

ENHANCED LOCAL ANESTHETIC ACTION OF MEPIVACAINE FROM THE BIOADHESIVE GELS

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ABSTRACT

Mepivacaine, an amide-type local anesthetic, has been used to relieve local pain. Among the many drug delivery systems, transdermal drug delivery has some advantages, as it provides controlled drug delivery for an extended period of time. To develop new gel formulations that have suitable bioadhesion, the bioadhesive force of hydroxypropyl methylcellulose (HPMC) was assessed using an auto-peeling tester. The effect of drug concentration on drug release from 2% HPMC gel was studied using synthetic cellulose membrane at 37±0.5°C. The drug concentrations tested were 0.5, 1, 1.5, 2, and 2.5%. The effect of temperature on drug release from the 2% drug gel was evaluated at 27, 32, 37 and 42°C. To increase the skin permeation of mepivacaine from HPMC gel, enhancers such as saturated and unsaturated fatty acids, pyrrolidones, propylene glycol derivatives, glycerides, and non-ionic surfactants were incorporated into the mepivacaine-HPMC gels. The enhancing effect of the enhancer on drug permeation was then examined in the modified Keshary-Chien cell. For the efficacy study, the anesthetic action of the formulated mepivacaine gel containing enhancer and vasoconstrictor was evaluated with the tail-flick analgesimeter. Among the various kinds of HPMC, HPMC-K100M gel showed the highest viscosity and bioadhesive force. As the viscosity of the HPMC gels increased, the bioadhesive forces increased. Increasing the drug concentration or temperature increased the drug release rate. Among the enhancers used, polyoxyethylene 2-oleyl ether showed the greatest enhancement of permeation. Based on the area under the efficacy curve of the rat tail flick test curve, mepivacaine gel containing polyoxyethylene 2-oleyl ether and tetrahydrozoline showed prolonged and increased local anesthetic action compared to the control. For bioadhesive mepivacaine gels with enhanced local anesthetic action, mepivacaine gels containing penetration enhancer and vasoconstrictor could be developed with the bioadhesive polymer, HPMC.

Keywords: Mepivacaine; gels; penetration enhancer; tail flick analgesimeter; local anesthetic action.

INTRODUCTION

Local anesthetics are widely used in surgical, obstetric and dental patients for chronic pain therapy and the control of postoperative pain (Dahm *et al.*, 2000; Colombin *et al.*, 2006). To relieve local pain, mepivacaine, an amide-type local anesthetic characterized by its long action and high therapeutic power, has been used (Bronaugh *et al.*, 1989).

Among many drug delivery systems, transdermal drug delivery has the advantages of providing controlled drug delivery for an extended period of time. Despite these important advantages, the greatest obstacle to transdermal drug delivery is the stratum corneum (SC), the uppermost layer of the skin that provides the rate limiting step for drug transport (Verma *et al.*, 2003). The use of a transdermal penetration enhancer is a simple and effective method for overcoming this obstacle. Indeed, dermal penetration can be improved by using compounds that have been proven to be effective enhancers on skin (Akimoto and Nagase, 2002).

With applications such as ointments and creams, it is difficult to expect their effects to last for a significant period of time because they are easily removed by wetting, movement and contact (Kleeh, 1999). Therefore, new formulations that have suitable bioadhesion are required. To develop an enhanced local anesthetic mepivacaine gel containing an enhancer, the *in vitro* permeation characteristics and the *in vivo* local anesthetic action were investigated. In this study, the viscosity and bioadhesive force were determined with various kinds of hydroxypropyl methylcellulose (HPMC), a bioadhesive gelling agent (Wu *et al.*, 1998). The effects of temperature and polymer and drug concentrations on drug release were evaluated. To improve permeability through the skin, various enhancers, such as non-ionic surfactants, glycols, glycerides and fatty acids, were incorporated into the gels. The mepivacaine gel containing the best enhancer based on the skin permeation studies was then formulated and evaluated for its local anesthetic effect. Mepivacaine gel containing polyoxyethylene 2-oleyl ether was prepared and the local anesthetic effects of the drug gels were evaluated in the tail flick anesthetic test.

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MATERIALS AND METHODS

Materials

Mepivacaine hydrochloride was supplied from Hanlim Pharm. Co. Ltd. (Korea). HPMC-K100M, HPMC-K10M, and HPMC-K4M were obtained from DOW Chemical Co. Ltd. (USA). Myristic acid, linoleic acid, lauric acid, oleic acid, caprylic acid, stearic acid, palmitic acid, 1-methyl-2-pyrrolidone, 2-pyrrolidone, polyoxyethylene-2-stearyl ether, polyoxyethylene-23-lauryl ether, polyoxyethylene-2-oleyl ether, and polyethylene glycol were purchased from Sigma-Aldrich Co. (USA). Oleyl macrogol-6 glycerides, caprylocaproyl macrogol-8 glycerides, propylene glycol laurate, and propylene glycol monolaurate were gifts from Gatteföse (France). Methanol (HPLC grade) was from J. T. Baker Inc. (USA). All reagents of analytical grade were used without further purification.

Measurement of viscosity and bioadhesive strength

The viscosity of each gel was measured using Haake viscometer (Japan). The sensor was inserted into a gel sample in the MV II cup. The sensor was adjusted to a shear rate of 1.8 1/s and sample equilibration took approximately 45s. The viscosity of the sample was then determined by multiplying the observed reading. Adhesive capacity was determined by measuring the maximum detachment force and the adhesion work was determined using auto peeling tester (CK Trading Co. Ltd. Korea). Cyanoacrylate adhesive was used to fix the intestinal mucosa to the upper and lower support. The HPMC gel was placed on both supports. Upon contact between the gel-intestinal mucosa, a force was applied for five minutes. The detachment procedure was carried out at a speed of 150 mm/min until the complete detachment of the components was achieved. The adhesion work was calculated in gf (gram force).

Preparation of mepivacaine-HPMC gels containing an enhancer

Two grams of HPMC was dissolved in hot water to make 35g. 2% mepivacaine and 5% enhancer were added with vigorous stirring to the above HPMC solution and water was added to make 100g.

HPLC determination of mepivacaine

Mepivacaine was assayed by HPLC method. The HPLC system consisted of a pump (Knauer, DE/K-120, USA.), ultraviolet detector (Waters 484, USA), C₁₈ column (250 x 4.6 mm, 5 μ m), degasser, and integrator (D520A, Youngin Scientific Co., Ltd., Korea). The mobile phase was composed of acetonitrile, methanol, and water (70:20:20, v/v/v). A flow rate of 1.0 mL/min yielded an operation pressure of ~1000 psi. The UV detector was operated at a wavelength of 214 nm. Under these conditions, the mepivacaine peak appeared at a retention time of 7.4 min.

In vitro release of mepivacaine from the HPMC gel through the cellulose membrane

The *in vitro* release of mepivacaine from the HPMC gels was determined using phosphate buffer solution (pH 7.4) as a receptor in the modified Keshary-Chien cell. The diameter of the cell was 2 cm, providing an effective constant area of 3.14 cm² between the cellulose membrane and the 20-ml bulk solution. A synthetic cellulose membrane (SPECTRA/POR[®] MW 12-14,000) was mounted on the receptor compartment of the diffusion cell. Five grams of prepared mepivacaine-HPMC gel was placed in intimate contact with the cellulose membrane and the donor cap was covered with parafilm and clamped. The sampling port was sealed with parafilm to prevent evaporation of the receptor medium. The receptor solution was maintained at 37°C with a circulating water bath and stirred with a magnetic stirring bar. The donor compartment was maintained at ambient temperature. The effect of drug concentration on its release from the gel was studied at drug concentrations of 0.5, 1, 1.5, 2, and 2.5% (w/w), and the effects of temperature on drug release were assessed at 27, 32, 37 and 42°C in a thermostated water bath. The total sample (20 mL) in the receptor compartment was withdrawn at predetermined intervals to maintain a sink condition and then immediately replaced by the same amount of fresh phosphate buffer solution (pH 7.4).

Skin preparation

A male rat (Sprague Dawley rat strain) was sacrificed by excess etherization. The hair of the abdominal area was carefully removed with an electric clipper. A square section of the abdominal skin was excised. After incision, the adhering fat and other visceral debris in the skin were carefully removed from the undersurface with tweezers. The excised skin was used immediately.

Permeation of mepivacaine from the HPMC gel containing an enhancer through rat skin

The freshly excised full-thickness skin sample was mounted on the receptor site of the diffusion cell with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment. Appropriate amounts of gel were placed on the stratum corneum side, covered with a round glass plate and clamped. The receptor medium, phosphate buffer solution (pH 7.4), was maintained at 37°C by a circulating water bath. The total sample was withdrawn at predetermined time intervals and immediately replaced by an equal volume of fresh medium. Permeation quantities of mepivacaine were analyzed by HPLC at 214 nm. Each data point represents the average of three determinations.

The cumulative amount of mepivacaine permeating the rat skin was plotted against time (min). A linear profile was observed for 2 h and the slope of the linear portion of

the curve was determined by linear regression. The effectiveness of penetration enhancers was defined as the enhancement factor (EnF). EnF was calculated using the following equation:

$$\text{EnF} = (\text{flux of HPMC gels containing enhancers}) / (\text{flux of the control})$$

Tail flick anesthetic test

The heat radiant tail flick assay, developed by D'Amour and Smith in the 1940s (D'Amour and Smith, 1941), is an experimental model for thermo-pain quantification. In this test, the rodent tail is exposed to a light source (radiant heat) and the latency of tail withdrawal from the heat source is recorded and analyzed. Depending on the experimental settings, the tail flick technique can be used to determine the basal nociception level, the analgesic effectiveness of pharmacological agents, or the tolerance formation. The tail flick assay is a valuable method due to its simplicity, reproducibility, its relatively low variation, and minimal apparatus requirement. Moreover, a unique feature of the tail flick method versus other thermo-pain quantification methods, such as the hot plate or Hargreaves method, is that the detected end-point, i.e., a flicking tail, is primarily, if not exclusively, a simple, spinally mediated reflex (Ossipov and Gebhart, 1996). The absence of complex behavior (e.g., paw-licking in hot-plate test) is advantageous because it avoids the confounding effects arising from scoring or interpreting more complicated behaviors. In some cases, the tail flick test can even be carried out in lightly anesthetized animals (Ness et al., 1986).

The rats were divided into four groups of three rats each: control gel group, mepivacaine gel group, mepivacaine gel containing enhancer group, mepivacaine gel containing enhancer and vasoconstrictor group.

Each rat was fixed on a tail-flick-test apparatus with a portion of the tail, 10cm from its tip, exposed to heat from a projector lamp. A single control switch simultaneously activated the light and a timer. The timer stopped automatically upon a recognized tail flick. The time interval between switching on the light to the flick of the tail was recorded. A 50-second cut-off time was used to prevent thermal injury. A 50-mg dose of drug gel was applied at the root of the tail on midline. The tail flick test was started after administration, and the test was performed every 5 min until the duration time fell to that of the blank.

The area under the effective curve (AUEC_{0→120min}) from time zero to 120 min for the rat tail flick test was calculated using the linear trapezoidal rule. The efficacy factor in local anesthetic effects of mepivacaine after topical application of mepivacaine gel containing polyoxyethylene 2-oleyl ether was compared with that of

control gel without any additives. The efficacy factor (EfF) was calculated using the following equation:

$$\text{EfF} = (\text{AUEC of mepivacaine gels containing enhancer}) / (\text{AUEC of the control gel})$$

RESULTS

Effects of type of HPMC and concentration on the bioadhesive forces and viscosity

The viscosity of K4M, K10M and K100M HPMC gels at 1.5% concentration was 6.26 mPa·s, 12.83 mPa·s and 26.64 mPa·s, respectively. The bioadhesive force of K4M, K10M and K100M HPMC gels at 1.5% concentration was 0.03, 0.06 and 0.12 N, respectively. Among the various types of HPMC, HPMC-K100M gel showed the highest viscosity and bioadhesive force. As the viscosity of the HPMC gels increased, the bioadhesive forces increased (fig. 1).

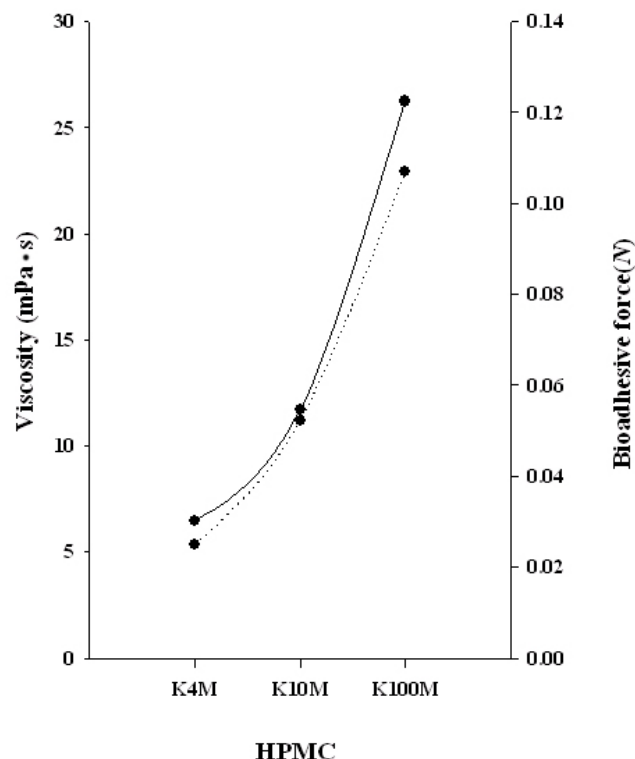


Fig. 1: Bioadhesive forces and viscosity of various kinds of HPMC at 1.5% concentration

...●... bioadhesive forces, -●- viscosity

Based on these experiments, HPMC-K100M showed the highest bioadhesive force and was selected for the bioadhesive gel formulation. To develop a new gel formulation that has suitable bioadhesion, an easy determination of viscosity could be used instead of bioadhesive force assessment.

Effect of mepivacaine concentration on drug release

The effect of mepivacaine concentration on drug release across synthetic cellulose membrane was studied using the prepared HPMC gels at $37 \pm 0.5^\circ\text{C}$. The concentrations tested were 0.5, 1, 1.5, 2 and 2.5%. As the concentration of mepivacaine in the gels increased, the drug release increased. As shown in fig. 2, the drug permeation followed Fick's law and exhibited concentration-dependent passive diffusion. From these experiments, 2% concentration of mepivacaine was chosen for the anesthetic gel formulation.

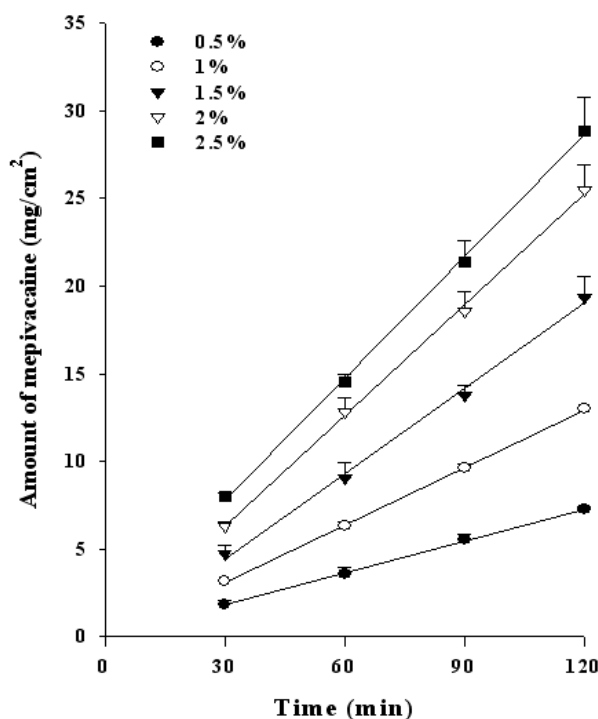


Fig. 2: Effect of mepivacaine concentration on drug release through cellulose membrane from the HPMC gels.

Effect of temperature on drug release

The effect of temperature on the release of mepivacaine from the gel formulations was evaluated at 27, 32, 37, and 42°C . All experiments were carried out at least in triplicate. A linear relationship was observed between the logarithm of the permeability coefficient (P) and the reciprocal temperature (fig. 3). The slope was used to calculate the activation energy (E_a) for drug diffusion. The intercept was used to calculate the pre-exponential term.

The permeability coefficient is then defined by:

$$P = \frac{\text{Flux}}{\text{Solubility}} \quad (1)$$

$$P = P_0 \cdot e^{-\frac{E_a}{RT}} \quad (2)$$

$$\log P = \log P_0 - \frac{E_a}{R \cdot 2.303 \cdot 1000} \cdot \frac{1000}{T} \quad (3)$$

$$\text{Slope} = - \frac{E_a}{R \cdot 2.303 \cdot 1000} \quad (4)$$

$$E_a = - \text{Slope} \times R \times 2.303 \times 1000 \text{ cal} \\ = - \text{Slope} \times 1.987 \times 2.303 \text{ kcal} \quad (5)$$

As expected from Equation 5, the activation energy (E_a) of drug permeation, which was calculated from the slope of $\log P$ versus $1000/T$ plots, was 7.27 kcal/mol for a 2% loading dose. This result clearly indicates that the release of drug from the gels is an energy-linked process (Miyazaki *et al.*, 1984).

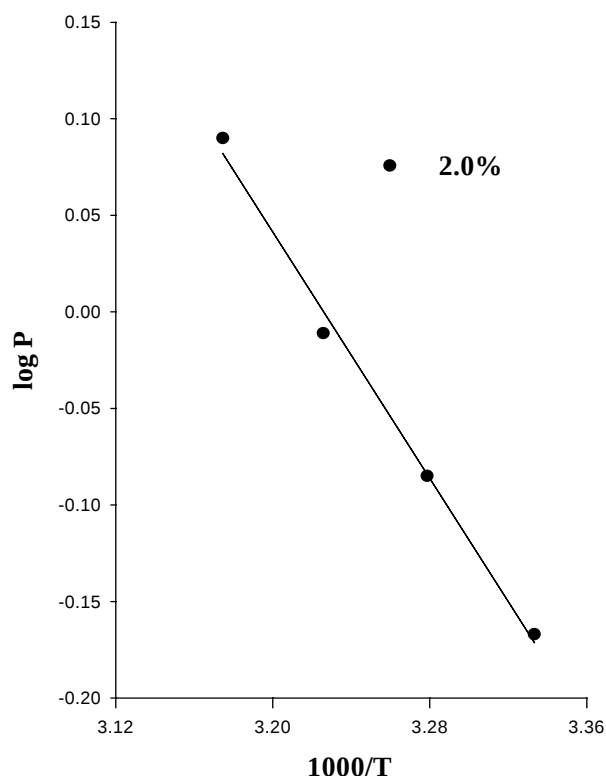


Fig. 3: Effect of temperature on drug release from HPMC gels containing 2% loading dose

Effect of enhancers on the permeation of mepivacaine across rat skin

To increase the skin permeation of mepivacaine from the HPMC gels, various enhancers such as saturated or unsaturated fatty acids, pyrrolidones, propylene glycol derivatives, glycerides, or non-ionic surfactants, were incorporated into the mepivacaine-HPMC gels, respectively.

Enhancement factors of various enhancers are given in table 1. Among the enhancers used in this test, polyoxyethylene 2-oleyl ether (Brij 92) showed the greatest enhancing effects.

DISCUSSION

Effect of enhancers on the permeation of mepivacaine

Skin is a complex, dynamic, layered organ that has many functions beyond its role as a barrier to the environment. The highly organized structure of the stratum corneum (SC) forms a barrier to substance penetration. Through interaction with skin components, penetration enhancers, accelerants, or promoters can increase fluidity in the intercellular lipid lamellae, causing the SC to swell and/or leach out structural components, thus increasing drug penetration through the barrier membrane (Magnusson and Runn, 1999). The enhancement factors of various enhancers are shown in table 1.

Fatty acids are currently receiving much attention as penetration enhancers (Tanojo *et al.*, 1997; Oh *et al.*, 1998). Fatty acids, via interactions with intercellular lipid domains, promote the skin permeation of drugs with a wide range of polarities. The efficacy of fatty acids is intrinsically linked to their structure, with differences evident between saturated and unsaturated forms and those of different hydrocarbon chain length (Kandimalla *et al.*, 1999). Unsaturated fatty acids, particularly those in *cis* conformation and with C₁₈ chain lengths, are more effective enhancers than their saturated counterparts, promoting the permeation of penetrants such as naloxone (Aungst *et al.*, 1986) and flurbiprofen (Chi *et al.*, 1995). When compared with the control, the saturated fatty acid enhancers slightly increased the permeation rate. Among the saturated fatty acids, myristic acid showed the highest

permeation rate. Among the unsaturated fatty acids like oleic acid and linoleic acid, oleic acid showed significantly increased permeation of mepivacaine from HPMC gels. A saturated fatty acid group increased the permeation rate more than an unsaturated fatty acid group. Surfactants have been reported to enhance the permeability of drugs (Lopez *et al.*, 2000; Shin *et al.*, 2001; Shokre *et al.*, 2001). They affect the permeability characteristics of several biological membranes, including skin (Florence *et al.*, 1994; Lopez *et al.*, 2000), and can thus enhance the skin penetration of other compounds present in the formulation. In skin pre-treated with non-ionic surfactant, the SC was loosely layered and the intercellular spaces were wide (Shin *et al.*, 2001). Therefore, in recent years, surfactants were used to enhance the permeation rates of several drugs. Among the non-ionic surfactants used, polyoxyethylene 2-oleyl ether (Brij 92) showed the greatest enhancing effects.

Caprylocaproyl macrogol-glyceride (Labrasol) increases the passive transport of drug molecules. It exhibits high tolerance and low toxicity. It is included as a pharmaceutical excipient in European Pharmacopoeia (2008). Oleyl macrogol-6 glyceride (Labrafil) is a PEG derivative that is used as a co-surfactant in pharmaceutical systems such as microemulsions. This substance is biocompatible and biodegradable (Gao *et al.*, 1995). Among the glycerides, oleyl macrogol-6 glyceride induces significant permeation of mepivacaine. Propylene glycol (PG) is widely used as a vehicle for penetration enhancers and permeates well through the human stratum corneum. PG readily permeates the skin and in so doing

Table 1: Enhancement factor for various enhancers

Enhancer	Flux (/cm ² /hr)	EnF
Control	11.90±1.86	1
Polyoxyethylene 2-stearyl ether	19.12±5.37	1.61
Polyoxyethylene 23-lauryl ether	15.06±3.96	1.27
Polyoxyethylene 2-oleyl ether	21.41±6.31	1.80
Oleic acid	14.06± 1.61	1.18
Linoleic acid	11.93 ±0.38	1.00
Myristic acid	18.44±2.94	1.55
Palmitic acid	13.48 ±1.01	1.13
Lauric acid	14.55± 0.93	1.22
Stearic acid	12.27± 1.40	1.03
Caprylocaproyl macrogol-8 glycerides	17.04±0.72	1.43
Oleyl macrogol-6 glycerides	13.03±0.16	1.09
Propylene glycol mono caprylate	17.89±0.77	1.50
Propylene glycol monolaurate	15.37±0.43	1.29
Propylene glycol laurate	13.16±0.87	1.11
2-pyrrolidone	12.25±0.97	1.03
N-methyl-2-pyrrolidone	15.56±2.33	1.31
Polyvinyl-pyrrolidone	10.77±1.50	0.91

may carry the drug molecules across (Adrian and Barry, 2004).

Pyrrolidones have been used as penetration enhancers in human skin for hydrophilic and lipophilic permeants. In terms of their mechanism of action, the pyrrolidones partition well into the human corneum stratum. Within the tissue, they may alter the solvent nature of the membrane and pyrrolidones have been used to generate 'reservoirs' within skin membranes. Such a reservoir effect offers the potential for sustained release of a permeant from the stratum corneum over an extended time period (Jungbauer *et al.*, 2001).

Tail-flick anesthetic test of mepivacaine gel containing an enhancer

In percutaneous permeation studies, polyoxyethylene 2-oleyl ether showed the greatest enhancing effects. The anesthetic action of the formulated gel was evaluated by the rat tail-flick analgesic meter. Table 2 shows the $AUEC_{0 \rightarrow 120min}$ of the rat-tail flick test for anesthetic gels. From the AUEC (area under the efficacy curve) of the rat tail flick test curve, the AUEC of mepivacaine gel containing polyoxyethylene 2-oleyl ether was 2394.08sec.min, while that of mepivacaine gel without enhancer was 1933.42sec.min and of that containing polyoxyethylene 2-oleyl ether and tetrahydrozoline was 2946sec.min. According to the rat tail flick test, mepivacaine gels containing polyoxyethylene 2-oleyl ether showed maximal anesthetic action at 5 minutes, while that for mepivacaine gels occurred at 13 minutes (fig. 4). The efficacy of mepivacaine gel containing polyoxyethylene 2-oleyl ether was about 1.94-fold that of mepivacaine gels without polyoxyethylene 2-oleyl ether. The mepivacaine gel containing polyoxyethylene 2-oleyl ether and tetrahydrozoline showed a 2.36-fold increase in anesthetic activity compared to the control gel without any additives.

These results suggest that the bioadhesive mepivacaine-HPMC gel containing permeation enhancer and vasoconstrictor could be developed for enhanced local anesthetic action.

Table 2: The comparison of $AUEC_{0 \rightarrow 120min}$ from the rat tail flick test for mepivacaine gel with or without enhancer

	AUEC (sec · min)	Efficacy factor
Control	1234.75±34.41	1
Mepivacaine gel	1933.42±45.52	1.57
Mepivacaine gel containing polyoxyethylene 2-oleyl ether	2394.08±201.31*	1.94
Mepivacaine gel containing polyoxyethylene 2-oleyl ether and tetrahydrozoline	2946.56±302.51*	2.39

Each value represents the mean ± S.D. (n=3). *p < 0.05

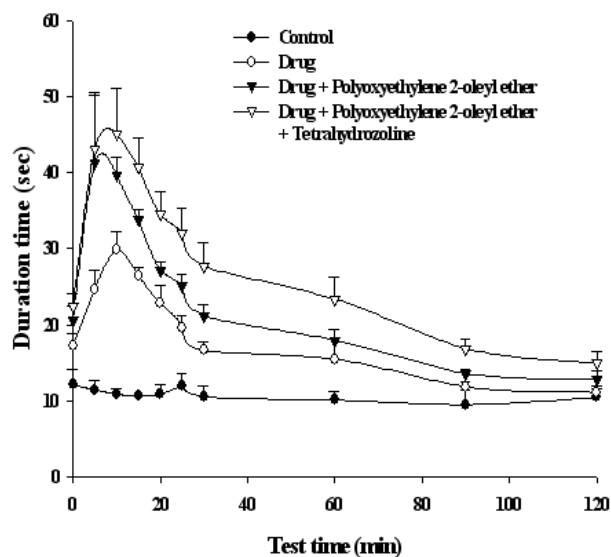


Fig. 4: Tail flick test of 2% mepivacaine gels

CONCLUSION

Increasing the drug concentration or temperature increased the drug release rate. Among the enhancers used, polyoxyethylene 2-oleyl ether exhibited the greatest enhancing effects on drug permeation through skin. From the local anesthetic study of mepivacaine gel, mepivacaine gel containing polyoxyethylene 2-oleyl ether and tetrahydrozoline produced a 2.36-fold increase in anesthetic activity compared to the control gel without any additives. These results suggest that the bioadhesive mepivacaine-HPMC gel containing permeation enhancer and vasoconstrictor could be developed for enhanced local anesthetic action.

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