia.

Preparation of histological sections

The animals in each group ($n = 7$/group) were intraperitoneally anesthetized with 60 mg/kg of pentobarbital sodium (JW Pharm. Co., Ltd., Republic of Korea) and sacrificed at 5 days after tGCI. Brains of the animals were transcardially rinsed with 0.1 M phosphate-buffered saline (PBS) (pH 7.4) and fixed with 4% paraformaldehyde (pH 7.4). The brains were removed and serially cut into 30-μm thickness of coronal sections in a cryostat (Leica, Germany). The brain sections were obtained from 7 animals/group, and adjacent sections in each animal were used for histo- and immunohistochemical staining for histological analysis described below.

Cresyl violet (CV) staining

The sections were stained with CV to observe cellular damage in the hippocampus, as we described previously (Lee et al. 2016). In brief, 1.0% (*w*/*v*) CV acetate (Sigma, St. Louis, MO, USA) solution was made, and glacial acetic acid (Sigma-Aldrich, St. Louis, MO, USA) was added to this solution. The sections were stained with CV solution at room temperature and washed with distilled water. The stained sections were dehydrated in serial ethanol (50%, 70%, 80%, 90%, 95% and 100%) baths. After dehydration, the sections were mounted with Canada Balsam (Kanto, Tokyo, Japan).

F-J B histofluorescence staining

The sections were stained with F-J B to observe neuronal death in the DG, as described previously (Lee et al. 2017). In short, the sections were immersed in a solution containing 1% of sodium hydroxide, transferred to a solution containing 0.06% of potassium permanganate, incubated with a solution containing 0.0004% of F-J B (Histochem, Jefferson, AR, USA), and placed on slide warmer (about 50 °C) for reaction.

Immunohistochemistry

The sections were immunostained with NeuN (a marker for neurons) for neurons or ionized calcium binding adapter molecule 1 (Iba-1) for microglia according to our published method (Lee et al. 2017). In short, the sections were incubated with diluted mouse anti-NeuN (1:1000, Chemicon, Temecula, CA, USA), and rabbit anti-Iba-1 (1:800, Wako, Osaka, Japan).

After rinsing them, they were exposed to biotinylated goat anti-mouse or rabbit immunoglobulin G (1:250, Vector Laboratories Inc., Burlingame, CA, USA) and streptavidin peroxidase complex (1:200, Vector). Finally, the reacted sections were visualized with 3, 3′-diaminobenzidine tetrahydrochloride (Sigma).

Data analysis

To quantitatively analyze of neuronal damage/death, five sections/animal were selected with 120 μm interval (anteroposterior −1.4 to −2.2 mm of the gerbil brain atlas) (Radtke-Schuller et al. 2016). NeuN-immunoreactive ($^+$) and F-J B-positive ($^+$) cells were counted as previously described (Song et al. 2018). In short, digital images of NeuN$^+$ and F-J B$^+$ cells were obtained under a light microscope (BX53, Olympus, Germany) and an epifluorescent microscope (Carl Zeiss, Göttingen, Germany) with blue excitation light (450–490 nm), respectively. The cells were obtained in a 250 X 250 μm square at the same area of each subregion and counted by averaging total numbers using an image analyzing system (software: Optima 6.5, CyberMetrics, Scottsdale, AZ).

To quantitatively analyze microgliosis, five sections/animal were selected and analyzed as previously described (Lee et al. 2018). In brief, digital image of Iba-1$^+$ cells was captured like the above-mentioned method. The obtained image was presented as a ratio of relative optical density (ROD), which was calibrated as % using Adobe Photoshop (version 8.0) and analyzed using NIH Image 1.59 software.

Statistical analysis

The data obtained in this study represent means ± SEM. Differences between the means among the groups were statistically analyzed by one-way analysis of variance (ANOVA) with a post hoc Bonferroni's multiple comparison test to elucidate tGCI-related differences among all groups. Statistical significance was considered at $P < 0.05$.

Results

CV$^+$ cells

In the sham group, CV$^+$ cells were easily shown in the gerbil hippocampus: CV$^+$ cells were densely located in the pyramidal layer of the CA1–3 and granule cell layer of the DG (Fig. 1a). In this study, we found difference in CV staining at 5 days after tGCI according to the group. In the 5- and 10-min tGCI groups, damaged CV$^+$ cells were observed in the pyramidal layer in the CA1 alone (Fig. 1b and c), and, in the 15-min tGCI group, damaged CV$^+$ cells were found in the CA2/3 as wells as CA1 (Fig. 1d). In the repeated 5-min tGCI group, CV$^+$ cells in the pyramidal layer of the CA1–3 were damaged, and CV$^+$ cells in the granule cell layer of the DG showed weak CV staining (Fig. 1e).

Based on previous studies (Yu et al. 2012), cells in the pyramidal layer of the CA1–3 (called CA1–3 pyramidal cells or neurons) were lost in gerbils after a 15-min tGCI like the finding in this study. However, we first found that cells in the granule cell layer of the DG (called granule cells) were weakly stained with CV in the repeated 5-min tGCI group. With this respect, we, in this study, focused on the damage/death of granule cells after repeated 5-min tGCI.

NeuN$^+$ cells

In the sham group, NeuN$^+$ cells in the DG were crowded in the granule cell layer and scattered in the polymorphic layer (Fig. 1f). In the 5-, 10- and 15-min tGCI groups, the distribution pattern and numbers of NeuN$^+$ granule cells were not significantly changed at 5 days after tGCI compared to those in the sham group; however, NeuN$^+$ polymorphic cells were gradually decreased with the longer time of single tGCI (Fig. 1g-i and p). In the repeated tGCI group, NeuN$^+$ polymorphic cells were hardly observed, and NeuN$^+$ granule cells were significantly decreased (37 ± 3.2 cells) compared to those (104 ± 4.1 cells) in the sham group (Fig. 1j and p).

F-J B$^+$ cells

In this study, we used F-J B histofluorescence staining because it is a very useful technique to detect the neuronal death. No F-J B$^+$ cells were observed in the DG of the sham group (Fig. 1k). In the 5-, 10-, and 15-min tGCI groups, also, no F-J B$^+$ granule cells were found; however, F-J B$^+$ polymorphic cells (5 ± 1.4 cells) in the 5-min tGCI group, and their numbers were gradually increased with the longer time of single tGCI (Fig. 1l-n, and q). In the repeated 5-min tGCI group, numbers of F-J B$^+$ polymorphic cells were further increased (16 ± 1.5 cells), and many F-J B$^+$ granule cells (76 ± 5.2 cells) were observed (Fig. 1o and q).

Iba-1$^+$ microglia

Iba-1$^+$ microglia, in the DG of the sham group, was mainly observed in the polymorphic and granule cell layers, and they had a small round cell body, which projected thin and long ramified branches (Fig. 2a). In the 5-min tGCI group, Iba-1$^+$ microglia were hypertrophied, and their processes were short and thickened, showing that their ROD was significantly increased (242% of the sham group) (Fig. 2b and f). In the other tGCI groups, ROD was significantly increased with the longer time of single tGCI (Fig. 2c, d and f). In the repeated 5-min tGCI group, Iba-1$^+$ microglia were more hypertrophied, and

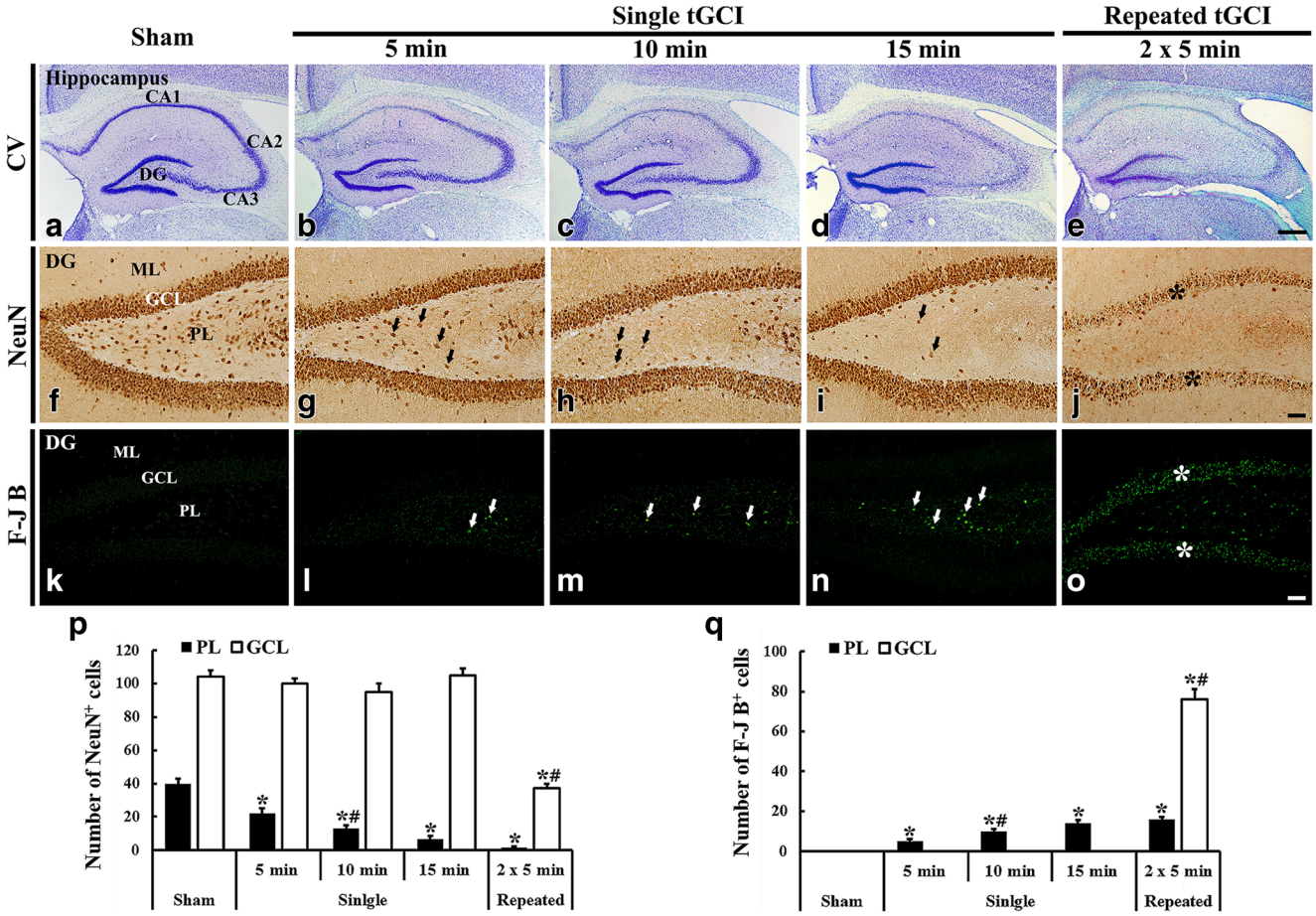

Fig. 1 Representative images of CV staining (**a**-**e**), NeuN immunohistochemistry (**f**-**j**), F-J B histofluorescence staining (**k**-**o**) in the hippocampus including DG of the sham-operated (**a**, **f**, **k**), single tGCI-operated (5-min, **b**, **g**, **l**; 10-min, **c**, **h**, **m**; 15-min, **d**, **i**, **n**) and repeated tGCI-operated (2 × 5-min, **e**, **j**, **o**) groups at 5 days after tGCI. In the single 5-, 10-, and 15-min tGCI-operated groups, $NeuN^+$ cells (black arrows) are decreased and F-J B^+ cells (white arrows) are increased with the longer time of single tGCI in the polymorphic layer, not in the granule cell layer. In the repeated 2 × 5-min tGCI-operated group, $NeuN^+$ cells are rarely observed and many F-J B^+ cells are detected in the polymorphic layer. Also, in this group, significant decrease of $NeuN^+$ cells and many F-J B^+ cells is observed in the granule cell layers (asterisks). CA, cornu ammonis; DG, dentate gyrus; GCL, granule cell layer; ML, molecular layer; PL, polymorphic layer. Scale bar = 400 μm (**a**-**e**), 50 μm (**f**-**o**). (**p** and **q**) Mean numbers of $NeuN^+$ and F-J B^+ cells in the polymorphic and granule cell layers of the DG ($n = 7$ in each group; $^*P < 0.05$ vs. sham-operated group, $^\#P < 0.05$ vs. preceding group). The bars indicate the means ± SEM

the ROD was significantly higher (149%) than that in the 15-min tGCI group (Fig. 2e and f).

Discussion

Granule cells in the hippocampal DG have been considered to be highly resistant to tGCI, because they survive from transient ischemic injury that kills other cell types of the DG, such as polymorphic cells (Ahn et al. 2016; Moon et al. 2009). In our present experiment, neuronal death was observed in the polymorphic layer of the DG at 5 days after a 5-min tGCI, showing that the death of polymorphic cells was progressively increased with longer time of tGCI (10 and 15 min of tGCI). However, neuronal death in the granule cell layer was not observed in all single tGCI groups. These findings are consistent with our previous study that showed that neuronal death in the DG occurred only in the polymorphic layer following various durations of single tGCI in the gerbil (Yu et al. 2012). In this study, we first found that massive neuronal death was shown in granule cell layers as well as in polymorphic cells of the gerbils subjected to 2 episodes of 5-min tGCI with 1-h interval. There is some experimental evidence that repeated tGCI results in adverse cumulative effects on ischemic brain damage, although it does not present the degeneration of granule cells in the DG. For example, Tomida et al. (1987) reported that 3 episodes of 5-min tGCI at different time intervals produced severer neuronal damage in some gerbil brain areas, such as the hippocampus, cerebral cortex, and striatum than that in single 15-min tGCI, suggesting that post-ischemic hypoperfusion might play a crucial role in the cumulative effect of repeated tGCI. In addition, they found that post-ischemic hypoperfusion was most distinct at 1-h recirculation following 5-min tGCI and that neuronal damage induced by 3 repeated

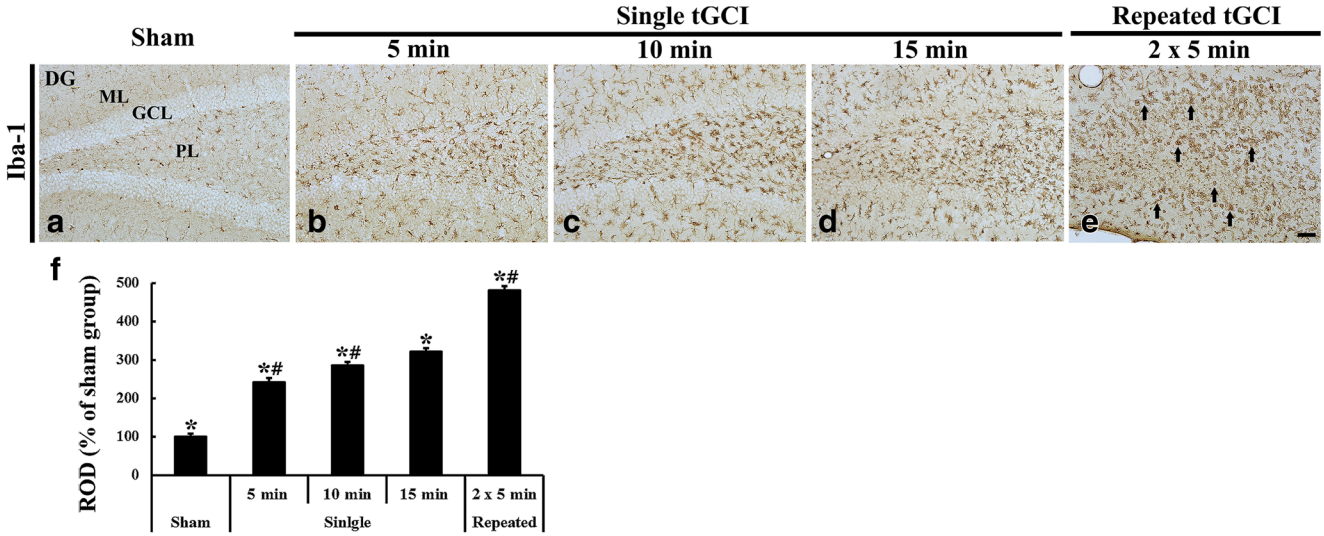

Fig. 2 Representative images of Iba-1-immunohistochemistry in the hippocampal DG of the sham-operated (**a**), single tGCI-operated (5-min, **b**; 10-min, **c**; 15-min, **d**) and repeated tGCI-operated (2 × 5-min, **e**) groups at 5 days after tGCI. In the single 5-, 10-, and 15-min tGCI-operated groups, Iba-1$^+$ microglia are hypertrophied and mainly distributed in the polymorphic layer. In the repeated 2 × 5-min tGCI-operated group, hypertrophied Iba-1$^+$ microglia (arrows) are significantly increased and scattered in all layers. GCL, granule cell layer; ML, molecular layer; PL, polymorphic layer. Scale bar = 50 μm. **f** ROD of Ib-1$^+$ structure in the DG ($n = 7$ in each group; $^*P < 0.05$ vs. sham-operated group, $^\#P < 0.05$ vs. preceding group). The bars indicate the means ± SEM

5-min tGCI was severest when tGCI was repeated with 1-h intervals (Tomida et al. 1987). Furthermore, Kato and Koqure (1990) reported that 3 or 5 episodes of 2-min tGCI with 1-h intervals elicited the destruction of most of CA1 pyramidal neurons in gerbils, although single 2-min tGCI did not evoke noticeable neuronal damage (Kato and Kogure 1990). Thus, based on the above-mentioned reports and our present findings, we insist that cumulative brain damage following 2 repeated 5-min tGCI with 1-h interval could elicit the death of granule cells in the DG, which was able to be found by F-J B histofluorescence staining, although longer time of single tGCI (10- and 15-min) did not result in the death of DG granule cells. To the best of our knowledge, this is the first study to show the death /loss of DG granule cells in a gerbil model of repeated tGCI.

A key pathological feature of cerebral ischemia is the activation of microglia in ischemic brain regions (Weinstein et al. 2010). In general, brain ischemia-induced microglia activation has been considered to play a detrimental role because activated microglia are a major source of a variety of neurotoxic substances, including free radicals and pro-inflammatory mediators which are closely linked to ischemic neuronal degeneration (Gregersen et al. 2000; Hur et al. 2010). In this study, our result showed that Iba-1$^+$ microglia were activated, showing that Iba-1 immunoreactivity was gradually increased with longer duration of single tGCI. In the repeated tGCI group, Iba-1 immunoreactivity was significantly higher than that in the 15-min tGCI group which showed the highest microgliosis among all single tGCI groups. Our results are in line with a previous study that showed that microglia are activated in a graded fashion in response to the severity of neuronal injury in the rat hippocampus after tGCI (Kato et al. 1995). Thus, it is likely that the degree of Iba-1 immunoreactivity after repeated tGCI might be severer than that after longer duration of single tGCI, which might be closely associated with the degree of neuronal degeneration in the DG.

In conclusion, repeated 5-min tGCI caused the death of granule cells in the gerbil DG, which was not observed after single tGCI for 10 and 15 min. In this respect, gerbils subjected to repeated 5-min tGCI can be used as an useful tool for study on the mechanism of granule cell death. In addition, this model could be used for study on neurogenesis in the hippocampus because the death of granule cells under pathological conditions could offer a stimulus for the proliferation of granule cell precursors in the hippocampal DG (Gould and Tanapat 1997; Spanswick et al. 2011).

Acknowledgements This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01321101)" Rural Development Administration, Republic of Korea, by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT &Future Planning (NRF-2017R1A2B4009079), and by the National Research Foundation of Korea (NRF) grant funded by the Korea government(MSIP; Ministry of Science, ICT & Future Planning) (NRF-2017R1C1B5075773).

Compliance with ethical standards

Conflict of interest All authors state that there is no conflict of interest.

References

Ahn JH et al (2016) Long-term observation of neuronal degeneration and microgliosis in the gerbil dentate gyrus after transient cerebral ischemia. J Neurol Sci 363:21–26. https://doi.org/10.1016/j.jns.2016.02.015

Araki T, Kato H, Kogure K (1990) Neuronal damage and calcium accumulation following repeated brief cerebral ischemia in the gerbil. Brain Res 528:114–122

Benjamin EJ et al (2018) Heart disease and stroke statistics-2018 update: a report from the american heart association circulation. Circulation 137:e67–e492. https://doi.org/10.1161/CIR.0000000000000558

Dietrich WD, Halley M, Valdes I, Busto R (1991) Interrelationships between increased vascular permeability and acute neuronal damage following temperature-controlled brain ischemia in rats. Acta Neuropathol 81:615–625

Ginsberg MD, Busto R (1989) Rodent models of cerebral ischemia. Stroke 20:1627–1642

Gould E, Tanapat P (1997) Lesion-induced proliferation of neuronal progenitors in the dentate gyrus of the adult rat. Neuroscience 80:427–436

Gregersen R, Lambertsen K, Finsen B (2000) Microglia and macrophages are the major source of tumor necrosis factor in permanent middle cerebral artery occlusion in mice. J Cereb Blood Flow Metab 20:53–65. https://doi.org/10.1097/00004647-200001000-00009

Hur J, Lee P, Kim MJ, Kim Y, Cho YW (2010) Ischemia-activated microglia induces neuronal injury via activation of gp91phox NADPH oxidase. Biochem Biophys Res Commun 391:1526–1530. https://doi.org/10.1016/j.bbrc.2009.12.114

Kato H, Kogure K (1990) Neuronal damage following non-lethal but repeated cerebral ischemia in the gerbil. Acta Neuropathol 79:494–500

Kato H, Kogure K, Araki T, Itoyama Y (1995) Graded expression of immunomolecules on activated microglia in the hippocampus following ischemia in a rat model of ischemic tolerance. Brain Res 694:85–93

Kirino T, Sano K (1984) Selective vulnerability in the gerbil hippocampus following transient ischemia. Acta Neuropathol 62:201–208

Lee JC et al (2016) New GABAergic neurogenesis in the hippocampal CA1 region of a gerbil model of long-term survival after transient cerebral ischemic injury. Brain Pathol 26:581–592. https://doi.org/10.1111/bpa.12334

Lee JC et al (2017) Neuroprotection of ischemic preconditioning is mediated by thioredoxin 2 in the hippocampal CA1 region following a subsequent transient cerebral ischemia. Brain Pathol 27:276–291. https://doi.org/10.1111/bpa.12389

Lee JC et al (2018) Brain ischemic preconditioning protects against moderate, not severe, transient global cerebral ischemic injury. Metab Brain Dis 33:1193–1201. https://doi.org/10.1007/s11011-018-0231-5

Lim JS et al (2015) Brain somatic mutations in MTOR cause focal cortical dysplasia type II leading to intractable epilepsy. Nat Med 21:395–400. https://doi.org/10.1038/nm.3824

Martinez NS et al (2012) Global brain ischemia in Mongolian gerbils: assessing the level of anastomosis in the cerebral circle of Willis. Acta Neurobiol Exp (Wars) 72:377–384

Moon JB et al (2009) Neuronal degeneration and microglial activation in the ischemic dentate gyrus of the gerbil. J Vet Med Sci 71:1381–1386

Olsson T, Wieloch T, Smith ML (2003) Brain damage in a mouse model of global cerebral ischemia. Effect of NMDA receptor blockade. Brain Res 982:260–269

Radtke-Schuller S, Schuller G, Angenstein F, Grosser OS, Goldschmidt J, Budinger E (2016) Brain atlas of the Mongolian gerbil (*Meriones unguiculatus*) in CT/MRI-aided stereotaxic coordinates. Brain Struct Funct 221(Suppl 1):1–272. https://doi.org/10.1007/s00429-016-1259-0

Schmidt-Kastner R, Freund TF (1991) Selective vulnerability of the hippocampus in brain ischemia. Neuroscience 40:599–636

Schmued LC, Hopkins KJ (2000) Fluoro-Jade B: a high affinity fluorescent marker for the localization of neuronal degeneration. Brain Res 874:123–130

Soler EP, Ruiz VC (2010) Epidemiology and risk factors of cerebral ischemia and ischemic heart diseases: similarities and differences. Curr Cardiol Rev 6:138–149. https://doi.org/10.2174/157340310791658785

Song M et al (2018) Chronic high-fat diet-induced obesity in gerbils increases pro-inflammatory cytokines and mTOR activation, and elicits neuronal death in the striatum following brief transient ischemia. Neurochem Int 121:75–85. https://doi.org/10.1016/j.neuint.2018.09.009

Spanswick SC, Epp JR, Sutherland RJ (2011) Time-course of hippocampal granule cell degeneration and changes in adult neurogenesis after adrenalectomy in rats. Neuroscience 190:166–176. https://doi.org/10.1016/j.neuroscience.2011.06.023

Tomida S, Nowak TS, Jr, Vass K, Lohr JM, Klatzo I (1987) Experimental model for repetitive ischemic attacks in the gerbil: the cumulative effect of repeated ischemic insults. J Cereb Blood Flow Metab 7:773–782. https://doi.org/10.1038/jcbfm.1987.133

Traystman RJ (2003) Animal models of focal and global cerebral ischemia. ILAR J 44:85–95

Weinstein JR, Koerner IP, Moller T (2010) Microglia in ischemic brain injury. Future Neurol 5:227–246. https://doi.org/10.2217/fnl.10.1

Wible CG (2013) Hippocampal physiology, structure and function and the neuroscience of schizophrenia: a unified account of declarative memory deficits, working memory deficits and schizophrenic symptoms. Behav Sci (Basel) 3:298–315. https://doi.org/10.3390/bs3020298

Yu DK et al (2012) Neuronal damage in hippocampal subregions induced by various durations of transient cerebral ischemia in gerbils using Fluoro-Jade B histofluorescence. Brain Res 1437:50–57. https://doi.org/10.1016/j.brainres.2011.12.029