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ORIGINAL ARTICLE

Evaluation of in vitro transdermal permeation, mass spectrometric imaging, and in vivo analgesic effects of pregabalin using a pluronic lecithin organogel formulation in mice

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Abstract

In clinical practice, pregabalin is orally administered for neuropathic pain, but causes severe central nervous system side effects, such as dizziness, which results in dose limitation or discontinuation. To reduce the central side effects of pregabalin, we developed four pregabalin preparations for transdermal application: 0.4% aqueous solution, pluronic lecithin organogel (PLO gel), hydrophilic cream, and lipophilic cream. Transdermal permeabilities of pregabalin among the four formulations were compared in vitro using hairless mouse skin. The longitudinal distribution of pregabalin within the skin was analyzed using mass spectrometric (MS) imaging. Furthermore, the in vivo analgesic effects of the formulations were evaluated using the von Frey filament test in a mouse model of diabetic neuropathy (DN). The PLO gel showed the highest permeability of pregabalin, followed by the aqueous solution, and no permeation was observed in the two cream formulations. The MS imaging analysis showed that pregabalin was distributed up to the dermis in the PLO gel 1 h after application, while the aqueous solution was distributed near the epidermis. A significant analgesic effect ($p < .05$) was observed 1.5 h after PLO gel application in the DN model mice, but the aqueous solution had no effect. This study indicated for the first time that pregabalin penetrated beyond the skin epidermis up to the dermis, from the PLO gel formulation, and that the application of this formulation exhibited an in vivo analgesic effect in the mouse model of DN.

KEYWORDS
analgesic effect, MS imaging, PLO gel, pregabalin, transdermal permeation, von Frey test

1 | INTRODUCTION

According to the revised International Association for the Study of Pain definition, painful neuropathy or peripheral neuropathic pain (PNP) is the primary neurological condition of pain[1] PNP often becomes chronic with marked long-term reduction in quality of life.[2] Oral pregabalin has been recommended for the treatment of neuropathic pain associated with diabetic peripheral neuropathy and postherpetic neuralgia.[3] It reduces neuronal excitability in the central nervous system by reversibly binding to the alpha-2-delta subunits of Ca^{2+} channels.[4] Pregabalin has been reported to suppress peripheral ectopic afferent discharges caused by nerve injury, and this mechanism may also be involved in its analgesic effect on neuropathic pain.[5,6] Compared with other drugs, pregabalin has been reported to show one of the highest pain reductions.[7] However, it has side effects, such as dizziness, somnolence (sleepiness), and edema, which may be severe enough to be either dose-limiting or lead to discontinuation in clinical practice.[8] In a previous study, transdermal application of an aqueous pregabalin solution was reported to be effective against neuropathic pain in rats and mice.[9] Since the central nervous system (CNS)-mediated side effects of orally administered pregabalin are expected to be avoided or minimized by transdermal delivery of the drug, topical application *via* the transdermal route would substantially benefit patients. We employed a pluronic lecithin organogel (PLO gel) formulation of pregabalin and compared its permeability and analgesic effect with an aqueous solution and cream formulations.

A PLO gel is a microemulsion-based gel formulation that can enhance transdermal delivery of hydrophilic and lipophilic drugs across the stratum corneum, as reported for various drugs.[10] Bryson et al. reported the analgesic effect of the topical administration of gabapentin PLO gel in preclinical formalin pain hamster models.[11] Gabapentin has a similar mechanism of action and pharmacological effect as pregabalin and has relatively comparable physicochemical properties, such as molecular weight, logP, and melting point. Therefore, a PLO gel preparation of pregabalin may also have an analgesic effect. However, there has been no scientific evidence to show that pregabalin can permeate through the stratum corneum down into the skin dermis.

In this study, we prepared four types of 0.4% pregabalin formulations: aqueous solution, PLO gel, and hydrophilic and lipophilic creams. We compared the in vitro transdermal permeability and in vivo analgesic effects of these formulations. The in vitro transdermal permeability of pregabalin was evaluated using Franz diffusion cells and hairless mouse skin. Imaging analysis using mass spectrometry (MS) was performed for the first time to evaluate pregabalin penetration within the skin. Furthermore, the in vivo analgesic effects of the pregabalin PLO gel were assessed using a von Frey filament test in a diabetic disorder mouse model and compared with the aqueous solution.

2 | MATERIALS AND METHODS

2.1 | Materials and animals

Pregabalin capsules were from Pfizer. The PLO gel was from Pharmedica Enterprise. Hydrophilic cream, plastic base, and purified lanolin were from Nipro, Taisho Pharmaceutical, and Nikko Pharmaceutical, respectively. Both 5-fluorouracil and streptozotocin were from Sigma-Aldrich. D-phosphate buffered saline (PBS) (–) powder, KH_2PO_4, $NaHPO_4$, propylene glycol, and acetonitrile were from Fujifilm Wako. Laboskin prepared from the back skin of hairless mice (♂, 7Ws) was purchased from Hoshino Animal Laboratory.

2.2 | Preparation of transdermal formulations of pregabalin

Preparation of the 0.4% aqueous pregabalin solution: A pregabalin capsule (150 mg) was decapsulated and added to 15 ml of purified water. The solution was stirred for 10 min using a water bath shaker (Taitec) and centrifuged at 100*g* at 4°C for 10 min. The supernatant was collected and deemed to be a 1% aqueous solution. This was then, diluted with purified water to make a 0.4% aqueous solution.

Preparation of 0.4% pregabalin PLO gel: A 1% aqueous solution of pregabalin (0.5 ml) described above was mixed with 0.208 ml of propylene glycol. This solution was then, mixed with 0.458 ml of the oil phase solution of a PLO gel kit, and the aqueous phase solution of the kit was added to make a total amount of 1250 mg. The oil phase solution contained lecithin, isopropyl palmitate, and sorbic acid. The aqueous phase solution contained poloxamer 407, potassium sorbate, and water.

Preparation of 0.4% pregabalin hydrophilic cream: A 1% aqueous solution of pregabalin (0.5 ml) was mixed with 0.208 ml of propylene glycol. Then, a hydrophilic cream was added to a total of 1250 mg.

Preparation of 0.4% lipophilic cream: The 1% aqueous solution of pregabalin (0.5 ml) was mixed with 5 mg purified lanolin. Then, a plastic base was added to a total of 1250 mg.

2.3 | In vitro evaluation of pregabalin skin permeability

The skin permeability of pregabalin was evaluated using a Franz diffusion cell (Hanson Research). Hairless mouse skin on the dermal side was mounted to contact the receptor compartment on a Franz diffusion cell. The donor/diffusion area was 0.65 cm^2, and the receptor compartment was filled with PBS (7 ml). Before using, the skin was slowly thawed, cut into appropriately sized pieces, and defrosted in PBS. The receptor compartment of each cell was maintained at 32°C under synchronous continuous stirring using a magnetic stirrer at 600 rpm. The procedure was performed while confirming that there were no air bubbles between the skin and the receptor compartment.

Each pregabalin preparation (310 mg) was applied to hairless mouse skin. At each time point (1, 2, or 3 h), 1 ml of a receptor sample was withdrawn from the sampling port and immediately replaced with an equal volume of PBS. An internal standard (IS) solution (20 μl of 0.01 mg/ml 5-FU) was added to each sample (180 μl) and the mixture was vortexed for 10 min. The samples were then analyzed using the validated HPLC-UV method described below.

2.4 | Determination of pregabalin concentration using high-performance liquid chromatography-ultraviolet detection (HPLC-UV)

The HPLC system consisted of a Prominence series (Shimadzu) equipped with LC20AT, CTO20A, and CBM-20A coupled with UV-VIS detection (SPD-20A). Data collection and analysis were performed using LC solution software (Shimadzu). Separation was achieved on a TSK gel ODS-100V column (150 × 4.6 mm, 5 μm, TOSOH) at a flow rate of 1.0 ml/min. The mobile phase was acetonitrile: 10 mM KH_2PO_4/10 mM $NaHPO_4$ (pH 6.9) (3:97).[12] The IS solution was prepared by dissolving 5-FU in purified water to a concentration of 0.01 mg/ml. The injection volume was 20 μl, and UV detection was performed at 200 nm. The calibration curve was linear, with an R^2 value of more than 0.99.

2.5 | MS imaging for evaluating pregabalin penetration into the skin

We prepared four skin samples for the following treatments: 0.4% pregabalin PLO gel (1 and 3 h after application) and 0.4% pregabalin aqueous solution (1 and 3 h after application). Skin samples were frozen at −80°C, sliced to a thickness of 10 μm to make skin depth section, and placed on an ITO-coated glass slide (Matsunami Glass). The sliced samples were coated to 0.7 μm thickness with α-cyano-4-hydroxycinnamic acid (CHCA) using the sublimation method by iMLayer (Shimadzu). After the matrix was coated, MS imaging was performed using iMScope *TRIO* (Shimadzu) to evaluate the penetration of pregabalin into the skin. The conditions of MS imaging are shown in Table 1. The tissue sections were uniformly coated with a matrix ionization aid, irradiated with a laser, ionized for MS.

2.6 | Preparation of a mouse model of diabetic neuropathy (DN)

Male C57BL/6J mice were maintained in a 12/12 h light/dark cycle with free access to food and water. A single dose of streptozotocin (8 mg/ml, 25 ml/kg) in saline water was administered intraperitoneally to each mouse. After 1 week, blood was collected from the tail vein, and the blood glucose level was measured using a glucose meter (Medisafe mini GR102; Terumo) under isoflurane anesthesia. Mice with blood glucose levels exceeding 400 mg/dl were defined as DN model mice.[13]

TABLE 1 Measurement conditions of iMScope *TRIO*

MS parameter	
Measuring pitch (μm)	10
Ion polarity	Positive
Mass range	*m/z* 156–163
Accumulations (count/pixel)	1
Sample voltage (kV)	3.5
Detector voltage (kV)	2.1
No. of laser shots	50
Laser frequency (Hz)	1000
Laser irradiation diameter setting (μm)	10
Laser intensity	24

Note: MS parameter of the iMScope *TRIO*. All skin samples were measured under the same conditions using these MS parameters.

2.7 | von Frey filament test for evaluating the in vivo analgesic effects of pregabalin formulations in DN model mice

The von Frey filament test, which has been considered as a standard method to evaluate allodynia in vivo,[14,15] was performed according to a previous study[13] with some modifications. Under isoflurane anesthesia, PLO gel without pregabalin (control) was applied to the back of the right foot and 0.4% pregabalin PLO gel was applied to the back of the left foot. (Figure 1) The amount of PLO gel used was 40 mg. Regarding the aqueous solution, 40 μl of purified water (control) and 0.4% aqueous pregabalin solution were applied to the back of the left and right foot of the mice, respectively, under isoflurane anesthesia. After 30 min of anesthesia, each mouse was acclimatized to a meshed table in a transparent box until further evaluation. The von Frey filament test was performed using an Ugo Basile Dynamic Planter Anesthesiometer 37450 (Ugo Basile Biological Research Apparatus Company). The filament (0.5 mm diameter) was applied to each target area (back paw) at 1 g/s, and the time until withdrawal motion was measured (time to reaction). Measurements were repeated three times for each point, and the average value was used for the analysis. The test was performed at 23 ± 2°C and a humidity of 50% ± 20%.

2.8 | Statistical analysis

Statistical analyses for comparison were conducted using Student's *t*-test between two groups. Statistical significance was set at $p < .05$. Statistical analyses were performed using JMP Pro version 14 (SAS Institute Japan).

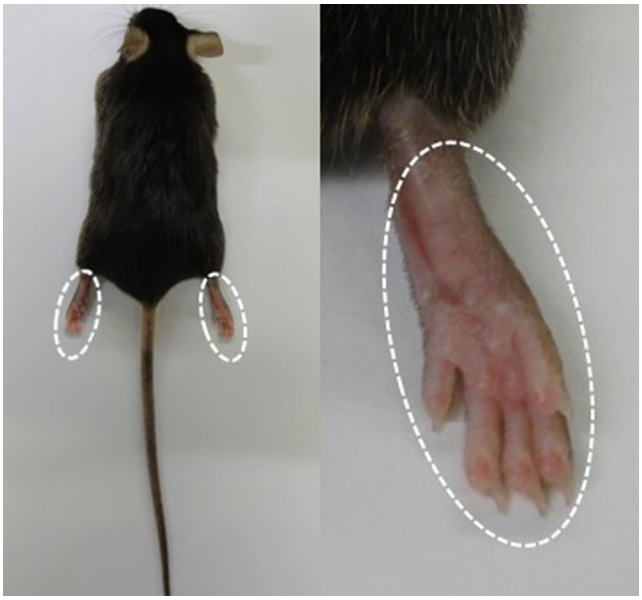

FIGURE 1 The locations of the topical application. The formulations were applied to the back of the feet in DN model mice. The locations we applied are circled with a dotted line. The photo on the right is an enlargement of the photo on the left

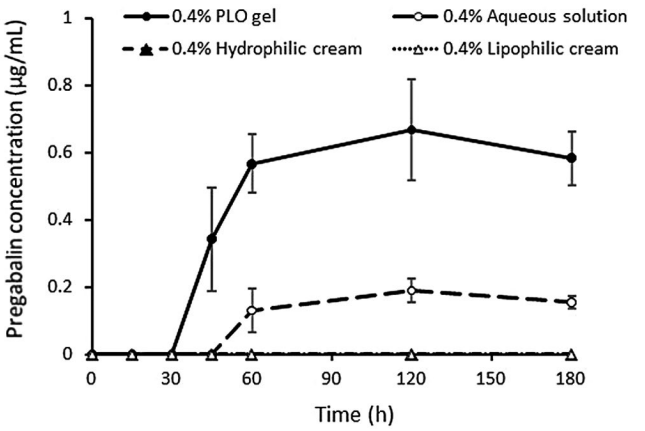

FIGURE 2 Skin permeability of 0.4% pregabalin formulations. Each value is expressed as the mean ± SEM ($n = 5$ for PLO gel and aqueous solution, $n = 4$ for hydrophilic and lipophilic creams) $^*p < .05$, versus aqueous solution. In vitro skin permeability of pregabalin was observed in both the aqueous solution and PLO gel formulations. Pregabalin in the 0.4% PLO gel formulation permeated approximately four times more than in the aqueous solution. Skin permeation was not observed in the hydrophilic and lipophilic cream formulations

3 | RESULTS

3.1 | In vitro skin permeability evaluation of pregabalin

The skin permeability of pregabalin was assessed in the aqueous solution and PLO gel formulations. Pregabalin was first detected in the receiver flask 45 min after the application of the PLO gel, and was detected 1 h after the application of the aqueous solution. Figure 2 shows that pregabalin in the 0.4% PLO gel formulation permeated approximately four times more than the aqueous solution. Skin permeation was not observed in either hydrophilic or hydrophobic cream formulations.

3.2 | MS imaging for evaluating pregabalin skin penetration

In the in vitro transdermal permeability studies, permeation of pregabalin was observed in the aqueous solution and the PLO gel (Figure 2), so MS imaging was performed for these two formulations to evaluate the penetration depth of pregabalin within the skin. Pregabalin-derived peaks were observed at m/z 160.134 in both formulations as shown in Figure 3. The MS imaging on the right panel and the optical image of the left panel in (A)–(D) are the same sections. Figure 4 shows the distribution of pregabalin in the skin after application. Pregabalin was mainly found in the epidermis, whereas it was distributed to the dermis in the PLO gel. Both formulations appeared to increase the amount of pregabalin deposited over time (1 to 3 h after application).

3.3 | von Frey filament test for evaluating the in vivo analgesic effects of pregabalin formulations in DN mouse model

The analgesic effects after transdermal application of 0.4% pregabalin PLO gel or an aqueous solution of pregabalin are shown in Figure 5. In the 0.4% pregabalin PLO gel, a significant analgesic effect was observed 1.5 h after drug application ($p = .0491$). This effect was also observed 3 h after drug application, but there was a slight trend toward significance ($p = .0650$). Concerning the aqueous solution of pregabalin, no significant analgesic effect was observed at either 1.5 or 3 h after transdermal application ($p = .497$ and .361, respectively).

4 | DISCUSSION

Hydrophilic compounds generally show low permeability through the skin with the stratum corneum (outer layer of the skin) being the primary barrier. In this study, we prepared transdermal formulations of pregabalin using various bases to improve its transdermal permeability. Due to the high solubility of pregabalin in water, the maximum concentration of pregabalin in an aqueous solution is 12 mg/ml (1.2% w/v) at 25°C.[16] We prepared pregabalin formulations from a 1% pregabalin aqueous solution by mixing with bases or additives, so the final concentration of the pregabalin preparations evaluated in this study was set at 0.4%.

In the in vitro skin permeability experiments using Franz diffusion cells, permeation of pregabalin was observed 45 min after the application of the PLO gel and 1 h for the aqueous solution. The results indicated higher skin permeation of the PLO gel than

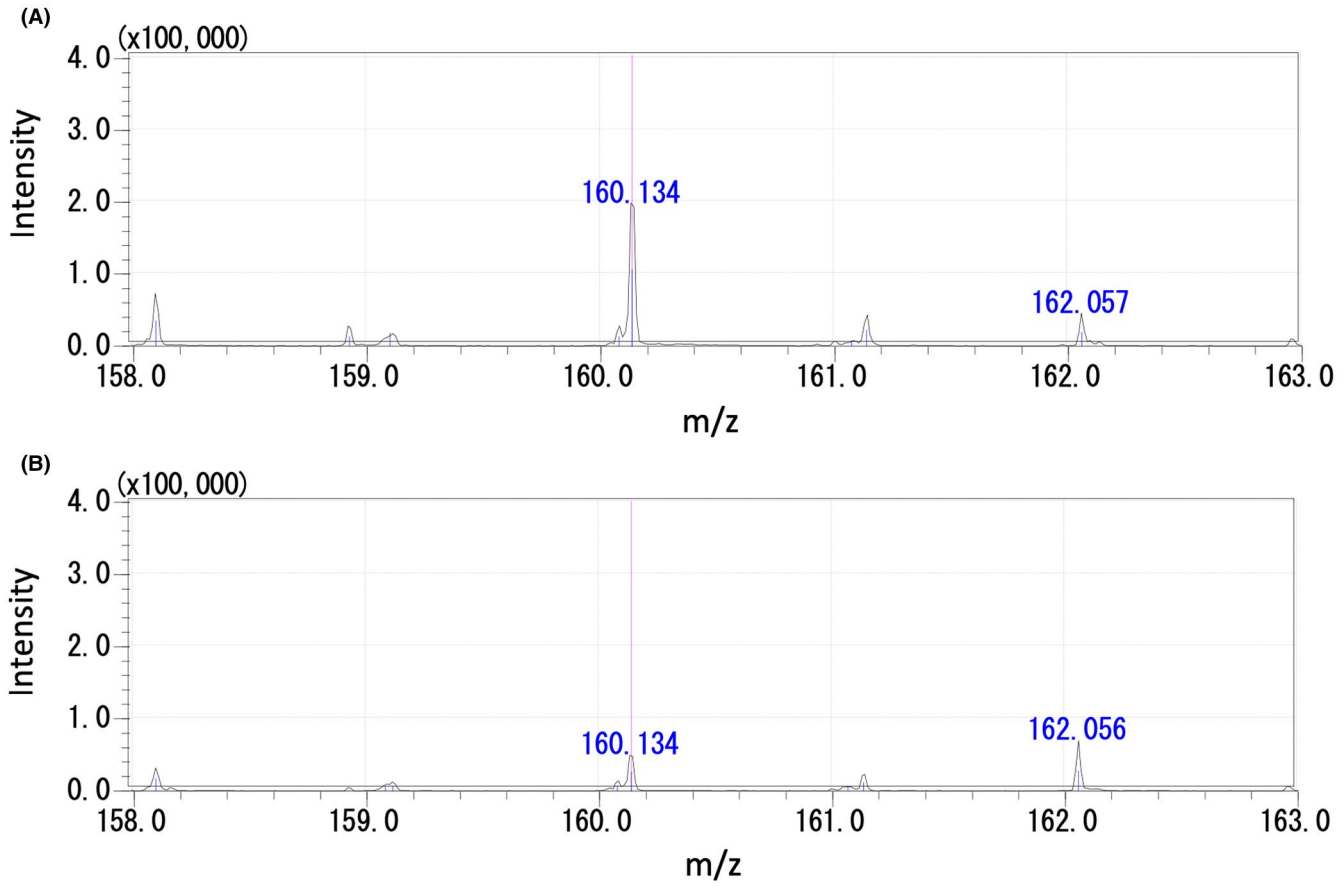

FIGURE 3 Mass spectra of pregabalin after application. (A) 0.4% PLO gel (B) 0.4% aqueous solution in the 0.4% PLO gel and 0.4% aqueous solution formulations, a pregabalin peak was confirmed at 160.134, and a pregabalin fragment peak was confirmed near 162.056 in the mass spectrum. This confirmed that pregabalin was present in the 0.4% PLO gel and 0.4% aqueous solution formulations

the aqueous solution (Figure 2). The base of the PLO gel preparation contained poloxamer 407, lecithin, and isopropyl palmitate to improve drug skin permeability.[17] Poloxamer 407 is a block copolymer composed of a polyoxypropylene chain (POP) and polyoxyethylene acid (POE) as a surfactant. It has amphiphilic properties and binds to pregabalin to release it from the base.[18] Lecithin is a phospholipid that functions as an absorption enhancer by softening the stratum corneum and creating gaps in its structure. Isopropyl palmitic acid is an ester with the excellent spreading ability and enhances the effects of the formulations by solubilizing lecithin. These components increased drug diffusion in the base and the affinity for the skin, which may have enhanced the skin permeability of pregabalin.

In contrast, the permeation of pregabalin was not observed in the cream formulations. In the case of the hydrophilic cream preparation, it was considered that it had a high affinity with pregabalin, Hence, it was difficult for pregabalin to distribute from this preparation into the skin. Although the lipophilic cream preparation was thoroughly mixed with pregabalin, it is likely that pregabalin could not diffuse easily within it because of the hydrophilic properties of pregabalin, which resulted in the low permeability of this preparation.

The skin permeability of the PLO gel of gabapentin, which has similar physicochemical properties as pregabalin, was measured using a Franz diffusion cell.[11] In a previous study, 1 h the application of 300 mg of gabapentin PLO gel to porcine skin, 0.125 ± 0.118 μg/cm^2 gabapentin was found to have penetrated the skin. Gabapentin PLO gel was more permeable than the other formulations used in that study. In the present study, after 1 h after the application of 310 mg of pregabalin PLO gel to hairless mouse skin, the concentration of pregabalin in the receptor compartment was 0.568 μg/ml. This amount corresponded to 2.246 μg/cm^2, considering the volume and area of the Franz diffusion cell. It is speculated that the penetration of pregabalin into the skin has higher than gabapentin, but further investigation is necessary due to the different experimental conditions and not a direct comparison.

The pregabalin PLO gel did not have an increased penetration 2 and 3 h after application when it showed a steady state. On the contrary, the gabapentin PLO gel of the previous study showed an increase at 2 and 3 h after application, so there is a difference in the permeation behavior between pregabalin and gabapentin. The PLO gel of pregabalin has a higher immediate effect than that of gabapentin, and it may be easier to apply in clinical settings.

MS imaging indicated the differences in pregabalin skin penetration between the aqueous solution and the PLO gel. There is a report that the thickness of the epidermis is 7–9 μm, and that of the dermis is 360–380 μm in mice.[19] In the aqueous solution,

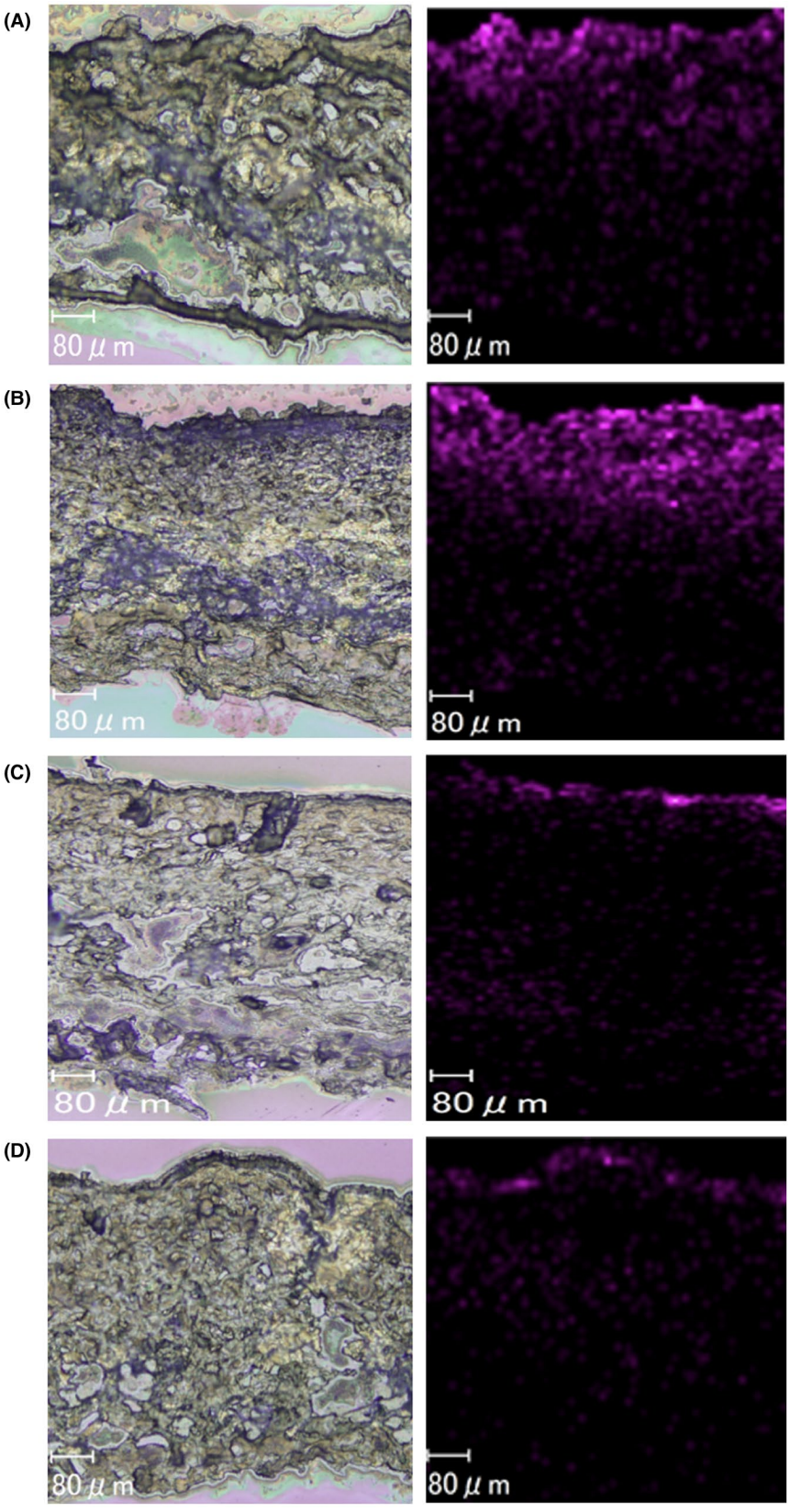

FIGURE 4 Distribution of pregabalin after application. (A) 1 h after application of 0.4% PLO gel (B) 3 h after application of 0.4% PLO gel (C) 1 h after application of 0.4% aqueous solution (D) 3 h after application of 0.4% aqueous solution. From the photographs of the skin, the extent to which pregabalin penetrated was confirmed. The intra-skin MS-images of pregabalin are indicated in pink in the right panels, which are matching with the optical images of the skin longitudinal sections. In (A, B), it was confirmed that pregabalin in 0.4% PLO gel formulation was distributed in the vicinity of the dermis over time. In (C, D), pregabalin in 0.4% aqueous solution was distributed near the epidermis over time

pregabalin was distributed mainly near the epidermis; however, in the PLO gel, pregabalin penetrated the dermis, where the skin nerves are located. This result suggests that pregabalin in the PLO gel can penetrate the dermis, affecting the peripheral nerves in the skin. In contrast, since the aqueous solution penetrated only near the epidermis, it could not reach the peripheral nerves, so an analgesic effect was unlikely to occur. The difference in the distribution properties of pregabalin between the PLO gel and the

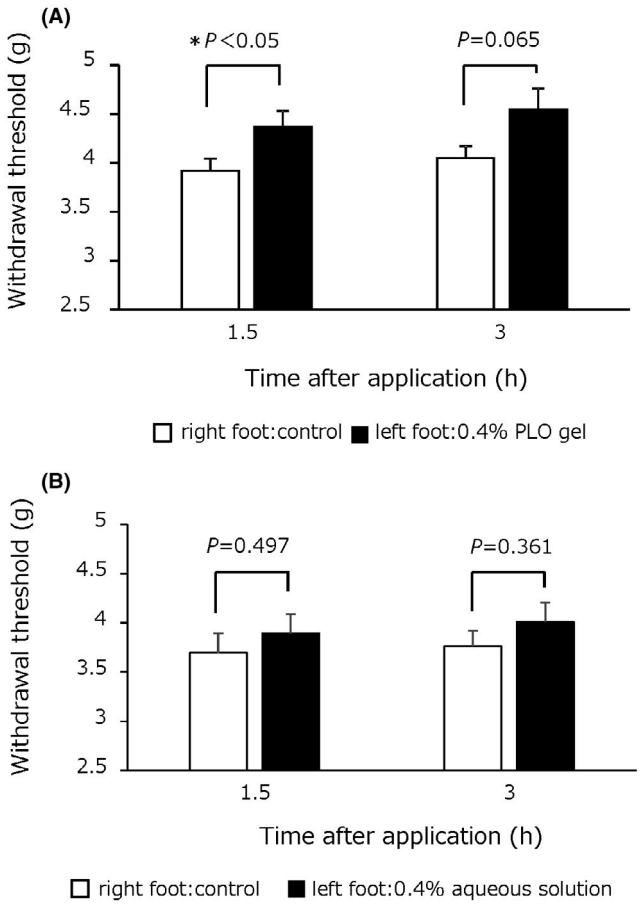

FIGURE 5 Analgesic effects of pregabalin in mouse models of diabetic neuropathy. (A) 0.4% pregabalin PLO gel ($n = 13$), (B) 0.4% pregabalin aqueous solution ($n = 13$) $^{*}p < .05$ versus control. 0.4% PLO gel and 0.4% aqueous solution of pregabalin were applied to mouse models of diabetic neuropathy at the left foot (control) and right foot (test formulation), and the analgesic effects were examined. A significant analgesic effect was observed 1.5 h after application of the 0.4% PLO gel, and a nonsignificant trend was observed after 3 h. There were no analgesic effects in the 0.4% aqueous solution at 1.5 and 3 h after application

aqueous solution, as demonstrated via MS imaging, supports the results of the pharmacological experiment in this study. Pregabalin in the PLO gel was distributed and accumulated in the dermis 3 h after application. However, no significant analgesic effect was observed 3 h after application in the pharmacological study, while the analgesic effect tended to remain ($p = .0650$) (Figure 5). This time discrepancy can be attributed to the differences in the experimental conditions, in the in vivo study, the sink condition (i.e., washout effect due to blood flow) may have helped the drug permeate continuously through the skin. The intensity of the pregabalin-associated MS imaging appeared to be corresponding to the in vivo analgesic effect.

Some studies have evaluated the analgesic effect of systemic pregabalin administration using the von Frey filament test.[7,13,20] In a previous study of the analgesic effect after intraperitoneal administration of 10 mg/kg pregabalin to DN model mice, the time to react was reported to be 3.29 ± 0.74 s for the control and 4.59 ± 0.59 s for pregabalin.[13] It is difficult to directly compare the values of time to react because the administration route of pregabalin was different; however, the value for the control observed in our study virtually agrees with the previous value noted above.

Some studies have also reported the analgesic effect of transdermal pregabalin preparations, that is, an aqueous solution or PLO gel of pregabalin.[9,21] According to one report,[9] an aqueous solution of pregabalin (0.25 or 0.75%) had a significant analgesic effect 1.5 h after application in a spinal nerve ligation model in mice. This previous study did not clearly state the amount applied, and it was performed using different pain models from the one used in the present study, which might have led to different results. According to another report,[21] topical application of a 10% pregabalin PLO gel to the infraorbital nerve territory in the vibrissae area significantly increased the threshold of tactile allodynia. There are some reports for the expression of alpha-2-delta containing voltage-dependent calcium channels on the skin,[22,23] and it is considered that the action of pregabalin is mediated by them.

In the present study, transdermal administration of a 0.4% pregabalin PLO gel was shown to have an analgesic effect in diabetic neuropathy for the first time, which was found at lower and, more clinically viable concentrations.

In this study, we applied the drug to mouse skin to evaluate the permeability of pregabalin. However, when the drug is applied to human skin in clinical settings, skin permeability may change due to differences between mice and human skin. In addition, although this was only a single-dose study, pregabalin PLO gels may be applied repeatedly in clinical settings, and further research on the analgesic effects and side effects of repeated applications is necessary.

In conclusion, this study indicated for the first time that pregabalin penetrated beyond the skin epidermis up to the dermis, from the PLO gel formulation, and that the application of this formulation exhibited an in vivo analgesic effect in the mouse model of diabetic neuropathy.

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DISCLOSURE

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Nagao, Tajima, Sugiyama, Yoshikawa, Nobe, and Sato H participated in the research design. Nagao, Shinouchi, Shibata, and Yamamoto conducted experiments. Nagao and Sato VH performed data analysis. Nagao, Tajima, Sugiyama, Shinouchi, Shibata, Sato VH, and Sato H wrote or contributed to the writing of the manuscript.

ETHICS STATEMENT

All experiments were conducted in accordance with the regulations of the Committee of Animal Care and Welfare of the Showa

University (permit number: 29008) and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health.

DATE AVAILABILITY STATEMENT

We share the date and other artifacts supporting the results in the paper by archiving them in an appropriate public repository.

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REFERENCES

1. Treede RD, Jensen TS, Campbell JN, et al. Neuropathic pain: redefinition and a grading system for clinical and research purpose. *Neurology*. 2008;70:1630-1635.
2. O'Connor AB. Neuropathic pain: quality-of-life impact, costs and cost effectiveness of therapy. *Pharmacoeconomics*. 2009;27:95-112.
3. JCQHC. Guidelines for the Pharmacologic Management of Neuropathic pain 2nd Edition 2016. Accessed May 30, 2021. Pharm acologic-Management-of-Neuropathic-pain-ENGver.pdf. Available at (jcqhc.or.jp)
4. Taylor CP, Angelotti T, Fauman E. Pharmacology and mechanism of action of pregabalin: the calcium channel alpha2-delta (alpha2-delta) subunit as a target for antiepileptic drug discovery. *Epilepsy Res*. 2007;73:137-150.
5. Ney JP, Devine EB, Watanabe JH, Sullivan SD. Comparative efficacy of oral pharmaceuticals for the treatment of chronic peripheral neuropathic pain: meta-analysis and indirect treatment comparisons. *Pain Med*. 2013;14:706-719.
6. Freynhagen R, Serpell M, Emir B, et al. A comprehensive drug safety evaluation of pregabalin in peripheral neuropathic pain. *Pain Pract*. 2015;15:47-57.
7. 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*. 2001;95:1473-1479.
8. Ochi Y, Harada T, Suzuki M, et al. Mechanism of action and clinical efficacy of pregabalin (Lyrica® capsule), a novel analgesic for peripheral neuropathic pain. *Jpn J Pharm Palliat Care Sci*. 2011;4:53-64.
9. Fukasawa H, Muratake H, Nagae M, Sugiyama K, Shudo K. Transdermal administration of aqueous pregabalin solution as a potential treatment option for patients with neuropathic pain to avoid central nervous system-mediated side effects. *Biol Pharm Bull*. 2014;37:1816-1819.
10. Murdan S. A review of pluronic lecithin organogel as a topical and transdermal drug delivery system. *Hosp Pharm*. 2005;12:267-270.
11. Bryson E, Asbill S, Sweitzer S. Skin permeation and antinociception of topical gabapentin formulations. *Int J Pharm Compd*. 2014;18:504-511.
12. Ciavarella AB, Gupta A, Sayeed VA, Khan MA, Faustino PJ. Development and application of a validated HPLC method for the determination of gabapentin and its major degradation impurity in drug products. *J Pharm Biomed Anal*. 2007;43:1647-1653.
13. Shinouchi R, Shibata K, Hashimoto T, Jono S, Hasumi K, Nobe K. SMTP-44D improves diabetic neuropathy symptoms in mice through its antioxidant and anti-inflammatory activities. *Pharmacol Res Perspect*. 2020;8:e00648.
14. Valsecchi AE, Franchi S, Panerai AE, Rossi A, Sacerdote P, Colleoni M. The soy isoflavone genistein reverses oxidative and inflammatory state, neuropathic pain, neurotrophic and vasculature deficits in diabetes mouse model. *Eur J Phamacol*. 2011;650:694-702.
15. Jang Y, Song H-K, Yeom MY, Jeong DC. The immunomodulatory effect of pregabalin on spleen cells in neuropathic mice. *IARS*. 2012;115:830-836.
16. Cid. NCBI. 54869771 (Pregabalin)-PubChem 2007. Accessed January 31, 2021. https://pubchem.ncbi.nlm.nih.gov/compound/5486971
17. Belgamwar VS, Pandey MS, Chauk DS, Surana SJ. Pluronic lecithin organogel. *Asian J Pharm*. 2008;2:134-138.
18. Almeida H, Amaral MH, Lobão P, Lobo JMS. Pluronic® F-127 and pluronic lecithin organogel (PLO): main features and their applications in topical and transdermal administration of drugs. *J Pharm Pharm Sci*. 2012;15:592-605.
19. Denda M, Sato J, Masuda Y, et al. Exposure to a dry environment enhances epidermal permeability barrier function. *J Invest Dermatol*. 1998;111:858-863.
20. Park HJ, Joo HS, Chang HW, et al. Attenuation of neuropathy-induced allodynia following intraplantar injection of pregabalin. *Can J Anaesth*. 2010;57:664-671.
21. Plaza-Villegas F, Heir G, Markman S, et al. Topical pregabalin and diclofenac for the treatment of neuropathic orofacial pain in rats. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2012;114:449-456.
22. Kitamura R, Andoh T, Mizoguchi S, Saito Y, Takahata H, Kuraishi Y. Gabapentin inhibits bortezomib-induced mechanical allodynia through supraspinal action in mice. *J Pharmacol Sci*. 2014;124:502-510.
23. Sann SB, Xu L, Nishimune H, et al. Neurite outgrowth and in vivo sensory innervation mediated by a CaV2.2–laminin β2 stop signal. *J Neurosci*. 2008;28:2366-2374.

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