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Research paper

Lidocaine/multivalent ion complex as a potential strategy for prolonged local anesthesia

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ABSTRACT

Local anesthetics are adopted in clinical practice to manage the perioperative and/or postoperative pain. However, their relatively short duration of action limit their ability to meet clinical needs. Herein, we prepared a lidocaine/multivalent ion complex (*ic*LD) using aqueous solutions containing positively charged LD and a multivalent counter-ion as a system for producing prolonged anesthesia. The results of the in vitro and in vivo experiments indicated that the *ic*LD facilitates prolonged LD release even without adjuvants and thus provides nerve blockade for a long duration of action (~14 h) without further increase in neurotoxicity than the LD itself. These findings suggested that the *ic*LD could be a practical strategy for effectively controlling perioperative and/or postoperative pain in clinical practice.

1. Introduction

Most patients who undergo surgical treatment are likely to experience moderate to severe pain [1], making the management of perioperative/postoperative pain a major concern of both surgeons and patients [2]. Appropriate pain control decreases the physical and economic burdens placed on patients and their families [3,4], and minimizes morbidity after surgery [5]. Since 1884, when cocaine was first introduced to relieve pain during operations, various local anesthetics (e.g., lidocaine, chloroprocaine, mepivacaine, bupivacaine, ropivacaine) have been developed and adopted in clinical practice to manage perioperative/postoperative pain [6]. However, their relatively short duration of action (e.g., lidocaine's effect lasts only 1–2 h [7]) continues to limit their ability to meet clinical needs. To overcome this barrier, multiple injections of these local anesthetics are commonly used; however, the additional injections can themselves inflict severe pain and even result in patient apprehension. For more reliable anesthesia, continuous infusion of the anesthetic agent through a catheter has been adopted to provide a constant concentration of the drug at the target site; however, limitations to this approach include the cost of the infusion device, the need for hospitalization, the risk of infection, and sometimes irreversible muscle damage [8,9]. In recent years, the administration of adjuvant agents such as epinephrine [10], clonidine [11,12], and dexamethasone [13,14] has been reported as a way of prolonging anesthetic action; an additional measure involves the use of delivery matrices, such as liposomes [15–18], microemulsions [19], microspheres [20–22], microcrystals [23], cross-linked hydrogels [24], thermosensitive hydrogels [25,26], thermosensitive nanogels [27], nanoparticles [28,29], an aqueous polymer solution [7], a liquid-type polymeric matrix [30], a solid-type polymeric matrix [31], a bioadhesive film [4,32,33], an interpenetrating polymer network (IPN) matrix [34], lipid-protein-sugar particles [35], a fatty-acid dimer-based polymer [36], and ceramic-based granules [37]. Among these options, the liposome-based delivery system (multivesicular liposomal bupivacaine, or Exparel®) has been approved for human use. In the rat model, Exparel® has been shown to approximately double the duration of action of sciatic nerve blockade (4 h, vs. 2 h with bupivacaine alone [18]), because of the sustained release of bupivacaine from the liposome. Despite the promising outcomes when anesthesia can be prolonged through the use of adjuvants and delivery matrices, certain hurdles need to be surmounted before these supplemental agents can be widely applied in clinical practice [13,18,38]. Such hurdles include the insufficient duration of action (with respect to adjuvants) and the fact that anesthetic actions are not reproducible owing to different (uncontrolled) drug release patterns with each fabrication batch [13,18,38]. Based on a

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review of the literature, we hypothesized that if a local anesthetic system could be developed that offered extended (long-term) release and a reliable rate of release for prolonged anesthesia, it might expand the clinical use of local anesthetic agents and appropriately control perioperative/postoperative pain.

In our previous study [39,40], we reported that an ionized drug and a multivalent counter-ion can form a complex that offers sustained release of the drug despite the lack of an additional delivery matrix. We also recognized that such a drug/multivalent ion complex might be a simple way to deliver a drug over time, thus prolonging its therapeutic effects. Therefore, our main goals were to fabricate a local anesthetic/multivalent ion complex that would allow sustained release of the anesthetic from the complex and to conduct a feasibility study to determine whether this complex could be part of a strategy to prolong anesthetic action (by extending the release period of the drug). Fig. 1 shows a schematic diagram of the expected mechanism involved in the formation of a complex between a positively charged drug and a multivalent counter-ion, as well as its release profile over time.

Lidocaine hydrochloride (LD), which is an ionized drug in an aqueous solution, was selected as a model local anesthetic to estimate whether or not the local anesthetic/multivalent ion complex could offer prolonged anesthesia. LD is commonly used to control acute or chronic pain, has relatively low toxicity compared with other local anesthetics [41], and has a positive charge in an aqueous solution that allows it to form an ion complex with multivalent counter-ions. We therefore prepared a lidocaine/multivalent ion complex (*ic*LD) by simply mixing two aqueous solutions containing positively charged LD and a multivalent counter-ion (PO_4^{3-} dissociated from potassium phosphate [K_3PO_4]) (see Fig. 1) and investigated its yield, release profile, and cytotoxicity. We also estimated the duration of anesthesia and neurotoxicity of *ic*LD in an animal model (i.e., sciatic nerve block in the rat).

2. Materials and methods

2.1. Materials

Lidocaine hydrochloride (LD; Sigma, USA) as a positively charged anesthetic and potassium phosphate tribasic (K_3PO_4; Daejung, Republic of Korea) as a negatively charged trivalent ion source were used to fabricate a lidocaine/multivalent ion complex (*ic*LD). Water was purified using a Milli-Q purification system (Millipore Co., USA).

2.2. Fabrication of lidocaine/multivalent ion complex (icLD)

The LD aqueous solution (20 mg/mL, pH ~ 5.4; i.e., the concentration used in clinical applications) and the K_3PO_4 aqueous solution (200 mg/mL, pH ~ 12.9) were prepared, respectively. Both transparent solutions were mixed with the same volume at room temperature (pH ~ 12.7). The *ic*LD precipitate (powder) created by the complex formed between LD and trivalent PO_4^{3-} was obtained after thoroughly washing with excess water (pH ~ 7.0) to remove free LD and K_3PO_4 and was then vacuum-dried for 24 h. For cell culture and animal study, all procedures to obtain the *ic*LD powder were conducted under an aseptic environment. To measure the actual LD in the *ic*LD, the *ic*LD powder (1 mg) was completely dissociated in water (1 mL) for 24 h and the amount of LD was determined using high performance liquid chromatography (HPLC; Shimadzu LC-2010A HPLC System, Japan). The HPLC conditions were as follows: column, Shodex C18 (150 × 4.6 mm, 5 μm particle; Shodex, Japan); mobility phase, acetonitrile/water (7/3, v/v); flow rate, 0.8 mL/min; UV/Vis detector; and wavelength, 254 nm. Residual K^+ ion in the *ic*LD powder, which can lead to hyperkalemia (high levels of potassium in the blood) was also estimated using an inductively coupled plasma-optical emission spectrometer (ICP-OES) (PerkinElmer, Optima 8300, USA).

2.3. Drug release study

Each white powder (4 mg) of the free LD and the *ic*LD (actual LD, ~3.64 mg) was loaded in a 15 mL conical tube. And 4 mL of phosphate buffered saline (PBS; pH ~ 7.4) was added in the conical tube, and then incubated at 37 °C for 24 h. At predetermined time intervals (0.5, 1, 3, 6, 9, 12, 15, 18, 21 and 24 h), the total supernatant in each tube was carefully sampled using a micropipette to prevent loss of the *ic*LD and fresh PBS was replaced to the same volume. The amount of released LD in the sampled medium was determined by HPLC by means of the same protocol as described above.

2.4. Cytotoxicity of icLD

The cytotoxicity of the free LD and of the *ic*LD at different drug concentrations (1.25, 1.50, 1.75, 2.00, 2.25 and 2.50 mg/mL) was estimated by means of total dissociation (or dissolution) in a cell culture medium [Dulbecco's modified Eagle's medium (DMEM)] (Gibco Laboratories, Gaithersburg, MD). The free LD and *ic*LD were completely dissociated (or dissolved) at 37 °C with mild shaking (100 rpm) for 24 h, and whole media containing LD were collected. To compare the cytotoxicity of the different LD concentrations dissociated (or dissolved) from each specimen (free LD and *ic*LD), we chose the rat pheochromocytoma cell line (PC12) (Korean Cell Line Bank, Republic of Korea) as a model [42]. The PC12 cells in the DMEM culture medium supplemented with 10% fetal bovine serum (FBS) (Gibco BRL, Grand Island, NY) and 1% penicillin G (Sigma) were seeded onto 24-well cell culture plates (Corning Inc., Corning, NY) at a density of 1×10^5 cells/well. At 24 h after seeding, the culture medium was removed from the plates and replaced with the medium containing the different concentrations of LD (1 mL) with 10% FBS, and 1% penicillin G added. After incubation for 24 h, cell viability was estimated by CCK-8 assay according to the manufacturer's instructions. Normal cell culture medium without LD was used as the control (with 100% cell viability).

2.5. Animal study

2.5.1. Application of icLD solution

Sprague-Dawley (SD) rats (weight ~300 g) were used to estimate the duration of anesthetic effect and neurotoxicity of the free LD and of the *ic*LD. A total of 36 rats were used for the analyses. The rats were divided into four groups (9 rats per group) according to the type of anesthetic delivered to the sciatic nerve: (i) normal group (no anesthetic); (ii) the LD 10-mg group [0.5 mL application of free LD-dissolved (dispersed) solution (20 mg/mL in PBS, which is the concentration used in the clinical setting)]; (iii) the *ic*LD 50-mg group [0.5 mL application of *ic*LD-dispersed solution (100 mg/mL in PBS); actual LD, ~45.5 mg)]; and (iv) the *ic*LD 100-mg group [0.5 mL application of *ic*LD-dispersed solution (200 mg/mL in PBS); actual LD, ~91.0 mg)]. The *ic*LD groups with different amount were used to determine the appropriate dose of the *ic*LD, in terms of anesthetic duration and neurotoxicity. The application volume selected to achieve sciatic nerve blockade in the rat was based on previous reports [3,18]. All animal experiments were approved by the Animal Care Committee of Dankook University of Korea (DKU-14-020), and all procedures were performed according to the appropriate guidelines. With the rats under inhalation anesthesia using 2% isoflurane (Hana Pharmaceutical Co., Republic of Korea), the sciatic nerve on the left side was exposed by a lateral