

Post-stroke pain hypersensitivity induced by experimental thalamic hemorrhage in rats is region-specific and demonstrates limited efficacy of gabapentin

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ABSTRACT

Intractable central post-stroke pain (CPSP) is one of the most common sequelae of stroke, but has been inadequately studied to date. In this study, we first determined the relationship between the lesion site and changes in mechanical or thermal pain sensitivity in a rat CPSP model with experimental thalamic hemorrhage produced by unilateral intra-thalamic collagenase IV (ITC) injection. Then, we evaluated the efficacy of gabapentin (GBP), an anticonvulsant that binds the voltage-gated Ca^{2+} channel $\alpha 2\delta$ and a commonly used anti-neuropathic pain medication. Histological case-by-case analysis showed that only lesions confined to the medial lemniscus and the ventroposterior lateral/medial nuclei of the thalamus and/or the posterior thalamic nucleus resulted in bilateral mechanical pain hypersensitivity. All of the animals displaying CPSP also had impaired motor coordination, while control rats with intra-thalamic saline developed no central pain or motor deficits. GBP had a dose-related anti-allodynic effect after a single administration (1, 10, or 100 mg/kg) on day 7 post-ITC, with significant effects lasting at least 5 h

for the higher doses. However, repeated treatment, once a day for two weeks, resulted in complete loss of effectiveness (drug tolerance) at 10 mg/kg, while effectiveness remained at 100 mg/kg, although the time period of efficacious analgesia was reduced. In addition, GBP did not change the basal pain sensitivity and the motor impairment caused by the ITC lesion, suggesting selective action of GBP on the somatosensory system.

Keywords: central post-stroke pain; intracerebral hemorrhage; intra-thalamic collagenase injection; mechanical pain hypersensitivity; gabapentinoids; anti-allodynic effect

INTRODUCTION

Stroke is the second most common cause of death and is the leading cause of adult disability worldwide^[1]. In China, stroke is also the leading cause of death and long-term adult disability, and has an age-adjusted prevalence of 260 to 719 per 100 000 for all ages^[2,3]. Clinically, stroke is classified into two major subtypes, ischemic and hemorrhagic. In a recent INTERSTROKE study,

ischemic stroke (IS) accounted for 78%, while intracerebral hemorrhagic stroke (ICH) accounted for 22% in developed countries^[1]. However, the proportion of ICH is significantly higher in China than in western countries, accounting for 44%–51% of all stroke patients^[3,4].

Central post-stroke pain (CPSP) is one of the most troublesome sequelae of stroke and can be caused by a primary lesion affecting the central somatosensory system (at any level of the ascending pathways from the medulla to the cortex) following both IS and ICH^[5–12]. CPSP has been identified in 1%–50% of total patients after stroke of both IS and ICH origins^[7,13–17]; however, CPSP is more common in patients with a thalamic stroke, and varies from 11% to 32%^[18–23]. CPSP patients most often present with spontaneous and/or evoked pain (hyperalgesia and allodynia) or paresthesia/dysesthesia in a persistent and/or paroxysmal form^[5–7,24]. The distribution, onset time, and clinical characteristics of CPSP vary substantially among patients^[7–12].

The treatment of CPSP remains an unmet clinical challenge due to its resistance to both pharmacological and non-pharmacological therapies in about half of CPSP patients^[9–12]. The situation has not changed even for patients with peripheral neuropathic pain. For instance, although antidepressants and anticonvulsants are frequently recommended as the first-line drugs for neuropathic pain in general^[25–28], a large proportion of patients are left with insufficient pain relief due to a limited improvement, according to a review of 174 randomized placebo-controlled trials^[29]. To the best of our knowledge, well-designed clinical trials for evaluation of the pharmacological efficacy of antidepressants and anticonvulsants in patients with CPSP are scarce^[30,31], leading to a shortage of knowledge on the clinical use of pharmacological therapy. Moreover, the underlying mechanisms of CPSP remain largely unknown due to the lack of experimental studies in animal models^[5,32–35].

The analgesic efficacy of gabapentin (GBP), an $\alpha 2\delta$ -binding anticonvulsant, has been extensively evaluated, and it has been approved in both preclinical and clinical studies for the treatment of various forms of peripheral neuropathic pain (for reviews see refs 25–28). Anticonvulsants (or antiepileptic drugs) have also been recommended for the treatment of central neuropathic pain, especially that associated with spinal cord injury^[36–38] (for

reviews see refs 30, 39, 40). However, the effectiveness of $\alpha 2\delta$ -binding anticonvulsants (GBP and pregabalin) for the treatment of CPSP has been less studied in either preclinical or clinical research, despite the documentation of other antiepileptic drugs such as carbamazepine, lamotrigine, and phenytoin^[30,40].

The aims of the present study were: (1) to determine the relationship between lesion site and changes in pain sensitivity of each individual animal following experimental ICH; and (2) to evaluate the pharmacological efficacy of GBP in animals with CPSP hypersensitivity. To determine whether GBP induces tolerance, the dose-effect of repeated GBP injection (once a day for 14 days) was also evaluated.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 280–320 g were provided by the Laboratory Animal Center of the Fourth Military Medical University (FMMU). Rats were housed in a climate-controlled room (22–26°C) under a light/dark cycle of 12 h/12 h with access to food and water *ad libitum*. Somatic functional evaluations were carried out between 09:00 and 18:30. The rats were acclimated to test boxes for >30 min on each day before the first testing. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996), and followed the ethical guidelines for pain research in conscious animals of the International Association for the Study of Pain. This study was approved by the Animal Care and Use Committee of FMMU. The number of animals used and their suffering were minimized.

Animals were randomly divided into three groups for establishment of the CPSP model with thalamic hemorrhage: (1) naïve rats without any treatment ($n = 6$); (2) rats receiving intra-thalamic microinjection of saline (ITS) ($n = 8$); and (3) rats receiving intra-thalamic microinjection of collagenase (ITC) ($n = 90$, 16 for histological analysis, 27 for single GBP administration, 28 for repeated GBP administration and 19 for Rota-rod test).

Surgery

Surgery was performed according to the methods described

previously^[34,41]. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and then securely fixed in a stereotaxic instrument (Narishige Scientific Instrument Lab, Japan). After a midline incision, an opening was made in the right skull with a dental drill. An intra-thalamic microinjection of collagenase type IV (Sigma-Aldrich China, Shanghai) or saline was made into the ventral basal complex (VBC) and posterior thalamic nucleus (bregma -3.48 mm anteroposterior; 3.6 mm lateral to the midline, and 6.2 mm ventral to the brain surface) on the right side according to the stereotaxic coordinates (Paxinos and Watson, 2005). A 0.5 μ L microinjection syringe filled with collagenase or saline was lowered into the region of interest, followed (5 min later) by slow administration of ITC (0.025 IU collagenase dissolved in 0.25 μ L saline) or ITS (0.25 μ L saline) over a period of 10 min. The syringe remained for 5 min after each injection to prevent spread of the agent to the brain surface. Then the needle was slowly withdrawn, the skin closed using 4.0 sutures, and all rats were allowed to recover in individual cages for at least 7 days. Naïve rats were fed under the same conditions in a parallel manner.

Experimental Design

The experimental procedure is shown in Fig. 1. Quantitative sensory tests with von Frey filaments and a radiant heat stimulator were performed on both hind-paws 1 day before, and on days 7, 14, 21, and 28 after ITC or ITS. Naïve rats were also tested in the same way as a negative control.

For the pharmacological assessment of GBP (kindly provided by Jiangsu Nhwa Pharmaceutical Co., Ltd., China), only rats that had developed pain hypersensitivity were examined between 7 and 21 days after ITC. GBP (1, 10, and 100 mg/kg) or vehicle (saline) was injected intraperitoneally. Single injection of GBP was made on day 7 after ITC. Repeated administrations (once a day for two weeks) of GBP were made between 7 and 21 days after ITC to determine whether long-term use of GBP induces tolerance. For each set of pharmacological evaluations, the time-course effect of GBP was recorded for 1–24 h for single administration (on day 7 after ITC) or 1–5 h for repeated administration (on days 14 and 21 after ITC or days 7 and 14 after GBP). The dose, time course, and route of administration of GBP were based on previous reports of its effects on other neuropathic pain models^[42–45].

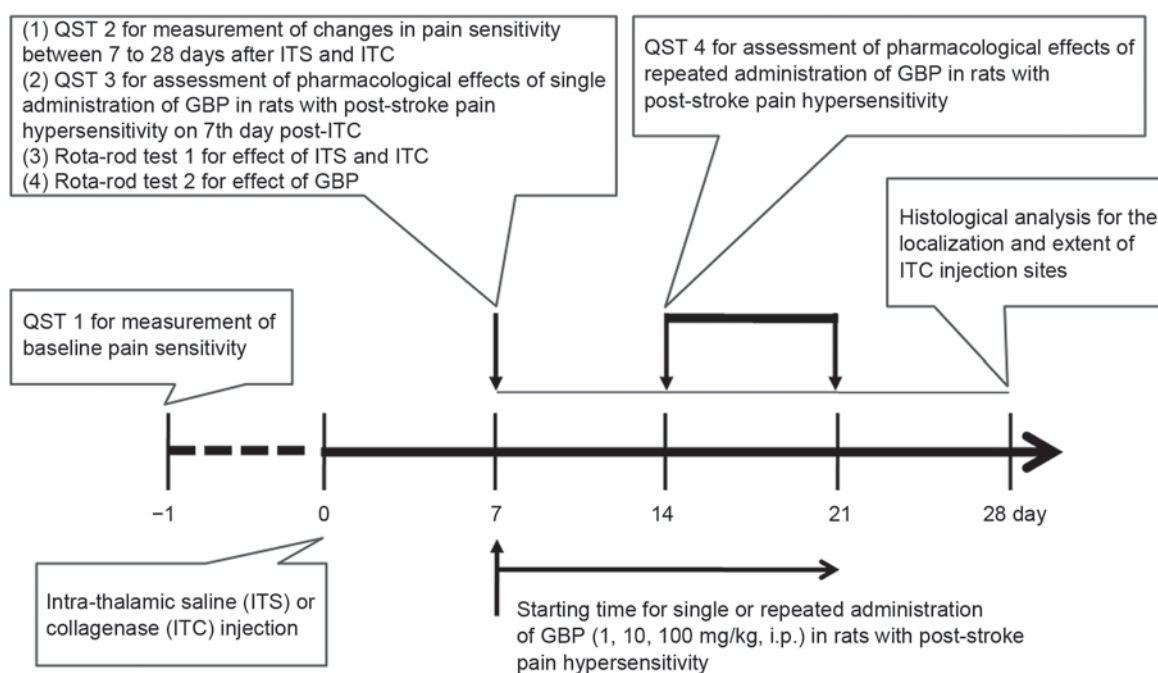


Fig. 1. Timeline of the experimental procedure. GBP, gabapentin; ITC, intra-thalamic collagenase injection; ITS, intra-thalamic saline injection; QST, quantitative sensory test.

To test whether GBP has any effect on baseline pain sensitivity, the highest dose (100 mg/kg, i.p.) was administered to naïve rats. Meanwhile, the effects of intrathalamic injection on motor coordination were also evaluated in both naïve rats and those receiving ITS and ITC (Rota-rod test 1 in Fig. 1). The pharmacological effects of GBP on motor coordination were also evaluated in rats with CPSP hypersensitivity induced by ITC (Rota-rod test 2 in Fig. 1).

Behavioral Tests

All the behavioral tests were performed by experimenters blinded to the treatment. The rats were acclimated to test boxes for >30 min on each of the 5 pre-test days.

Mechanical Sensitivity

Mechanical sensitivity was evaluated with von Frey monofilaments as described previously^[46]. Rats were placed on a metal mesh floor in a plastic chamber and mechanical stimuli were applied using monofilaments with ascending bending forces of 0.8 g, 2–20 g at 2-g increments, 25, 30, 45 and 60 g. Each monofilament was applied 10 times (once every several seconds) to the plantar area of each hind-paw to induce a withdrawal reflex. The bending force of the monofilament able to elicit a 50% withdrawal response was expressed as the paw withdrawal mechanical threshold (PWMT, g).

Thermal Sensitivity

The thermal sensitivity was determined by measuring the withdrawal latency of the hind-paws in response to radiant heat^[46]. The rat was placed on the surface of a 2-mm-thick glass plate in the same plastic chamber. Five heat stimuli repeated at inter-stimulus intervals of 10 min generated by a TC-1 radiant heat stimulator (RTY-3; Xi'an Bobang Technologies of Chemical Industry Co. Ltd., China) were applied to the plantar area of each hind-paw. The latency was determined as the time from the beginning of the heat stimulus to the appearance of a hind-paw withdrawal reflex. The last three values were averaged as the mean paw-withdrawal thermal latency (PWTL, s). Baseline latencies were established at 14–20 s to allow a sufficient window for the detection of possible hypersensitivity. A maximal cutoff was set at 30 s to avoid tissue injury.

Rota-rod Test

Motor coordination was tested on a Rota-rod treadmill (Ugo Basile, Italy)^[47,48]. The speed of the Rota-rod was set

to increase from 6 to 30 r/min within 2 min. The animals were placed on the treadmill and the timers started with the onset of acceleration and automatically stopped when the animal fell off, with a cutoff time of 300 s. The test was repeated six times at inter-test intervals of 30 min. The first three times were used for accommodation to the apparatus, and the last three values were recorded for further analysis.

Histological Localization of Injection Sites

After completion of the study, all the animals were sacrificed with an overdose of sodium pentobarbital and perfused transcardially with sterile saline followed by 4% paraformaldehyde. The brains were removed and stored in 30% sucrose for two days. Frozen sections (25 µm) were cut in the coronal plane and subjected to Nissl staining. The localization and extent of the lesions were observed under a light microscope (BX51 TR, Olympus, Japan). Photomicrographs were captured by a computer-based microscope CCD camera (DP-70, Olympus) and processed by Image-Pro-Plus 5.1 (Olympus). A typical example is shown in Fig. 2A.

Statistical Analysis

Data were analyzed using GraphPad Prism version 5 (GraphPad, San Diego, CA) and all data are expressed as mean ± SEM. To check the drug tolerance, percent maximum possible effect (% MPE) was calculated: % MPE = $(PWMT_{h \text{ post-GBP}} - PWMT_{pre-GBP}) / (PWMT_{baseline} - PWMT_{pre-GBP}) \times 100\%$, in which h means hours after GBP administration. Differences in values over time for each group were tested using *t*-tests and one-way or two-way repeated ANOVA, followed by individual *post-hoc* comparisons (Tukey or Bonferroni test). *P* < 0.05 was considered as statistically significant.

RESULTS

Central Post-stroke Pain Hypersensitivity in Rats Induced by Experimental Thalamic Hemorrhage

Of 16 rats that received unilateral ITC injection and were histologically examined, 44% (7/16) had no changes in either mechanical or thermal pain sensitivity (#10–16 in Fig. 3), while 56% (9/16) developed bilateral mechanical pain hypersensitivity (#1–9 in Fig. 4). Among the nine rats with CPSP, two showed accompanying contralateral thermal pain hypersensitivity lasting for 14 days (#1) and 28 days

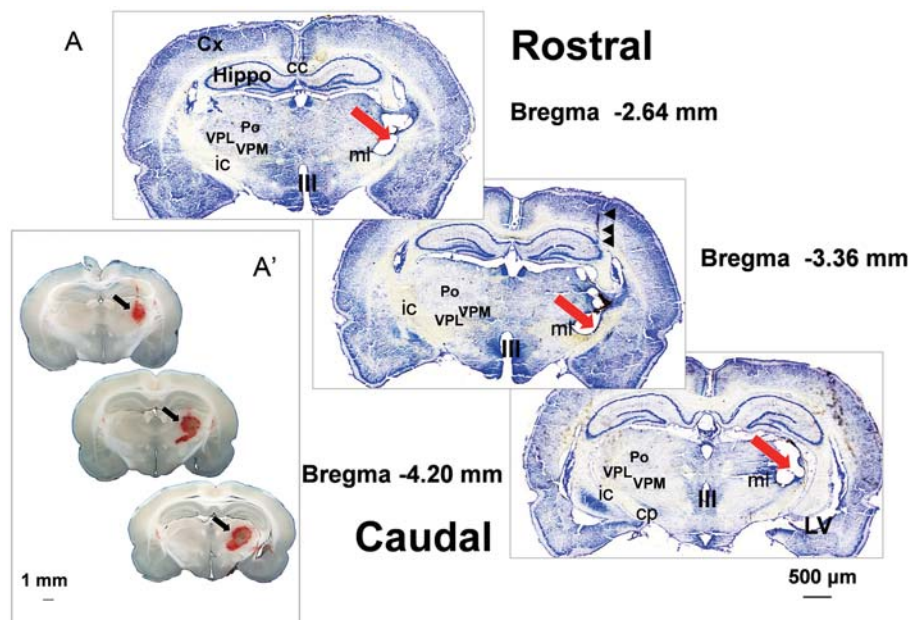


Fig. 2. Photomicrographs showing representative examples of the injection site and the spatial extent of the hemorrhagic lesion induced by intra-thalamic collagenase (ITC) microinjection. **A**, Nissl-stained sections (rostral-caudal coronal, 25 μ m) from a rat with central post-stroke pain hypersensitivity (CPSP) 28 days after ITC. The area of the injection site and hemorrhagic lesion is mainly confined to the ventroposterior lateral nucleus of the thalamus, the posterior thalamic nucleus, and part of the internal capsule (arrows). The injection track is indicated by arrowheads. **A'** shows that the hemorrhagic lesion site (arrows) can be clearly localized on unstained brain slices (rostral-caudal coronal, 500 μ m) from another rat 1 day after ITC injection. III, 3rd ventricle; cc, corpus callosum; cp, cerebral peduncle; Cx, cerebral cortex; Hippo, hippocampus; ic, internal capsule; LV, lateral ventricle; ml, medial lemniscus; Po, posterior thalamic nuclear group; VPL, ventral posterolateral nucleus of the thalamus; VPM, ventral posteromedial nucleus of the thalamus; scale bars, 500 μ m for **A**, 1 mm for **A'**.

(#6), while only one rat (#5) showed thermal hypoalgesia on day 28 post-ITC (Fig. 4). In the remaining six rats (#2–4, #7–9), there was less change in thermal pain sensitivity of the hind-paws from 7–28 days post-ITC (Fig. 4). None of the naïve rats ($n = 6$) and rats that received unilateral ITS injection ($n = 8$) developed pain hypersensitivity to mechanical or heat stimuli (Fig. 5). In addition, the ITC-induced bilateral mechanical pain hypersensitivity was identified on day 7 post-operation and the reduced PWMT remained unrelieved until 28 days of observation, suggesting a chronic, persistent bilateral mechanical allodynia in this model (Fig. 5).

In each animal that had developed bilateral mechanical pain hypersensitivity, the region of the VBC, equivalent to the ventroposterior lateral nucleus of the thalamus (VPL)/ventroposterior medial nucleus of the thalamus (VPM), the medial lemniscus and/or the posterior thalamic nucleus (Po) were all or partially involved in the ITC injection sites

and area of the lesion (Fig. 4 and Table 1). However, in the remaining 7 rats that failed to develop pain hypersensitivity, the ITC injection sites and the lesions were mostly confined to the following regions: the internal capsule (#10–12), the middle third of the thalamus (#13), and the lateral and medial geniculate bodies (#14–16) (Fig. 3 and Table 1).

Among the nine animals that developed bilateral mechanical pain hypersensitivity after ITC injection, seven showed significant deficits in motor coordination in the Rota-rod test compared with the naïve rats ($n = 6$) and rats receiving ITS injection ($n = 7$) (Table 2).

Effects of Gabapentin on Central Post-stroke Mechanical Pain Hypersensitivity Induced by Thalamic Hemorrhage

The time-course of the anti-allodynic effects of GBP was first evaluated in the group of rats that developed bilateral mechanical hypersensitivity on day 7 after ITC injection (see QST 3 in Fig. 1). In this experiment, each rat received a

Table 1. Summary of the loci of primary (core) and secondary (surrounding) hemorrhagic lesions in rats with and without central post-stroke pain (CPSP) hypersensitivity following intra-thalamic collagenase microinjection

Rats with CPSP			Rats without CPSP		
Rat No.	Core	Surrounding	Rat No.	Core	Surrounding
#1			#10		
R:	Rt	ic/VL		ic	Rt
M:	VPL/ml	Rt/ic		ic	Rt
C:	VPL/VPM/ml	Rt/ic/ZI		ic	SubG/VLG/DLG
#2			#11		
R:	ic	Rt		ic	Rt/CPu/EGP
M:	VPL/VPM/ml	Rt/ic		ic	Rt/CPu
C:	VPL/VPM/ml	eml/Rt/DLG/ZI		VPM/VPL/ml	Po/LPLR/DLG/ZI/Rt/ic
#3			#12		
R:	ic	Rt/VPL/ZI		ic	Rt
M:	ic	VPL/VPM/ml/ZI/VM		ic	Rt
C:	VPL/VPM/ml	DLG/Po/VPPC/ZI/Rt		ic	Rt/ZI
#4			#13		
R:	ic	Rt		Po/LPMR/LPLR	PF/CL/DG/CA1
M:	ic	VPL/ml/Rt		LPMR/LPMC/Po	ZI/PLH/PR/DG/CA1
C:	VPL/VPM/ml	LPLR/Po/Rt/DLG			
#5			#14		
R:	ic	Rt/ml/VPL		VLG	
M:	VPL/VPM/ml	LDVL/Po/Rt		VLG	
C:	VPL/VPM/ml	LPLR/DLG/Po		MGD/MGV	
#6			#15		
R:	VPL	Rt/ic		LPLC/LPMC	Po
M:	VPL/VPM/ml	LDVL/Po/Rt/ic		VLG	MGV/DLG
C:	VPL/VPM/ml	LPLR/DLG/Po/Rt/ic/ZI		MGD/MGV	DLG/VLG
#7			#16		
R:	Po	LDVL		VLG	DLG
M:	Po	LDVL			
C:	Po/VPM	LPMC/LPLC/LPMR		MGD/MGV	LPMC/LPLC/DLG
#8					
R:	VPL	Rt/ic			
M:	VPL/VPM/ml	Po			
C:	VPM/Po	ZI/str/ml			
#9					
R:	Rt/ic	VPL			
M:	VPL/ml/Rt	VPM/ic			
C:	VPM	str/DLG/VLG/ZI			

C, caudal; CA1, CA1 field of the hippocampus; CL, centrolateral thalamic nucleus; CPu, caudate-putamen (striatum); DG, dentate gyrus; DLG, dorsal lateral geniculate nucleus; EGP, external globus pallidus; eml, external medullary lamina; ic, internal capsule; LDVL, laterodorsal thalamic nucleus (ventrolateral); LPLC, lateral posterior thalamic nucleus (laterocaudal); LPLR, lateral posterior thalamic nucleus (laterorostral); LPMC, lateral posterior thalamic nucleus (mediocaudal); LPMR, lateral posterior thalamic nucleus (mediorostral); M, middle; MGD, medial geniculate nucleus (dorsal); MGv, medial geniculate nucleus (ventral); ml, medial lemniscus; PF, parafascicular thalamic nucleus; PLH, peduncular part of lateral hypothalamus; Po, posterior thalamic nuclear group; R, rostral; str, superior thalamic radiation; PR, prerubral field; SubG, subgeniulate nucleus; VL, ventrolateral thalamic nucleus; VLG, ventral lateral geniculate nucleus; VM, ventromedial thalamic nucleus; VPL, ventral posterolateral thalamic nucleus; VPM, ventral posteromedial thalamic nucleus; VPPC, ventral posterior nucleus of the thalamus (parvocellular); Rt, reticular thalamic nucleus; ZI, zona incerta.

Table 2. Rota-rod treadmill assessment of motor coordination in the naïve, intra-thalamic saline (ITS) and intra-thalamic collagenase (ITC) injection groups

Trial Number	Naïve (s)	ITS (s)	ITC (s)
1	118.14 ± 13.09	127.57 ± 13.27	50.00 ± 7.19***
2	121.29 ± 11.53	130.14 ± 14.73	48.57 ± 7.83***
3	128.71 ± 11.70	134.14 ± 17.70	56.43 ± 7.16**

****P* < 0.01, *****P* < 0.001 vs ITS, *n* = 6, 7, and 7 for each group. Rota-rod treadmill assessment was carried out on day 7 after intra-thalamic injection.

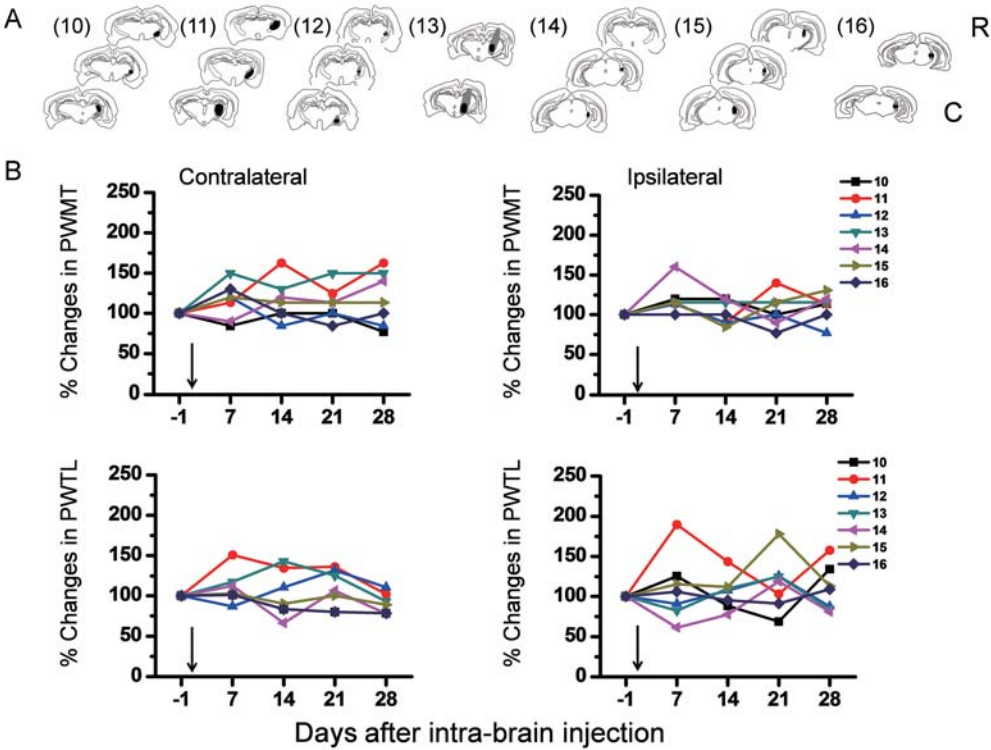


Fig. 3. Individual analyses of the localized hemorrhagic lesions in seven rats that failed to develop central post-stroke pain hypersensitivity following intra-thalamic collagenase (ITC) microinjection. A, histology-based schematic reconstructions of the lesion in the diencephalon of the seven rats. B, no changes in mechanical or thermal pain sensitivity in each corresponding rat in A after ITC injection. The black areas in A represent the site of tissue damage surrounded by a secondary lesion area (gray). C, caudal; PWMT, paw-withdrawal mechanical threshold; PWTL, paw-withdrawal thermal latency; R, rostral. Numerals in parentheses in A correspond to those for the curves in B. Arrows, day of ITC injection.

single injection of each of the doses of GBP (1, 10, and 100 mg/kg, i.p., 6–8 animals for each dose). Compared with vehicle, single administration of GBP produced a dose-dependent anti-allodynic effect in the hypersensitive rats. The anti-allodynic effect of GBP reached a peak at 1 h after administration and was maintained at a significant level for at least 5 h at higher doses (10 and 100 mg/kg) (Fig. 6A).

The lowest dose (1 mg/kg) did not have any significant anti-allodynic effect throughout the observation period (Fig. 6A). The anti-allodynic effect of GBP disappeared completely by 24 h after administration.

The time-course of the long-term anti-allodynic effect of GBP was then evaluated in another group of rats that had developed chronic bilateral mechanical

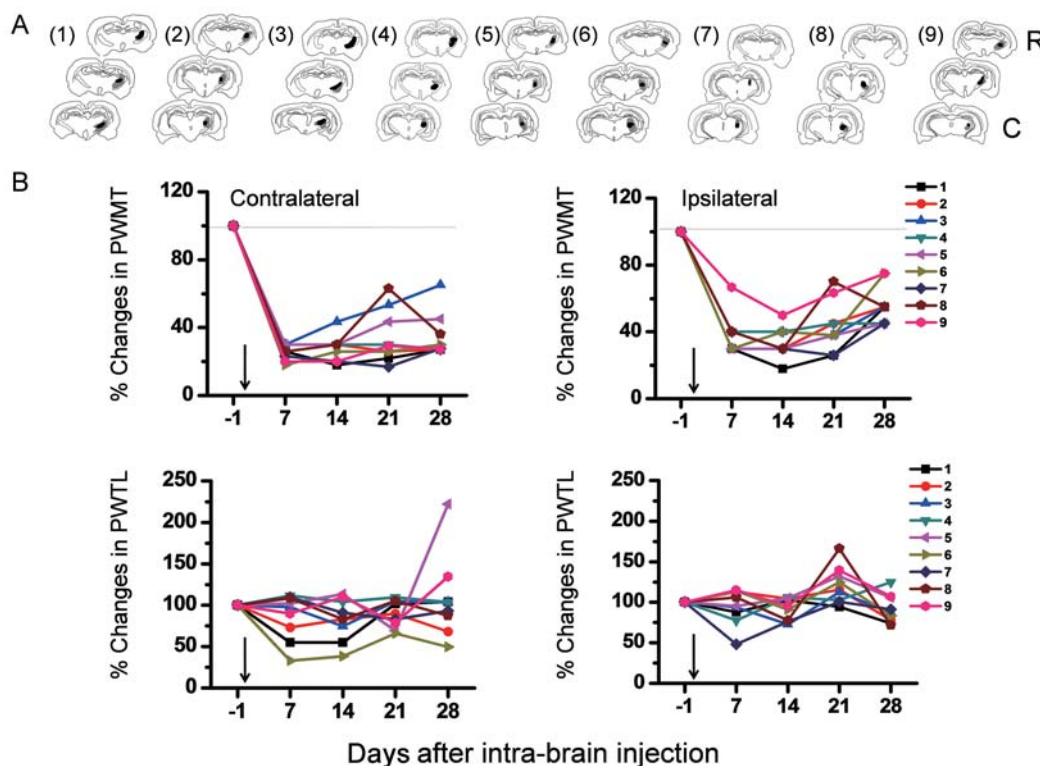


Fig. 4. Individual analyses of the relationship between the localized hemorrhagic lesion and the development of post-stroke pain hypersensitivity in rats following intra-thalamic collagenase (ITC) microinjection. A, histology-based schematic reconstruction of the lesion in nine rats. B, development of bilateral mechanical pain hypersensitivity in each corresponding rat in A and changes in thermal pain sensitivity in rats (#1, #5, #6) after ITC injection. The black area in A represents the site of tissue damage surrounded by the secondary lesion area (gray). C, caudal; PWMT, paw-withdrawal mechanical threshold; PWTL, paw-withdrawal thermal latency; R, rostral. Numerals in parentheses in A correspond to those for the curves in B. Arrows, day of ITC injection.

Table 3. Rota-rod treadmill assessment of pharmacological effects of a single intra-peritoneal injection of gabapentin (GBP) on motor coordination in naïve rats and in rats with intra-thalamic collagenase (ITC) injection

Trial Number	Naïve (s)		ITC (s)	
	Vehicle	GBP	Vehicle	GBP
1	156.00 ± 22.68	140.83 ± 15.36	86.67 ± 12.76	84.00 ± 8.78
2	154.00 ± 21.00	157.33 ± 19.82	98.17 ± 22.01	74.17 ± 13.36
3	179.20 ± 26.60	173.17 ± 20.53	94.67 ± 14.04	93.17 ± 16.06

The highest dose of GBP (100 mg/kg) was used ($n = 6$ for each group).

hypersensitivity. In this chronic setting, each rat received chronic administration of GBP (1, 10, or 100 mg/kg, i.p., 6–8 animals for each dose) once a day for two weeks from day 7 after ITC. QST assessment was performed in each rat with post-stroke pain hypersensitivity on days

7 and 14 after drug administration (14 and 21 days after ITC injection) (Fig. 6B, C, Fig. 7, also see QST 4 in Fig. 1). The dose-dependent pattern of the anti-allodynic effect remained relatively unchanged until 7 days after GBP administration. However, this pattern was disrupted after 14

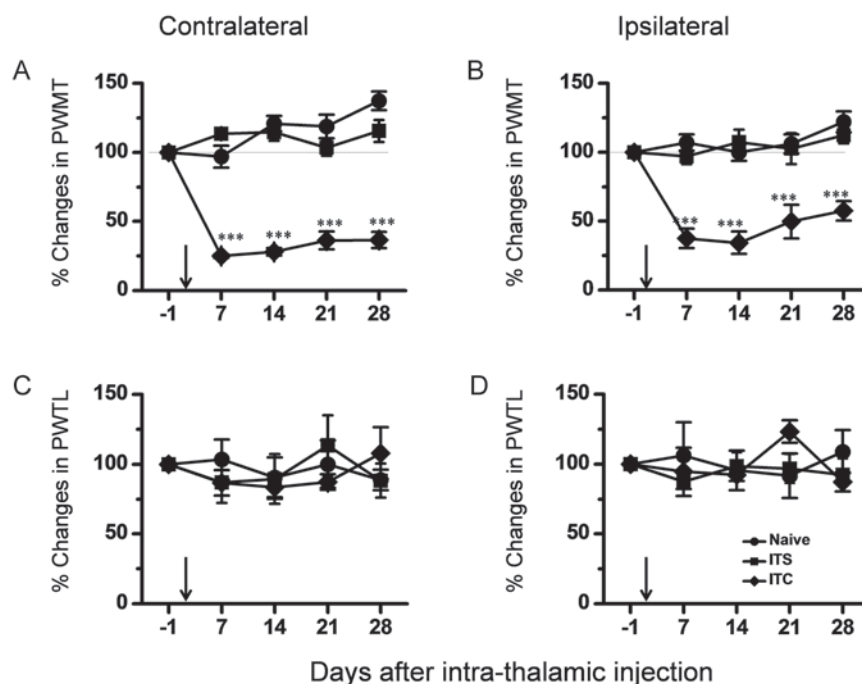


Fig. 5. Averaged effects of intra-thalamic collagenase (ITC, $n = 9$)-induced hemorrhagic lesions on mechanical (A and B) and thermal (C and D) pain sensitivity of both hind-paws in rats with central post-stroke mechanical pain hypersensitivity. Naïve rats ($n = 6$) and rats receiving intra-thalamic saline injection (ITS, $n = 8$) served as controls. PWMT, paw-withdrawal mechanical threshold; PWTL, paw-withdrawal thermal latency. *** $P < 0.001$ ITC vs ITS and Naïve.

days, when the anti-allodynic effect disappeared completely at 10 mg/kg although the effect of 100 mg/kg remained relatively unchanged (Fig. 6B, C). The time-related drug tolerance (% MPE) was clearly seen at 10 mg/kg (Fig. 7A), while a decreased time-course of drug efficacy also occurred with the highest dose used (100 mg/kg) (Fig. 7B).

Finally, single or repeated administration of GBP at the highest dose (100 mg/kg) did not produce any changes in baseline pain sensitivity in naïve rats compared with vehicle administration (Fig. S1). Moreover, single administration of GBP at 100 mg/kg had no significant effect on the motor coordination of naïve rats (Table 3). No reversal effect was produced by a single administration of GBP at 100 mg/kg on the motor coordination deficit in rats with CPSP (Table 3).

DISCUSSION

Central Post-stroke Pain Hypersensitivity of Experimental Thalamic Hemorrhage Origin

In the present study, we re-examined the thermal and

mechanical pain sensitivity in rats with experimental thalamic hemorrhage induced by local ITC injection according to a previously-reported method^[34]. The most prominent characteristic of the model of CPSP in the current study was the development of bilateral mechanical, but not thermal, pain hypersensitivity in both hind-paws. Case-by-case analysis of the relationship between the lesion site and the occurrence of pain hypersensitivity showed that this type of CPSP phenomenon is region-related and caused by unilateral damage of the dorsal column-medial lemniscus-VPL/VPM-Po system, a structural complex that mediates somatosensory functions in both humans and rodents^[49]. Although damage of the internal capsule and the reticular thalamic nucleus was also seen in rats with CPSP, these structures can be excluded because there was less change in either mechanical or thermal pain sensitivity in rats with severe damage mainly confined to these structures (see #10–11 in Fig. 3 and Table 1). This result strongly supports the new definition of central neuropathic pain: pain caused by a lesion or disease of the central somatosensory

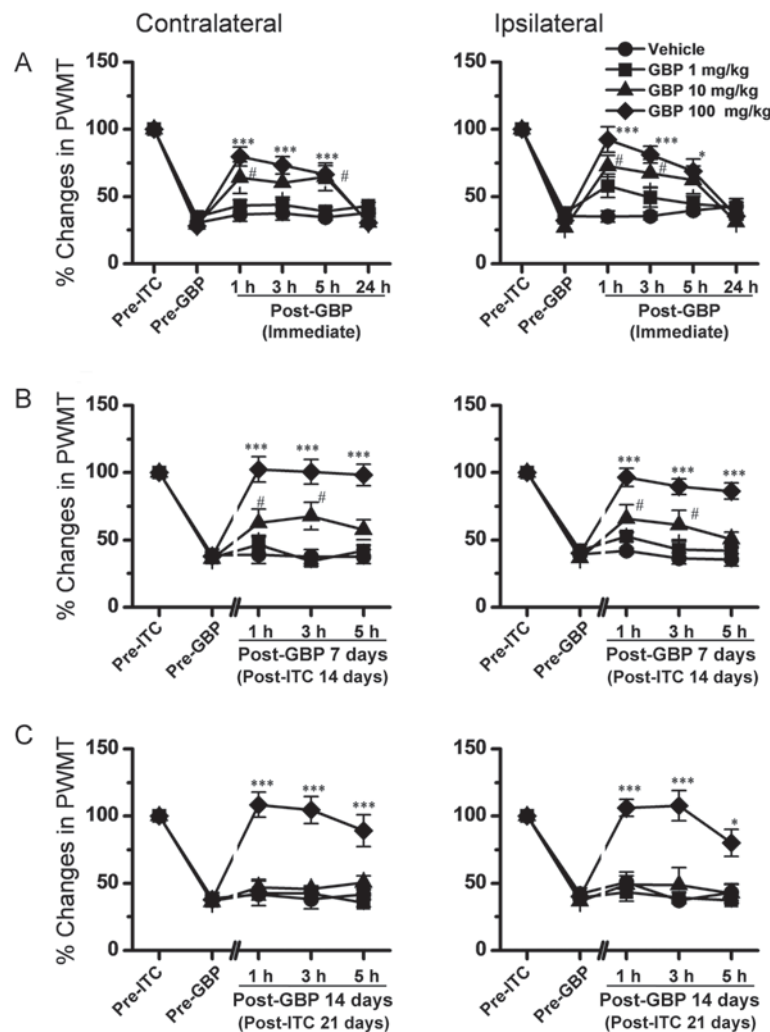


Fig. 6. Effects of systemic gabapentin (GBP) on central post-stroke mechanical pain hypersensitivity caused by a thalamic hemorrhagic lesion. Time-course of the anti-allodynic effect of single administration of GBP (1, 10, and 100 mg/kg, i.p.) 7 days after intra-thalamic collagenase (ITC) injection (A); after repeated administration (once a day for 7 days) 14 days after ITC injection (B); and after repeated administration (once a day for 14 days) 21 days after ITC injection (C). Pre-GBP, just prior to GBP administration 7 days after ITC injection; Pre-ITC, one day prior to ITC injection; PWMT, paw-withdrawal mechanical threshold; Post-GBP, just after GBP administration; *** $P < 0.001$, * $P < 0.05$, 100 mg/kg GBP vs vehicle; # $P < 0.05$, 10 mg/kg GBP vs vehicle. $n = 6-8$ animals for each group.

nervous system^[7,27,50,51]. Our animal data also correlate well with well-designed neuroimaging studies defining the lesion site in CPSP patients^[52-55]. In a comparative study of the thalamic lesion sites between patients with and without CPSP^[53], the damage of more posterior, inferior, and lateral parts of the VPL and the anterior pulvinar (equivalent to the Po in rodents) was highly correlated with the occurrence of CPSP. Moreover, the size of the damaged area may also be relevant to the occurrence of CPSP^[55]. Although we did

not measure the exact volume of the lesions in ITC-induced thalamic damage, the rats with bilateral mechanical pain hypersensitivity had a larger hematoma and secondary surrounding injury than those without CPSP (see Figs. 3 and 4).

So far, CPSP caused by experimental IS or ICH has rarely been studied in animals^[56-59]. The lack of studies on the validity and sensitivity of animal models of CPSP has largely hindered advances in understanding the underlying

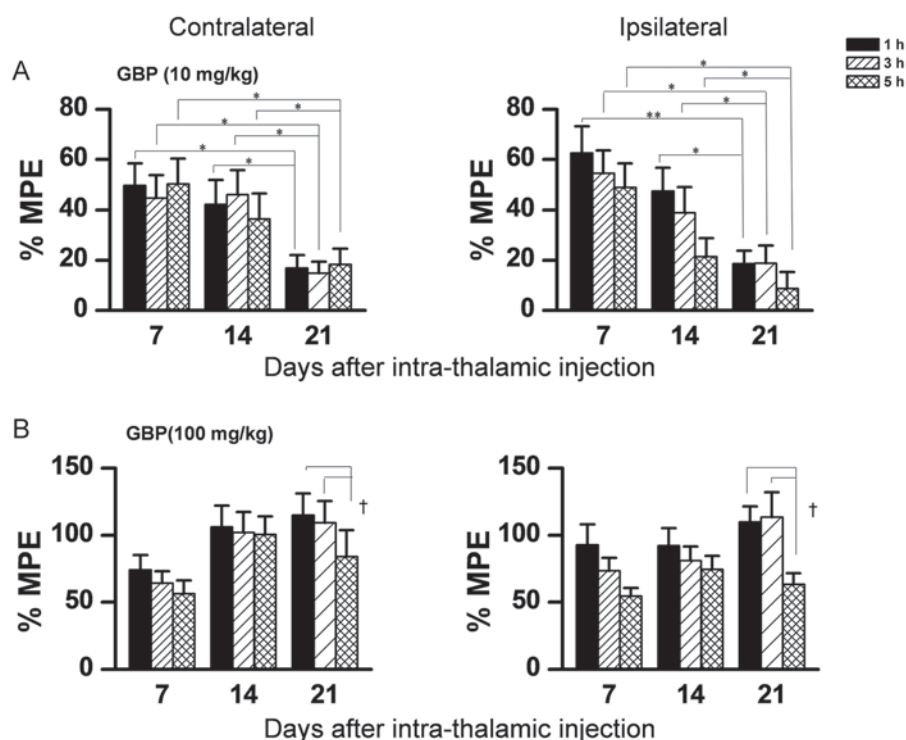


Fig. 7. Tolerance to repeated administration of gabapentin (GBP) of central post-stroke mechanical pain hypersensitivity caused by a thalamic hemorrhagic lesion. **A**, time-related decrease in % MPE after 14 days of repeated administration of GBP (10 mg/kg, i.p.). **B**, time-related decrease in effective time-course of GBP (100 mg/kg, i.p.) after 14 days of repeated administration. * $P < 0.05$, ** $P < 0.01$, † $P < 0.05$, $n = 6-8$ for each group.

mechanisms and the development of therapeutic approaches. Recently, efforts have been made to develop rodent CPSP models, but the behavioral outcomes are various due to the different methods and measurements adopted. Different outcomes were reported in the two existing studies of CPSP caused by ITC injection in rats. In the first report, contralateral thermal (hot plate) and mechanical (weight-bearing score and toe-pinch), but not cold (acetone), hypersensitivity were identified during 7–21 days post-ITC^[34], while in the second, bilateral mechanical (von Frey) and cold (acetone), but not thermal (Hargreaves's test with radiant heat), hypersensitivity were identified during 2–32 days post-ITC^[32]. In a new mouse model of CPSP caused by ITC and measured using a Dynamic Plantar Anesthesiometer and a Paw Thermal Stimulator^[33], bilateral mechanical and contralateral thermal pain hypersensitivity were found. Taken together with our current findings, experimental thalamic hemorrhagic stroke caused by ITC injection results in consistent

bilateral mechanical pain hypersensitivity while thermal pain hypersensitivity might be inconsistent. The reasons for differences in behavioral outcomes are not clear, but are likely to be due to the lesion site in the thalamus, again speaking to the need for careful mapping of each individual infarct. Because there is no detailed description of the relationship between the lesion sites and the pain behaviors through case-by-case analysis in the previous reports, a comparison between different studies is not possible. Nonetheless, based on our case-by-case analysis, we propose that the inconsistency of the changes in thermal pain sensitivity and consistency of the changes in mechanical pain sensitivity are likely due to damage of the medial lemniscus-VPL/VPM/Po system and sparing or partial damage to the spinothalamic tract, which is known to be responsible for the transmission of thermal pain information. Involvement of the dorsal column-medial lemniscus system in the development of mechanical allodynia is strongly supported by a previous study showing

that the terminations of the dorsal columns are important to the manifestation of tactile allodynia, but not thermal hyperalgesia^[60]. Actually, positive or negative thermal pain hypersensitivity is a common phenomenon in patients with CPSP^[61]. It has also been proposed that the VMpo is a specific relay for pain and temperature sensation^[62,63]. In humans, a non-VBC locus lying more posteriorly in the thalamus has been demonstrated to be important for thermo-algesic transmission^[64]. Collectively, it is reasonable to expect a lack of thermal hyperalgesia in rats with lesions confined to the medial lemniscus-VPL/VPM/Po, as in our current study.

Furthermore, no limping, foot-dragging, or uneven gait was observed in rats after ITC injection, but the total time on the Rota-rod treadmill was reduced (Table 2). The impairment of motor coordination in rats with thalamic hemorrhage is likely due to proprioceptive dysfunction or motor deficit rather than mechanical pain hypersensitivity, because the highest dose of GBP (100 mg/kg) had good anti-allodynic efficacy but did not reverse the motor coordination deficit (Table 3).

Anti-allodynic Efficacy and Tolerance to Gabapentin in Rats with Central Post-stroke Pain Hypersensitivity Caused by Thalamic Hemorrhage

GBP, a member of a class of anticonvulsants, has a high-affinity binding site on the $\alpha 2\delta$ subunit of the voltage-gated Ca^{2+} channel (VGCC) in the CNS^[64-66]. GBP modulates neurotransmission presynaptically by inhibiting the release of various neurotransmitters from hyper-excitable or pathophysiological cells *via* blocking the trafficking of the $\alpha 2\delta 1$ subunit to the presynaptic membrane^[67-70]. In many models of peripheral neuropathic pain, the $\alpha 2\delta 1$, but not the $\alpha 2\delta 2$ (another GBP binding site) subunit of VGCC is significantly up-regulated in both dorsal root ganglia (DRG) and the spinal dorsal horn and contributes to the development of allodynia and hyperalgesia^[71-74]. GBP has therefore been proposed to alleviate neuropathic pain by impairing the trafficking of $\alpha 2\delta 1$ to the presynaptic terminals of DRG neurons, leading to a reduction of Ca^{2+} influx and neurotransmitter release in the dorsal horn, and inhibition of central sensitization^[68,75].

In the present study, the anti-allodynic effectiveness of GBP was confirmed in rats with central post-stroke mechanical pain hypersensitivity caused by experimental

thalamic hemorrhage. The onset and duration of the anti-allodynic effect of GBP following a single intraperitoneal injection were dose-related and in line with its known pharmacokinetic and pharmacodynamic characteristics^[44,76-78]. The anti-allodynic effect of GBP is not likely to be attributable to the side-effect (sedation) induced by high doses in our study, because no effect on motor coordination was observed after administration of 100 mg/kg, indicating that GBP is more tolerable than ketamine that has severe motor side-effects^[32]. The current results provide evidence for the beneficial use of GBP for the treatment of CPSP in patients after thalamic hemorrhage, in parallel with recent research^[35]. However, despite possible side-effects of GBP, such as dizziness and drowsiness, the most intriguing problem we found here was tolerance after repeated administration; this was not reported in the latest research due to the lack of evaluation of dose and time-course effects^[35]. The phenomena of GBP tolerance can be characterized as: (1) loss of anti-allodynic effectiveness after two weeks of administration of a low effective dose (i.e., 10 mg/kg, i.p.), as used in our study; and (2) shortened time-course of anti-allodynic effectiveness after repeated administration of the highest dose (i.e., 100 mg/kg, i.p.). Actually, these phenomena have frequently been noted in patients with CPSP (personal communications with patients with CPSP), but unfortunately so far this problem has been neglected, even for other patients with neuropathic pain. Experimentally, GBP tolerance has been shown in a rat model of bortezomib-induced painful neuropathy^[79]. In that study, it was reported that administration of GBP (100 mg/kg by gavage every day for 3 weeks) results in a mild anti-allodynic effect on day 4 after treatment, followed by a complete loss of effectiveness afterward, strongly supporting the existence of tolerance to GBP in the treatment of neuropathic pain.

So far, it is not clear where and how GBP acts to produce anti-allodynic effects in this animal model of CPSP, since the systemic route of administration can result in anti-allodynic efficacy at both spinal and supraspinal sites^[66,69]. Moreover, the molecular and cellular mechanisms underlying central post-stroke mechanical pain hypersensitivity remain unclear. Thus, research into the underlying mechanisms of CPSP will be of particular significance for understanding its development and the anti-allodynic effectiveness and tolerance to the drugs

(e.g., gabapentinoids) used as the first line of clinical treatment of central neuropathic pain^[25-28]. Since it has been proposed that the development of CPSP might be caused by sensitization, disinhibition, and/or neuroplastic changes above the diencephalon^[7], the level of VGCC $\alpha 2\delta$ subunit expression and the binding sites of [³H] GBP in the dorsal column-medial lemniscus-VPL/VPM/Posterior cerebral cortex system should be examined in both human patients and animals with CPSP. Tolerance to GBP in patients and animals with CPSP might be due to changes in VGCC $\alpha 2\delta$ expression levels in hyperexcitable neurons with advances in the pathological process. As for the relationship between the level of $\alpha 2\delta 1$ expression and anti-allodynic effectiveness, it has been clearly demonstrated that the anti-allodynic effectiveness of a certain dose of GBP is attenuated at a time when $\alpha 2\delta 1$ subunit protein is abundant^[80]. The other possibility is that GBP tolerance might be due to changes in pharmacokinetic and pharmacodynamic characteristics in patients and animals with CPSP. All the above presumptions need further study in this animal model of CPSP.

Using the same CPSP model in mice, a single dose of diclofenac (30 mg/kg, i.p.), morphine (3 mg/kg, i.p.), and pregabalin (10 mg/kg, i.p.) was unable to reverse mechanical and thermal pain hypersensitivity during the period of observation^[33]. The lack of therapeutic improvement of pain in patients with CPSP by pregabalin (150 to 600 mg/day) has also been demonstrated in a 13-week, randomized, double-blind, multicenter, placebo-controlled trial^[31]. These pre-clinical and clinical results for pregabalin, another $\alpha 2\delta$ -binding anticonvulsant, are surprising and not conclusive because the authors did not investigate the dose-effect relationship. In contrast, however, in the same report, amitriptyline, lamotrigine, and minocycline (inhibitors of microglial cell activation) were effective in suppressing pain hypersensitivity in mice with CPSP^[33]. Moreover, ketamine was able to relieve mechanical and cold pain hypersensitivity only when a high dose (25 mg/kg) was used in the same rat model of CPSP^[32].

In conclusion, our data demonstrate that CPSP produced by ITC injection is a region-specific and sensitive model for the evaluation of anti-allodynic drugs, when investigated using case-by-case histological analysis, and provides a useful experimental tool for studying both the mechanisms underlying CPSP and its treatment.

SUPPLEMENTAL DATA

Supplemental data include one figure and can be found online at <http://www.neurosci.cn/epData.asp?id=217>.

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