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Short communication

P-glycoprotein inhibitors improve effective dose and time of pregabalin to inhibit intermittent cold stress-induced central pain

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ABSTRACT

Pregabalin (PGB) is a valuable therapeutic drug against chronic pain. Here we attempted to perform the combinatorial drug therapy with P-glycoprotein (P-gp) inhibitors to lower therapeutic dosage of PGB in the intermittent cold stress-induced fibromyalgia-like pain model. Single intracerebroventricular (i.c.v.) PGB injection exerted long-lasting anti-hyperalgesic effects for 72 h, while the effect of PGB given intraperitoneally (i.p.) disappeared within 3 h. Importantly, the pretreatment with P-gp inhibitors markedly prolonged the PGB (i.p.) effects, which lasted for 72 h. These results suggest that the combinatorial treatment with P-gp inhibitor enables the prolongation of dose-interval for PGB.

Pregabalin (PGB) is a central nervous system (CNS) targeting drug clinically used for epilepsy, trigeminal neuralgia, diabetic peripheral neuropathy and fibromyalgia (FM) (1, 2). High affinity and selective binding to voltage-gated calcium channels alpha2-delta (α2δ) subunits (3) is underlie the biological activity of PGB. Indeed PGB is known to modulate the voltage-dependent calcium channels gating dynamics and reduce calcium-dependent presynaptic release of neurotransmitters (4), leading to anti-hyperalgic or anti-allodynic actions (5). Although PGB is therapeutically valuable against neuropathic pain (NP) and FM, frequently observed side effects including peripheral edema limit their effective clinical use (6). Thus specific brain targeting or lowered therapeutic dosage is important therapeutic strategy with reduced adverse side effects.

CNS-partition of PGB is carefully regulated by influx and efflux transporters at blood–brain barrier (BBB) (7, 8). Especially, P-glycoprotein (P-gp), one of the ATP-binding cassette transporters is known to be involved (7). Here we investigated the effect of P-gp inhibitor on PGB-mediated anti-hyperalgesia and anti-allodynia phenotypes in intermittent cold stress (ICS)-induced FM-like pain model in mice.

Male C57BL/6J mice (TEXAM Corporation, Nagasaki, Japan) (20–25 g) were used. The mice were housed in a room (22 ± 3 °C) with free access to a standard laboratory diet and tap water. All procedures were approved by the Nagasaki University Animal Care Committee, and complied with the fundamental guidelines for the proper conduct of animal experiments and related activities in academic research institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Pregabalin was given by Pfizer (Ann Arbor, MI), while valspodar (Val) were purchased from Sigma–Aldrich Japan (Tokyo, Japan). These drugs were dissolved in physiological saline for intraperitoneal (i.p.) injection or in artificial cerebrospinal fluid (aCSF) for intrathecal (i.t.) and intracerebroventricular (i.c.v.) injection. Val was injected subcutaneously (s.c.) 0.5 h prior to PGB injection. The i.t. injection was given into the space between spinal L5 and L6 segments, according to the method described by Hylden and Wilcox (9).

Mice were exposed to intermittent cold stress (ICS), as previously described (10). We designated day 3 following the onset of the stress exposure as day 1 post-stress exposure (P1). Mice in the control group were maintained at 24 °C for all 3 days without any analgesics. Partial ligation of the sciatic nerve (SNL) was performed under pentobarbital (50 mg/kg) anesthesia, following the methods of Malmberg and Basbaum (11). The nociception test was performed 5 days after SNL.

In the thermal paw withdrawal tests, the nociception threshold was determined using thermal stimulator (IITC Inc., Woodland

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Hills, CA, USA) as previously described (12). A cut-off time of 20 s was set to avoid tissue damage. The mechanical paw pressure test was performed using a Transducer Indicator (Model 1601; IITC Inc.) as previously described (12). A cutoff pressure of 20 g was set to avoid tissue damage. ED50 was calculated based on the linear dose–response equation using 2 different dose/response points that include 50% maximum effect between them.

Statistical analyses were performed using one-way analysis of variance with the Tukey–Kramer multiple comparison *post-hoc* test. The criterion of significance was set at $p < 0.05$. All results are expressed as means ± standard error of the mean (SEM).

First we determined the anti-hyperalgesic effect of PGB in SNL model mice. As Fig. 1A shows, systemic PGB injection (30 mg/kg, i.p.) significantly inhibited the decrease in paw withdrawal latency (PWL) in SNL mice, indicating an anti-thermal hyperalgesic effect of PGB (i.p.). The effect of PGB (i.p.) was disappeared at 3 h after injection (Fig. 1A). Furthermore, PGB (i.p.) showed dose-dependent increase in the PWL in the dose range of 3–30 mg/kg (ED50 = 14.0 mg/kg). This corresponds with the previous report using other neuropathic pain model showing anti-cold allodynic effect of PGB (13). Similar effect was observed in the mechanical paw pressure test (Supplemental Fig. 1A). To determine the role of CNS in PGB effects, mice were injected with PGB (30 μg) via i.c.v. However, it did not show any effect (Fig. 1B). On the other hand, i.t. PGB (30 μg) showed significant anti-thermal hyperalgesic effect in SNL mice (Fig. 1C). The dose-dependent effect of PGB (i.t.) was also confirmed (ED50 = 1.8 μg).

Next we determined the anti-hyperalgesic effect of PGB in ICS model mice. As Fig. 1D shows, PGB injection (1 mg/kg, i.p.) significantly increased the PWL, indicating an anti-thermal hyperalgesic effect of PGB (i.p.) on ICS-induced FM-like pain. Furthermore, PGB (i.p.) showed dose-dependent increase in PWL in the dose range of 0.1–1 mg/kg (ED50 = 0.3 mg/kg). Similar results were obtained in mechanical paw pressure test (Supplemental Fig. 1B). To determine the role of CNS in PGB effect on ICS, mice were injected with PGB via i.c.v. at the dose of 1 μg. Pregabalin (i.c.v.) significantly increased the PWL in ICS exposed mice up to 72 h after injection (Fig. 1E). The dose-dependent effect of PGB (i.c.v.) was also confirmed in the dose range of 0.1–0.3 μg (ED50 = 0.2 μg). On the other hand, i.t. PGB (10 μg) showed significant effect only at 0.5 h after injection (Fig. 1F). The dose-dependent effect of PGB (i.t.) was also confirmed in the dose range of 1–10 μg (ED50 = 5.7 μg).

We have previously reported ICS exposure induces persistent hyperalgesia that last over 19 days after the stress (14). To determine the effect of PGB on ICS exposure-induced persistent hyperalgesia, mice were repeatedly injected with PGB (i.c.v.) at every 3 days starting on P5, and the effect of PGB was determined on the same days. As shown in Fig. 2A, on P5, PGB injection (i.c.v., 1 μg) significantly increased the PWL of ICS exposed mice up to 3 h in thermal paw withdrawal test. Similar effects were observed on P8 and P11. Importantly, PWL was gradually increased and completely returned to the normal (i.e., control mice) levels even after the cessation of repeated PGB (i.c.v.) injection (i.e., P14 to P20) (Fig. 2B). Similar results were obtained in the mechanical paw pressure test (Supplemental Fig. 2A). To determine whether the delivery of PGB to the brain is important for their therapeutic effects, we used specific inhibitor of P-gp, one of the ATP-binding cassette transporter that regulate effective CNS drug delivery at BBB. As P-gp inhibitors, we used Val, a Cyclosporine A-analog lacking immunosuppressant effects (28). In thermal paw withdrawal test, pretreatment of Val (3 mg/kg, s.c.) markedly prolonged the anti-thermal hyperalgesic effect of PGB (1 mg/kg, i.p.) up to 48 h after

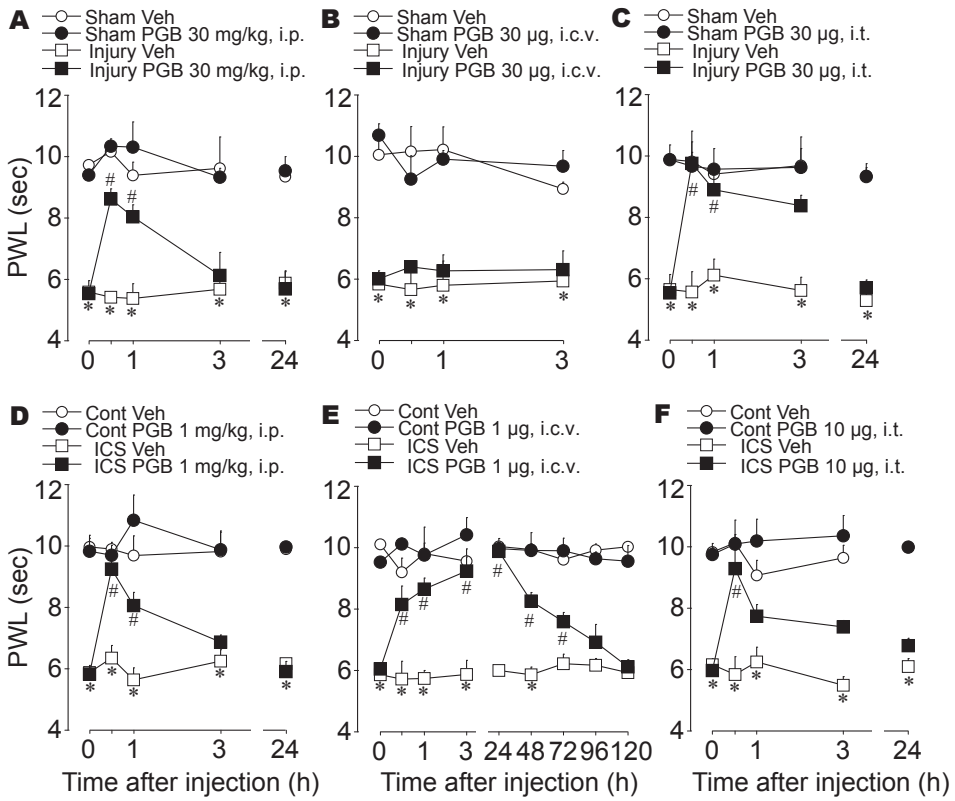

Fig. 1. Blockade of ICS but not of SNL-induced hyperalgesia by supra-spinal PGB. Thermal hyperalgesia was determined by the measurement of PWL in SNL (A–C) or ICS (D–F) mice on 5 days (P5) after the onset of stress. The test was performed at the indicated periods after the i.p. (30 mg/kg) (A), i.c.v. (30 μg) (B), i.t. (30 μg) (C), i.p. (1 mg/kg) (D), i.c.v. (1 μg) (E) and i.t. (10 μg) (F) PGB injection. Data are expressed as means ± SEM. *$p < 0.05$, vs. Sham Veh (A–C) or Cont Veh (D–E); #$p < 0.05$, vs. Injury Veh (A–C) or ICS Veh (D–F).

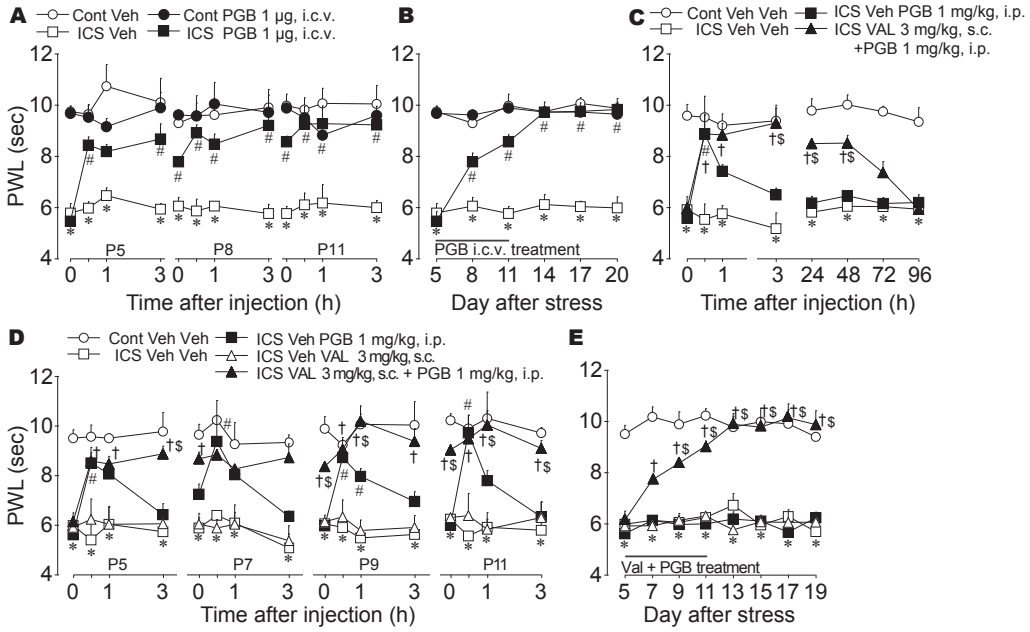

Fig. 2. Complete blockade of ICS-induced thermal hyperalgesia by repeated i.c.v. PGB treatment or combinatorial treatments of PGB with P-gp inhibitor. Thermal hyperalgesia was determined in ICS exposed mice on 5 days (P5) (A, C and D) or indicated days (B and E) after the onset of ICS. Repeated i.c.v. PGB injection (A and B) and i.p. PGB injection (D and E) was performed every 3 days (P5–P11) and every 2 days (P5–P13), respectively. Val was injected systemically (s.c., 3 mg/kg) 30 min before the PGB (1 mg/kg, i.p.) injection (C–E). Data are expressed as means ± SEM. $^{*}p < 0.05$, vs. Cont Veh; $\#p < 0.05$, vs. ICS Veh (A and B); $^{*}p < 0.05$, vs. Control Veh Veh; $\#p < 0.05$, $\dagger p < 0.05$, vs. ICS Veh Veh, $\$p < 0.05$, vs. ICS Veh PGB (C–E).

injection (Fig. 2C), indicating that P-gp inhibition can significantly enhance and prolong the systemic PGB effect.

Finally we determined the therapeutic effect of combination of P-gp inhibitor and systemic PGB on ICS exposure-induced persistent hyperalgesia. Mice were repeatedly injected with PGB (1 mg/kg, i.p.) with or without pretreatment of Val (3 mg/kg, s.c.) at every other day from P5 to P11. As shown in Fig. 2D, anti-hyperalgesic effect of PGB (i.p.) was significantly enhanced by pretreatment of Val on P5 to P11. Importantly, basal PWL was gradually increased and completely returned to the normal (i.e., control mice) levels even after the cessation of repeated Val + PGB injection (i.e., P13 to P19) (Fig. 2E). Similar results were obtained in the mechanical paw withdrawal test (Supplemental Fig. 2B).

As we showed here, we used two types of pain model. One is a classic NP model (11) and the other is a novel pain model of FM syndrome with the generalized chronic pain phenotype of FM patients (10). Since the majority of FM patients have no nerve lesions demonstrable, the FM-associated pain can be maintained by a number of different mechanisms from the classic NP that is initiated by nervous system lesions or dysfunction leading to a different pharmacotherapeutic features between them. Here we found that the ED50 in the thermal paw withdrawal test was 14.0 mg/kg with PGB (i.p.) in the NP model, which was 47-fold higher than that in the ICS model (0.3 mg/kg, i.p.). As the anti-hyperalgesic activity substantially disappears in both cases at 3 h after the single injection, the difference of potency looked initially attributed to the severity of pain. However, it was found that the discrepancy is more complicated from the findings that potent PGB actions in the NP model were observed with the ED50 (1.8 μg) by i.t. injection, but no significant action with i.c.v. injection of 30 μg PGB, while the actions in the ICS model were also observed with i.t. injection (ED50 = 5.7 μg), and with i.c.v. injection (ED50 = 0.2 μg), respectively. Furthermore, the significant anti-hyperalgesia action was observed as late as 72 h after the i.c.v. injection in the ICS model but not in SNL model. These results suggest that ICS-induced hyperalgesia is closely related to the disturbance in the brain, while the mechanisms located in peripheral nerves to spinal cord may also have some modest contribution to the hyperalgesia and PGB effects, equivalent to the levels in the SNL model. Since the target molecule of PGB such as α2δ subunit is expressed in the CNS as well as peripheral nervous system (15), the stress-related changes in the levels of these molecules in the supraspinal and spinal might differ between SNL model and ICS model, while it needs to be determined.

Interestingly, the repeated i.c.v. treatments of PGB and the repeated i.p. treatment in combination with P-gp inhibitor gradually elevated the basal thermal and mechanical threshold to the naïve mouse levels even after the cessation of treatments, suggesting that the chronic pain status was completely cured. Since it is known that chronic pain consists of the first acute pain caused by various stimuli that trigger the secondary pain (16), i.e., the vicious cycle of pain, the repeated i.c.v. treatments of PGB and the repeated i.p. treatment in combination with P-gp inhibitor may inhibit not only the first but also the secondary pain, while the detailed mechanisms are needed to be determined. Furthermore, the present results suggest PGB is likely acting in the brain to exert its effect against FM-associated pain and the combination therapy of PGB and P-gp inhibitors may be clinically effective. It would be needed to determine whether the distribution or transport of PGB into the brain is increased by the pretreatment of P-gp inhibitor.

In conclusion, here, we succeeded in demonstrating the therapeutic effect of PGB on FM. Additionally, we enabled the prolongation of dose-interval for PGB by using P-gp inhibitor and achieved prolonged and complete pain relief and recovery from the FM by repeated injections as therapeutic advantages for PGB.

Conflict of interest

There is no conflict of interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jphs.2016.01.002.

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