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Cysteinyl leukotriene receptor 1 partially mediates brain cryoinjury in mice[1]

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Key words

cysteinyl leukotriene receptor; antagonist; pranlukast; cryoinjury; neuroprotection

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Abstract

Aim: To determine whether the cysteinyl leukotriene receptor 1 ($CysLT_1$ receptor) modulates brain cryoinjury and whether the $CysLT_1$ receptor antagonist pranlukast exerts a time-dependent protective effect on cryoinjury in mice. **Methods:** Brain cryoinjury was induced by applying a liquid nitrogen-cooled metal probe to the surface of the skull for 30 s. Brain lesion, neuron density, and endogenous IgG exudation were observed 24 h after cryoinjury. Transcription and the expression of the $CysLT_1$ receptor were detected by RT-PCR and immunoblotting, and the localization of the receptor protein by double immunofluorescence. **Results:** The mRNA and protein expressions of the $CysLT_1$ receptor were upregulated in the brain 6–24 h after cryoinjury, and the $CysLT_1$ receptor protein was primarily localized in the neurons, not in the astrocytes or microglia. Pre-injury treatments with multi-doses and a single dose of pranlukast (0.1 mg/kg) attenuated cryoinjury; postinjury single dose (0.1 mg/kg) at 30 min (not 1 h) after cryoinjury was also effective. **Conclusion:** The $CysLT_1$ receptor modulates cryoinjury in mice at least partly, and postinjury treatment with its antagonist pranlukast exerts the protective effect with a therapeutic window of 30 min.

Introduction

Cysteinyl leukotrienes (CysLT, including leukotriene C_4 [LTC_4], LTD_4, and LTE_4), 5-lipoxygenase metabolites of arachidonic acid, are potent inflammatory mediators and are involved in cerebral ischemia[1,2] and brain trauma[3]. The actions of CysLT are mediated by G protein-coupled receptors, namely the $CysLT_1$ and $CysLT_2$ receptors[4]. Recently, we reported that the pre-ischemic or postischemic treatment with the $CysLT_1$ receptor antagonists, pranlukast and montelukast, protect against cerebral ischemia in rats and mice[5–8]. Pranlukast also exerts a protective effect on *N*-methyl-*D*-aspartate (NMDA)-induced brain injury in mice[9]. Moreover, we have found that the expression of the $CysLT_1$ receptor is increased in the brain after focal cerebral ischemia in rats and mice[10,11] or NMDA injury in mice[9]. The expression of the $CysLT_1$ receptor is induced in the neuron- and glial-appearing cells in the human brain by traumatic injury[12]. These findings indicate that the $CysLT_1$ receptor mediates brain injury. However, the exact changes in the $CysLT_1$ receptor expression in the brain with traumatic injury are still unknown.

To clarify the role of the $CysLT_1$ receptor in traumatic brain injury (TBI), we recently investigated the effect of pranlukast on brain cryoinjury. Brain cryoinjury (also called cold injury) is a well-established model that can mimic some of the characteristics of TBI and the related repair responses; for example, vasogenic brain edema[13,14], inflammation[15–19], and the disruption of the blood-brain barrier (BBB)[20,21]. We found that pre-injury treatment with pranlukast dose-dependently protected mouse brain from cryoinjury, suggesting that the $CysLT_1$ receptor might mediate TBI[22]. However, it is not known whether pranlukast exerts protective effects when it is administered after cryoinjury, and how the $CysLT_1$ receptor expression changes after cryoinjury. Because most pathophysiological changes of TBI are similar to those of cerebral ischemia[23], we hypothesize that the $CysLT_1$ receptor may have changes after TBI, similar to those after cerebral ischemia, and postinjury treatment with pranlukast may

have protective effects on TBI.

To test this hypothesis, in the present study we observed the expression and localization of the $CysLT_1$ receptor in the mouse brain, and further investigated the time-dependent effect of pranlukast on cryoinjury. As a positive control, minocycline, a semi-synthetic tetracycline with central anti-inflammatory activity[24,25], was used in this study because it can protect mice against TBI[26].

Materials and methods

Materials Pranlukast was a gift from Dr Masami TSUBOSHIMA (Ono Pharmaceutical Co, Osaka, Japan). Minocycline was purchased from Syowa Hakko (Tokyo, Japan). Chloral hydrate, biotinylated anti-mouse IgG antibody, and 2,3,5-triphenylterazolium chloride (TTC) were from Sigma (St Louis, MO, USA). The reagents for RT-PCR were from TaKaRa (Kyoto, Japan). The polyclonal rabbit anti-human $CysLT_1$ antibody was from Cayman Chemicals (Ann Arbor, MI, USA). The mouse monoclonal antibodies against neuronal nuclei (NeuN), glial fibrillary acidic protein (GFAP) and CD11b, GAPDH, fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG, and Cy3-conjugated goat anti-mouse IgG were from Chemicon International (Temecula, CA, USA). Biotinylated goat anti-rabbit IgG, horseradish peroxidase streptavidin and 3,3'-diaminobenzidine (DAB) were from Zhongshan Biotechnology (Beijing, China).

Animals Male Kunming mice weighting 25–30 g (Shanghai Experimental Animal Center, China, Certificate No 22-001004) were used in this study. All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The mice were housed under a controlled temperature (22±1 °C), 12 h light/dark cycle, and allowed free access to food and water.

Cryoinjury and drug treatment The mice were anesthetized with an ip injection of chloral hydrate (400 mg/kg) and placed on a stereotaxic frame (SR-5, Narishige, Tokyo, Japan). Brain cryoinjury was induced according to a reported method[18] with modifications. Briefly, the scalp was incised on the midline to expose the skull. A metal probe (100 g in weight, 3 mm tip diameter) cooled in liquid nitrogen was applied to the surface of the intact skull above the right parietal lobe (1.5 mm lateral to the midline, -3 mm from the bregma) for 30 s. The rectal temperature was measured and maintained at 37±0.5 °C with a heating pad and a heating lamp during the surgery. Incisions were sutured after cryoinjury, and the mice were kept in a recovery box with heating lamps to maintain body temperature and then returned to their cages.

In the multidose group, the mice were pretreated by ip injections of pranlukast (0.01 and 0.1 mg/kg) or minocycline (45 mg/kg) once a day for 3 consecutive days before cryoinjury; the last doses were given 30 min before cryoinjury (total of 4 doses). In the single dose groups, the mice were pretreated 30 min before cryoinjury or post-treated 30 min or 1 h after cryoinjury with the same doses of both drugs. In the control groups, saline (5 mL/kg) was ip injected at the same time as the drug treatment groups.

Determination of lesion volume and brain edema The mice were anesthetized with chloral hydrate and decapitated 24 h after cryoinjury. The brains were quickly removed and dissected into 1 mm-thick coronal slices. The slices were stained with 0.5% TTC at 37 °C for 30 min, and then fixed in a 10% buffered formalin solution. The stained slices, with the caudal facing upwards, were photographed with a digital camera (FinePix S602 Zoom, Fuji, Tokyo, Japan) and recorded on a computer. The lesion and hemisphere area of each slice were determined by an image analysis program (AnalyPower1.0, Zhejiang University, Hangzhou, China). The lesion volume was calculated as follows: lesion volume=lesion area× thickness (1 mm), and the summation of the lesion volumes of all brain slices was the total lesion volume. Brain edema was indirectly evaluated as a percentage increase of the lesioned hemisphere volume.

Pathohistological examination In another series, the mice were anaesthetized with chloral hydrate and then perfused transcardially with 4% paraformaldehyde after a saline prewash. The brains were removed, postfixed in 4% paraformaldehyde overnight, and then transferred to 30% sucrose and submerged for 3–7 d. Serial 10 μm-thick coronal sections were cut by cryomicrotomy (CM1900, Leica, Wezlar, Germany). The sections were immunostained with a mouse monoclonal antibody against NeuN (a specific marker of neurons) to detect neuron density as described later. Since the neurons in the lesion core almost completely disappeared (data not shown), we observed the neurons in the periphery of the lesion, neocortex layers III and IV (1.8–2.0 mm caudal from bregma). Endogenous IgG immunostaining was performed to detect the disruption of the BBB[27]. The brain sections were sequentially reacted with biotinylated anti-mouse IgG antibody (1:500), horseradish peroxidase streptavidin (1:200), and DAB. The optical gray scales in the immunostained sections were detected with an image analyzer (Imagetool 2.0, University of Texas, Health Science Center, San Antonio, TX, USA). IgG exudation was evaluated as the percentage increase of the gray scales of the injured hemisphere, $IgG\%=(G_i-G_0)/G_0\times100\%$. Here, G_i=the gray scale of the injured hemisphere and G_0=the gray scales

of the contralateral hemisphere.

RT-PCR The mouse cerebral cortex from the injured hemisphere and the contralateral cortex were dissected on ice at the indicated time points, and stored at -70 °C until use. The total RNA was extracted from the tissue samples using Trizol reagents (Invitrogen, Calsbad, CA, USA) according to the manufacture's protocol. For the cDNA synthesis, aliquots of total RNA (2 μg) were mixed with 0.2 μg random hexamer primer, 20 U RNasin, 1 mmol/L dNTP, and 200 U M-MuLV reverse transcriptase in 20 μL of the reverse reaction buffer. The mixture was incubated at 42 °C for 60 min, and then at 72 °C for 10 min to inactivate the reverse transcriptase.

PCR was performed on an Eppendorf Master Cycler (Eppendorf, Hamburg, Germany). The mixture was as follows: 1 μL RT-cDNA temple was dissolved in 20 μL reaction mixture containing 1×PCR buffer, 200 μmol/L dNTP, 1.5 mmol/L $MgCl_2$, 20 pmol of each primer, and 0.5 U *Taq* DNA polymerase. Cycling parameters were as follows: 94 °C for 2 min, followed by 33 cycles of 94 °C for 30 s, 63 °C for 30 s, and 72 °C for 30 s, with a final extension step of 72 C for 10 min. The primer sequences for the mouse $CysLT_1$ receptor were derived from the published cDNA sequence[28]: 5'-CAA CGA ACT ATC CAC CTT CACC-3' as sense, and 5'-AGC CTT CTC CTA AAG TTT CCAC-3' as antisense (product size 164 bp). The primers for β-actin were 5'-GTC GTA CCA CAG GCA TTG TGA TGG-3' as sense, and 5'-GCA ATG CCT GGG TAC ATG GTG-3' as antisense (product size 490 bp). The amplification products were separated by electrophoresis on a 2% agarose gel containing ethidium bromide and photographed. The optical density of the bands was determined by an image analysis system (Bio-Rad, Richmond, CA, USA). The amounts of the $CysLT_1$ receptor mRNA were calculated as the ratios of the $CysLT_1$/β-actin.

Immunoblotting analysis The mice were sacrificed and the cerebral cortex of the injured hemisphere were quickly dissected on ice at the indicated time points, then stored at -70 °C until use. The brain samples were homogenized; the homogenates were then centrifuged at 15 000×*g* at 4 °C for 30 min and the supernatant was harvested. The protein samples (80 μg) were separated by 12% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked with 5% bovine serum albumin and then incubated with a rabbit polyclonal antibody against the human $CysLT_1$ receptor (1:2000) or the mouse monoclonal antibody against GAPDH (1:5000) at 4 °C overnight. After repeated washing, the membranes were incubated with peroxidase-conjugated goat anti-rabbit IgG (1:2000). Finally, the protein bands were visualized by enhanced chemiluminescence. The protein bands were scanned by a Laser Densitometer and analyzed by Met Imaging Series 5.0 (Bio-Rad, USA). The amounts of the $CysLT_1$ receptor protein were calculated as the ratios of the $CysLT_1$/GAPDH. The antibody against the human $CysLT_1$ receptor had been confirmed to be specific for mouse brain tissue[9].

$CysLT_1$ receptor specific immunohistochemical analysis To visualize the localization of the $CysLT_1$ receptor in different cell types, double immunofluorescence was employed on the 10 μm-thick sections. Briefly, non-specific binding of IgG was blocked with 5% normal goat serum for 2 h at room temperature. Each section was incubated overnight at 4 °C with a mixture of rabbit polyclonal antibody against the $CysLT_1$ receptor and mouse monoclonal antibodies against NeuN, GFAP (a specific marker of astrocytes) or CD11b (a specific marker of microglia). Then the sections were incubated with the mixture of FITC-conjugated goat anti-rabbit IgG and Cy3-conjugated goat anti-mouse IgG and observed under a fluorescence microscope (Olympus BX51, Tokyo, Japan).

Statistical analysis All values are presented as mean±SD. One-way ANOVA (Student-Newman-Keuls) was performed for statistical analysis using the SPSS 10.0 software package for Windows (SPSS, Chicago, IL, USA). $P<0.05$ was considered statistically significant.

Results

Brain injury Pretreatments for 3 d before cryoinjury with multidoses of pranlukast (0.1 mg/kg) and minocycline (45 mg/kg) significantly reduced the lesion volume and brain edema 24 h after cryoinjury. In the single dose groups with the 2 drugs, pretreatment at 30 min before cryoinjury or post-treatment at 30 min after cryoinjury had protective effects as well. However, post-treatment with pranlukast at 1 h after cryoinjury did not show any significant protective effects, but minocycline was still effective. Pranlukast 0.01 mg/kg was not effective at any dosing regimen (Figure 1). We then administered single doses of these agents 30 min after cryoinjury in the following experiments.

The density of the NeuN-positive neurons was substantially decreased in the periphery of the lesion 24 h after cryoinjury. Pranlukast (0.1 mg/kg) and minocycline (45 mg/kg) significantly attenuated the neuron loss ($P<0.01$, Figure 2). The endogenous IgG exudation was found in the injured cortexes, indicating BBB disruption. Pranlukast (0.1 mg/kg) and minocycline (45 mg/kg) significantly reduced IgG exudation ($P<0.01$, Figure 3).

Expression of the $CysLT_1$ receptor The expression of the $CysLT_1$ receptor in the injured cortexes was significantly

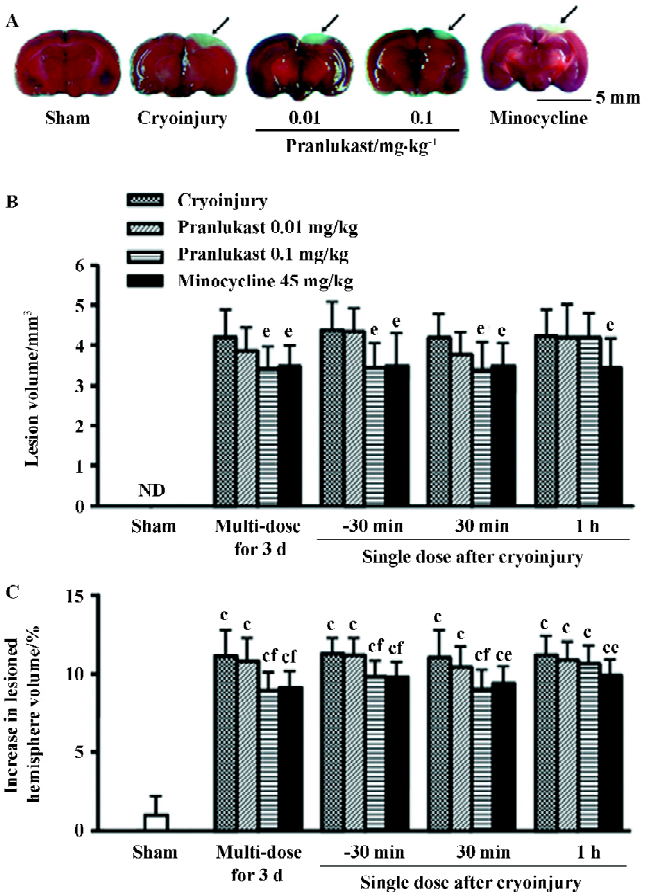

Figure 1. Time-dependent effect of pranlukast or minocycline on lesion volume and brain edema 24 h after cryoinjury in mice. (A) TTC-stained brain slices show the cortical lesions (arrows) in the mice pretreated with multi-doses. (B) lesion volumes and (C) percentage increases in the injured hemisphere volume (brain edema) are reported as mean±SD; n=5 mice for sham operation or n=8 mice for each cryoinjured group. ^{c}P<0.01 *vs* sham operation, ^{e}P<0.05 and ^{f}P<0.01 *vs* cryoinjury alone, one-way ANOVA. ND, not detectable.

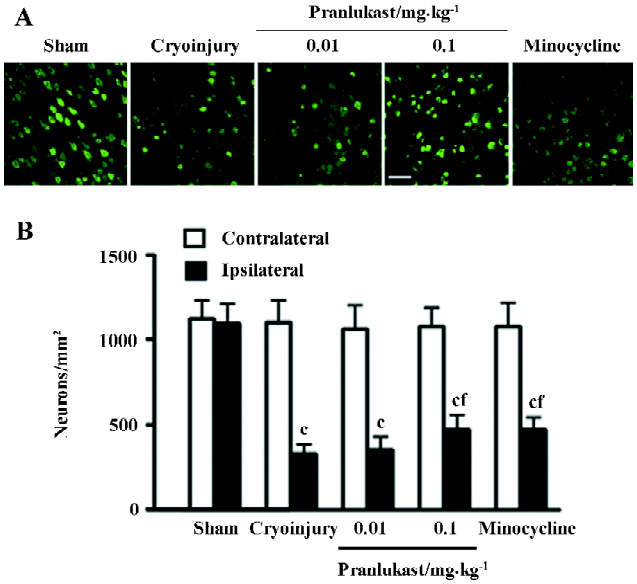

Figure 2. Effect of postinjury (30 min) single dose of pranlukast or minocycline on neuron density 24 h after cryoinjury in mice. (A) microphotographs show the loss of NeuN-positive neurons in the periphery of the lesion after cryoinjury. (B) neuron densities are reported as mean±SD; n=5 mice for sham operation or 8 mice for each cryoinjured group. ^{c}P<0.01 *vs* sham operation, ^{f}P<0.01 *vs* cryoinjury alone, one-way ANOVA. Scale bar=25 μm.

increased at 6, 12, and 24 h, and then recovered 48 h after cryoinjury (Figure 4B). The expression in the contralateral cortexes was not changed 48 h after cryoinjury (Figure 4A). The expression of the $CysLT_1$ receptor protein in the injured cortexes was also significantly increased 6, 12, and 24 h after cryoinjury (Figure 4C). Minocycline (45 mg/kg), not pranlukast (0.01 and 0.1 mg/kg), significantly inhibited the increased expression (P<0.05, Figure 5). The double immunofluorescence showed that the $CysLT_1$ receptor immunore-

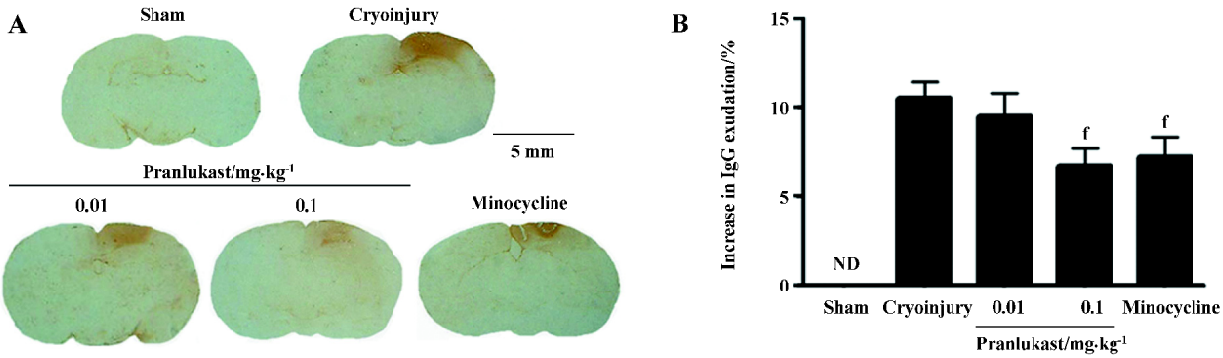

Figure 3. Effect of postinjury (30 min) single dose of pranlukast or minocycline on endogenous IgG exudation 24 h after cryoinjury in mice. (A) photographs show IgG immunoreactivities. (B) percentages of IgG exudation are reported as mean±SD; n=5 mice for sham operation or 8 mice for each cryoinjured group. ^{f}P<0.01 *vs* cryoinjury alone, one-way ANOVA. ND, not detectable.

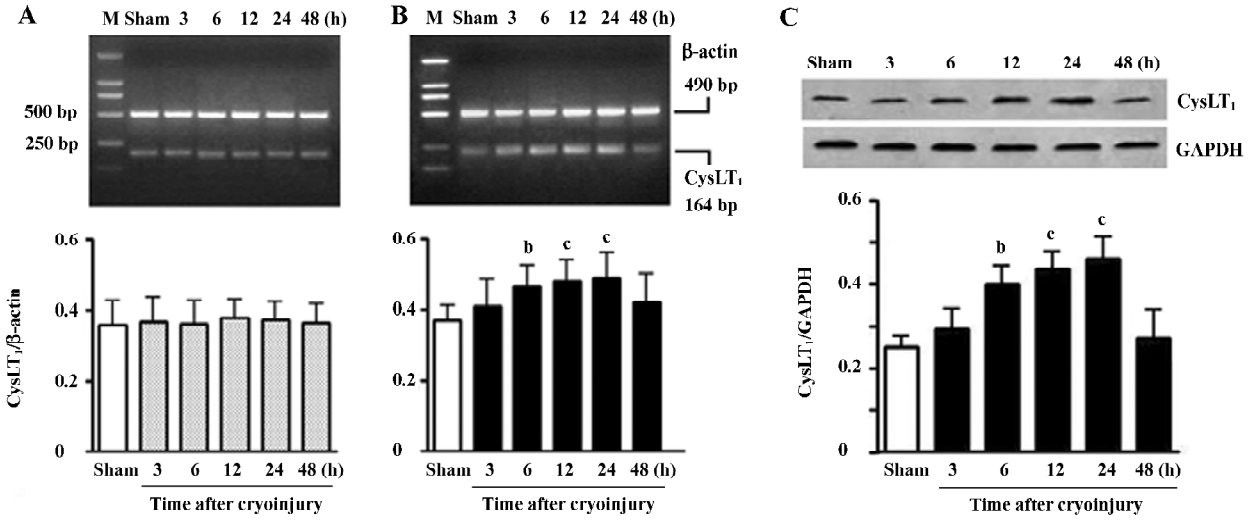

Figure 4. Time course of the expression of the $CysLT_1$ receptor after cryoinjury in the mouse brain. (A) RT-PCR analysis shows that transcription was unchanged in the contralateral cortexes, (B) expression was increased in the injured cortexes 6, 12, and 24 h after cryoinjury. (C) immunoblot analysis shows the increased protein (43 kDa) expression in the injured cortexes 6, 12, and 24 h after cryoinjury. Values are reported as mean±SD; n=5 mice for sham operation, or n=8 mice for each cryoinjured group. $^bP<0.05$ and $^cP<0.01$ *vs* sham operation, one-way ANOVA.

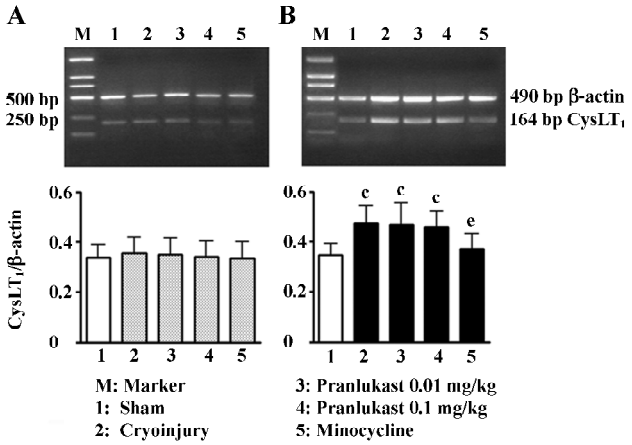

Figure 5. Effect of postinjury (30 min) single dose of pranlukast or minocycline on the expression of the $CysLT_1$ receptor in the brain 24 h after cryoinjury in mice. (A) both pranlukast and minocycline (45 mg/kg) did not change the expression of the $CysLT_1$ receptor mRNA in the contralateral cortexes. (B) minocycline, not pranlukast, inhibited the increased expression in the injured cortexes. Values are reported as mean±SD; n=5 mice for sham operation or 8 mice for each cryoinjured group. $^cP<0.01$ *vs* sham operation, $^eP<0.05$ *vs* cryoinjury alone, one-way ANOVA.

activity was primarily localized in the NeuN-positive neurons (Figure 6A), but less in the GFAP-positive astrocytes in the periphery of the lesion 24 h after cryoinjury (Figure 6B). Moreover, the amount of CD11b-positive microglia was increased, but they expressed less of the $CysLT_1$ receptor (Figure 6C).

Discussion

The present study indicates that the $CysLT_1$ receptor mediates brain cryoinjury. This mediation is evidenced by the fact that the $CysLT_1$ receptor expression is enhanced after cryoinjury, and its antagonist pranlukast exerts a protective effect on cryoinjury. These findings further confirm that the $CysLT_1$ receptor mediates not only ischemic brain injury, but also traumatic brain injury.

The most important finding is that transcription and the protein expression of the $CysLT_1$ receptor in the brain are enhanced 6–24 h after cryoinjury, and the increased $CysLT_1$ receptor is primarily localized in the neurons. This result is similar to, but somewhat different from that found in focal cerebral ischemia in mice[10]. Focal cerebral ischemia-increased expression of the $CysLT_1$ receptor peaked at 24 h and was maintained for 48 h[10], while cryoinjury-increased expression was limited to 6–24 h. This difference may be due to the smaller lesion induced by cryoinjury than that by focal cerebral ischemia. However, the increased $CysLT_1$ receptor was similarly localized in the neurons, not in the astrocytes and microglia, in the periphery of the lesion 24 h after focal cerebral ischemia and cryoinjury. This finding indicates that the $CysLT_1$ receptor plays a role in neuronal damage in the acute phase of injury as we reported in rat focal

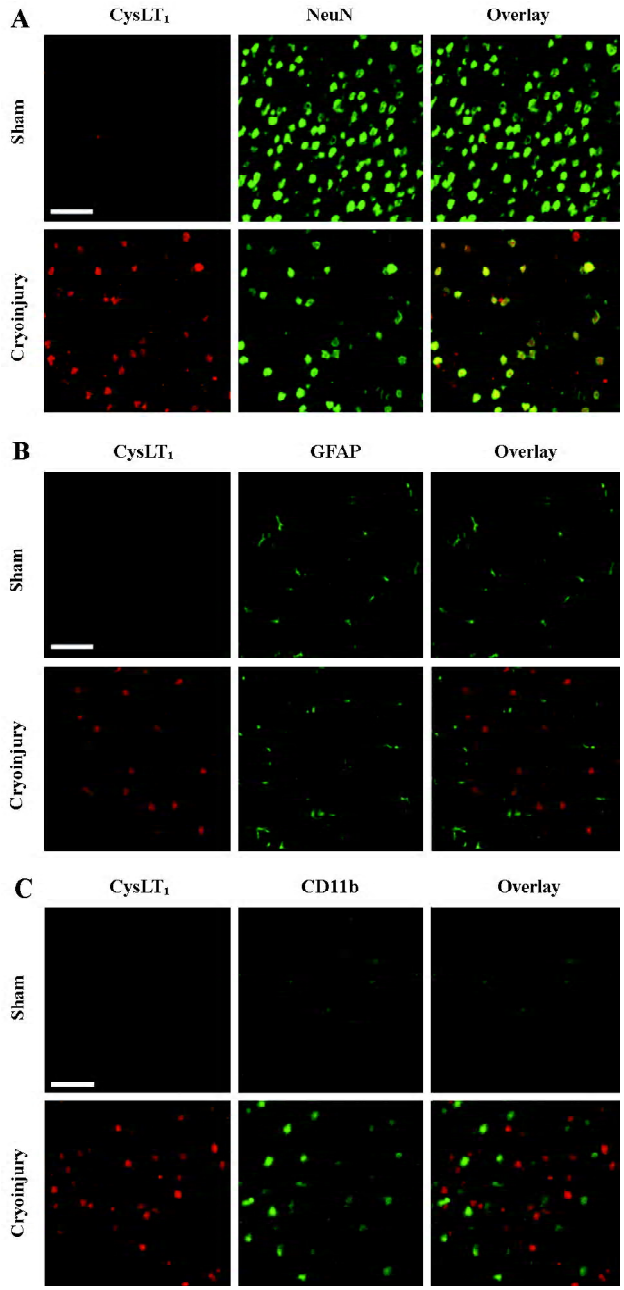

Figure 6. Double immunofluorescence for the localization of the $CysLT_1$ receptor protein 24 h after cryoinjury in the mouse brain. (A) $CysLT_1$ receptor is primarily localized in NeuN-positive neurons in the periphery of the lesion; (B) receptor is less localized in GFAP-positive astrocytes; and (C) in CD11b-positive microglia. Scale bars=50 μm.

cerebral ischemia[11].

We can not exactly explain why the $CysLT_1$ receptor is upregulated after cryoinjury. However, 1 possible mechanism may be excitotoxicity after cryoinjury. Cryoinjury can induce the endogenous excitatory acid glutamate release[29]. The released glutamate might activate the NMDA receptor and produce the resultant damage because the NMDA receptor antagonist dizocilpine (MK-801) can reduce cryoinjury[30]. Moreover, we found that NMDA microinjection induced the upregulation of the $CysLT_1$ receptor, and the increased receptor was localized in the neurons[9]. Therefore, NMDA receptor activation (excitotoxicity) may be an intermediate triggering step of the cryoinjury-induced $CysLT_1$ receptor expression. Since ischemic brain injury is also evoked by excitotoxicity[31], the enhanced $CysLT_1$ receptor expression might be a common consequence in both ischemic and traumatic brain injury. Of course, postinjury upregulation of the $CysLT_1$ receptor may be also induced by various unknown factors secondary to cryoinjury.

Another finding is that pranlukast, a selective antagonist of the $CysLT_1$ receptor, exerts protective effects on cryoinjury with a therapeutic window of 30 min. We found dose-dependent protective effects of pretreatment with multidoses of pranlukast on cryoinjury in mice, and the most effective dose was 0.1 mg/kg[22]. The present study further reveals its effect of post-treatment with a single dose of pranlukast; the therapeutic window (30 min) is the same as that found in focal cerebral ischemia in mice[5]. Because postinjury treatments are clinically important, pranlukast administered in a short duration after brain injury may be an effective treatment of brain injury. However, pranlukast did not inhibit the expression of the $CysLT_1$ receptor in the brain after cryoinjury, which was inhibited after NMDA injury[9].

The control agent minocycline exerts the protective effect on cryoinjury with a wider therapeutic window of at least 1 h, which is consistent with that (4 h) in focal cerebral ischemia in rats[25]. Interestingly, minocycline inhibited the increased expression of the $CysLT_1$ receptor, suggesting a new aspect of its anti-inflammatory ability in addition to the inhibition of microglial cell activation[32], apoptotic cascades in neurons[33,34], 5-lipoxygenase activation in rat pheochromocytoma PC12 cells[35,36], and the activated p38 mitogen-activated protein kinase in microglial cells[37].

In summary, we found that $CysLT_1$ receptor is up-regulated in the brain and localized in neurons after cryoinjury in mice, and the postinjury treatment with $CysLT_1$ receptor antagonist pranlukast exerts protective effects with a therapeutic window of 30 min. These findings indicate that the $CysLT_1$ receptor modulates cryoinjury at least partly, and $CysLT_1$ receptor antagonist(s) may possess therapeutic potential in the treatment of brain injury, including ischemic and traumatic brain injury.

Acknowledgments

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References

1 Di Gennaro A, Carnini C, Buccellati C, Ballerio R, Zarini S, Fumagalli F, *et al*. Cysteinyl-leukotrienes receptor activation in brain inflammatory reactions and cerebral edema formation: a role for transcellular biosynthesis of cysteinyl-leukotrienes. FASEB J 2004; 18: 842–4.

2 Rao AM, Hatcher JF, Kindy MS, Dempsey RJ. Arachidonic acid and leukotriene C4: role in transient cerebral ischemia of gerbils. Neurochem Res 1999; 24: 1225–32.

3 Dhillon HS, Dose JM, Prasad MR. Regional generation of leukotriene C4 after experimental brain injury in anesthetized rats. J Neurotrauma 1996; 13: 781–9.

4 Brink C, Dahlen SE, Drazen J, Evans JF, Hay DW, Nicosia S, *et al*. International Union of Pharmacology XXXVII. Nomenclature for leukotriene and lipoxin receptors. Pharmacol Rev 2003; 55: 195–227.

5 Yu GL, Wei EQ, Zhang SH, Xu HM, Chu LS, Zhang WP, *et al*. Montelukast, a cysteinyl leukotriene receptor-1 antagonist, dose- and time-dependently protects against focal cerebral ischemia in mice. Pharmacology 2005; 73: 31–40.

6 Zeng LH, Zhang WP, Wang RD, Wang PL,Wei EQ. Protective effect of ONO-1078, a leukotriene antagonist, on focal cerebral ischemia in mice. Yao Xue Xue Bao 2001; 36: 148–50. Chinese.

7 Zhang WP, Wei EQ, Mei RH, Zhu CY, Zhao MH. Neuroprotective effect of ONO-1078, a leukotriene receptor antagonist, on focal cerebral ischemia in rats. Acta Pharmacol Sin 2002; 23: 871–7.

8 Zhang LH, Wei EQ. Neuroprotective effect of ONO-1078, a leukotriene receptor antagonist, on transient global cerebral ischemia in rats. Acta Pharmacol Sin 2003; 24: 1241–7.

9 Ding Q, Wei EQ, Zhang YJ, Zhang WP, Chen Z. Cysteinyl leukotriene receptor 1 is involved in *N*-methyl-*D*-aspartate-mediated neuronal injury in mice. Acta Pharmacol Sin 2006; 27: 1526–36.

10 Zhang YJ, Zhang L, Ye YL, Fang SH, Zhou Y, Zhang WP, *et al*. Cysteinyl leukotriene receptors CysLT1 and CysLT2 are involved in acute neuronal injury after focal cerebral ischemia in mice. Acta Pharmacol Sin 2006; 27: 1553–60.

11 Fang SH, Wei EQ, Zhou Y, Wang ML, Zhang WP, Yu GL, *et al*. Increased expression of cysteinyl leukotriene receptor-1 in the brain mediates neuronal damage and astrogliosis after focal cerebral ischemia in rats. Neuroscience 2006; 140: 969–79.

12 Zhang WP, Hu H, Zhang L, Ding W, Yao HT, Chen KD, *et al*. Expression of cysteinyl leukotriene receptor 1 in human traumatic brain injury and brain tumors. Neurosci Lett 2004; 363: 247–51.

13 Kawai N, Kawanishi M, Nagao S. Treatment of cryoinjury-induced brain edema with a nonspecific matrix metalloproteinase inhibitor MMI270 in rats. Acta Neurochir Suppl 2003; 86: 291–5.

14 Murakami K, Kondo T, Yang G, Chen SF, Morita-Fujimura Y, Chan PH. Cryoinjury in mice: a model to study mechanisms of brain edema and neuronal apoptosis. Prog Neurobiol 1999; 57: 289–99.

15 Knerlich F, Schilling L, Gorlach C, Wahl M, Ehrenreich H, Siren AL. Temporal profile of expression and cellular localization of inducible nitric oxide synthase, interleukin-1beta and interleukin converting enzyme after cryogenic lesion of the rat parietal cortex. Brain Res Mol Brain Res 1999; 68: 73–87.

16 Maeda M, Ampo K, Kiryu-Seo S, Konishi H, Ohba N, Kadono C, *et al*. The p53-independent nuclear translocation of cyclin G1 in degenerating neurons by ischemic and traumatic insults. Exp Neurol 2005; 193: 350–60.

17 Flentjar NJ, Crack PJ, Boyd R, Malin M, de Haan JB, Hertzog P, *et al*. Mice lacking glutathione peroxidase-1 activity show increased TUNEL staining and an accelerated inflammatory response in brain following a cold-induced injury. Exp Neurol 2002; 177: 9–20.

18 Sewell DL, Nacewicz B, Liu F, Macvilay S, Erdei A, Lambris JD, *et al*. Complement C3 and C5 play critical roles in traumatic brain cryoinjury: blocking effects on neutrophil extravasation by C5a receptor antagonist. J Neuroimmunol 2004; 155: 55–63.

19 Shin T, Ahn M, Kim H, Moon C, Kang TY, Lee JM, *et al*. Temporal expression of osteopontin and CD44 in rat brains with experimental cryolesions. Brain Res 2005; 1041: 95–101.

20 Methia N, Andre P, Hafezi-Moghadam A, Economopoulos M, Thomas KL, Wagner DD. ApoE deficiency compromises the blood brain barrier especially after injury. Mol Med 2001; 7: 810–5.

21 Nag S, Papneja T, Venugopalan R, Stewart DJ. Increased angiopoietin2 expression is associated with endothelial apoptosis and blood-brain barrier breakdown. Lab Invest 2005; 85: 1189–98.

22 Qian XD, Wei EQ, Zhang L, Sheng WW, Zhang WP, Wang ML, *et al*. Pranlukast, a cysteinyl leukotriene receptor-1 antagonist, protects mice against brain cold injury. Eur J Pharmacol 2006; 546: 35–40.

23 Bramlett HM, Dietrich WD. Pathophysiology of cerebral ischemia and brain trauma: similarities and differences. J Cereb Blood Flow Metab 2004; 24: 133–50.

24 Blum D, Chtarto A, Tenenbaum L, Brotchi J, Levivier M. Clinical potential of minocycline for neurodegenerative disorders. Neurobiol Dis 2004; 17: 359–66.

25 Yrjanheikki J, Tikka T, Keinanen R, Goldsteins G, Chan PH, Koistinaho J. A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. Proc Natl Acad Sci USA 1999; 96: 13 496–500.

26 Sanchez Mejia RO, Ona VO, Li M, Friedlander RM. Minocycline reduces traumatic brain injury-mediated caspase-1 activation, tissue damage, and neurological dysfunction. Neurosurgery 2001; 48: 1393–401.

27 Schmidt-Kastner R, Meller D, Bellander BM, Stromberg I, Olson L, Ingvar M. A one-step immunohistochemical method for detection of blood-brain barrier disturbances for immunoglobulins in lesioned rat brain with special reference to false-positive labelling in immunohistochemistry. J Neurosci Methods 1993; 46: 121–32.

28 Ogasawara H, Ishii S, Yokomizo T, Kakinuma T, Komine M, Tamaki K, *et al*. Characterization of mouse cysteinyl leukotriene receptors mCysLT1 and mCysLT2: differential pharmacological properties and tissue distribution. J Biol Chem 2002; 277: 18 763–8.

29 Saito N, Chang C, Kawai K, Joo F, Nowak TS Jr, Mies G, *et al*. Role of neuroexcitation in development of blood-brain barrier and oedematous changes following cerebral ischaemia and traumatic brain injury. Acta Neurochir Suppl (Wien) 1990; 51: 186–8.

30 Gorgulu A, Kiris T, Unal F, Turkoglu U, Kucuk M, Cobanoglu S. Protective effect of the N-methyl-D-aspartate receptor antagonists, MK-801 and CPP on cold-induced brain oedema. Acta Neurochir (Wien) 1999; 141: 93–8.

31 Lee JM, Grabb MC, Zipfel GJ, Choi DW. Brain tissue responses to ischemia. J Clin Invest 2000; 106: 23–31.

32 Yrjanheikki J, Keinanen R, Pellikka M, Hokfelt T, Koistinaho J. Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. Proc Natl Acad Sci USA 1998; 95: 15769–74.

33 Chen M, Ona VO, Li M, Ferrante RJ, Fink KB, Zhu S. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. Nat Med 2000; 6: 797–801.

34 Zhu S, Stavrovskaya IG, Drozda M, Kim BY, Ona V, Li M, *et al*. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. Nature 2002; 417: 74–8.

35 Song Y, Wei EQ, Zhang WP, Zhang L, Liu JR, Chen Z. Minocycline protects PC12 cells from ischemic-like injury and inhibits 5-lipoxygenase activation. Neuroreport 2004; 15: 2181–4.

36 Song Y, Wei EQ, Zhang WP, Ge QF, Liu JR, Wang ML, *et al*. Minocycline inhibits NMDA-induced 5-lipoxygenase activation and cell injury in PC12 cells. Brain Res 2006; 1085: 57–67.

37 Tikka TM, Koistinaho JE. Minocycline provides neuroprotection against *N*-methyl-*D*-aspartate neurotoxicity by inhibiting microglia. J Immunol 2001; 166: 7527–33.