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Analgesic effects of the novel $\alpha_2\delta$ ligand mirogabalin in a rat model of spinal cord injury

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Mirogabalin, which is a novel ligand for the $\alpha_2\delta$ subunit of voltage-gated calcium channels, is under development for the treatment of pain associated with diabetic peripheral neuropathy and postherpetic neuralgia. Mirogabalin possesses unique binding characteristics to $\alpha_2\delta$ subunits and potent and long-lasting analgesic effects in peripheral neuropathic pain models. In the present study, we investigated the analgesic effects of mirogabalin in a rat model of spinal cord injury as an experimental animal model for central neuropathic pain. The spinal cord injury model was established by acute compression of the spinal cord at the T6/7 level with a microvascular clip in male rats. Twenty-eight days after spinal cord injury, the animals received the test compound orally, and the paw withdrawal threshold to mechanical stimulation was determined using the von Frey test at 0 (before administration), 2, 4, 6, 8, and 24 h after administration. The area under the curve of the paw withdrawal threshold (paw withdrawal threshold AUC) was also calculated. In rats subjected to spinal cord injury, mechanical allodynia was demonstrated by a decreased paw withdrawal threshold. A single oral administration of mirogabalin (2.5, 5, or 10 mg/kg) significantly increased the paw withdrawal threshold. The effects of mirogabalin were still significant 6 or 8 h after administration. The paw withdrawal threshold AUC was significantly higher in the treated animals than in the control group. In conclusion, mirogabalin showed potent and long-lasting analgesic effects in a rat model of spinal cord injury and may therefore provide effective pain relief for patients with central neuropathic pain.

1. Introduction

Gabapentinoids, such as pregabalin and gabapentin, are selective ligands for the $\alpha_2\delta$ subunit of voltage-gated calcium channels (Li et al. 2011; Alexander et al. 2015) and exert various pharmacological effects, including analgesia and anticonvulsant and anxiolytic activities (Sills 2006; Dooley et al. 2007; Stahl et al. 2013). They also have analgesic effect in neuropathic pain, and several scientific associations and guidelines recommend pregabalin and gabapentin as first-line drugs for the treatment of neuropathic pain (Finnerup and Jensen 2007; O'Connor and Dworkin 2009; Attal et al. 2010; Bril et al. 2011; Dworkin et al. 2013; Cohen et al. 2015; Cruccu and Truini 2017).

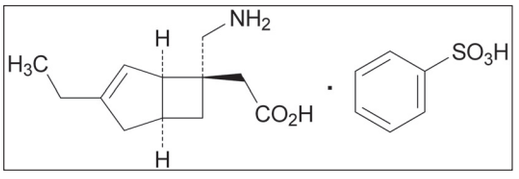

Fig. 1: Chemical structure of mirogabalin besylate.

Mirogabalin ([(1*R*,5*S*,6*S*)-6-(aminomethyl)-3-ethylbicyclo[3.2.0]hept-3-en-6-yl]acetic acid) is a novel ligand for the $\alpha_2\delta$ subunit of voltage-gated calcium channels (the chemical structure is shown in Fig. 1) and is under development for the treatment of pain associated with diabetic peripheral neuropathy and postherpetic neuralgia. We have previously reported that mirogabalin has unique binding characteristics to $\alpha_2\delta$ subunits and potent and long-lasting analgesic effects in peripheral neuropathic pain models (Domon et al. 2018). In the present study, we investigated the analgesic effects of mirogabalin in a rat model of spinal cord injury (SCI) as an experimental animal model for central neuropathic pain.

2. Investigations, results and discussion

The SCI model was established as acute compression of the spinal cord in rats. Twenty-eight days after SCI, the animals received the test compound, and the paw withdrawal threshold to mechanical stimulation was determined using the von Frey test. Fig. 2 shows the time-course changes in the paw withdrawal threshold (A) and the paw withdrawal threshold AUC (B). In the SCI model rats, mechanical allodynia was demonstrated by a significantly decreased paw withdrawal threshold. Oral administration of mirogabalin (2.5, 5, or 10 mg/kg) significantly increased the paw withdrawal threshold. The effects of mirogabalin peaked 4 h after administration and persisted until 6 or 8 h after administration. The paw withdrawal threshold $AUC_{0\text{-}8h}$ and $AUC_{0\text{-}24h}$ in the mirogabalin treatment groups were significantly higher than those in the vehicle control group.

In our preliminary study (N = 5, data not shown), oral administration of pregabalin had no effect on the paw withdrawal threshold at 10 mg/kg, and it increased the threshold at 30 mg/kg. The effects of 30 mg/kg pregabalin peaked 4 h after administration and returned to the vehicle control level after 8 h. Pregabalin has also been shown to increase the paw withdrawal threshold in a mouse model of SCI at intraperitoneal doses of 10 and 30 mg/kg (Tanabe et al. 2009). We previously reported that mirogabalin had more potent and longer-lasting analgesic effects than pregabalin in peripheral neuropathic pain models, such as rats subjected to partial sciatic nerve ligation and streptozotocin-induced diabetic rats (Domon et al. 2018). The greater binding affinity of mirogabalin for the $\alpha_2\delta$-1 subunit is thought to contribute to its potent and long-lasting analgesic effects (Domon et al. 2018). A phase II proof-of-concept study

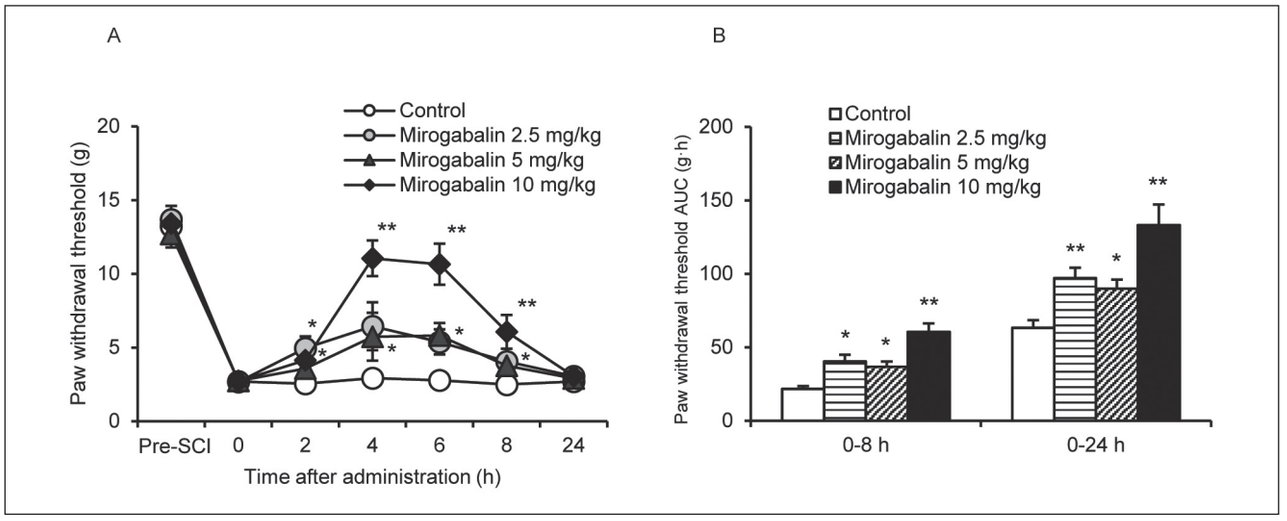

Fig. 2: Analgesic effects of mirogabalin in rats subjected to spinal cord injury (SCI). A: Time-course changes in the 50% paw withdrawal threshold. B: Paw withdrawal threshold AUC. Mirogabalin besylate was administered orally (10 mL/kg). The control group received distilled water. Dose levels are expressed for the free form of the drug. Each value represents the mean ± standard error (N = 10). *$P < 0.05$, **$P < 0.01$: Significantly different from the control group by Steel's test. Wilcoxon's signed-rank test revealed significant differences between the pre-SCI and post-SCI assessments (Day -1 vs. Day 28, $P < 0.0001$, N = 40).

demonstrated that mirogabalin was effective and well-tolerated in patients with diabetic peripheral neuropathy (Vinik et al. 2014; Merante et al. 2017). In the present study, mirogabalin had potent and long-lasting analgesic effects in a rat model of SCI, which was used as an experimental animal model for central neuropathic pain. The effective doses of mirogabalin and pregabalin in the SCI model were similar to those in peripheral neuropathic pain models (Table). Pregabalin has been licensed in many countries and is widely used for the treatment of central and peripheral neuropathic pain (Finnerup and Jensen 2007; O'Connor and Dworkin 2009; Cruccu and Truini 2017). Like pregabalin, mirogabalin is expected to have beneficial effects on central neuropathic pain.

Table: Effective doses of mirogabalin and pregabalin in rat neuropathic pain models

		Mirogabalin	Pregabalin
Central Neuropathic pain	SCI model	2.5, 5, and 10 mg/kg	30 mg/kg
Peripheral Neuropathic pain	PSL model	3 and 10 mg/kg	10 and 30 mg/kg
	STZ-diabetic model	2.5, 5, and 10 mg/kg	10, 20, and 40 mg/kg
(Domon et al. 2018)		(ED_{50} = 4.4 mg/kg)	(ED_{50} = 26.8 mg/kg)

Dose levels of test compounds are expressed for the compound in free form.
SCI: spinal cord injury
PSL: partial sciatic nerve ligation
STZ: streptozotocin

In conclusion, mirogabalin had potent and long-lasting analgesic effects in a rat model of SCI. Mirogabalin may provide effective pain relief for patients with central neuropathic pain.

3. Experimental

3.1. Chemicals

Mirogabalin besylate (code number: DS-5565) was synthesised by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Mirogabalin besylate was dissolved in distilled water (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) and administered orally at a volume of 10 mL/kg. The dose levels presented reflect that of the free form of the drug. All other reagents were of analytical grade and obtained from conventional commercial sources.

3.2. Animals

We used 6-week-old male Crl:CD(SD) rats (Charles River Laboratories Japan, Inc., Kanagawa, Japan). The animals were housed under conditions of regulated temperature (20–28 °C) and relative humidity (30–80 %) in a room with a 12 h light-dark cycle (lights on 7:00–19:00 h). A commercial diet (CRF-1, Oriental Yeast Co., Ltd.; Tokyo, Japan) and tap water were available *ad libitum*. The animals were randomly allocated to the study groups using a computed randomisation procedure based on paw withdrawal threshold and body weight. All procedures for pain assessment were conducted by an experimenter blinded to the treatment conditions.
All experimental procedures were performed in accordance with the Basic Guidelines for the Use of Experimental Animals in Institutions under the Jurisdiction of the Ministry of Health, Labour and Welfare (Notification No. 0601001 of the Science Bureau, Japanese Ministry of Health, Labour and Welfare, June 1, 2006), the Guidelines for Animal Experimentation by the Animal Care and Use Committee at Hamamatsu Pharma Research, Inc. and the Guideline of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd.

3.3. Preparation of SCI model

The SCI model was established as described in previous reports (Rivlin and Tator 1978; Hama and Sagen 2007). Under isoflurane anaesthesia, a laminectomy was performed to expose spinal cord segments T6/7. The spinal segment in the area within T6/7 was compressed for 60 s using a micro-vascular clip (#61-0196, Harvard Apparatus; Holliston, MA). After SCI surgery, the bladder was manually expressed twice daily until spontaneous bladder evacuation resumed (about 1 w post-surgery). Locomotor function was scored using a 21-point Basso, Beattie, and Bresnahan locomotor rating scale (BBB score, Basso et al. 1995) on the day before SCI (Day -1), and 1, 7, and 27 days after SCI. The exclusion criteria for the BBB score were "≥7 in both hind limbs" on Day 1 and "<7 in both hind limbs" on Day 27. No rats met the exclusion criteria on Day 1, and 3 rats were excluded on Day 27.

3.4. Assessment of pain response

Twenty-eight days after SCI, the 50% paw withdrawal threshold to mechanical stimulation was determined using von Frey filaments (North Coast Medical Inc.; Gilroy, CA). Rats subjected to SCI with paw withdrawal thresholds of 4 g or lower were selected and assigned to treatment groups. The rats received the test compound orally, and the von Frey test was conducted 0 (before administration), 2, 4, 6, 8, and 24 h after administration. The areas under the curves of the paw withdrawal threshold (paw withdrawal threshold $AUC_{0\text{-}8h}$ and $AUC_{0\text{-}24h}$) were calculated using the trapezoidal method.

3.5. Statistical analysis

Data are presented as the mean±standard error. Statistical analyses of the paw withdrawal threshold at each time point and the paw withdrawal threshold AUC were performed using the Steel test. Wilcoxon's signed-rank test was used to compare pre-SCI and post-SCI results (Day -1 vs. Day 28). Differences were considered significant when $P < 0.05$. SAS Analytics Pro version 9.3 (SAS Institute Japan Ltd., Tokyo, Japan) and EXSUS Version 8.0 (CAC Croit Corporation, Tokyo, Japan) were used for these analyses.

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