NOTE *Pharmacology*

Effect of Mexiletine on Dynamic Allodynia Induced by Chronic Constriction Injury of the Sciatic Nerve in Rats

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ABSTRACT. Static and dynamic allodynia occurred in a rat model of neuropathic pain induced by chronic constriction injury (CCI) of the sciatic nerve. Static allodynia was detected within 1 day after the CCI surgery, and persisted for 28 days. Dynamic allodynia displayed a slower course of development with a late onset, and statistically significant changes were achieved between 14 and 28 days after the surgery. Mexiletine at 10 and 30 mg/kg, s.c. produced a significant and dose-dependent inhibition of CCI-induced static and dynamic allodynia on day 14 post-surgery. Pregabalin, used as a reference drug, also significantly inhibited both static and dynamic allodynia at 30 and 60 mg/kg, p.o. These findings rationalize the clinical use of mexiletine for treatment of neuropathic pain.
KEY WORDS: dynamic allodynia, mexiletine, neuropathic pain, pregabalin, static allodynia.

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Peripheral nerve injury causes neuropathic pain that is characterized by an exaggerated sensory response to non-noxious stimuli, referred to as mechanical allodynia [15]. Recent studies have revealed that 2 distinct types of mechanical allodynia can be detected in animals and humans, i.e., static allodynia which is evoked by the application of increasing pressure on the skin, and dynamic allodynia that is evoked by lightly stroking the surface of the skin [9, 13]. The sensory hyperexcitability induced by peripheral nerve injury is associated with a concomitant generation of spontaneous action potential in the primary afferent fibers at the site of nerve injury and related dorsal root ganglia. Early studies have demonstrated that an accumulation of voltage-gated sodium channels occurred at the sites of nerve injury in rats [6, 12]. In addition, sodium channel blockers, such as lidocaine, were shown to decrease spontaneous action potential arising from damaged fibers with significant inhibition of the concomitant mechanical sensitivity [5]. These findings indicate that mechanical allodynia in neuropathic pain is attributed, at least in part, to an augmented expression of voltage-gated sodium channels at the sites of nerve injury.

Mexiletine, a subtype-nonselective sodium channel blocker, is reported to be effective on both static and dynamic allodynia in clinical practice [2], and prescribed for treating patients with painful diabetic neuropathy. The anti-allodynia effect of mexiletine has been also investigated in rats, and the majority of evidence has shown that this non-selective sodium channel blocker can effectively inhibit the static allodynia induced by peripheral nerve injury in rats [1, 7]. No experimental study, however, has been conducted to date to determine the effect of mexiletine on dynamic allodynia in rat models of neuropathic pain. In the present study, we examined its effects on dynamic and static components of mechanical allodynia induced by chronic constriction injury of the sciatic nerve in rats. Pregabalin, a standard analgesic drug for neuropathic pain, was used as a reference drug.

Male Sprague-Dawley rats (Charles River, Hino, Japan) were housed in pairs with free access to food and water under conditions of constant temperature (23 ± 2°C) and humidity (55 ± 15 %) with a 12-hr light/dark cycle (lights on 7:00 a.m.). All procedures were approved by the Animal Ethics Committee at the Pfizer Global Research and Development Nagoya Laboratories (Japan) according to the guidelines of Laboratory Animal Welfare. The chronic constriction injury (CCI) of the sciatic nerve was made using the 4–0 chromic gut suture (ETHICON, U.S.A.) according to the method of Bennett and Xie [4]. Sham operation was performed in the same manner except for a nerve ligation.

The rats were examined for both static and dynamic allodynia at specified time intervals following the CCI surgery, as described previously [9]. In short, static allodynia was evaluated by application of von Frey hairs (VFH, Semmes-Weinstern Monofilaments, North Coast Medical Inc.) to the planter surface of the affected left hind paw in ascending order of force (0.16, 0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 10.0, 15.0 and 26.0 g) till a withdrawal response occurred, and the lowest force required to elicit a paw withdrawal response was recorded as the paw withdrawal threshold (g). After static allodynia was determined, dynamic allodynia was assessed in the following manner; the planter surface of the left hind paw was lightly stroked with a cotton bud until a paw withdrawal response was observed, and the paw withdrawal latency (sec) was recorded as an index of dynamic allodynia. If no reaction was exhibited within 15 sec, the procedure was terminated and animals were assigned this withdrawal time. Care was taken to perform these proce-

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dures in fully habituated rats.

Pregabalin (synthesized at Pfizer Global Research and Development) and mexiletine (Sigma, St. Louis, U.S.A.) were dissolved in physiological saline, and the volume of administration was kept constant at 1 m*l*/kg of body weight. The data of paw withdrawal threshold are non-parametric, and thus analyzed using a Kruskal-Wallis test followed by an individual Mann-Whitney U-test or just an individual Mann-Whitney U-test where there were only 2 groups [9]. The data of paw withdrawal latency were subjected to a one-way ANOVA followed by a Dunnett's *t*-test or a *t*-test where there were only 2 groups.

Neither static nor dynamic allodynia occurred in sham-operated control rats throughout the entire observation period (Fig. 1). In CCI rats, static allodynia could be detected on day 1 post-surgery, as evidenced by significant decrease in the paw withdrawal threshold to the static punctuate stimuli produced by von Frey hairs. On the other hand, dynamic allodynia followed a slower course of development, and the statistically significant reduction in paw withdrawal latency to a cotton bud stimulus occurred between 14 and 28 days post-surgery. Based on these findings, the drug testing was carried out on day 14 post-surgery.

Mexiletine dose-dependently suppressed both static and dynamic allodynia induced by CCI at doses ranging from 3 to 30 mg/kg, s.c. (Fig. 2). The anti-allodynia effects pro-

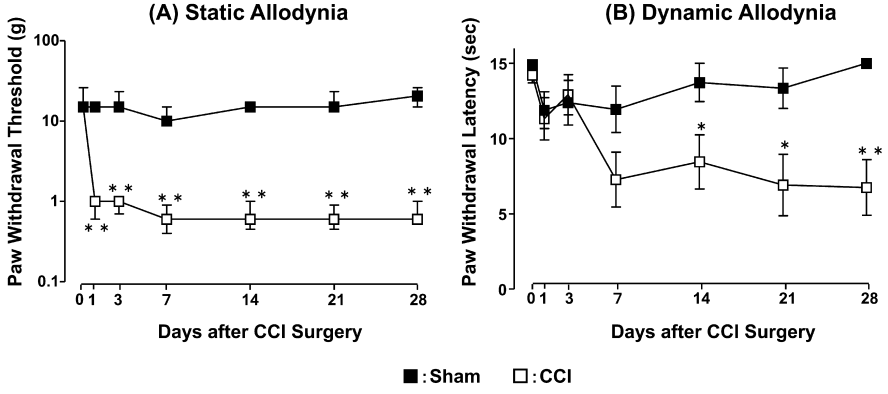

Fig. 1. Development of static (A) and dynamic (B) allodynia induced by chronic constriction injury of the sciatic nerve in rats. Data for static allodynia are expressed as median value of paw withdrawal threshold (g) in 10 animals per group, and vertical bars represent 1st and 3rd quartiles. Data for dynamic allodynia are expressed as mean value of paw withdrawal latency (sec) in 10 animals per group, and vertical bars represent SEM. * P<0.05, ** P<0.01 significantly different from sham-operated control group.

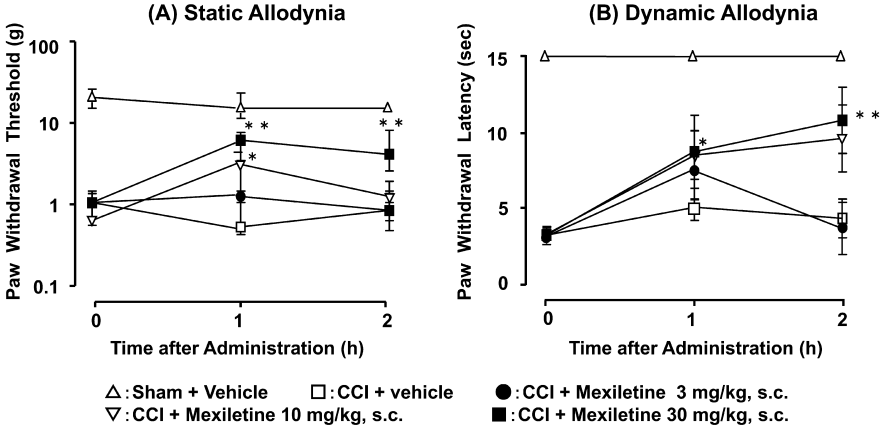

Fig. 2. Effects of mexiletine on CCI-induced static (A) and dynamic (B) allodynia in rats on day 14 post-CCI surgery. Data for static allodynia are expressed as median value of paw withdrawal threshold (g) in 8 animals per group, and vertical bars represent 1st and 3rd quartiles. Data for dynamic allodynia are expressed as mean value of paw withdrawal latency (sec) in 8 animals per group, and vertical bars represent SEM. * P<0.05, ** P<0.01 significantly different from vehicle treated control group.

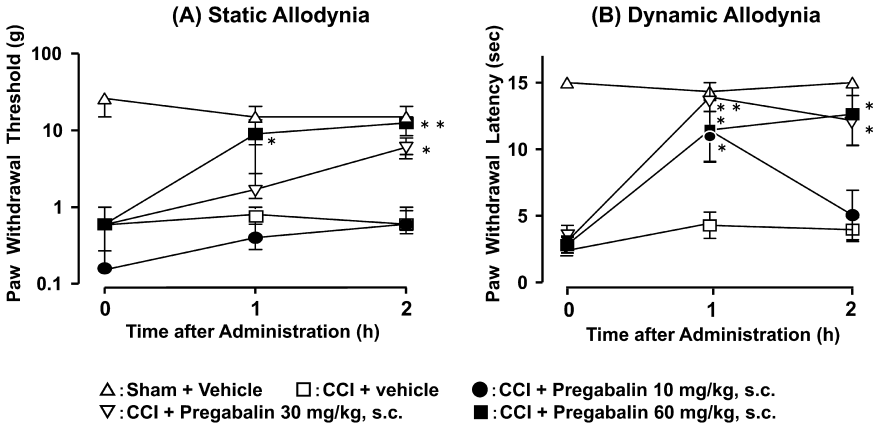

Fig. 3. Effects of pregabalin on CCI-induced static (A) and dynamic (B) allodynia in rats on day 14 post-CCI surgery. Data for static allodynia are expressed as median value of paw withdrawal threshold (g) in 8 animals per group, and vertical bars represent 1st and 3rd quartiles. Data for dynamic allodynia are expressed as mean value of paw withdrawal latency (sec) in 8 animals per group, and vertical bars represent SEM. * P<0.05, ** P<0.01 significantly different from vehicle treated control group.

duced by the highest dose of the sodium channel blocker remained statistically significant for 2 hr after administration. On the other hand, pregabalin produced a significant inhibition of CCI-induced static allodynia at doses of 30 and 60 mg/kg, p.o. at 2 hr post-dosing (Fig. 3). The same doses of pregabalin also significantly suppressed CCI-induced dynamic allodynia either 1 or 2 hr after administration.

Previous studies have demonstrated that mexiletine could alleviate static allodynia and hyperalgesia in multiple neuropathic pain models, including the rat CCI model [1, 7, 11]. These studies, however, have not determined the effect of mexiletine on the dynamic component of mechanical allodynia. In this study, we clearly demonstrated that mexiletine, like pregabalin, could significantly inhibit dynamic as well as static allodynia in the rat model of CCI-induced neuropathic pain. In a recent study, it was reported that oral mexiletine inhibited static, but not dynamic, allodynia in mice with postherpetic pain induced by herpes simplex virus type-1 [16]. The precise reason why the mode of action of mexiletine differed between the animal models remains unknown, but may be attributed to the differences in the species and methods to produce neuropathy.

Static and dynamic allodynia have been claimed to be mediated by small unmyelinated and large myelinated primary neurons, respectively, in patients with neuropathic pain [13]. Similarly, CCI-induced static allodynia is reported to be mediated by small myelinated Aδ-fibers, while dynamic allodynia is signaled by large myelinated Aβ-fibers [9]. A multitude of sodium channel subtypes are known to be involved in activation of these primary neurons, and recent studies have indicated that rat Aβ-fibers express both tetrodotoxin-sensitive (TTX-s) and resistant (TTX-r) currents [8], whereas TTX-s channel is the only type of functional sodium channel in mammalian Aδ-fibers [14]. It is generally acknowledged that subtype-nonselective sodium channel blockers, such as lidocaine and its oral congener mexiletine, inhibit TTX-s channels with higher potency, compared to TTX-r channels [17]. Previous *in vivo* electrophysiological study using the rat sciatic nerve demonstrated that Aδ-fibers were more susceptible to lidocaine in comparison with Aβ-fibers [10]. Although the relationship with the expressed sodium channel subtypes remains unclear, this differential nerve block has been implicated in the preferential inhibition by mexiletine of static allodynia in a mouse model of postherpetic neuropathic pain [16]. In this study, we found that the efficacy of mexiletine on the CCI-induced dynamic allodynia appeared to be essentially comparable to that on static allodynia, indicating that the two components of CCI-induced mechanical allodynia are equally susceptible to the sodium channel blocker at doses tested.

Our data also demonstrate that pregabalin, used as a reference drug, is capable of inhibiting both static and dynamic allodynia induced by CCI in rats, as has been previously reported [9]. The mechanism by which pregabalin exerts its antinociceptive activities against CCI-induced mechanical allodynia remains to be fully investigated, evidence has accumulated to indicate that it acts through potently binding to the $\alpha 2\delta$ subunit of voltage-gated calcium channels to reduce depolarization-evoked calcium influx at the nerve terminals, and consequently suppresses the release of excitatory neurotransmitters, including glutamate, noradrenaline, substance P and calcitonin gene-related peptide [3].

In summary, results presented here provided the first preclinical evidence that a subtype non-selective sodium channel blocker mexiletine, like pregabalin, could alleviate dynamic as well as static allodynia induced by CCI in rats, rationalizing its use in clinical practice for treatment of neu-

ropathic pain.

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