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Potent analgesic effects of a putative sodium channel blocker M58373 on formalin-induced and neuropathic pain in rats

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Abstract

M58373, 4-[2-(4-hydroxy-4-{[*N*-(4-isopropoxyphenyl)-*N*-methylamino]methyl}piperidin-1-yl)ethyl]benzonitrile monohydrochloride, is a novel compound, which has an inhibitory activity on neurotoxin binding to the site 2 of voltage-gated sodium channels. In this study, we investigated the effects of M58373 on substance P release from sensory neurons in vitro and pain behaviors/responses in rats, compared with mexiletine. M58373 (1–10 μM) inhibited veratridine-induced release of substance P from dorsal root ganglion cells. In the formalin test, oral M58373 (0.3–10 mg/kg) reduced the time spent in nociceptive behaviors only in the late phase. In the neuropathic pain model, oral M58373 (1–10 mg/kg) attenuated mechanical allodynia and heat hyperalgesia in the nerve-injured paw without affecting normal responses in the uninjured paw. In contrast, oral mexiletine (10–100 mg/kg) had a narrow therapeutic dose range in both models because of the adverse effects on the central nervous system. These results suggest that M58373 is a favorable prototype for novel anti-neuropathic pain agents.

Keywords: Neuropathic pain; Sodium channel; Dorsal root ganglion; Substance P; Nerve injury

1. Introduction

Peripheral nerve injury causes neuropathic pain which can be manifested as allodynia (pain response to non-noxious stimulus), hyperalgesia (increased response to noxious stimulus) and spontaneous pain (Woolf and Mannion, 1999). This hyperexcitability observed in neuropathic pain results at least partly from the accumulation of voltage-gated sodium channels at the nerve-injured site (England et al., 1994; Matzner and Devor, 1994). In clinical practice, several local anesthetics, anticonvulsants and antiarrhythmics are currently used to manage neuropathic pain (Tanelian and Brose, 1991; Kalso, 2005). These drugs commonly bind to sodium channels to prevent the influx of sodium ion into cells (Clare et al., 2000). In addition, several antidepressants and neuroleptics used for neuropathic pain have been shown to inhibit cell excitability evoked by an alkaloid, veratridine, which activates the voltage-gated sodium channel (Deffois et al., 1996; Urenjak and Obrenovitch, 1996).

To search for a novel anti-neuropathic pain agent, we carried out veratridine-induced cytotoxicity assay using the mouse neuroblastoma cell line (Neuro-2A). This cytotoxicity is caused by the activation of sodium channels (Hamasaki et al., 1996). Consequently, we found M58373, 4-[2-(4-hydroxy-4-{[*N*-(4-isopropoxyphenyl)-*N*-methylamino]methyl}piperidin-1-yl)ethyl]benzonitrile monohydrochloride (Fig. 1), which potently inhibited the cell death.

In this study, we examined the effects of M58373 and a subtype-nonselective sodium channel blocker, mexiletine, on veratridine-induced release of substance P from rat dorsal root ganglion cells in vitro. We also investigated their effects in the rat formalin test and neuropathic pain model in vivo. The data obtained have suggested that M58373 has a wider therapeutic dose range than mexiletine.

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2. Materials and methods

2.1. Animals

All experiments were performed in accordance with the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983). In addition, all experimental procedures mentioned below were approved by the Institutional Animal Use Committee of our laboratory.

Young adult male Wistar Hannover rats (Charles River Laboratories Japan, Inc. and Clea Japan, Inc.) at the age of 6–9 weeks were used for the experiments. They were kept in an air-conditioned and pathogen-free room with temperature of 23±2 °C and humidity of 55±10% on a regulated 12-h light/dark cycle. They had free access to standard laboratory chow (CE-2; Clea Japan, Inc.) and drinking water. When the compound was given orally, they were fasted overnight with free access to drinking water.

2.2. Compounds

M58373 was synthesized in our laboratory. Mexiletine hydrochloride (racemate) was purchased from Sigma Chemical Co. In vitro experiments, these compounds were dissolved in pure water, and then the solutions were diluted to the final concentration with the medium. In vivo experiments, they were dissolved in 0.5 w/v% hydroxypropylmethylcellulose and given orally in a volume of 10 ml/kg. The following reagents were used: tetrodotoxin (Sankyo Co., Ltd.); collagenase A (Roche Diagnostics); trypsin (Worthington Biochemical Co.); nerve growth factor-7S, veratridine, captopril, phosphoramidon sodium (Sigma Chemical Co.); substance P enzyme immunoassay kit (Cayman Chemical). In all experiments, an equal volume of the vehicle was used as the control.

2.3. Culture of rat dorsal root ganglion cells

Dorsal root ganglion cells were prepared according to the method of Inoue et al. (1999). The rats were decapitated, and the spinal columns were surgically removed. The columns were bilaterally incised under a dissecting microscope, and then the dorsal root ganglia were aseptically removed. The ganglia were digested with 0.125 w/v% collagenase and 0.1 w/v% trypsin in Hanks' balanced salt solution. Enzymatic reaction was terminated by adding 2 ml of Hanks' solution containing 10% heat-inactivated fetal bovine serum. The ganglia were mechanically dissociated by trituration through a Pasteur pipette until tissue fragments were no longer visible. After washing in Hanks' solution, the ganglion cells were suspended in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 100 ng/ml nerve growth factor. The cells were placed in 48-well culture plates coated with poly-L-lysine and maintained in the medium at 37 °C in an atmosphere of 5% CO_2 for 3 days. Our preliminary experiments have shown that the dorsal root ganglion cells are positively immunostained for substance P and sensitive to capsaicin (100–300 nM) exposure.

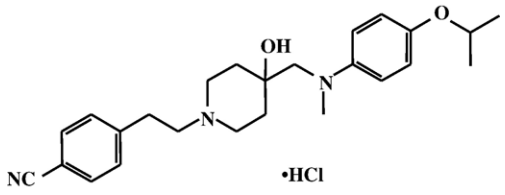

Fig. 1. Chemical structure of M58373.

2.4. Determination of substance P release

The release experiments were performed according to the method of Kessler et al. (1983). The dorsal root ganglion cells were preincubated in Dulbecco's modified Eagle's medium with 25 mM HEPES, 2 mg/ml bovine serum albumin and peptidase inhibitors (10 μM captopril and 10 μM phosphoramidon) containing a test compound at 37 °C for 10 min. After the medium was removed, the cells were incubated in the medium containing 10 μM veratridine and a test compound at 37 °C for 30 min. And then the medium supernatant was collected as the sample for immunoassay.

The substance P content in the supernatant was determined using substance P enzyme immunoassay kit. Optical density at 405 nm was measured with Thermomax microplate reader (Molecular Devices, CA, USA). The concentration of substance P was expressed as picogram per well (pg/well). The limit of detection was 4.1 pg/well. The data presented here are based on 3 independent experiments. Our preliminary experiments have shown that M58373 does not release substance P from these cells by itself.

2.5. Formalin test

The experiment was performed according to our previous report (Akada et al., 2005). The rats were initially acclimated to the acryl cages (Muromachi Kikai, Tokyo, Japan) for 15 min before the formalin injection. Fifty microliters of 0.5% formaldehyde solution in saline was subcutaneously injected into the plantar surface of the rat's left hind paw. Nociceptive behaviors were quantified by measuring the time spent in licking/biting the injected paw every 5 min with a stopwatch. Changes in the time spent in nociceptive behaviors are biphasic. The first reaction between 0 and 10 min after the formalin injection was considered as the early phase, whereas the second reaction between 10 and 45 min after it as the late phase. Compounds were given orally 30 min before the formalin injection (that is, about 1 h before the peak of the late phase). At the end of the experiment, the formalin-injected rats were killed with CO_2 gas.

2.6. Chronic constrictive injury model of neuropathic pain

Chronic constrictive injury model was produced according to the method of Bennett and Xie (1988). The rats were anesthetized with 45 mg/kg, i.p. of sodium pentobarbital. The