Biochemical and Biophysical Research Communications 441 (2013) 457–462

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Connexin-43 hemichannels contribute to the propagation of μ-calpain-mediated neuronal death in a cortical ablation injury model

Yasuhiro Ishii, Kaori Shintani-Ishida, Ken-ichi Yoshida *

Department of Forensic Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

ARTICLE INFO

Article history:
Received 11 October 2013
Available online 26 October 2013

Keywords:
Traumatic brain injury
Calpain
Hemichannel
Astrocyte
Connexin-43
Neuronal death

ABSTRACT

We investigated the role of the astrocytic and neuronal hemichannels (HCs) in the spread of cortical neuronal death in a rat cortical injury model. Over time (by 6 h), propidium iodide (PI)-positive cells with labeling either with anti-neuron specific enolase or anti-parvalbumin (indicating GABAnergic interneurons) antibody spread in the deep cortical layers adjacent to the injury and co-localized with activated μ-calpain. Connexin (Cx)-43, glial fibrillary acidic protein (GFAP), activated μ-calpain and α-fodrin breakdown product (FBP) increased post-injury, peaking at 1 h, in the injury and adjacent areas. GFAP-Cx43-positive reactivated astrocytes exhibited similar distribution to the dead neurons. Cx43 and Cx36 primarily comprise HCs in the astrocyte and neuron, respectively. Ethidium bromide (EtBr) uptake was enhanced post-injury, and confirmed in the Cx43- and Cx36-positive cells. A Cx43-HC inhibitor Gap26 prevented the opening of the Cx43-HC and Cx36-HC, μ-calpain activation, α-fodrin proteolysis and death in the deep cortical neurons. Collectively, opening of the astrocytic Cx43-HC and neuronal Cx36-HC would induce the regional spread of cortical neuronal death through μ-calpain activation in the rat brain injury model.

1. Introduction

Brain injuries induce intracellular Ca^{2+} overloading and μ-calpain activation [1,2], which result in necrosis through proteolysis of cytoskeletal proteins such as α-fodrin [3,4]. In the *in vivo* models of cortical injury, immunostaining of activated μ-calpain and α-fodrin breakdown products (FBP) were detected in the early phase [5]. Additionally, the spread of FBP was inhibited by calpain inhibitors [6], and the expansion of neuronal injury was blocked by μ-calpain knockdown [7]. These findings support the critical role of μ-calpain activation in secondary neuronal death after brain injury.

Transcripts of the connexin (Cx) gene family form hexamers called connexons in the membrane [8]. Open connexons in the nonjunctional plasma membrane, referred to as hemichannels (HCs), promote the release of ATP and glutamate in response to depolarization and ischemia [9,10]. The docking of two connexons at cell–cell appositions results in the formation of gap junction (GJ) and promotes intercellular transfer of ions and small molecules [8]. Dramatic changes in the GJ communication have been reported in reactivated astrocytes with glial fibrillary acidic protein (GFAP) up-regulation, in association with the enhanced expression of Cxs [11].

It depends on the experimental setting whether spatial and functional interactions between astrocytes and neurons through GJ or HC are protective or deleterious to the neurons [11]. In astrocyte-neuron co-cultures, astrocytic GJ prevents neuronal death following oxidative stress NMDA challenge [12], whereas astrocytic GJ promotes the propagation and amplification of cell death [13]. Pharmacological or genetic blockade of Cx43 inhibits the expansion of secondary neuronal injury in the hippocampal slices [14] and the spinal cord [15]. Meanwhile, Cx36 is the primary Cx in neurons, particularly in the GABAnergic inhibitory interneurons, and can form GJ [16]. In cortical impact injury in mice, secondary striatal death depends on the expression of Cx36 [17].

Increasing evidence favors a crucial role of HCs in reactivated astrocytes in the secondary neuronal death. Cx43-HC enhances the generation of inter-astrocytic Ca^{2+} waves through ATP release upon pharmacological stimulation [18] and induces neuronal death upon metabolic inhibition [19]. Recently, astrocytic Cx43-HC and neuronal HC comprised of pannexin-1 or Cx36 have been implicated in the spread of *in vitro* neuronal injury following exposure to amyloid-β peptide (Aβ), cytokines and hypoxia-reoxygenation in co-cultures [20,21].

This study aims to investigate whether the activation of HC or GJ in the astrocytes and the neurons promotes the spread of *in vivo* secondary neuronal death after injury through μ-calpain activation and elucidate which Cx isoforms are involved in this process.

* Corresponding author. Fax: +81 3 5841 3366.
E-mail address: kyoshida@m.u-tokyo.ac.jp (K.-i. Yoshida).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.10.091

2. Materials and methods

2.1. Materials

Carbenoxolone (CBX), propidium iodide (PI), and ethidium bromide (EtBr) were purchased from Sigma–Aldrich (St. Louis, MO), Gap26 from Seven Biotec (Kidderminster, Worcester, UK), MedGel® from MedGEL (Kyoto, Japan), and OCT compound from Sakura Finetek (Torrance, CA).

The rabbit anti-μ-calpain and rabbit anti-FBP antibodies were kindly provided by Dr. K. Inomata (Tokyo Metropolitan Institute for Gerontology). The rabbit anti-Cx43 antibody (71-0700) was purchased from Invitrogen (Carlsbad, CA), the rabbit anti-GFAP antibody (AB5804) from Millipore (Billerica, MA), the anti-neuron specific enolase antibody (NSE, AB951) from Chemicon (Mansfield, TX), the anti-parvalbumin antibody from Millipore (Billerica, MA), Iba-1 from Wako Pure Chemical (Osaka, Japan).

2.2. The rat cortical ablation model

Animal experiments were performed according to a protocol approved by the Ethics Committee on Animal Experiments at The University of Tokyo, Graduate School of Medicine and followed the Committee's Guideline for Animal Experiments. Adult male Sprague–Dawley rats (200–250 g) were anesthetized with an i.p. injection of pentobarbital (64.8 mg/kg). A 5 × 5 mm^2 midline craniotomy was made with a rhomboid suture at the rear end. A 4 × 4 mm^2 ablation injury that was 2 mm deep with 3 coronal incisions within the area was made with a scalpel blade through the craniotomy, following a previously described method with some modifications [22] (Fig. 1A). In parallel experiments, a reagent or the vehicle (5 μl each) were applied to the injury site at the ipsilateral and contralateral cortical surfaces, respectively and covered with 5 × 5 mm gels (MedGel®, PI5-9480E53) soaked with the reagent or the vehicle (20 μl each). After the specified time, the injured area and the adjacent area anterior to the injury were harvested *en bloc* (4 × 4 × 2 mm) and frozen at −80 °C.

2.3. Western blot analysis

The frozen tissue (0.04 g) was homogenized in 0.4 ml ice-cold STE buffer containing 320 mM sucrose, 10 mM Tris–HCl (pH 7.4), 1 mM EDTA, 50 mM NaF, 1 mM Na_3VO_4 and complete protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany), and underwent Western blotting. Protein concentrations were determined using a Coomassie Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA). The lysates (5 μg protein/lane) were separated by SDS–PAGE on 7.5% gels (μ-calpain and fodrin) and 15% gels (others). After transfer, the nitrocellulose membrane was blocked with 5% nonfat dry milk in Tris-buffered saline (TBS-T; 150 mM NaCl, 10 mM Tris–HCl, pH 7.4, 0.05% Tween-20) for 1 h, washed with TBS-T, and incubated with primary antibodies against activated μ-calpain (1:10,000), α-fodrin (1:1000), Cx43 (1:500), GFAP (1:1000), or actin (1:5000). Peroxidase-conjugated anti-mouse or -rabbit IgG antibodies (Promega, Madison, WI) were used for chemiluminescence detection.

2.4. Cell death

PI penetrates necrotic cells via the disrupted membranes and emits fluorescent signals, which is not observed in the intact cells. PI-positive neurons were shown in hippocampal slices after impact injury [14]. We employed this method to determine the spreading of dead cells. PI (10 μM) in physiological saline (5 μl) was immediately applied to the injury, and a gel soaked with PI (10 μM, 20 μl) was overlaid. The sagittal planes in the area adjacent to the edges of the injury site (at the left bar-line) were examined for PI-positive cells. The ratio of PI-positive to DAPI-positive (whole) cells was calculated in 3 randomly-selected 5 μm sections. Fluorescent images were acquired and analyzed with Image-Pro Plus software (Media Cybernetics, Bethesda, MD). Pixel intensity in 1.2 × 1.2 mm^2 area

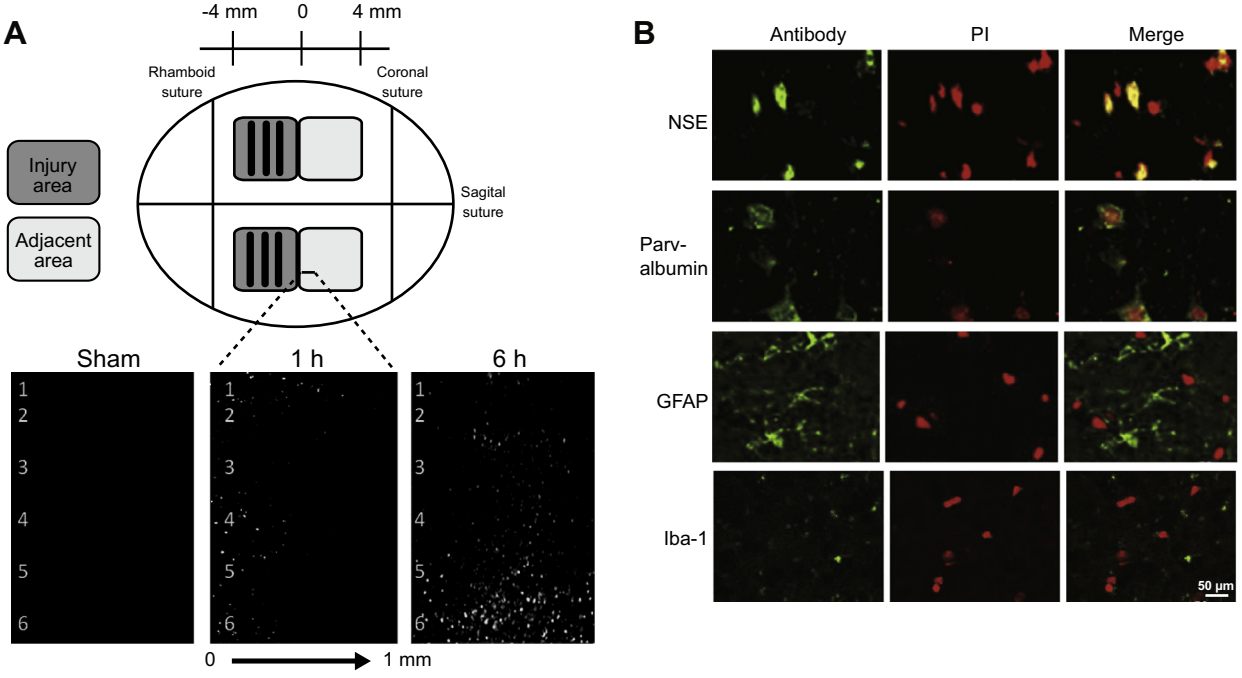

Fig. 1. The model of brain ablation injury, and the distribution and identification of PI-positive dead cells. (A) PI-positive dead cells (evident at 6 h but not 1 h post-injury) spread rightward from the injury margin (left bar-line) to the adjacent areas in the 5–6 cortical layers (40×). (B) Immunofluorescence of PI-NSE (in neurons), PI-parvalbumin (in GABAnergic inhibitory neurons), PI-GFAP (in astrocytes) and PI-iba 1 (in microglia) in the deep cortical layers 6 h post-injury indicates the death of NSE- or parvalbumin-positive neurons.

(within a standard size box) was obtained from the cortical layers adjacent to the injury. Gains and black (negative signal) levels were standardized for each experiment.

2.5. HC activity

To monitor the HC activity, EtBr (5 μM) in physiological saline (5 μl) was applied to the injury immediately, and a gel soaked with EtBr (5 μM, 20 μl) was laid over the site for 1 h. EtBr-positive cells in the 5-μm sections were imaged with rhodamine filters. The ratio of EtBr-positive to DAPI-positive cells, within a $2 \times 2\ mm^2$ area (standard box) adjacent to the injury, as described in the PI assay, was used to assess the HC activity.

2.6. Immunofluorescence microscopy

Under anesthesia, rats were perfused with PBS and 4% paraformaldehyde via trans-cardiac cannulation, and the brain was excised and postfixed with 8% paraformaldehyde for 2 h. After cryoprotection with 30% sucrose in PBS, the tissues were immersed in OCT compound, frozen in liquid nitrogen and stored at −80 °C.

Cryosections (10 μm thick) were placed on Siran II glass slides, washed with TBS, blocked with 5% BSA in TBS for 1 h, and incubated with anti-Cx-43 (1:500), anti-activated μ-calpain (1:500), anti-GFAP (1:1000), or anti-NSE (1:1000) antibodies in 1% BSA at 4 °C overnight. The sections were then washed with PBS and incubated with anti-rabbit IgG or anti-mouse IgG secondary antibodies (each diluted 1:500) in 1% BSA for 2 h. Fluorescent images were acquired using the Axioskope 2 fluorescence microscope (CarlZeiss, Gottingen, Germany).

2.7. Statistical analysis

The results are expressed as the means ± SE. Data were analyzed using ANOVA with post hoc analysis using Dunnet's test at 99% confidence levels.

3. Results

3.1. Neuronal cell death in deep cortical layers post-injury

PI-positive dead cells, reflecting necrosis, spread from the margins of the injury (left bar-line) to the adjacent areas in the deep (5–6) cortical layers (rightward) 6 h post-injury, but were not detected 1 h post-injury (Fig. 1A). In the sham (contralateral) brain, PI-positive cells were not detected in any cortical layer (Fig. 1A).

In order to identify the type of the dead cells, NSE was used for neurons, parvalbumin for the GABAnergic interneurons [16], GFAP for astrocytes [23], and Iba-1 for microglia. The PI fluorescence co-localized with NSE and parvalbumin fluorescence in the deep cortical layers in the adjacent area 6 h post-injury (Fig. 1B), indicating neuronal cell death. Compared to the PI-NSE-positive cells, the PI-parvalbumin-positive cells were larger, and consistent with the morphology and localization of the GABAnergic interneuron [16]. The lack of co-localization of PI with GFAP or Iba-1 (Fig. 1B) ruled out the death of astrocytes and microglial cells.

3.2. Increase in Cx43 and GFAP expression in astrocytes, and inhibition of secondary neuronal death by HC inhibitors post-injury

As reported in the infarct penumbra [24], an increase in the expression of Cx43 and GFAP was markedly increased 6 h post-injury in the deep cortical layers in the adjacent area (Fig. 2A). Wes-

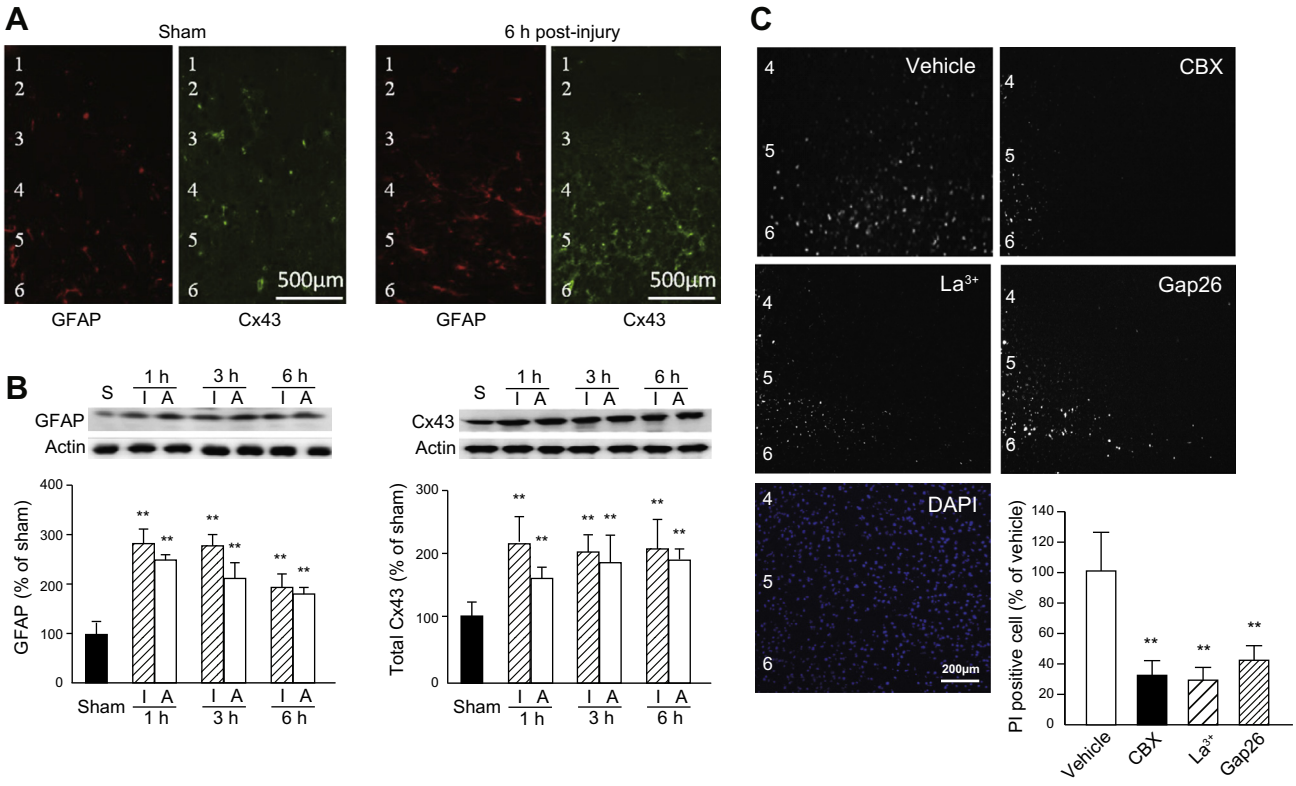

Fig. 2. Post-injury spreading of Cx43-GFAP-immunofluorescence in astrocytes and the effects of HC inhibitors on neuronal cell death. (A) Immunofluorescent images of GFAP (red) and Cx43 (green) in the sham and 6 h post-injury sections. (B) Western blots show an increase in GFAP and Cx43 levels in the injured (I) and adjacent (A) areas. The results are expressed as means ± SE, $n = 4$ (**Indicates $P < 0.01$ vs. sham, Dunnet's test). (C) CBX, La^{3+} or Gap26 prevented the spread of PI-positive cells, as quantified by the ratio of PI-positive to DAPI-positive cells (**Indicates $P < 0.01$ vs. vehicle, Dunnet's test).

tern blotting confirmed the rapid and sustained increase in the expression of Cx43 and GFAP, peaking at 1 h post-injury, in the injury and adjacent areas (Fig. 2B).

The PI-positive dead neurons and the GFAP-positive reactivated astrocytes were both localized in the deep cortical layers (Figs. 1A and 2A). Additionally, the extended images of PI-NSE (indicating neurons as predominant dead cells) and PI-GFAP (indicating dead cells and reactivated astrocytes in the same field) (Fig. 1B) suggest that the interaction of the reactivated astrocytes and the neurons contributes to the secondary neuronal death in the deep cortical layers (Figs. 1 and 2).

The distribution of the PI-positive dead cells in the area adjacent to the injury was quantified as the ratio of PI-positive to DAPI-positive cells in the fixed area adjacent to the margins of the injury (left bar-line) (Fig. 2C). CBX inhibits GJ and HC, and lanthanum (La^{3+}) blocks HC, irrespective of the Cx isoforms. Gap26, a peptide mimetic of the Cx43 extracellular loop, is a relatively specific inhibitor of Cx43-HCs [25,26]. CBX (150 mM), La^{3+} (200 μM) and Gap26 (200 μM) prevented the spread of the PI-positive cells (secondary injury) 6 h post-injury by 70–80% in the adjacent areas (Fig. 2C), supporting the involvement of Cx43-HCs in the spread of secondary neuronal death.

3.3. The opening of astrocyte and neuronal HCs post-injury

The Cx43-HC in reactivated astrocyte has been implicated in the neuronal death in the co-cultures of astrocytes and neurons after treatment with Aβ or cytokines or after ischemia [20,21]. EtBr penetrates the cell through HC, but cannot traverse intact cell membranes. We found that injury induced the spread of EtBr fluorescence in the adjacent cortical layers 1 h post-injury (Fig. 3A), when the increase in Cx43 expression peaked (Fig. 2B). The 1 h time point was selected because the PI-positive cells were not detected in the adjacent area (Fig. 1A), ruling out the potential uptake of EtBr through the disrupted membranes. The ratio of the EtBr-positive cells to DAPI-positive cells in the fixed area in the cortical layers adjacent to the injury was used to assess the HC activity (Fig. 3A). Gap26 prevented the HC activity (Fig. 3A). We then examined the Cx species that comprise the HC opened in the deep cortical layers. Cx43 and Cx36 are the primary Cx of astrocytes and neurons, respectively [27]. We found EtBr uptake in the Cx43-positive cells and the Cx36-positive cells post-injury, implying the opening of astrocytic Cx43-HC and neuronal Cx36-HC (Fig. 3B). Gap 26 prevented the post-injury opening of the Cx43-HC and Cx36-HC (Fig. 3B). Given that Gap26 is a relatively specific inhibitor of Cx43-HC [25,26], we speculate that Gap 26 inhibited not only the astrocytic Cx43-HC but also the neuronal Cx36-HC that might have been opened by a factor released through the Cx43-HC.

3.4. The spread of μ-calpain activation and α-fodrin proteolysis to the adjacent cortical area post-injury

Brain injury induces neuronal cell death through the activation of calpain and calpain-dependent proteolysis of α-fodrin [2,3]. Western blotting demonstrated an increase in the levels of activated μ-calpain in the injury and adjacent areas, which reached a peak at 1 h and remained elevated for over 6 h (Fig. 4A). Likewise, FBP increased in the injury and adjacent areas, peaking at 1 h (Fig. 4B). CBX, La^{3+} and Gap26 treatment prevented the increase in activated μ-calpain and FBP in the adjacent area 1–6 h post-injury (Fig. 4A and B), supporting the Cx43-HC-dependent spreading of μ-calpain activation in the adjacent area. In the injury area, CBX, La^{3+} and Gap26 reduced the FBP but not the activated μ-calpain (Fig. 4A and B), suggesting that a Cx43-HC-dependent process is required for α-fodrin proteolysis but not for μ-calpain activation.

3.5. Post-injury spread of μ-calpain activation on PI-positive cells

To address the relationship between μ-calpain activation and cell death post-injury, we examined double fluorescence of activated μ-calpain and PI in the deep cortical layers 6 h post-injury.

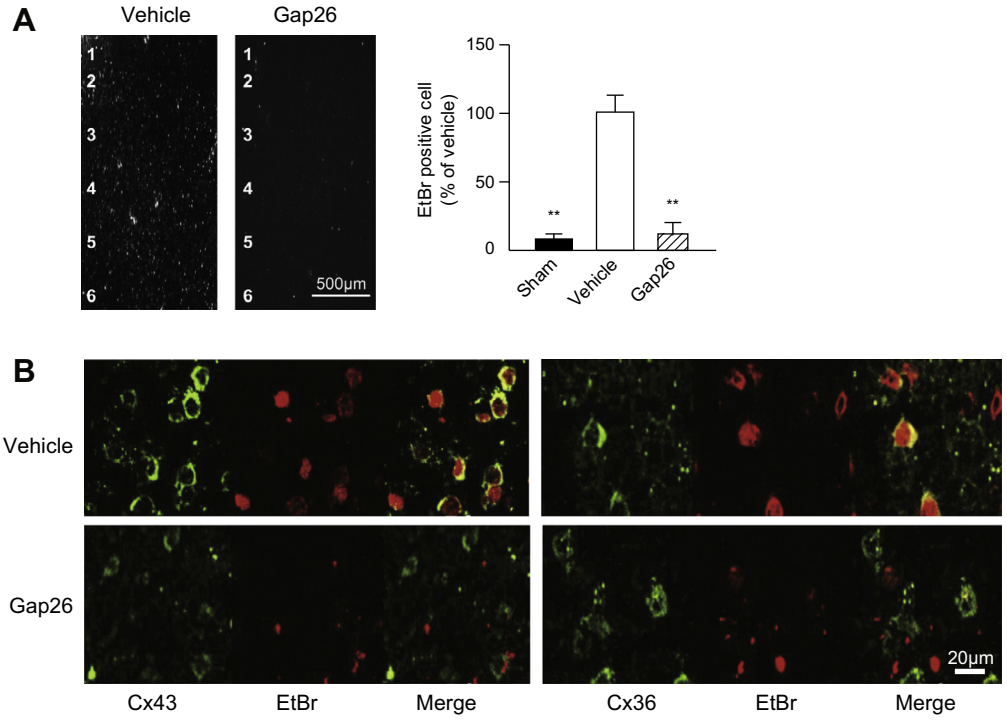

Fig. 3. Spreading of HC opening in the adjacent cortical layers 1 h post-injury and its inhibition by Gap26. (A) HC activity, quantified as the ratio of EtBr-positive to the whole DAPI-positive cells, was enhanced 1 h post-injury and was inhibited by Gap26 (**Indicates $P < 0.01$ vs. vehicle, Dunnet's test). (B) EtBr fluorescence was enhanced in Cx43-positive and Cx36-positive cells in the deep cortical layers 6 h post-injury, and was inhibited by Gap26.

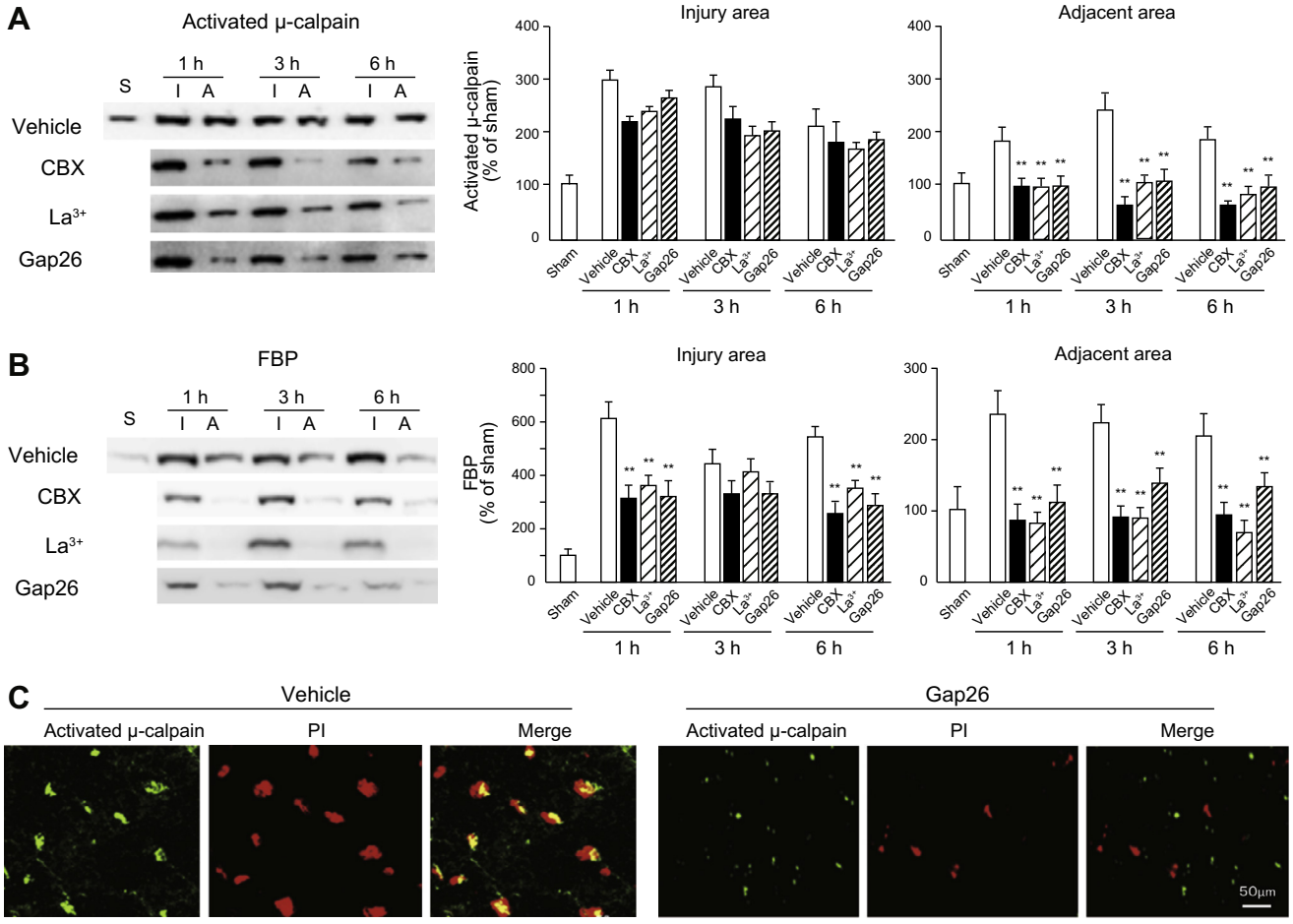

Fig. 4. Western blotting analysis and quantification of the temporal change in activated μ-calpain (A) and α-fodrin breakdown product (FBP) (B) in the injury and adjacent areas, 1, 3 and 6 h post-injury, and the effects of CBX, La^{3+} and Gap26. The results are expressed as means ± SE, $n = 4$ (**Indicates $P < 0.01$ vs. vehicle, Dunnet's test). Panel C shows merged fluorescent images of activated μ-calpain and PI in the adjacent deep cortical layers 6 h post-injury and the effect of Gap26 (400×).

Fluorescent images showed the co-localization of the activated μ-calpain and the PI-positive cells, which was inhibited by Gap26 (Fig. 4C). These results suggest the coordinated propagation of μ-calpain activation and neuronal death through the opening of the astrocytic Cx43-HC in the deep cortical layers.

4. Discussion

We demonstrated that *in vivo* cortical injury induces Cx43-HC opening in the reactivated astrocytes, Cx36-HC opening and μ-calpain activation in the neurons, and spreading of neuronal death in the deep cortical layers of the adjacent area. This is the first demonstration of the link between the spread of reactivated astrocytes with Cx43-HC opening and the μ-calpain-mediated secondary neuronal death, each of which has been known as independent phenomena.

The extended images of PI-NSE (dead neurons) and PI-GFAP (dead cells and reactivated astrocytes in the same field) (Fig. 1B) support the known spatial interaction between the dead neurons and the reactivated astrocytes [11] in the deep cortical layers post-injury (Figs. 1A and 2A). The activated μ-calpain and PI-double positive cells were also found in the same region (Fig. 4C). Cx43 is the primary Cx in the astrocyte [27]. Besides GFAP up-regulation in reactivated astrocytes, we confirmed the increase in Cx43 levels (Fig. 2) and the Cx43-HC opening (Fig. 3) 1 h post-injury in the deep cortical layers. Previous studies on injuries in the hippocampal slices [14] support the critical role of Cx43 in the expansion of secondary neuronal death. Additionally, reactivated astrocytes with enhanced Cx43 expression are supposed to promote neuronal death through enhanced inter-cellular communications, either though GJ or HC [11].

It is supposed that CBX and La^{3+} are non-specific inhibitors for Cx-GJ/HC and Cx-HC, respectively. Gap26 is a relatively specific inhibitor for Cx43-HC, even though it may inhibit Cx43-GJ at higher doses with longer periods of application [25,26]. In this study, the striking inhibition of the EtBr uptake in the adjacent area at a low dose and an early time-point (1 h) after injury (Fig. 3) supports that Gap26 inhibited Cx43-HC. Given the Cx43 expression in astrocytes but not neurons [27], Gap26, CBX and La^{3+} inhibited μ-calpain activation, α-fodrin proteolysis (Fig. 4A and B) and death in neurons (Fig. 2) in the peri-injury area. Gap 26 also diminished double fluorescence of PI and activated μ-calpain in neurons post-injury (Fig. 4C). These findings suggest that the astrocytic Cx43-HC indirectly contributes to the μ-calpain-mediated secondary neuronal death in the adjacent area post injury.

The propagation of the inter-astrocytic Ca^{2+} waves via Cx43-HC-mediated release of ATP [18] would induce the spread of the Ca^{2+}-overloading and μ-calpain activation in neurons post-injury (Fig. 4). Consistently, μ-calpain activation peaked at 1 h in the injury area and at 3 h in the adjacent area, and Gap26 prevented the propagation of μ-calpain activation to the adjacent area (Fig. 4). Recently, Choo et al. reported that *in vivo* hippocampal trauma and *in vitro* astrocyte stretch trigger inter-astrocyte Ca^{2+} waves, which contribute to expansion of the neuronal death through purinergic receptors including P2Y1 [28]. In hippocampal slices, ATP activates P2Y1 receptors and increases Ca^{2+} in the astro-

cytes and interneurons [29]. Additionally, glutamate receptor antagonists inhibit calpain-mediated cytoskeletal proteolysis in focal cerebral ischemia through blocking neuronal Ca^{2+}-influx [30]. These findings suggest that the purinergic and/or glutamate receptors contribute to the post-injury Ca^{2+}-overload and calpain activation in neurons, through the opening of the neuronal HC.

Orellana et al. proposed that the reactivation of astrocytes induces opening of the astrocytic Cx43-HC and the neuronal HC comprised of Cx36 or pannexin-1 via a paracrine mechanism [20,21]. Meanwhile, pharmacological or genetic inhibition of Cx36 prevents secondary striatal neuronal death following cortical injury in mice [17]. In this study, given that La^{3+} inhibits Cx-HC but not pannexin-HC [31], La^{3+} would have inhibited the post-injury calpain activation (Fig. 4) and neuronal death (Fig. 2) through blocking Cx-HC rather than pannexin-HC. Given that Cx36 is the primary Cx in neurons [3,16,32], the EtBr uptake in the Cx36-positive cells 1 h post-injury (Fig. 3) imply the opening of the Cx36-HC in neurons. The Cx43-HC blocker Gap26 inhibited the opening of the Cx36-HC in neurons (Fig. 3) possibly through a paracrine mechanism. Consistently, Orellana et al. proposed that the reactivation of astrocytes induces the closing of GJ and opening of Cx43-HC, and the Cx43-HC-mediated secretion of ATP enhances the opening of the neuronal HC [20,21]. We identified a part of the PI-positive dead cells as the parvalbumin-positive GABAnergic interneurons (Fig. 1). Besides the regional reactivation of astrocytes, the GABAnergic interneurons may be involved in the spreading of the neuronal death via Cx36-HC in the deep cortical layers.

The results of this study suggest that the Cx43-HC inhibitors are useful in preventing the secondary injury derived from surface injuries such as brain contusions. The topical application of the drugs by use of gel could avoid the side effects induced by systemic administration of the drugs.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgment

This study was supported by JSPS KAKENHI Grant Numbers 22590628, 24659334.

References

[1] K.E. Saatman, J. Creed, R. Raghupathi, Calpain as a therapeutic target in traumatic brain injury, Neurotherapeutics 7 (2010) 31–42.
[2] T.C. Saido, M. Yokota, S. Nagao, I. Yamaura, E. Tani, T. Tsuchiya, K. Suzuki, S. Kawashima, Spatial resolution of fodrin proteolysis in postischemic brain, J. Biol. Chem. 268 (1993) 25239–25243.
[3] S. Fukuda, K. Harada, M. Kunimatsu, T. Sakabe, K. Yoshida, Postischemic reperfusion induces alpha-fodrin proteolysis by m-calpain in the synaptosome and nucleus in rat brain, J. Neurochem. 70 (1998) 2526–2532.
[4] R.M. Posmantur, A. Kampfl, W.C. Taft, M. Bhattacharjee, C.E. Dixon, J. Bao, R.L. Hayes, Diminished microtubule-associated protein 2 (MAP2) immunoreactivity following cortical impact brain injury, J. Neurotrauma 13 (1996) 125–137.
[5] E.D. Hall, P.G. Sullivan, T.R. Gibson, K.M. Pavel, B.M. Thompson, S.W. Scheff, Spatial and temporal characteristics of neurodegeneration after controlled cortical impact in mice: more than a focal brain injury, J. Neurotrauma 22 (2005) 252–265.
[6] R.T. Bartus, E.Y. Chen, G. Lynch, J.H. Kordower, Cortical ablation induces a spreading calcium-dependent, secondary pathogenesis which can be reduced by inhibiting calpain, Exp. Neurol. 155 (1999) 315–326.
[7] K.H. Yamada, D.A. Kozlowski, S.E. Seidl, S. Lance, A.J. Wieschhaus, P. Sundivakkam, C. Tiruppathi, I. Chishti, I.M. Herman, S.M. Kuchay, A.H. Chishti, Targeted gene inactivation of calpain-1 suppresses cortical degeneration due to traumatic brain injury and neuronal apoptosis induced by oxidative stress, J. Biol. Chem. 287 (2012) 13182–13193.
[8] J.C. Saez, V.M. Berthoud, M.C. Branes, A.D. Martinez, E.C. Beyer, Plasma membrane channels formed by connexins: their regulation and functions, Physiol. Rev. 83 (2003) 1359–1400.
[9] D.C. Spray, Z.C. Ye, B.R. Ransom, Functional connexin "hemichannels": a critical appraisal, Glia 54 (2006) 758–773.
[10] K. Shintani-Ishida, K. Uemura, K. Yoshida, Hemichannels in cardiomyocytes open transiently during ischemia and contribute to reperfusion injury following brief ischemia, Am. J. Physiol. Heart Circ. Physiol. 293 (2007) H1714–1720.
[11] C. Giaume, A. Koulakoff, L. Roux, D. Holcman, N. Rouach, Astroglial networks: a step further in neuroglial and gliovascular interactions, Nat. Rev. Neurosci. 11 (2010) 87–99.
[12] G. Zundorf, S. Kahlert, G. Reiser, Gap-junction blocker carbenoxolone differentially enhances NMDA-induced cell death in hippocampal neurons and astrocytes in co-culture, J. Neurochem. 102 (2007) 508–521.
[13] J.H. Lin, H. Weigel, M.L. Cotrina, S. Liu, E. Bueno, A.J. Hansen, T.W. Hansen, S. Goldman, M. Nedergaard, Gap-junction-mediated propagation and amplification of cell injury, Nat. Neurosci. 1 (1998) 494–500.
[14] M.V. Frantseva, L. Kokarovtseva, C.G. Naus, P.L. Carlen, D. MacFabe, V.J.L. Perez, Specific gap junctions enhance the neuronal vulnerability to brain traumatic injury, J. Neurosci. 22 (2002) 644–653.
[15] C. Huang, X. Han, X. Li, E. Lam, W. Peng, N. Lou, A. Torres, M. Yang, J.M. Garre, G.F. Tian, M.V. Bennett, M. Nedergaard, T. Takano, Critical role of connexin 43 in secondary expansion of traumatic spinal cord injury, J. Neurosci. 32 (2012) 3333–3338.
[16] K. Oguro, T. Jover, H. Tanaka, Y. Lin, T. Kojima, N. Oguro, S.Y. Grooms, M.V. Bennett, R.S. Zukin, Global ischemia-induced increases in the gap junctional proteins connexin 32 (Cx32) and Cx36 in hippocampus and enhanced vulnerability of Cx32 knock-out mice, J. Neurosci. 21 (2001) 7534–7542.
[17] A.B. Belousov, Y. Wang, J.H. Song, J.V. Denisova, N.E. Berman, J.D. Fontes, Neuronal gap junctions play a role in the secondary neuronal death following controlled cortical impact, Neurosci. Lett. 524 (2012) 16–19.
[18] C.E. Stout, J.L. Costantin, C.C. Naus, A.C. Charles, Intercellular calcium signaling in astrocytes via ATP release through connexin hemichannels, J. Biol. Chem. 277 (2002) 10482–10488.
[19] J.E. Contreras, H.A. Sanchez, E.A. Eugenin, D. Speidel, M. Theis, K. Willecke, F.F. Bukauskas, M.V. Bennett, J.C. Saez, Metabolic inhibition induces opening of unapposed connexin 43 gap junction hemichannels and reduces gap junctional communication in cortical astrocytes in culture, Proc. Natl. Acad. Sci. USA 99 (2002) 495–500.
[20] J.A. Orellana, N. Froger, P. Ezan, J.X. Jiang, M.V. Bennett, C.C. Naus, C. Giaume, J.C. Saez, ATP and glutamate released via astroglial connexin 43 hemichannels mediate neuronal death through activation of pannexin 1 hemichannels, J. Neurochem. 118 (2011) 826–840.
[21] J.A. Orellana, K.F. Shoji, V. Abudara, P. Ezan, E. Amigou, P.J. Saez, J.X. Jiang, C.C. Naus, J.C. Saez, C. Giaume, Amyloid beta-induced death in neurons involves glial and neuronal hemichannels, J. Neurosci. 31 (2011) 4962–4977.
[22] E.A. Eugenin, D. Eckardt, M. Theis, K. Willecke, M.V. Bennett, J.C. Saez, Microglia at brain stab wounds express connexin 43 and in vitro form functional gap junctions after treatment with interferon-gamma and tumor necrosis factor-alpha, Proc. Natl. Acad. Sci. USA 98 (2001) 4190–4195.
[23] M. Cronin, P.N. Anderson, J.E. Cook, C.R. Green, D.L. Becker, Blocking connexin43 expression reduces inflammation and improves functional recovery after spinal cord injury, Mol. Cell. Neurosci. 39 (2008) 152–160.
[24] T. Nakase, Y. Yoshida, K. Nagata, Enhanced connexin 43 immunoreactivity in penumbral areas in the human brain following ischemia, Glia 54 (2006) 369–375.
[25] W.H. Evans, S. Boitano, Connexin mimetic peptides: specific inhibitors of gap-junctional intercellular communication, Biochem. Soc. Trans. 29 (2001) 606–612.
[26] L. Leybaert, K. Braet, W. Vandamme, L. Cabooter, P.E. Martin, W.H. Evans, Connexin channels, connexin mimetic peptides and ATP release, Cell Commun. Adhes. 10 (2003) 251–257.
[27] J.E. Rash, T. Yasumura, K.G. Davidson, C.S. Furman, F.E. Dudek, J.I. Nagy, Identification of cells expressing Cx43, Cx30, Cx26, Cx32 and Cx36 in gap junctions of rat brain and spinal cord, Cell Commun. Adhes. 8 (2001) 315–320.
[28] A.M. Choo, W.J. Miller, Y.C. Chen, P. Nibley, T.P. Patel, C. Goletiani, B. Morrison, M.K. Kutzing, B.L. Firestein, J.Y. Sul, P.G. Haydon, D.F. Meaney, Antagonism of purinergic signalling improves recovery from traumatic brain injury, Brain 136 (2013) 65–80.
[29] M. Kawamura, C. Gachet, K. Inoue, F. Kato, Direct excitation of inhibitory interneurons by extracellular ATP mediated by P2Y1 receptors in the hippocampal slice, J. Neurosci. 24 (2004) 10835–10845.
[30] S.L. Minger, J.W. Geddes, M.L. Holtz, S.D. Craddock, S.W. Whiteheart, R.G. Siman, L.C. Pettigrew, Glutamate receptor antagonists inhibit calpain-mediated cytoskeletal proteolysis in focal cerebral ischemia, Brain Res. 810 (1998) 181–199.
[31] M.A. Retamal, N. Froger, N. Palacios-Prado, P. Ezan, P.J. Saez, J.C. Saez, C. Giaume, Cx43 hemichannels and gap junction channels in astrocytes are regulated oppositely by proinflammatory cytokines released from activated microglia, J. Neurosci. 27 (2007) 13781–13792.
[32] Y. Ma, H. Hioki, M. Konno, S. Pan, H. Nakamura, K.C. Nakamura, T. Furuta, J.L. Li, T. Kaneko, Expression of gap junction protein connexin36 in multiple subtypes of GABAergic neurons in adult rat somatosensory cortex, Cereb. Cortex 21 (2011) 2639–2649.