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Cell Viability Test for Alkalinized Lidocaine using the Three-dimensional Cultured Human Skin Model

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Abstract
Tissue damage by lidocaine and alkalinized lidocaine was studied using a three-dimensional cultured skin model. Water for injection, dibasic potassium hydrogen phosphate, and sodium bicarbonate were added to 4% lidocaine, and pH was measured. Dibasic potassium hydrogen phosphate and sodium bicarbonate were added to phosphate buffered saline (PBS) (pH 7.4) and adjusted so that they would be the same as the obtained pH (7.85, 7.90). Each of the test drugs were added to TESTSKINTM (LSE-d) and cultured, and were compared using the MTT method regarding cell viability.

The formazan absorbance decreased in the alkalinized lidocaine compared to PBS and alkalinized PBS. On the other hand, no significant difference was observed in formazan absorbance between lidocaine that was not alkalinized (pH 6.7) and PBS, so it is believed that the alkalinization of lidocaine may have a possibility of decreasing cell viability.

Key words: lidocaine, human skin model, cell viability, Lidocaine

Introduction
In anesthesia management for oral and maxillofacial surgery, a tracheotomy is frequently performed in view of postoperative airway obstruction. Tube discomfort and pains frequently appear due to the placement of endotracheal tubes after surgery. On the other hand, it is said that injecting lidocaine or alkalinized lidocaine into the cuff of an endotracheal tube instead of air causes the lidocaine to extravasate out of the cuff, thus reducing tube discomfort and pain (Huang et al., 1999; Altintas et al., 2000; Dollo et al., 2001; Estebe et al., 2004; Jean et al., 2005).

Lidocaine has a surface anesthetic action and is also frequently used in dental clinical practice, but there is no report on a study of tissue injury by alkalinized lidocaine in the skin or mucosa. In this study, we studied the survival rate of cells affected by lidocaine and alkalinized lidocaine using a three-dimensional cultured skin model.

Materials
Preparation of test solutions
Prior to the experiment, the pH of mixed solutions of 2 ml of 4% lidocaine and 4 ml of water for injection, 2 ml of 4% lidocaine and 4 ml of dibasic potassium hydrogen phosphate (K₂HPO₄), and 2
ml of 4% lidocaine and 4 ml of sodium bicarbonate (NaHCO₃) was measured. Based on the obtained pH values, dibasic potassium hydrogen phosphate and sodium bicarbonate were added to phosphate buffered saline (PBS) and adjusted so that they would have the same pH. In addition, PBS was used as a control drug.

**Experimental Groups**
Divided into the following six groups: Lidocaine + water for injection (pH 6.7); lidocaine + K₂HPO₄ (pH 7.85); lidocaine + NaHCO₃ (pH 7.90); PBS (pH 7.4); PBS + K₂HPO₄ (pH 7.85); and PBS + NaHCO₃ (pH 7.90).

**Methods**

**Cell viability test**
A three-dimensional cultured human skin model (TESTSKIN™, LSE-d, Toyobo, Japan; Fig.1) (Ramsamoq et al., 1998; Hada et al., 2005; Imai et al., 2008; Yamaguchi et al., 2008) was used for the purpose of studying cytopathy. 80 µl each of two specimens from each group was added to the air-exposed surface of a transwell having a diameter of 12 mm, totaling twelve wells, and cultured in an assay medium for 24 hours at 37°C. After washing, the human skin model (LSE-d) was transferred to new 12-well assay plates with 1mL of assay medium containing MTT (Sigma Chemical Co., MO, USA) and then as incubated for 3 hr at 37°C. At the end of staining, the exposed portion of the human skin models were excised using an 8 mm diameter skin biopsy punch and extracted in 0.3 mL isopropanol containing 0.04N HCl. The extracts (0.2 mL) were transferred to a 96 well plate and absorbance was read on a plate reader (SPECTRAmax PLUS, Molecular Devices, Toronto, Canada) at 570 nm with the instrument blanked on isopropanol extraction medium.

**Statistical analysis**
The absorbance was shown as the mean±standard deviation. For statistical processing, Kaleida Graph 4.0 (Synergy Software, PA, USA) for Windows was used, and after an analysis of variance was performed using two factor repeated measure ANOVA, a Fisher’s PLSD multiple comparison test was performed. Less than 1% of the hazard ratio was determined to be a significant difference.

**Results**
Very little Formazan pigment production was observed in Lidocaine + K₂HPO₄ or lidocaine + NaHCO₃. The absorbance of each of the test drugs was 0.126±0.004 for lidocaine + water for injection, 0.062±0.004 for lidocaine + K₂HPO₄, and 0.059±0.006 for lidocaine + NaHCO₃. The absorbance of PBS was 0.145±0.005, with 0.152±0.016 for PBS + K₂HPO₄ and 0.184±0.007 for PBS + NaHCO₃. Compared to the PBS, PBS + K₂HPO₄, and PBS + NaHCO₃, lidocaine + K₂HPO₄ and lidocaine + NaHCO₃ showed low values of formazan absorbance as well as significant differences. However, no significant difference was observed in formazan absorbance between PBS and lidocaine + water for injection (Fig. 2).
Discussion

It has been believed that the action of local anesthetic drugs is reversible and that tissue toxicity would not develop after a nerve block or infiltration anesthesia. However, in recent years, cauda equina syndrome (Rigler et al., 1991) that has developed after spinal anesthesia has been reported, as has damage of the corneal endothelium and the retina after surface anesthesia using a highly-concentrated local anesthetic drug for ophthalmic surgery (Guzey et al., 2001), and therefore the neural toxicity and local tissue toxicity of local anesthetic drugs have become apparent.

In general, local anesthetic drugs are alkalescent, but they are used in a form of hydrochloride when used for treatment in order to obtain solubility and stability. Adding sodium bicarbonate to lidocaine followed by alkalinization increases the ratio of a non-ionization-type of local anesthetic drug, which is important for diffusion, and enhances the anesthetic action. In clinical practice, for the purpose of reducing discomfort and pains due to an endotracheal tube, the usefulness of an injection of alkalinized lidocaine into the cuff has been reported (Dollo et al., 2001; Estebe et al., 2004; Jean et al., 2005). However, there are no reports of any studies on the toxicity of lidocaine and alkalinized lidocaine on the tracheal tissues.

The tunica mucosa is covered with pseudostratified ciliated columnar epithelium consisting of ciliated cells, goblet cells, and basal cells. The three-dimensional cultured skin model (TESTSKINFO) used in this study consists of a dermal layer and an epidermal layer comprising human skin fibroblast and collagen gel, and differs from component cells of the trachea mucosa, but using these involved a simple method of detecting colors for analyzing the number of living cells using the MTT method, and it is believed that they would be useful for assessing the tissue toxicity of liquid test drugs.

The pH of HCl-lidocaine is low, at 3.5–5, but when administered to tissues, it breaks down into ionization-type (cation) and non-ionization type (base) of molecules, and the ratio of cation to base is determined depending on the environment of the surrounding body fluid. It is believed that, if lidocaine is preliminarily alkalinized as in this study, the ratio of the base increases due to the ion-trapping effect (Wong et al., 1993). In this study, using a cultured human skin model, PBS was alkalinized so that the pH would become the same as alkalinized lidocaine, and the cytotoxicity was compared using the MTT method. Consequently, the formazan absorbance decreased in alkalinized lidocaine. MTT is broken down into formazan via a succinate-tetrazolium reductase system that belongs to mitochondrial respiratory chains and only shows activity in living cells. In addition, it is said that the amount of formazan

![Cell Viability of Alkalinized Lidocaine with LSE-d (mean±SD)](image)

*Significantrity difference from lido+water, PBS, PBS+K₂PO₄
pigment is directly proportional to the number of cells having metabolic activity in cultured cells (Watanabe et al., 2001), and it was indicated that the increase in the lidocaine base was significantly involved in a decrease in the survival rate of cells. However, no difference was observed in formazan absorbance between HCl-lidocaine (pH 6.7) and PBS (pH 7.4). On the other hand, compared to HCl-lidocaine (pH 6.7), the absorbance decreased in alkalinized lidocaine (pH 7.85, pH 7.9), resulting in having properties that damage tissues, indicating that there is a risk of lidocaine decreasing cell viability by being alkalinized.

The injection of alkalinized lidocaine into a tracheal tube cuff accelerates the permeability of lidocaine into the cuff, but it is believed that there is a risk that it might cause tracheal tissue damage.

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References


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