Transdermal Administration of Aqueous Pregabalin Solution as a Potential Treatment Option for Patients with Neuropathic Pain to Avoid Central Nervous System-Mediated Side Effects

Hiroshi Fukasawa,*,[a] Hideaki Muratake,[a] Marina Nagae,[b] Kiyoshi Sugiyama,[b] and Koichi Shudo[a]

[a] Research Foundation ITSUU Laboratory; 2–28–10 Tamagawa, Setagaya-ku, Tokyo 158–0094, Japan: and
[b] Department of Clinical Pharmacokinetics, Hoshi University; 2–4–41 Ebara, Shinagawa-ku, Tokyo 142–8501, Japan.

Pregabalin, (*S*)-3-isobutyl-*γ*-aminobutyric acid (GABA), is a widely used adjuvant therapy for patients with neuropathic pain, which is defined as chronic pain caused by lesions or diseases of the somatosensory nervous system. However, dizziness and somnolence (sleepiness) are common dose-limiting side effects, probably due to excessive sedative effects on higher centers of the central nervous system (CNS) which are involved in the anticonvulsant and analgesic actions of pregabalin. We speculated that transdermal delivery would minimize centrally mediated side effects. To test this idea, we evaluated the analgesic effects of pregabalin delivered through the transdermal route in animal models of neuropathic pain. Transdermally administered pregabalin increased the pain thresholds in response to mechanical stimuli in a partial sciatic nerve ligation model in rats and a spinal nerve ligation model in mice, and surprisingly also in normal animals. It is noteworthy that simple transdermal application of an aqueous solution of pregabalin is effective. This could be a useful treatment option to avoid or minimize the CNS-mediated side effects of orally administered pregabalin.

Key words   neuropathic pain; pregabalin; transdermal administration

Neuropathic pain is chronic pain caused by lesion or disease of the somatosensory nervous system.[1–3] It is characterized by allodynia (pain in response to normally innocuous stimuli), hyperalgesia (increased pain evoked by noxious stimuli) and spontaneous pain. Treatment of neuropathic pain remains a clinical challenge, because the pathophysiology of neuropathic pain is complex and the underlying mechanisms remain poorly understood.[4,5] Although chronic neuropathic pain often responds unsatisfactorily to opioids and non-steroidal anti-inflammatory drugs (NSAIDs), it can be treated effectively with antidepressants and antiepileptics as adjuvant analgesics.[5] The (*S*)-isomer of 3-isobutyl-*γ*-aminobutyric acid (GABA), pregabalin, which was identified as an antiepileptic in the early 1990's, is now widely used for treatment of diabetic peripheral neuropathy, postherpetic neuralgia, spinal cord injury nerve pain, fibromyalgia and partial onset seizures.[6–8] Unfortunately, though, central nervous system (CNS)-mediated side effects such as dizziness and somnolence (sleepiness) are common in pregabalin-treated patients and have a significant impact on their quality of life.[9] Therefore, other treatment options that overcome these problems would provide substantial benefits to patients. Topical application through the transdermal route is one possible option to reduce systemic drug exposure and penetration into the brain, and thereby to minimize CNS-mediated side effects. In this study, therefore, we evaluated the analgesic effects of transdermally administered pregabalin in rats and mice.

MATERIALS AND METHODS

Chemicals   Pregabalin (free base) used for experiments in rats was obtained from Sequoia Research Products Ltd. (Pangbourne, U.K.). Pregabalin (crystallized free base) used for experiments in mice was synthesized at ITSUU Laboratory.[10]

Animals   Male CD rats at 4 weeks of age were purchased from Charles River Laboratories (Yokohama, Japan). Male ICR mice at 4 weeks of age were purchased from Japan SLC, Inc. (Hamamatsu, Japan). The animals were maintained under appropriate conditions and allowed access to food and water *ad libitum*. Animal experiments were performed according to the guidelines of the Science Council of Japan and also with the approval of the local animal ethics committees of Hoshi University, Mitsubishi Chemical Medience Corporation (Kumamoto, Japan), and ITSUU Laboratory.

Neuropathic Pain Models and Measurement of Pain Threshold   Mechanical allodynia was induced by partial sciatic nerve ligation (PSL) in rats.[11] In brief, the left sciatic nerve of male Sprague-Dawley (SD) rats at 5 weeks of age was partially (1/2—1/3) ligated under anesthesia. To test the effects of transdermally administered pregabalin, 1 mg of pregabalin dissolved in 0.1% Tween-80 at 10 mg/mL was applied to both hind paws 17 d after surgery and allowed to dry. An Elizabethan collar was put around the neck of each rat to prevent paw-licking for 40 min after administration (*i.e.*, until 20 min before the first postadministration von Frey test). In normal rats, 1 mg of pregabalin was dissolved in methanol at 10 mg/mL and applied in the same way, but only to the left paw. The hind paw withdrawal responses of PSL or normal rats to a series of calibrated von Frey filaments were measured on both sides to quantify the pain threshold at 1, 3, and 5 h after administration.

In mice, mechanical allodynia was induced by means of the spinal nerve ligation (SNL) procedure.[12] In brief, the right L5 and L6 spinal nerves of male ICR mice at 5 weeks of age were tightly ligated under anesthesia and the animals were used for tests 28 d after surgery. Sham mice were subjected to similar procedures except for the spinal nerve ligation. The right lower legs of SNL or sham mice were dipped into

*To whom correspondence should be addressed.   e-mail: hfukasawa@itsuu.or.jp; hfwork@zav.att.ne.jp

a solution of 2.5 or 7.5 mg/mL pregabalin in pure water for 5 min and allowed to dry. The pain threshold of the right paw was measured by using von Frey filaments at 30, 60, 90 and 180 min after administration.

RESULTS AND DISCUSSION

Pregabalin has been reported to show analgesic activity in various animal models of neuropathic pain.[13,14] In our previous work, we evaluated the analgesic action of novel GABA derivatives containing silicon–carbon bonds and pregabalin in the PSL model (so-called Seltzer model) and the SNL model (so-called Chung model).[10,15] In those studies, we found that orally administered pregabalin significantly increased the withdrawal threshold not only on the ipsilateral operated side (anti-allodynic effect), but also on the contralateral non-operated side. This increase of pain threshold on the contralateral side, which was observed with a delay compared to the ipsilateral side, can be considered as representing hypoalgesic action due to excessive sedative action on the pain-related higher centers after distribution of pregabalin into the brain. Therefore, we evaluated the analgesic action of transdermally administered pregabalin to elucidate whether transdermal delivery would minimize centrally mediated side effects of pregabalin.

In PSL rats, whose unilateral sciatic nerve was partially ligated, mechanical allodynia and hyperalgesia can be assessed in terms of the decrease of the mechanical threshold for paw withdrawal response evoked by mechanical stimuli. Pregabalin was transdermally administered to both hind paws of PSL rats by spreading pregabalin solution (1 mg in 100 μL) in 0.1% Tween-80 all over each paw. Although a vehicle group was not included in this initial experiment, we considered that the vehicle would not have had any significant effect on the pain threshold, because Tween-80 is a safe non-ionic surfactant that is widely used in transdermal delivery formulations. The dose (total of 2 mg per rat) is equivalent to a systemic dose of 7 mg/kg, which is considered the minimum effective dose of orally or subcutaneously administered pregabalin in rats.[14,16] On the ipsilateral (operated) side, the pain threshold was significantly increased after topical administration and peaked at 1 h (Fig. 1). It was still significantly increased at 3 h after administration. Interestingly, the pain threshold on the contralateral (non-operated) side was also significantly increased and peaked at 1 h after administration (Fig. 1). This increase of the contralateral pain threshold above its normal level can be regarded as representing a hypoalgesic effect. In contrast to the hypoalgesic effect observed in the contralateral paw when pregabalin was orally administered, the effect on the contralateral side in transdermally administered PSL rats was not delayed compared to that on the ipsilateral side. The similar time-courses of efficacy on the ipsilateral and contralateral sides suggest that the sites of action of transdermally administered pregabalin may be the same on both sides, whether peripheral or central. It is noteworthy that a rather small amount of transdermally administered pregabalin was effective, although the delivery route and vehicle were not optimized.

Because transdermally administered pregabalin showed a hypoalgesic effect on the non-operated side in PSL rats, we next evaluated the effects of transdermal administration of pregabalin in normal rats. Pregabalin solution (1 mg/100 μL
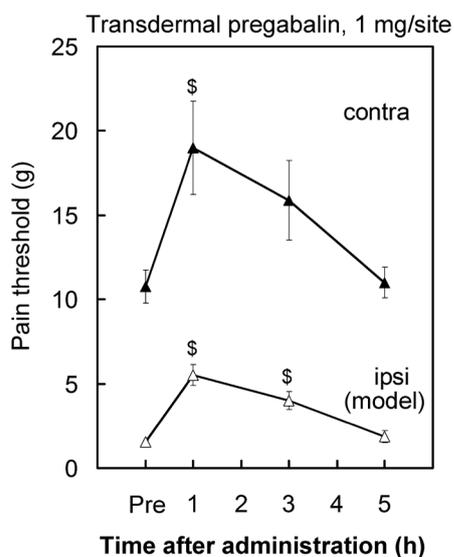
Fig. 1. Analgesic Effects of Transdermally Administered Pregabalin in PSL Rats

Mechanical allodynia was induced by partial ligation of the left sciatic nerve in male SD rats. Paw withdrawal thresholds were measured in both hind paws by stimulation with von Frey filaments at 1, 3 and 5 h after administration. One milligram of pregabalin dissolved in 0.1% Tween-80 at 10 mg/mL was transdermally administered 17 d after surgery. Pregabalin solution was spread and rubbed all over the hind paw and allowed to dry. An Elizabethan collar was put around the neck of each rat to prevent paw-licking for 40 min after administration (*i.e.*, until 20 min before the first postadministration von Frey test). Data for the left operated paw (ipsi) and right non-operated paw (contra) are shown in open and closed triangles, respectively. Data are expressed as geometric mean±S.E.M. (n=8, each group). Statistical analysis was done by using Excel with Analyse-it (Analyse-it Software, Ltd., U.K.). $^{\$}p$<0.05, Dunnett's test (*vs.* pre) after repeated-measures ANOVA (p<0.05) in each group.
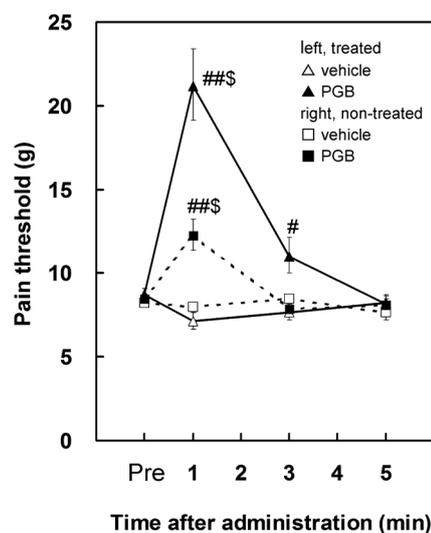
Fig. 2. Analgesic Effects of Transdermally Administered Pregabalin in Normal Rats

For transdermal administration, 1 mg of pregabalin dissolved in methanol at 10 mg/mL was applied to the left hind paw of normal SD rats by spreading and rubbing all over the paw, and allowed to dry. An Elizabethan collar was put around the neck of each rat to prevent paw-licking for 40 min after administration (*i.e.*, until 20 min before the first postadministration von Frey test). Data for the left treated paw and right non-treated paw are shown as triangles and squares, respectively. Data for the vehicle control and pregabalin (PGB) are shown as open and closed symbols, respectively. Data are expressed as geometric mean±S.E.M. (n=8, each group). Statistical analysis was done by using Excel with Analyse-it (Analyse-it Software, Ltd., U.K.). $^{\#\#}p$<0.01, $^{\#}p$<0.05 in the *t*-test with Bonferroni correction (*vs.* vehicle). $^{\$}p$<0.05 in Dunnett's test (*vs.* pre) after repeated-measures ANOVA (p<0.05) in each group.
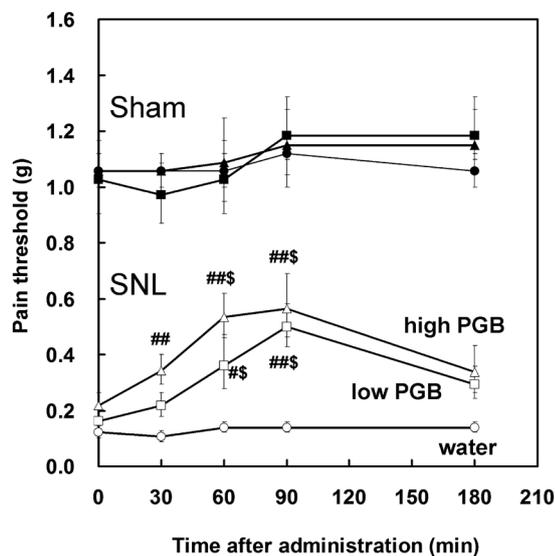
Fig. 3.   Analgesic Effects of Transdermally Administered Pregabalin in SNL Mice

Mechanical allodynia was induced by ligation of the right L5 and L6 spinal nerves in male ICR mice. The mice were used for tests at 28 d after surgery. The right paw of SNL or sham mice was dipped into a solution of 2.5 or 7.5 mg/mL pregabalin in pure water for 5 min and allowed to dry. The pain threshold of the right paw was measured by using von Frey filaments at 30, 60, 90 and 180 min after administration. Data in SNL and sham mice are shown as open and closed symbols in a) and b), respectively. Data for 2.5 mg/mL pregabalin (low PGB), 7.5 mg/mL pregabalin (high PGB) and vehicle control are shown as squares, triangles and circles, respectively. Data are expressed as geometric mean±S.E.M. (n=6, each group). Statistical analysis was done by using Excel with Analyse-it (Analyse-it Software, Ltd., U.K.). $^{\#\#}p<0.01$, $^{\#}p<0.05$ in the t-test with Bonferroni correction (vs. vehicle). $^{\$}p<0.05$ in the Dunnett's test (vs. 0 min) after repeated-measures ANOVA ($p<0.05$) in each group.

methanol) was applied to the left hind paw of normal rats, and the paw withdrawal thresholds were measured on both sides. The pain threshold of the treated paw was significantly increased and peaked at 1 h (Fig. 2). The pain threshold of the untreated right paw was also significantly increased simultaneously at 1 h after administration, but to a lesser extent than that of the left paw (Fig. 2). It is possible that this could be a behavioral effect associated with dulled sensation in the left paw. It is also possible that transdermally administered pregabalin would be partially transferred to plasma and distributed to the right paw. In either case, significant distribution of pregabalin to the brain seems unlikely to play a role, because the peak time of increase of the pain threshold of the right paw was not delayed from that of the left paw, in contrast to the case of orally administered pregabalin in PSL rats.

We further evaluated the analgesic effects of transdermally administered pregabalin by using the SNL model in mice. Pregabalin was dissolved at 2.5 or 7.5 mg/mL in pure water, and the right paw of SNL mice was dipped into the solution for 5 min and allowed to dry. Transdermal administration of 7.5 mg/mL aqueous pregabalin solution significantly increased the pain threshold at 30, 60, and 90 min after administration (Fig. 3, SNL/high PGB). Its efficacy peaked at 90 min. We found that 2.5 mg/mL pregabalin solution also increased the pain threshold significantly at 60 and 90 min after transdermal application, but to a lesser extent (Fig. 3, SNL/low PGB). Therefore, the analgesic efficacy of transdermally administered pregabalin is dose-dependent. In sham mice, no increase of the pain threshold was observed. This is in contrast to the hypoalgesic effect observed in normal rats treated transder-

mally with pregabalin. Although the reason for this discrepancy remains unclear, one reason might be a difference of sensitivity to mechanical stimuli between rats and mice. The differences in solvent (methanol, pure water) or manipulation (spreading and rubbing, dipping) could also be important. For example, when pregabalin is dissolved in a small volume of methanol and applied by spreading and rubbing over a rat's paw, it might be efficiently absorbed locally and then distributed systemically. By contrast, when pregabalin is applied by dipping a mouse's paw in aqueous solution, absorption may be slower or less effective. Further experiments to estimate the amount of absorbed pregabalin will be necessary to address this issue. Nevertheless, our results suggest that transdermally administered pregabalin can work locally at the treated area, at least in part. Plaza-Villegas et al. reported that neuropathic orofacial pain in rats was suppressed by means of external application of pregabalin in pluronic lecithin organogel and anhydrous gel base as vehicle to the infraorbital nerve territory in the vibrissae area to achieve a higher plasma concentration.[17] It is noteworthy that in our experiments transdermal application of a simple solution of pregabalin in water without any additive or surfactant for enhancing the penetration rate was effective.

In the present study, we evaluated the analgesic effects of transdermally administered pregabalin in PSL rats, normal rats and SNL mice. Since CNS-mediated effects should be delayed and bilateral, our results indicate that transdermally administered pregabalin exerted its analgesic action peripherally in our experiments, at least in part. It is currently considered that pregabalin exhibits its analgesic action by preferentially inhibiting central sensitization in the spinal cord and higher centers of the CNS, and penetration into the brain across the blood brain barrier would be essential in such a scenario. Indeed, specific binding of pregabalin to the α_2–δ subunit of voltage-gated calcium channel, which is expressed at the presynaptic terminal of neurons in the spinal cord and brain, is thought be responsible for the analgesic and anticonvulsant actions.[8,18,19] The present results raise the possibility that some peripheral target(s) other than α_2–δ protein might be involved in the analgesic action of pregabalin. Transdermal delivery of pregabalin could be an effective treatment option to minimize or avoid dose-limiting CNS-mediated side effects of orally administered pregabalin. Further characterization of the cutaneous absorption and subsequent tissue distribution will be necessary to elucidate the site of action of transdermally administered pregabalin.

REFERENCES

1) Jensen TS, Baron R, Haanpaa M, Kalso E, Loeser JD, Rice AS, Treede RD. A new definition of neuropathic pain. Pain, **152**, 2204–2205 (2011).
2) Treede RD, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, Griffin JW, Hansson P, Hughes R, Nurmikko T, Serra J. Neuropathic pain: redefinition and a grading system for clinical and research purposes. Neurology, **70**, 1630–1635 (2008).
3) Baron R, Binder A, Wasner G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. Lancet Neurol., **9**, 807–819 (2010).
4) O'Connor AB, Dworkin RH. Treatment of neuropathic pain: an overview of recent guidelines. Am. J. Med., **122** (Suppl.), S22–S32 (2009).

5) Dworkin RH, O'Connor AB, Audette J, Baron R, Gourlay GK, Haanpaa ML, Kent JL, Krane EJ, Lebel AA, Levy RM, Mackey SC, Mayer J, Miaskowski C, Raja SN, Rice AS, Schmader KE, Stacey B, Stanos S, Treede RD, Turk DC, Walco GA, Wells CD. Recommendations for the pharmacological management of neuropathic pain: an overview and literature update. *Mayo Clin. Proc.*, **85** (Suppl.), S3–S14 (2010).

6) Silverman RB, Andruszkiewicz R, Nanavati SM, Taylor CP, Vartanian MG. 3-Alkyl-4-aminobutyric acids: the first class of anticonvulsant agents that activates L-glutamic acid decarboxylase. *J. Med. Chem.*, **34**, 2295–2298 (1991).

7) Taylor CP, Vartanian MG, Yuen PW, Bigge C, Suman-Chauhan N, Hill DR. Potent and stereospecific anticonvulsant activity of 3-isobutyl GABA relates to *in vitro* binding at a novel site labeled by tritiated gabapentin. *Epilepsy Res.*, **14**, 11–15 (1993).

8) Stahl SM, Porreca F, Taylor CP, Cheung R, Thorpe AJ, Clair A. The diverse therapeutic actions of pregabalin: is a single mechanism responsible for several pharmacological activities? *Trends Pharmacol. Sci.*, **34**, 332–339 (2013).

9) Zaccara G, Gangemi P, Perucca P, Specchio L. The adverse event profile of pregabalin: a systematic review and meta-analysis of randomized controlled trials. *Epilepsia*, **52**, 826–836 (2011).

10) Muratake H, Ito A, Toda T, Suzuki H, Fukasawa H, Tsuda M, Inoue K, Sugiyama K, Shudo K. (*R*)- and (*S*)-4-Amino-3-(trimethylsilyl)-methylbutanoic acids ameliorate neuropathic pain without central nervous system-related side effects. *Bioorg. Med. Chem. Lett.*, **22**, 7602–7604 (2012).

11) Seltzer Z, Dubner R, Shir Y. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. *Pain*, **43**, 205–218 (1990).

12) Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain*, **50**, 355–363 (1992).

13) Tuchman M, Barrett JA, Donevan S, Hedberg TG, Taylor CP. Central sensitization and Ca(V)alpha(2)delta ligands in chronic pain syndromes: pathologic processes and pharmacologic effect. *J. Pain*, **11**, 1241–1249 (2010).

14) Bryans JS, Wustrow DJ. 3-Substituted GABA analogs with central nervous system activity: a review. *Med. Res. Rev.*, **19**, 149–177 (1999).

15) Fukasawa H, Muratake H, Ito A, Suzuki H, Amano Y, Nagae M, Sugiyama K, Shudo K. Silicon-containing GABA derivatives, silagaba compounds, as orally effective agents for treating neuropathic pain without central-nervous-system-related side effects. *ACS Chem. Neurosci.*, **5**, 525–532 (2014).

16) Chen SR, Xu Z, Pan HL. Stereospecific effect of pregabalin on ectopic afferent discharges and neuropathic pain induced by sciatic nerve ligation in rats. *Anesthesiology*, **95**, 1473–1479 (2001).

17) Plaza-Villegas F, Heir G, Markman S, Khan J, Noma N, Benoliel R, Patel J, Eliav E. Topical pregabalin and diclofenac for the treatment of neuropathic orofacial pain in rats. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.*, **114**, 449–456 (2012).

18) Field MJ, Cox PJ, Stott E, Melrose H, Offord J, Su TZ, Bramwell S, Corradini L, England S, Winks J, Kinloch RA, Hendrich J, Dolphin AC, Webb T, Williams D. Identification of the $\alpha 2$-δ-1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of pregabalin. *Proc. Natl. Acad. Sci. U.S.A.*, **103**, 17537–17542 (2006).

19) Li Z, Taylor CP, Weber M, Piechan J, Prior F, Bian F, Cui M, Hoffman D, Donevan S. Pregabalin is a potent and selective ligand for $\alpha_2\delta$-1 and $\alpha_2\delta$-2 calcium channel subunits. *Eur. J. Pharmacol.*, **667**, 80–90 (2011).