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Lidocaine inhibits neurite growth in mouse dorsal root ganglion cells in culture

Hiromi Hiruma[1], Hiroshi Maruyama[2], Zyun'ici B. Simada[3], Takashi Katakura[1], Sumio Hoka[2], Toshifumi Takenaka[4] and Tadashi Kawakami[1]

[1]Department of Physiology, Kitasato University School of Medicine, 1-15-1 Kitasato, Sagamihara, 228-8555, Email: hiruma@med.kitasato-u.ac.jp; [2]Department of Anesthesiology, Kitasato University School of Medicine, Sagamihara, 228-8555; [3]Phonetics Laboratory, Kitasato University School of Allied Health Sciences, Sagamihara, 228-8555; [4]Faculty of Physical Education, Kokushikan University, Tokyo, 206-0025, Japan

Abstract. Recent clinical and experimental studies suggest the effectiveness of lidocaine in blocking neuropathic pain. Because it has been demonstrated that the pathogenetic mechanisms of neuropathic pain involve morphological changes in afferent neuronal terminals onto spinal cord, we examined the effects of lidocaine on neurite growth in isolated mouse dorsal root ganglion cells in culture. Incubation for 2-42 h with various concentrations of lidocaine (0.006 mM, 0.6 mM, and 30 mM) reduced the number of cells exhibiting neurites. The effects were time- and dose-dependent. Lidocaine therefore may exert its pharmacological effect, at least in part, by changing neuronal structures derived from sensory neurons.

Key words: Lidocaine, neurite growth, neuropathic pain, mouse dorsal root ganglion cells, culture

Neuropathic pain that follows nerve damage is often severe and persistent even after complete healing of damage tissues. Recently, anatomical study has demonstrated that after peripheral nerve injury the central terminals of myelinated Aβ afferents, instead of unmyelinated C afferents, sprout into lamina II of the rat dorsal horn (Woolf et al. 1992), suggesting that the pathogenetic mechanisms of neuropathic conditions involve plasticity of neuronal structures within the spinal cord where peripheral sensory neurons are terminated. On the other hand, lidocaine has been shown to relieve a variety of neuropathic pains. Experimental study using a rat model of peripheral neuropathy has demonstrated that local treatment with lidocaine prior to injury blocks thermal hyperalgesia, and this blocking effect sustains for more than 1 week (Sotgiu et al. 1995). Recent clinical and experimental investigations have suggested that systemic administration blocks various types of neuropathic pain (Kastrup et al. 1987, Bach et al. 1990, Rowbotham et al. 1991, Abram et al. 1994, Chaplan et al. 1995, Ferrante et al. 1996, Puig et al. 1996, Wallace et al. 1997). Analgesic effects on neuropathic pain induced by 60 min-infusion of lidocaine last for several weeks in rats with tactile allodynia (Chaplan et al. 1995). Thus, we hypothesize that lidocaine could affect neuronal structures of afferent neurons. If this is true, lidocaine can permanently prevent pains developed by excessive or abnormal morphogenesis in sensory neurons. To test this hypothesis, we investigated the effects of lidocaine on neurite growth in isolated mouse dorsal root ganglion (DRG) cells in culture. We used various concentrations of lidocaine, 0.006 mM, 0.06 mM, 30 mM and 60 mM, relevant to the clinical use in both cases of administrations locally (≤1-2% = 43-85 mM) and systemically (plasma concentrations: 1.5-7 μg/ml = 0.006-0.03 mM). In addition, because a prolonged nerve block (12-16 h) has been reported to prevent hyperalgesia (Kissin et al. 1998), we also investigated the time-dependence of the effects of exposure to lidocaine for up to 42 h.

The somata of DRG cells were isolated from adult male c57BL/6 mice (8 weeks old). Mice were killed with ether. The ganglia were removed and incubated for 90 min at 37°C in F-12 medium containing 0.2% collagenase (Worthington Biochemical, Freehold, NJ). Subsequently, the ganglia were incubated for 15 min at 37° C with Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (g/l: KCl, 0.4; KH_2PO_4, 0.06; NaCl, 8; $Na_2HPO_4/7H_2O$, 0.09; D-glucose, 1; Phenol Red, 0.01; HEPES 3.6; NaOH, 0.3) containing 0.25% trypsin (Sigma Chemical Company, St. Louis, MO). Trypsin activity was then inhibited by addition of trypsin inhibitor (0.125 mg/ml, Sigma). After a rinse with enzyme-free medium, the ganglia were triturated with fire-polished pipettes. The cells were plated onto polylysine (Sigma)-coated 35-mm Petri dishes and were cultured in F-12 medium containing 10% fetal bovine serum and penicillin (100 units/ml)-streptomycin (100 g/ml) at 37°C under 5% CO_2 (pH 7.3). Following 24 h-incubation after plating, the culture medium was changed to fresh medium with or without lidocaine hydrochloride (Research Biochemicals International, Natric, MA), and cells were incubated again under conditions described above. In lidocaine-containing medium, pH was adjusted to 7.3 under culture conditions by adding NaOH. In order to investigate whether the effect of high osmolarity can be eliminated from that of lidocaine, the effects of 120 mM mannitol that produced the same osmolarity (+120 mOsm) as 60 mM lidocaine were also examined. Just before, and 2, 18 and 42 h after incubation with lidocaine, the number of cells exhibiting neurites was counted at x100 magnification in the same field of a phase contrast microscopy (TMD 300, Nikon, Tokyo, Japan). A neurite was identified as a process longer than two cell body diameters. Because higher concentrations of lidocaine caused cell death in our experiments, the number of survival cells was simultaneously analyzed, and percentages of cells with neurites compared with survival cells were calculated. Viability of cells was judged from their bright appearance under phase contrast. Student's *t*-test was used to test for statistical significances. Data are presented as mean ± SD.

The morphologies of DRG cells treated with lidocaine are demonstrated photographically in Fig. 1. In cells treated with lidocaine, neurite growth was prevented (Fig. 1b, d), or cell bodies were swollen or destroyed when higher doses of lidocaine were used (Fig. 1c). We found that the exposure to lidocaine resulted in a time- and dose-dependent decrease in cell survival (Fig. 2). Cells that were exposed to 60 mM lidocaine began to die within 10 min after the addition of lidocaine (data not shown) and most of cells died within 2 h. Cells incubated with 30 mM lidocaine survived only 30% after 2 h-incubation and almost no cell survived after 18 h-incubation. Lower doses of lidocaine used in this study were less toxic to cell survival. When incubated with 0.6 mM lidocaine, most of cells were alive 2 h after incubation, but 20% and 40% of cells died after 18- and 42-h incubation, respectively. Lidocaine at concentration of 0.006 mM

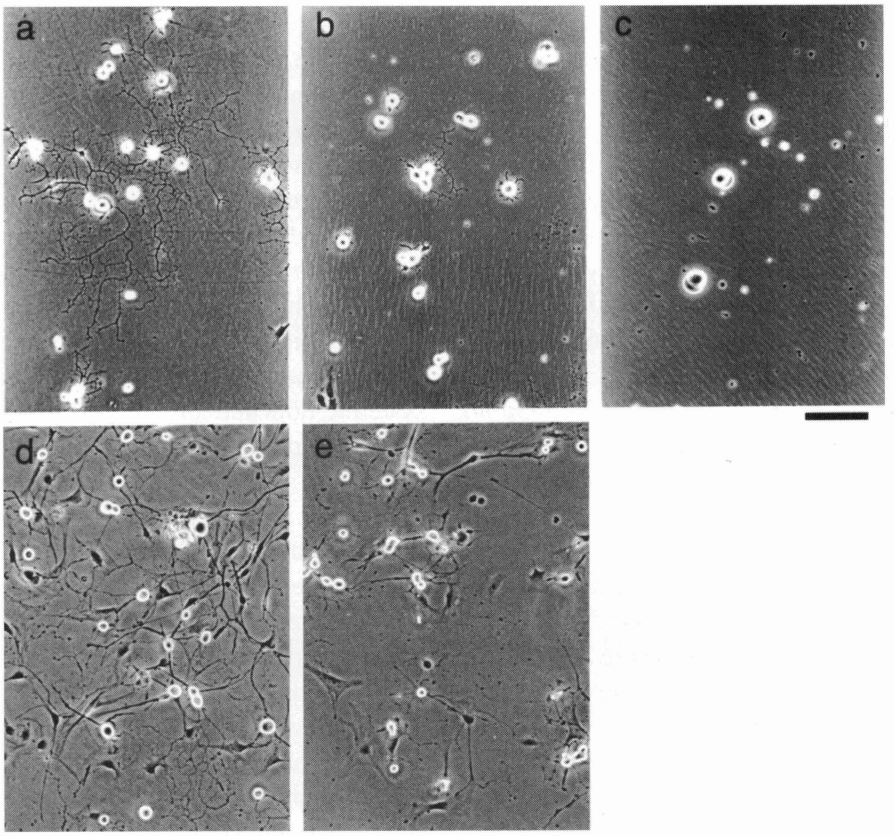

Fig. 1. Photographs of cultured mouse DRG cells treated with or without lidocaine. Cells were incubated in culture medium with or without lidocaine at 37°C under 5% CO_2. a, non-treated cells (control) 18 h after incubation. b, cells exposed to 0.6 mM lidocaine for 18 h. c, cells exposed to 60 mM lidocaine for 30 min. d, non-treated cells (control) 42 h after incubation. e, cells exposed to 0.006 mM lidocaine for 42 h. Scale bar; 100 μm.

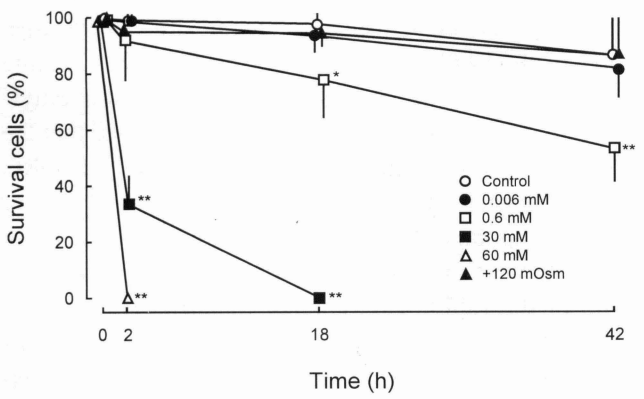

Fig. 2. Cell survival after the addition of various concentrations of lidocaine. Cells were incubated with 0.006 mM, 0.6 mM, 30 mM and 60 mM lidocaine or 120 mM mannitol that produced +120 mOsm (the same osmolarity as 60 mM lidocaine) at 37°C under 5% CO_2. Each point illustrates the mean (±SD) of the values obtained from five experiments. *P<0.05, **P<0.005 compared with the control (non-treated cells) at corresponding times.

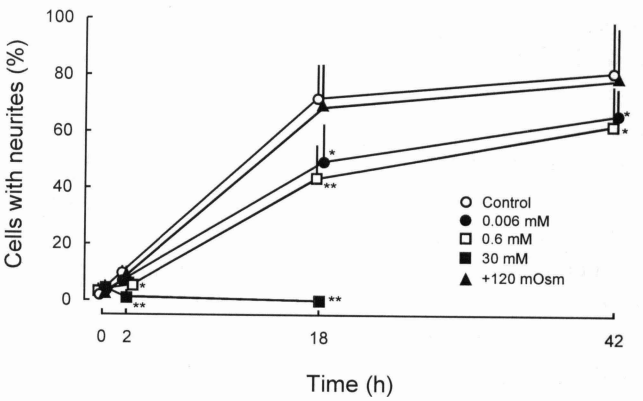

Fig. 3. The number of cells expressing neurite after the addition of various concentrations of lidocaine. Cells incubated with 0.006 mM, 0.6 mM and 30 mM lidocaine or 120 mM mannitol at 37°C under 5% CO_2 were used to analyze. A neurite was identified as a process longer than two cell body diameters. Each point illustrates the mean (± SD) of the values obtained from five experiments. *P<0.05, **P<0.005 compared to the control (non-treated cells) at corresponding times.

did not cause so extensive cell death even after 42-h incubation. Cell survival was not affected by 120 mM mannitol (+120 mOsm) throughout the observation period, therefore, the effect of lidocaine was not due to high osmolarity. Counts of cells exhibiting neurites among survival cells revealed that lidocaine decreased the number of cells with neurites in a time- and dose-dependent manner (Fig. 3). Cells incubated with 30 mM lidocaine revealed no sprouting of their neurite throughout an 18 h-incubation period. Incubation with 0.6 mM lidocaine significantly reduced the number of cells with neurites when observed 2, 18 and 42 h after incubation. Significant reduction was detected even in cells incubated with a much lower concentration (0.006 mM) of lidocaine for 18 and 42 h. High osmolarity (+120 mOsm) did not affect neurite growth, indicating that the effects of high osmotic pressure were eliminated from the inhibitory effect of lidocaine on neurite growth.

Toxic effect of lidocaine on neuronal cells, in relation to the neurologic deficit caused by local anesthetics, has been reported. In various types of neurons, exposure to lidocaine immediately blocks impulse conduction in concentrations 0.005% = 0.21 mM (Fink et al. 1972, Byers et al. 1973) and inhibits rapid axonal transport in higher concentrations (0.1-0.6% = 4.3-26 mM) (Fink et al. 1972, Anderson et al. 1973, Byers et al. 1973). Further increases in concentrations for a longer time (0.6% = 26 mM, 75-90 min) induce irreversible changes in neuronal ultrastructures such as microtubules and neurofilaments in the rabbit vagus nerve *in vitro* (Fink et al. 1972, Byers et al. 1973). More recent evidence has shown that high concentrations of lidocaine induce irreversible membrane depolarization of the crayfish giant axon (80 mM, 30 min) (Kanai et al. 1998) and result in cell death in cultured rat DRG cells (30 mM, ≥4 min) (Gold et al. 1998). In the last decade, systemic treatment of lidocaine has been emphasized to relieve neuropathic pain, although the concentrations around neurons reached by systemic infusion are much lower than that achieved when given locally. Therefore, it is now necessary to evaluate the pharmacological effect of lidocaine at lower concentrations than those previously studied. In the present study, we demonstrated that lidocaine at concentrations of not only 0.6 mM and 30 mM but also a much lower concentration, 0.006 mM, inhibited neurite growth in cultured mouse DRG cells. This is the first description of the inhibitory effect of lidocaine on axogenesis of sensory neurons and the effectiveness of lidocaine in micromoles concentration.

The results obtained in the present study may shed light on the analgesic mechanisms of lidocaine on neuropathic pain. In this study, we used isolated DRG cells whose neurites had once removed in the process of cell isolation. As described above, lidocaine inhibited the induction of neurite growth in these cells. Therefore, during a period of development of sensory structure as is in the genesis of neuropathic pain, lidocaine may be capable of inhibiting a sprouting of neurites. However, *in vivo* investigation will be required to address this issue.

Other possible mechanisms of the analgesic actions of systemic lidocaine on neuropathic pain have been suggested in previous studies using animal models. Intravenous infusion of lidocaine inhibits spinal sensitization induced by activation of C afferents (Abram et al. 1994, Omana-Zapata et al. 1997) and attenuates generation of action potentials at injury sites and in axotomized dorsal root ganglion (Devor et al. 1992, Omana-Zapata et al. 1997). Such actions of lidocaine on neuronal functions may be involved in the relief from neuropathic pain. However, if the analgesic action of lidocaine is prolonged for more than several weeks as supposed by previous studies (Chaplan et al. 1995, Sotgiu et al. 1995), the effect of lidocaine on neuronal morphology may be explainable for a long-term analgesic action of lidocaine.

At present, however, the cellular mechanism underlying the lidocaine-induced inhibition of neurite growth is not known. In this regard, some other investigators have supposed the neurotoxic mechanisms of lidocaine. Kanai et al. (1998) have proposed that the loss of resting potential caused a persistent neurologic deficit in crayfish giant cells. Gold et al. (1998) have described that the lidocaine-induced neurotoxicity on cultured rat DRG cells is caused by an increase in intracellular Ca^{2+} rather than by depolarization. It has been also reported that ultrastructures of cytoskeletons are destroyed by lidocaine in the rabbit vagus nerve *in vitro* (Byers et al. 1973). However, it is not known whether these mechanisms can be applied to the effects induced by low concentrations of lidocaine. In this regard, further studies will be required.

Finally, our results indicate that the duration of exposure to lidocaine may also be important to exert its effects. Exposure to lidocaine even at low concentrations resulted in the inhibition of neurite growth when the treatment duration was prolonged. As implicated in previous studies indicating that a prolonged nerve block effectively prevents hyperalgesia, the duration of exposure

to lidocaine may be considered as on important factor in the appropriate usage of lidocaine.

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