Binding Characteristics and Analgesic Effects of Mirogabalin, a Novel Ligand for the $\alpha_2\delta$ Subunit of Voltage-Gated Calcium Channels

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ABSTRACT

Mirogabalin ([1R,5S,6S]-6-(aminomethyl)-3-ethylbicyclo[3.2.0]hept-3-en-6-yl]acetic acid), a novel ligand for the $\alpha_2\delta$ subunit of voltage-gated calcium channels, is being developed to treat pain associated with diabetic peripheral neuropathy and postherpetic neuralgia. In the present study, we investigated the in vitro binding characteristics and in vivo analgesic effects of mirogabalin compared with those of pregabalin, a standard $\alpha_2\delta$ ligand. Mirogabalin showed potent and selective binding affinities for the $\alpha_2\delta$ subunits, while having no effects on 186 off-target proteins. Similar to pregabalin, mirogabalin did not show clear subtype selectivity ($\alpha_2\delta$-1 vs. $\alpha_2\delta$-2) or species differences (human vs. rat). However, in contrast to pregabalin, mirogabalin showed greater binding affinities for human $\alpha_2\delta$-1, human $\alpha_2\delta$-2, rat $\alpha_2\delta$-1, and rat $\alpha_2\delta$-2 subunits; further, it had a slower dissociation rate for the $\alpha_2\delta$-1 subunit than the $\alpha_2\delta$-2 subunit. Additionally, in experimental neuropathic pain models, partial sciatic nerve ligation rats and streptozotocin-induced diabetic rats, mirogabalin showed more potent and longer lasting analgesic effects. In safety pharmacological evaluations, mirogabalin and pregabalin inhibited rota-rod performance and locomotor activity in rats; however, the safety indices of mirogabalin were superior to those of pregabalin. In conclusion, mirogabalin shows potent and selective binding affinities for the human and rat $\alpha_2\delta$ subunits, and slower dissociation rates for the $\alpha_2\delta$-1 subunit than the $\alpha_2\delta$-2 subunit. It shows potent and long-lasting analgesic effects in rat models of neuropathic pain, and wider safety margins for side effects of the central nervous system. These properties of mirogabalin can be associated with its unique binding characteristics.

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). The predominant mechanism of action of gabapentinoids is inhibiting neurotransmitter release at the presynaptic endings of neurons. The inhibition of neurotransmitter (e.g., glutamate, substance P, and calcitonin gene–related peptide) release attenuates neuronal hyperexcitability in the brain and spinal cord and contributes to various pharmacological effects such as analgesic, anticonvulsant, and anxiolytic activity (Fehrenbacher et al., 2003; Sills, 2006; Dooley et al., 2007; Taylor et al., 2007; Stahl et al., 2013). In fact, pregabalin and gabapentin have been licensed and used in many countries for pain, epilepsy, and generalized anxiety disorders (Stahl et al., 2013). Several scientific associations and guidelines recommend pregabalin and gabapentin as the first-line drugs for the treatment of neuropathic pain (Argoff et al., 2006; Dworkin et al., 2007, 2013; Finnerup and Jensen, 2007; Attal et al., 2010; Brlt et al., 2011; Cohen et al., 2015). However, the clinical utility of pregabalin and gabapentin is limited by central nervous system (CNS) side effects such as dizziness and somnolence (Freeman et al., 2008; Ziegler, 2008; Goodman and Brett, 2017); thus, ABBREVIATIONS: AUC, area under the curve; AUC$_{0-24}$h, area under the curve of the 8-hour paw withdrawal threshold; AUC$_{0-12}$h, area under the curve of the 12-hour paw withdrawal threshold; CNS, central nervous system; $K_d$, dissociation constant; $K_{off}$, dissociation rate constant; mirogabalin, [[1R,5S,6S]-6-(aminomethyl)-3-ethylbicyclo[3.2.0]hept-3-en-6-yl]acetic acid; PK, pharmacokinetics; PSL, partial sciatic nerve ligation; STZ, streptozotocin.
there is an unmet need for further improvement in this class of drugs. Behavioral, neurochemical, and electrophysiological studies using transgenic mice have shown that the αδ-1 subunit of voltage-gated calcium channels contributes to analgesic effects (Field et al., 2006; Li et al., 2006), whereas the αδ-2 subunit contributes to CNS side effects (Barclay et al., 2001; Brill et al., 2004), suggesting that ligand selectivity for αδ-1 and αδ-2 might result in different clinical outcomes.

Mirogabalin ([1R,5S,6S]-6-(aminomethyl)-3-ethylbicyclo[3.2.0]-hept-3-en-6-yl)acetic acid) is a novel ligand for the αδ subunit of voltage-gated calcium channels that is being developed to treat pain associated with diabetic peripheral neuropathy and postherpetic neuralgia.

In the present study, we investigated the in vitro binding characteristics and in vivo analgesic effects of mirogabalin compared with those of pregabalin.

Materials and Methods

Chemicals

Mirogabalin besylate (code number DS-5565), mirogabalin (free-form of DS-5565), and pregabalin were synthesized by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). [3H]mirogabalin (specific radioactivity, 756 GBq/mmol) and [3H]pregabalin (specific radioactivity, 1280 GBq/mmol) were obtained from Sekisui Medical Co., Ltd. (Tokyo, Japan). The mirogabalin besylate and pregabalin were dissolved in distilled water. In the rota-rod and locomotor tests, mirogabalin besylate was suspended in a 0.5% methylcellulose solution because of its solubility limit. For an in vitro off-target profiling assay, mirogabalin besylate was dissolved in dimethylsulfoxide. The dose levels of test compounds are expressed as free form. All other reagents were of analytical grade and were obtained from conventional commercial sources. The chemical structures of mirogabalin besylate and pregabalin are shown in Fig. 1.

Cell Membranes

The cell membrane fraction containing each αδ subunit was prepared from the 293A stable cell line expressing each αδ subunit, as described in a previous report (Gee et al., 1996). Briefly, 293A cells (Thermo Fisher Scientific, Waltham, MA) were cotransfected with 5 μg of αδ subunit expression plasmid and 0.5 μg of pPURO (Clontech Laboratories, Inc., Mountain View, CA) by lipofection. The transfected cells were diluted and spread to 150-mm dishes. After 10 days in culture with Dulbecco’s modified Eagle medium (Thermo Fisher Scientific) containing 10% fetal bovine serum and 1 g/ml puromycin (Clontech Laboratories, Inc., Mountain View, CA) by lipofection, the cell membranes were 11.2, 10.7, 9.8, and 9.4 mg/ml, respectively.

Fig. 1. Chemical structures of mirogabalin besylate (top) and pregabalin (bottom).

In Vitro Binding Profile for the αδ Subunits

Saturation Assay.

The cell membranes prepared as described above were diluted with binding assay buffer (0.01 M HEPES, pH 7.5, and 0.1 M NaCl) on ice. The diluted cell membranes (final concentration of 0.1 mg protein/ml) and [3H]-labeled compound serial dilutions (final concentration of 0.59–100 nM [3H]-mirogabalin or 0.78–200 nM [3H]-pregabalin) were mixed in each well of a 96-U plate (MS-3296U; Sumitomo Bakelite Co., Ltd., Tokyo, Japan) and incubated for 4 hours at room temperature (N = 4). After incubation, the membranes from each well were collected in a 96-well UNIFILTER (GF/B Filter 7700–3303; Whatman Inc., Clifton, NJ) using vacuum manifold. Each filter was washed three times with 400 μl of binding assay buffer and dried overnight at room temperature. The radioactivity of the dried filter picked up from each well was counted by liquid scintillation counter (PerkinElmer Inc., Waltham, MA) after being immersed in 4 ml of Pico-Fluor 40 (PerkinElmer) in glass vial. Nonspecific binding was defined as the residual binding in the presence of 100 μM mirogabalin or 200 μM pregabalin.

Dissociation Kinetic Assay.

The cell membranes (final concentration of 0.1 mg protein/ml) and [3H]-labeled compound solutions (final concentration of 10 nM [3H]-mirogabalin or 20 nM [3H]-pregabalin) were incubated for 4 hours at room temperature, as described above. After that, the dissociation reaction was initiated by adding 2 μl of the corresponding unlabeled compound solution (10 mM mirogabalin or 20 mM pregabalin), and the reaction mixture was incubated for 0 hours (immediately), and for 0.5, 1, 2, 4, 6, and 10 hours at room temperature (N = 4). After incubation, the membrane-bound radioligand was recovered by filtration, and the radioactivity was

Animals

Male Crl:CD (SD) rats and F344/DuCrlCrlj rats (Charles River Laboratories Japan, Inc., Kanagawa, Japan), as well as B6SsN Scl rats (Japan SLC, Inc., Shizuoka, Japan) were used. The animals were housed under regulated conditions for temperature (19–26°C), relative humidity (35%–75%), and a 12-hour light/dark cycle (lights on from 7:00 AM to 7:00 PM), with a commercial diet and tap water available ad libitum. Animals were randomly allocated to the study groups by computed randomization procedure based on paw withdrawal threshold (neuropathic pain models) or random number generation (safety pharmacological evaluations). 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, Labor and Welfare (Notification Number 0601001 of the Science Bureau, Japanese Ministry of Health, Labor and Welfare, June 1, 2006), the Guidelines for Animal Studies (Nonclinical Research Center and Toxicological Science Division, Mitsubishi Chemical Medience Corporation), and the Guideline of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd.
The in vitro pharmacological activity of mirogabalin besylate on a total of 187 receptors, channels, transporters, and enzymes was evaluated by radioligand binding and enzyme assays using the standard protocols provided by Eurofins Panlabs Taiwan Ltd. (formerly MDS Pharma Services Taiwan Ltd., Taipei, Taiwan). The protocols consisted of the SpectrumScreen package, seven additional binding assays, and 14 enzyme assays. The primary assays were performed at a concentration of 50 $\mu$M in duplicate, and when significant responses ($\geq 50\%$ inhibition) were noted, the IC$_{50}$ values were determined in the follow-up assays.

### Binding parameters of $^3$H-mirogabalin and $^3$H-pregabalin for the $\alpha_2\delta$ subunits

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Parameter</th>
<th>Mirogabalin</th>
<th>Pregabalin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human $\alpha_2\delta$-1</td>
<td>$K_a$ [nM]</td>
<td>13.5 (11.9–15.4)</td>
<td>62.5 (55.6–71.4)</td>
</tr>
<tr>
<td></td>
<td>$B_{max}$ [pmol/mg]</td>
<td>50.2 (46.1–55.3)</td>
<td>46.5 (42.5–51.5)</td>
</tr>
<tr>
<td>Human $\alpha_2\delta$-2</td>
<td>$K_a$ [nM]</td>
<td>22.7 (20.8–24.4)</td>
<td>125.0 (76.9–333.3)</td>
</tr>
<tr>
<td></td>
<td>$B_{max}$ [pmol/mg]</td>
<td>22.3 (21.0–23.7)</td>
<td>22.0 (15.2–45.9)</td>
</tr>
<tr>
<td>Rat $\alpha_2\delta$-1</td>
<td>$K_a$ [nM]</td>
<td>27.0 (24.4–29.4)</td>
<td>142.9 (125.0–200.0)</td>
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<tr>
<td></td>
<td>$B_{max}$ [pmol/mg]</td>
<td>47.6 (44.5–51.6)</td>
<td>38.2 (31.4–49.7)</td>
</tr>
<tr>
<td>Rat $\alpha_2\delta$-2</td>
<td>$K_a$ [nM]</td>
<td>47.6 (37.0–62.5)</td>
<td>166.7 (142.9–250.0)</td>
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<tr>
<td></td>
<td>$B_{max}$ [pmol/mg]</td>
<td>63.3 (52.2–82.3)</td>
<td>46.8 (37.8–62.7)</td>
</tr>
</tbody>
</table>

### Dissociation kinetic parameters of $^3$H-mirogabalin and $^3$H-pregabalin for the $\alpha_2\delta$ subunits

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Parameter</th>
<th>Mirogabalin</th>
<th>Pregabalin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human $\alpha_2\delta$-1</td>
<td>$K_{off}$ [h$^{-1}$]</td>
<td>0.0627 (0.0423–0.0831)</td>
<td>0.5051 (0.4817–0.5286)</td>
</tr>
<tr>
<td></td>
<td>$t_{1/2}$ [h]</td>
<td>11.1 (8.3–16.4)</td>
<td>1.4 (1.3–1.4)</td>
</tr>
<tr>
<td>Human $\alpha_2\delta$-2</td>
<td>$K_{off}$ [h$^{-1}$]</td>
<td>0.2837 (0.2441–0.3233)</td>
<td>0.5103 (0.2603–0.7603)</td>
</tr>
<tr>
<td></td>
<td>$t_{1/2}$ [h]</td>
<td>2.4 (2.1–2.8)</td>
<td>1.4 (0.9–2.7)</td>
</tr>
<tr>
<td>Rat $\alpha_2\delta$-1</td>
<td>$K_{off}$ [h$^{-1}$]</td>
<td>0.0798 (0.0629–0.0966)</td>
<td>0.4929 (0.4297–0.5561)</td>
</tr>
<tr>
<td></td>
<td>$t_{1/2}$ [h]</td>
<td>8.7 (7.2–11.0)</td>
<td>1.4 (1.2–1.6)</td>
</tr>
<tr>
<td>Rat $\alpha_2\delta$-2</td>
<td>$K_{off}$ [h$^{-1}$]</td>
<td>0.3027 (0.2359–0.3695)</td>
<td>0.5266 (0.3937–0.6595)</td>
</tr>
<tr>
<td></td>
<td>$t_{1/2}$ [h]</td>
<td>2.3 (1.9–2.9)</td>
<td>1.3 (1.1–1.8)</td>
</tr>
</tbody>
</table>

$t_{1/2}$, dissociation half-life.
Spontaneous Locomotor Activity in Rats

Eighty male Fischer rats were divided into groups of eight. After oral administration of the test compound or vehicle (control), locomotor activity was measured for 1 hour using the SUPERMEX system (model SM-32; Muromachi Kikai Co., Ltd., Tokyo, Japan). Based on the time of peak effects of the test compounds in the rota-rod test, the pretreatment time was set at 6 hours for mirogabalin besylate and at 4 hours for pregabalin.

Statistical Analysis

For the in vitro saturation assay and dissociation kinetic assay, the $B_{max}$, dissociation constant ($K_d$), dissociation rate constant ($K_{off}$), and dissociation half-life, including their 95% confidence limits, were conducted at 0 hours (before administration), and at 2, 4, 6, 8, and 24 hours after administration.

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calculated. For paw withdrawal threshold AUC and locomotor activity, the statistical analysis was performed using Dunnett's multiple-comparison test. Rota-rod performance was analyzed by Fisher's exact probability test. In addition, the Spearman's correlation coefficient was calculated to evaluate the dose-response relationship from the paw withdrawal threshold AUC. Differences were considered significant when \( P < 0.05 \). The ED\(_{50}\) values and their 95% confidence limits were calculated by log-linear or logistic regression analysis and probit analysis. SAS software release 8.2 or 9.1.3 (SAS Institute Japan Ltd., Tokyo, Japan), EXSUS version 7.7 (CAC Croit Corporation, Tokyo, Japan), and Excel 2003 (Microsoft Japan Co., Ltd., Tokyo, Japan) were used for these analyses. Sample sizes were determined to provide >80% power based on the results of preliminary studies and historical data.

**Results**

**In Vitro Binding Profile for the \( \alpha_2\delta \) Subunits**

**Saturation Assay.** The binding parameters of \(^3\)H-mirogabalin and \(^3\)H-pregabalin for the \( \alpha_2\delta \) subunits are summarized in Table 1. The \( K_d \) values of mirogabalin for the

![Fig. 4. Analgesic effects of mirogabalin and pregabalin in STZ-induced diabetic rats. (A) Time-course changes of paw withdrawal threshold. (B) Paw withdrawal threshold AUC. Mirogabalin besylate and pregabalin were orally administered (2 ml/kg). The control group received distilled water. The dose levels of test compounds are expressed as free form. Each value represents the mean ± S.E.M. \((N = 9 \text{ or } 10)\. One rat in the group of pregabalin 10 mg/kg was excluded from the experiment because of an administration error on day 2. *\( P < 0.05 \); **\( P < 0.01 \), significantly different from the control group by Dunnett's multiple-comparison test. Spearman's correlation coefficient reveals significant dose-response relationships in AUC\(_{0-12 \text{ hours}}\) values as follows: mirogabalin (0.8669, \( P < 0.0001 \)) and pregabalin (0.7545, \( P < 0.0001 \)) on day 1; mirogabalin (0.8867, \( P < 0.0001 \)) and pregabalin (0.7255, \( P < 0.0001 \)) on day 3; mirogabalin (0.8724, \( P < 0.0001 \)) and pregabalin (0.8123, \( P < 0.0001 \)) on day 5. ED\(_{50}\) values and their 95% confidence limits were estimated as follows: mirogabalin 4.4 (3.6–6.9) and pregabalin 26.8 (21.3–32.5) on day 1; mirogabalin 3.1 (1.7–4.0) and pregabalin 22.4 (1.1–31.5) on day 3; mirogabalin <2.5 (95% confidence limits were not determined) and pregabalin 29.3 (25.3–34.5) on day 5.\)
The dissociation half-life of pregabalin from the human α2δ-1 and α2δ-2 subunits were estimated to be 11.1 and 2.4 hours, respectively. Further, neither compound exhibited subtype selectivity (a human Kd value of 16.0 nM. Mirogabalin had no effect on any other receptors, channels, transporters, or enzymes at 50 μM.

**In Vitro Off-Target Pharmacological Profile**

Mirogabalin showed binding affinity for the gabapentin binding site in rat cortical brain homogenates with the IC50 value of 16.0 nM. Mirogabalin had no effect on any other receptors, channels, transporters, or enzymes at 50 μM.

**Partial Sciatic Nerve Ligation Model in Rats**

Figure 3A shows the dose-response and time-course changes of paw withdrawal threshold in each treatment group; the results for the paw withdrawal threshold AUC are shown in Fig. 3B. Mirogabalin (3 and 10 mg/kg) and pregabalin (10 and 30 mg/kg) significantly increased AUC0–12 hours values in a dose-dependent manner. The effect of mirogabalin peaked at 4 hours after administration and remained there until 6 or 8 hours after administration. The effect of pregabalin peaked at 4 hours after administration and returned to the vehicle control level after 6 hours.

**STZ-Induced Diabetic Model in Rats**

Figure 4A shows the dose-response and time-course changes of paw withdrawal threshold in each treatment group; the results for the paw withdrawal threshold AUC are shown in Fig. 4B. The paw withdrawal threshold in vehicle control rats was lower than that in normal control rats. Mirogabalin (2.5, 5, and 10 mg/kg) and pregabalin (10, 20, and 40 mg/kg) significantly increased AUC0–12 hours values in a dose-dependent manner. The effects of mirogabalin and pregabalin peaked at 4 hours after administration, and there were no apparent differences in the maximum effects of both compounds. The analgesic effects of mirogabalin were enhanced by repeated dosing, whereas those of pregabalin showed no apparent changes. The ED50 values for mirogabalin on day 1, day 3, and day 5 were estimated to be 4.4, 3.1, and <2.5 mg/kg, respectively. The ED50 values for pregabalin on day 1, day 3, and day 5 were estimated to be 26.8, 22.4, and 29.3 mg/kg, respectively.

Figure 5 shows the dose-response and time-course changes in plasma concentrations of mirogabalin and pregabalin in each satellite group on day 5, and the inset shows the dose-proportionality of Cmax and AUC0–12 hours values. Table 3 summarizes the PK parameters on day 1 and day 5. The Cmax and AUC0–12 hours values of mirogabalin and pregabalin increased nearly dose proportionally. There were no apparent changes in these parameters for mirogabalin and pregabalin, even after repeated dosing.

**Rota-Rod Performance in Rats**

Figure 6 shows the dose-response and time-course changes of rota-rod performance in each treatment group. Mirogabalin had no significant effect on rota-rod performance at 1 and 3 mg/kg, and it significantly inhibited rota-rod performance at 10, 30, and 100 mg/kg. The maximum effect was observed 4–6
hours after administration, and the ED_{50} values were estimated to be 9.5 and 9.4 mg/kg, respectively, at 4 and 6 hours after administration. Pregabalin had no significant effect on rota-rod performance at 3 and 10 mg/kg, and it significantly inhibited rota-rod performance at 30, 100, and 300 mg/kg. The maximum effect was observed 4–6 hours after administration, and the ED_{50} value was considered to be 11.7 mg/kg. All animals recovered 24 hours after the administration of mirogabalin and pregabalin, at all dosage levels.

**Spontaneous Locomotor Activity in Rats**

Figure 7 shows the dose-response changes of locomotor activity in each treatment group. Mirogabalin had no effect on locomotor activity at 3 and 10 mg/kg, and it significantly decreased locomotor activity at 100 and 300 mg/kg. The ED_{50} values of mirogabalin and pregabalin were estimated to be 43.9 and 111.8 mg/kg, respectively.

**Safety Indices for CNS Side Effects in Rats**

Table 4 summarizes the CNS side-effect profile of mirogabalin and pregabalin. Safety indices were obtained by calculating the ratio between side-effect ED_{50} values (rota-rod ED_{50} or locomotor ED_{50}) and analgesic ED_{50} values on day 1 in the STZ study. In rota-rod performance, the safety indices were 2.1 for mirogabalin and 0.4 for pregabalin. The safety index 0.4 (under 1) represents a negative margin, indicating that pregabalin induced ataxia at lower doses than its analgesic doses. In locomotor activity, the safety indices were 10.0 for mirogabalin and 4.2 for pregabalin. Therefore, the safety indices of mirogabalin were superior to those of pregabalin in both tests.

**Discussion**

The α_{2}δ subunits are multifunctional and have been reported to affect calcium channel trafficking as well as the biophysical properties of calcium channel currents (Dolphin, 2012, 2013). Increased expression of α_{2}δ-1 mRNA and α_{2}δ-1 protein has been observed in the dorsal root ganglion and the spinal cord dorsal horn of rat models for neuropathic pain (Luo et al., 2001; Newton et al., 2001; Wang et al., 2002; Li et al., 2004; Bauer et al., 2009, 2010; Boroujerdi et al., 2011). In addition, knockdown of α_{2}δ-1 subunits by antisense has been reported to inhibit tactile allodynia in these rat models (Li et al., 2004; Boroujerdi et al., 2011). Transgenic mice overexpressing the α_{2}δ-1 subunit in neuronal tissues have been reported to exhibit behavioral hypersensitivity (tactile allodynia and thermal hyperalgesia) and electrophysiological hyperexcitability in the dorsal root ganglion and spinal dorsal horn neurons (Lie et al., 2006). The α_{2}δ-1 knockout mice have been reported to show markedly reduced behavioral sensitivity to mechanical and cold stimuli. The knockout mice also showed a delayed development of mechanical hypersensitivity after FSL and loss of the analgesic effect of pregabalin (Patel et al., 2013). Knockin mice expressing a mutant α_{2}δ-1 subunit that does not bind pregabalin or gabapentin have been reported to develop neuropathic pain that is insensitive to these drugs (Field et al., 2006). These findings indicate that the α_{2}δ-1 subunit has an important role in neuropathic pain states and that the analgesic effects of gabapentinoids are mediated through the α_{2}δ-1 subunit. In contrast, the α_{2}δ-2 subunit is dominantly expressed in the cerebellar Purkinje cells, and mutant mice with a α_{2}δ-2 subunit deletion have been reported to show ataxia, paroxysmal dyskinesia, and absence seizures (Barclay et al., 2001; Brodbeck et al., 2002; Brill et al., 2004; Ivanov et al., 2004; Donato et al., 2006). Furthermore, human epileptic encephalopathies associated with α_{2}δ-2 mutations have been reported (Edvardson et al., 2013; Pippucci et al., 2013). These distinct roles of α_{2}δ-1 and α_{2}δ-2 subunits suggest that ligand selectivity for α_{2}δ-1 and α_{2}δ-2 might bring about separate analgesic effects and CNS side effects.

In the present study, mirogabalin specifically bound to α_{2}δ subunits with high affinity at two-digit nanomolar concentrations and had no effects on a total of 186 off-target proteins at three orders of higher concentration (50 μM). The binding affinities of mirogabalin for the human α_{2}δ-1, human α_{2}δ-2, rat α_{2}δ-1, and rat α_{2}δ-2 subunits were greater than those of pregabalin, and neither compound showed subtype selectivity (α_{2}δ-1 vs. α_{2}δ-2) or species differences (human vs. rat). Interestingly, although the subtype selectivity of mirogabalin is not significant in K_{d} values, mirogabalin showed longer dissociation half-lives against the α_{2}δ-1 subunit than the α_{2}δ-2 subunit both in human and rat. On the other hand, pregabalin showed equal dissociation half-lives from the α_{2}δ-1 subunit and the α_{2}δ-2 subunit in both human and rat. The binding affinity of mirogabalin and pregabalin for the α_{2}δ-3 or α_{2}δ-4 subunit was not evaluated in the present study. Gabapentin and pregabalin have been reported to show no binding affinity for those subtypes (Marais et al., 2001; Qin et al., 2002; Taylor et al., 2007). To date, drugs showing significant binding to the α_{2}δ-3 or α_{2}δ-4 subunit have not yet been reported.

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**Table 3**

Pharmacokinetic parameters of mirogabalin and pregabalin in STZ-induced diabetic rats

Mirogabalin besylate and pregabalin were orally administered (2 ml/kg). Dose levels and concentrations of test compounds are expressed as free form. Each value represents the mean ± S.E.M. (N = 3).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>C_{max} (µg/ml)</th>
<th>AUC_{0-12 hours} (µg-h/ml)</th>
<th>Day 1</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mirogabalin</td>
<td>2.5</td>
<td>0.5 ± 0.1</td>
<td>2.6 ± 0.5</td>
<td>0.5 ± 0.1</td>
<td>1.9 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.5 ± 0.0</td>
<td>7.2 ± 0.8</td>
<td>1.0 ± 0.1</td>
<td>5.2 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3.2 ± 0.0</td>
<td>10.9 ± 0.5</td>
<td>2.5 ± 0.2</td>
<td>15.1 ± 2.7</td>
</tr>
<tr>
<td>Pregabalin</td>
<td>10</td>
<td>7.0 ± 1.9</td>
<td>42.4 ± 0.4</td>
<td>6.8 ± 0.5</td>
<td>46.3 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>11.6 ± 3.3</td>
<td>69.4 ± 7.0</td>
<td>14.9 ± 0.7</td>
<td>94.2 ± 17.6</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>20.3 ± 0.5</td>
<td>148.7 ± 13.7</td>
<td>26.7 ± 2.7</td>
<td>165.3 ± 16.7</td>
</tr>
</tbody>
</table>
In typical experimental neuropathic pain models, such as PSL rats and STZ-induced diabetic rats, mirogabalin showed more potent and longer lasting analgesic effects than pregabalin. The greater binding affinity of mirogabalin for the $\alpha_2\delta$-1 subunit is considered to contribute to its potent analgesic effects. Interestingly, unlike pregabalin, the analgesic effects of mirogabalin were enhanced by repeated administration in STZ-induced diabetic rats. In the mirogabalin treatment groups, the paw withdrawal threshold AUCs increased day by day without increases in the AUCs of plasma drug concentrations. Specifically, at 10 mg/kg mirogabalin, the paw withdrawal thresholds before administration on day 3 and day 5 were at the same level as those of normal controls and were higher than those on day 1, regardless of almost undetectable plasma drug concentrations. The above phenomena and differences from pregabalin might be potentially explained by the sustained binding affinity of mirogabalin for the $\alpha_2\delta$-1 subunit rather than its PK parameters.

Including the IC$_{50}$ and $K_d$ values, the dissociation half-life for a target protein is suggested to be an important factor with which to determine the duration of pharmacological effects and target selectivity in vivo (Copeland et al., 2006). In safety pharmacological evaluations, mirogabalin and pregabalin dose-dependently inhibited rota-rod performance and

**Fig. 6.** Effects of mirogabalin and pregabalin on rota-rod performance in rats. Mirogabalin besylate and pregabalin were orally administered (10 ml/kg). Control group received 0.5% methylcellulose solution. Dose levels of test compounds are expressed as free form. Each value represents the success rate of rota-rod performance (N = 8). *P < 0.05, significantly different from the control group by Dunnett’s multiple comparison. ED$_{50}$ value for mirogabalin was 9.4 mg/kg (95% confidence limits were not determined) at 4 hours and 9.4 mg/kg (95% confidence limits, 5.4–17.4) at 6 hours after administration, respectively. ED$_{50}$ values for pregabalin were 11.7 mg/kg (95% confidence limits were not determined) at 4 and 6 hours after administration.

**Fig. 7.** Effects of mirogabalin and pregabalin on spontaneous locomotor activity in rats. Mirogabalin besylate and pregabalin were orally administered (10 ml/kg). Control group received 0.5% methylcellulose solution. Dose levels of test compounds are expressed as free form. Each value represents the mean ± S.E.M. (N = 8). *P < 0.05, significantly different from the control group by Dunnett’s multiple comparison. ED$_{50}$ value for mirogabalin was 43.9 mg/kg (95% confidence limits, 35.1–55.9). ED$_{50}$ value for pregabalin was 111.8 mg/kg (95% confidence limits, 70.0–178.6).
locomotor activity in rats. These effects are the class effects of gabapentinois and have been suggested to be related to the CNS side effects observed in clinical practice. In both rota-rod and locomotor tests, the safety indices of mirogabalin were superior to those of pregabalin. As described above, several studies using transgenic mice have shown that the αδ-1 subunit plays an important role in the analgesic effects of gabapentinoids, whereas the αδ-2 subunit is related to CNS side effects. Mirogabalin had a longer dissociation half-life from the αδ-1 subunit than the αδ-2 subunit, in contrast to pregabalin. The unique binding characteristics of mirogabalin might contribute to the wider safety margin for CNS side effects as well as the long-lasting analgesic effects. The findings in the present study support the favorable outcomes obtained in the phase II proof-of-concept study of mirogabalin as a treatment for patients with diabetic peripheral neuropathy (Vinik et al., 2014; Merante et al., 2017). This phase II study demonstrated that mirogabalin was effective and well tolerated at 15, 20, and 30 mg/d, given either once or twice daily with or without titration.

In conclusion, mirogabalin shows potent and selective binding affinities for the human and rat αδ subunits, and a slower dissociation rate for the αδ-1 than for the αδ-2 subunit. It shows potent and long-lasting analgesic effects in rat models for neuropathic pain and a wider safety margin for CNS side effects. The unique binding characteristics of mirogabalin might contribute to its high analgesic efficacy and wide safety margin.

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Conducted experiments: Domon, Arakawa, Inoue, Matsuda, Takahashi, Yamamura, Kai, and Kitano.

Performed data analysis: Domon, Yamamura, and Kitano.

Wrote or contributed to the writing of the manuscript: Matsuda, Yamamura, Kai, and Kitano.

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