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Local Anesthetic Cream Prepared from Lidocaine-Tetracaine Eutectic Mixture

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Local anesthetic creams for the clinical treatment of conditions such as postherpetic neuralgia were prepared as an in-house formulation from the eutectic mixture of lidocaine-tetracaine (LT cream) using two eutectic mixtures of local anesthetic (EMLA) type bases. The LT formulation was compared with a lidocaine–prilocaine (LP cream) eutectic mixture formulated using the same base as EMLA. The chemical stability of lidocaine was examined in advance and was found to be stable for more than 3 months either in LT cream or in LP cream. The release rate of lidocaine from the formulated creams was examined using a cellulose ester membrane. The release rate of lidocaine from LT cream was similar to that from LP cream. The release rate of tetracaine was slightly slower than that of lidocaine in LT cream reflecting the larger molecular size of tetracaine. The penetration rate was examined *in vitro* using a Yucatan micropig skin. The penetration rate of lidocaine was similar between LT and LP creams. Infiltration anesthesia action examined in guinea pigs indicated that the difference between the two creams was statistically insignificant. The present study suggests the equivalence of the LT and LP creams as a local anesthetic and the potential of LT cream for clinical use either in the easy formulation or in the low-cost formulation.

Key words――lidocaine; tetracaine; prilocaine; eutectic mixture; local anesthetic; EMLA®

INTRODUCTION

Various therapeutic agents or nerve block treatments, such as nonsteroidal antiinflammatory drugs, opioid analgesics, mexiletine, *etc*. have been used for the clinical treatment of postherpetic neuralgia. However, there are some cases when the analgesic effect is insufficient or unanticipated side effects occur. Under such circumstances, the eutectic mixture of local anesthetic cream (EMLA®) has been developed especially for the children's pain associated with the needle insertion.[1)] EMLA is a typical, effective local anesthetic cream in which the active ingredient is a lidocaine-prilocaine eutectic mixture. Other commercially available local anesthetic ointments include LMX–4[2)] (or ELA-Max), S-Caine peel,[3)] and S-Caine patch.[4,5)] After lidocaine and prilocaine patents expired, in-house formulations of these drugs began to appear. The advantages of in-house formulations are that freshly prepared formulations are available and the contents of these highly efficacious ingredients are controllable, in addition to the economical advantage. Examples of EMLA-like formulations are the eutectic mixtures of lidocaine-prilocaine,[6)] the eutectic mixture of lidocaine-procaine,[7)] lidocaine-tetracaine[8,9)] and tetracaine alone.[10)] We have also attempted to formulate an EMLA-type cream as an in-house formulation. In our formulation, however, problems with prilocaine occurred. Prilocaine is available only as prilocaine hydrochloride and free prilocaine is commercially unavailable. Accordingly, free prilocane must be prepared from prilocaine hydrochloride prior to formulation. Such complex process to form a lidocaine-prilocaine eutectic mixture could lead to errors in the medical treatment. Accordingly, we have focused on the eutectic mixture of lidocaine-tetracaine as an alternative to the lidocaine-prilocaine mixture. Tetracaine is available not as a salt form but as a free reagent. In addition, tetracaine forms a eutectic mixture with lidocaine at a relatively low temperature of about 18°C and can easily be formulated as a monodispersed cream.

Two formulations of the combination of lidocaine

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and tetracaine have been reported: an in-house formulation (2.5% lidocaine and 2.5% tetracaine)[9] and manufactured S-caine peel and patch (7% lidocaine and 7% tetracaine).[3] The former has been reported in clinical application and no information on the physicochemical or pharmacokinetic properties was included. On the other hand, S-caine products have different formulations from EMLA cream and are for the treatment of different disorders.

This study was carried out to accumulate fundamental information leading to the clinical application of local anesthetic. Physicochemical and *in vivo* studies include the formulations of the lidocaine–tetracaine eutectic mixture, release properties of ingredients from formulated cream, penetration properties of the ingredients in model skin, and a papular method in the guinea pig.

Table 1. Composition of Local Anesthetic Cream Using Lidocaine-Tetracaine and Lidocaine-Prilocaine Eutectic Mixtures

LT cream	
Lidocaine	2.5 g
Tetracaine	2.5 g
Polyoxyethylene hydrogenated castor oil (HCO60)	1.9 g
Carboxypolymethylene (Carbopol 934P)	1.0 g
Ethyl parahydroxybenzoate	0.05 g
Ultrapure water	100 ml

LP cream	
Lidocaine	2.5 g
Prilocaine (free)	2.5 g
Polyoxyethylene hydrogenated castor oil (HCO60)	1.9 g
Carboxypolymethylene (Carbopol 934P)	1.0 g
Ethyl parahydroxybenzoate	0.05 g
Ultrapure water	100 ml

MATERIALS AND METHODS

Reagents Lidocaine, tetracaine, and prilocaine hydrochloride were purchased from Sigma-Aldrich Corp. The following reagents were used for the formulation: carboxypolymethylene (Carbopol 934P, Neveon) to enhance viscosity, polyoxyethylene hydrogenated castor oil (HCO60, Wako Pure Chemicals Industries) to enhance absorption, and ethyl parahydroxybenzoate as a preservative (Wako Pure Chemical Industries). Ultrapure water was prepared using the PURIC Model-R sysytem (Japan Organo).

Preparation of Iidocaine-tetracaine and Lidocaine-prilocaine Cream In-house lidocaine-prilocaine (LP) cream used as a reference was prepared as follows (Table 1). Before preparing the eutectic mixture, free prilocaine was prepared from prilocaine chloride. Free prilocaine thus prepared (2.5 g) and lidocaine (2.5 g) were mixed, and a eutectic mixture was prepared, and then HCO60 was added (A). Separately, ethyl parahydroxybenzoate was dissolved in ultrapure water, and Carbopol 934P was added and mixed well. Finally, the pH value was adjusted to 9.0 (B). LP cream was formulated by mixing (A) and (B).

Lidocaine-tetracaine (LT) cream using a similar base to that for LP cream was prepared as follows (Table 1). Lidocaine (2.5 g) and tetracaine (2.5 g) were mixed, a eutectic mixture was prepared, and then HCO60 was added (A). Separately, ethyl parahydroxybenzoate was dissolved in ultrapure water and Carbopol 934P was added and mixed well. Finally, the pH value was adjusted to 9.0 (B). LT cream was formulated by mixing (A) and (B).

Stability Test The stability of the formulated creams was examined in samples stored at room temperature under room fluorescent lighting. A prescribed amount of the sample was removed from several different parts of a container and the concentration change of lidocaine was monitored using HPLC condition I in Table 2 (1 week, 2 weeks, 1 month, 2 months, and 3 months after the formulation). The color and appearance change of the samples were also examined visually.

Release Rate to Receptor Solution Modified Franz-type diffusion cells were used to examine the drug release rate from the donor cream phase to the receptor solution phase.[11,12] A cellulose ester membrane with 0.3 μm pore size (ADVANTEC, Toyo Roshi Kaisha) was used as a permeating separator. The thickness of the base cream was adjusted to 1.0 mm and the effective area of the cellulose membrane was adjusted to 1.1 cm^2. Phosphate buffer solution 0.05 mol/l (pH 7.1) was used as a receptor solution in a volume of 11 ml. The diffusion cells were kept at 37°C and stirred with a Teflon disk rotator at 600 rpm. The upper space of the base cream was connected to the outside atmosphere through a capillary to avoid pressure difference. The lidocaine released from LT and LP creams was analyzed under HPLC condition I in Table 2. The lidocaine and tetracaine released from LT cream were analyzed using HPLC.

Table 2. HPLC Conditions to Detect Lidocaine and Tetracaine

HPLC condition I

Column	ODS colum (CAPCELL PAK C18 UG120 S 3 μm) 4.6 mm *I.D.* × 150 mml
Mobile phase	Phosphate buffer 0.02 mol/l (pH 3.0) : acetonitrile (11 : 9) mixture including dodecyl sodium sulfate 5.76 g in 2,000 ml of mixture
Detector	UV detector : 254 nm for lidocaine
Method	Internal standard method (benzophenone)
Flow rate	0.9 ml/min
Temperature	29℃

HPLC condition II

Column	ODS colum (CAPCELL PAK C18 UG120 S 3 μm) 4.6 mm *I.D.* × 150 mml
Mobile phase	Phosphate buffer 0.05 mol/l (pH 2.0) : acetonitrile (7 : 3)
Detecter	UV detector : 226 nm for lidocaine 303 nm for tetracaine
Method	Absolute calibration curve method
Flow rate	1.0 ml/min
Temperature	35℃

In this study, the different HPLC condition II in Table 2 was used for the simultaneous detection of lidocaine and tetracaine.

Penetration Study into Model Skin The skin of the Yucatan micropig (YMP) was used as model skin. YMP skin and human skin are known to have similar permeability. In addition, the skin area from one pig was sufficiently wide to avoid variation among different pieces.[11] YMP skin prepared from 5-month old female was purchased from Charles River Japan Inc. and stored at −80℃. The apparatus and procedure were similar to that in the release study. In this case, thawed and pretreated YMP skin was mounted on a diffusion cell instead of the cellulose membrane. Each formulated cream was spread on the upper side of the skin 1.0 mm thick. After 24 h, the skin was removed from the diffusion cell and the cream on the skin surface was washed out thoroughly using ultrapure water. Then the central circle of the skin sample (1.1 cm^2) was dissected from the outer residual. The dissected sample was segmented into the epidermides and corium. Each sample was homogenized in a phosphate buffer solution using a homogenizer (Polytron, Kinematica, Littau). The homogenized sample was centrifuged at 12 000 rpm for 10 min. The supernatant was filtered using a disposable syringe filter unit (DISMIC-25cs cellulose acetate, Toyo Roshi Kaisha) and subjected to HPLC. In this study, HPLC condition I in Table 2 was used.

Infiltration Anesthesia Action Infiltration anaesthesia action was examined *in vivo* in male guinea pigs[13] weighing 250–350 g. Formulated creams were applied on alopecic skin, washed off after 24 h, and the response of the guinea pigs was examined using the maculopapular method. The back skin surface was divided into four quadrants and the test cream and control creams were administrated alternatively. The response was examined 30 times either on the treated or on the control skin. The *t*-test was used to determine statistical significance between respones.

RESULTS AND DISCUSSION

Long Term Stability Test Long term stability of formulated creams was examined and the results are shown in Fig. 1. In this study, the lidocaine content was measured as a representative common ingredient. The overall standard deviation was about 3.6%. It can be concluded from the results that little degradation of lidocaine occurred in any of the cream samples examined within 3 months, as expected based on the atmospheric stability of lidocaine.

The appearance of the creams was also examined. No apparent change in color or the appearance of the liquid phase was observed in LT or LP creams after 3-month storage.

Release Rate of Lidocaine to Receptor Solution The direct release property from the donor cream phase into the receptor solution phase was examined to determine the diffusion rate of lidocaine and tetracaine molecules in LT and LP creams. First, the lidocaine content was measured as a representative common ingredient. The amount of lidocaine released was plotted *vs.* the square root of time, as shown in Fig. 2, assuming that the rate determining step was diffusion.[14] The linear portion that appeared in the initial stage of the plot strongly suggested that the release process was semi-infinite diffusion. It can be seen in Fig. 2 that the release properties of lidocaine in LT and LP creams are similar. The amount of lidocaine released from both creams was saturated after about 8 h of contact. The arrow in Fig. 2 indicates the nominal amount of lidocaine assuming that all lidocaine molecules were released into the receptor solution. The discrepancy between the nominal and the saturated amounts must be ascribed

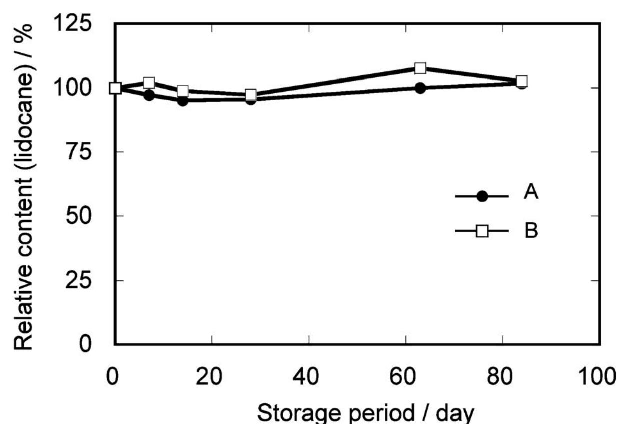

Fig. 1. Long-term Stability of Lidocaine
A, LP cream; B, LT cream.

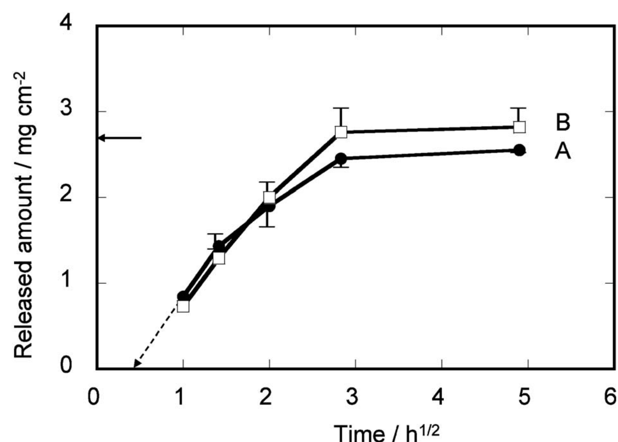

Fig. 2. Release Rate of Lidocaine from Cream Phase into Receptor Solution
A, LP cream; B: LT cream. Arrow indicates expected amount assuming that all lidocaine molecules were released into receptor solution. HPLC Condition I. Error bars indicate standard deviation.

to the error in the weighed amount of the applied cream, with thickness being as great as 1 mm.

The release rate was estimated from the slope of the linear portion of each curve assuming that the release process was diffusion controlled. The initial lag time was observed in a similar manner to that of Almeta and Myser ointments, although the time lag was much shorter than that of the latter two ointments.[12] The linear approximation was assumed on the data points from 0 $h^{1/2}$ to 2 $h^{1/2}$ including the hypothetical starting point at about 0.4 $h^{1/2}$. The release rate thus calculated was 1.3 mg $h^{-1/2}$ cm^{-2} and 1.2 mg $h^{-1/2}$ cm^{-2} for LT cream and LP cream, respectively.

The release rate was also compared between lidocaine and tetracaine in LT cream, as shown in

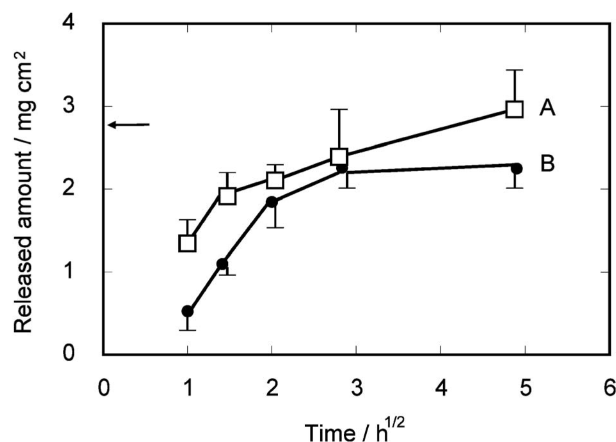

Fig. 3. Release Rate of Lidocaine and Tetracaine from LT Cream into Receptor Solution
Arrow indicates expected amount assuming that all lidocaine or tetracaine molecules were released into receptor solution. HPLC Condition II. Error bars indicate standard deviation.

Fig. 3. In this study, HPLC condition II was used for the simultaneous detection of lidocaine and tetracaine. The release rate of tetracaine was slower than that of lidocaine, while the saturated amount was similar within the limit of experimental error. The slow release rate of tetracaine must reflect the large molecular weight as well as the long molecular shape and resulted in the slow diffusion rate.

Penetration Property of Lidocaine into Model Skin Following the drug release study, the amount of lidocaine penetrated into actual skin was measured using model YMP skin. Only lidocaine was measured as a representative common ingredient. The amount of penetrated lidocaine was measured separately in the corium and epidermides because it was thought that lidocaine would remain in the epidermides layer and could not reach the corium layer. The results are summarized in Fig. 4. It was found that lidocaine passed through the epidermides and diffused to the inner layer of skin tissue. The amount of lidocaine penetrated was similar between LP and LT cream and no significant difference was observed in either the corium or epidermides.

The overall penetration process of a drug into the skin occurs in three steps: (i) diffusion in the cream phase to the interface; (ii) transfer across the interface and adsorption onto the skin surface; and (iii) penetration into the skin. The results in Figs. 2 and 4 were compared taking the different experimental procedures used to determine the release rate and penetration amount into consideration. The amount

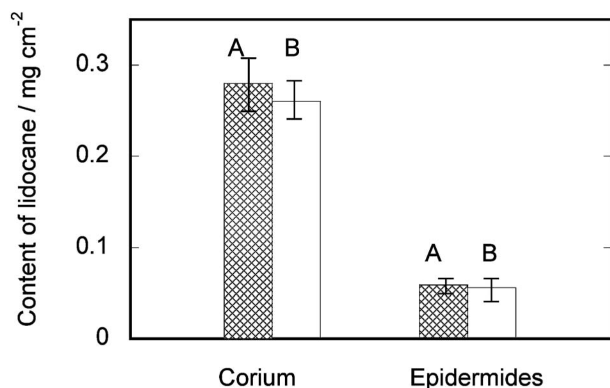

Fig. 4. Amount of Lidocaine Penetrated into Corium and Epidermides

A, LP cream; B, LT cream. $n=3$, $p>0.5$ LP cream *vs*. LT cream. HPLC Condition I was used. Error bars mean standard deviation.

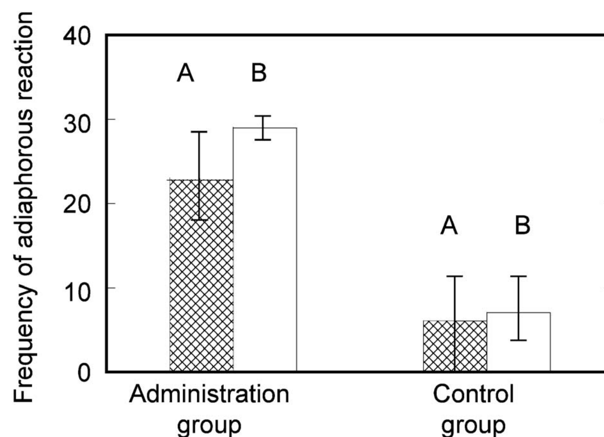

Fig. 5. Infiltration Anesthesia Action Test for LP Cream (A) and LT cream (B)

$n=4$, $p<0.01$. Error bars mean standard deviation.

of lidocaine released through the porous membrane was more than 2.5 mg cm^{-2} 4 h after contact with the solution phase and the amount penetrated was less than 0.3 mg cm^{-2} 24 h after the administration to YMP skin. Accordingly, it was reasonably concluded that the rate-determining step is the penetration through the epidermides layer rather than the diffusion rate in the cream phase or the adsorption onto the skin surface because the adsorption rate onto the skin must be rather fast.[15)]

Infiltration Anesthesia Action Infiltration anesthesia action was examined *in vivo* in guinea pigs to investigate clinical significance. After 30 pinprickings, the adiaphorous reaction was counted. The results are shown in Fig. 5. Although the frequency of adiaphorous reaction after the administration of LT cream appears larger than that of LP cream, these two local anesthetic creams were found to be statistically equivalent in both treated and control surface.

It was found in the present study that local anesthetic cream prepared using a lidocaine-tetracaine eutectic mixture had similar properties to that prepared using a lidocaine-prilocaine eutectic mixture in terms of fundamental clinical effects and the physicochemical properties. In addition, it was reported that the anesthetic effects and long-term effects of tetracaine are more potent than those of prilocaine.[8)] Subsequent clinical investigations in hospital are now necessary. If the clinical benefits of LT cream as an in-house formulation are confirmed, its easy formulation method as well as the economical benefit will be of great help for many patients with various types of disease.

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