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Development of the Bioadhesive Tetracaine Gels for Enhanced Local Anesthetic Effects

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ABSTRACT

To develop the new local anesthetic formulations with a suitable bioadhesive property, hydroxypropyl methylcellulose (HPMC)-based gel was formulated. As the drug concentration in the gels, and the temperature of surrounding solutions increased, the drug release increased. The activation energy of tetracaine permeation was 4.47 kcal/mol. The effects of permeation enhancers on the permeation rate of drug through skin were studied using various enhancers, such as the glycols, the nonionic surfactants, and the bile salts. Among the enhancers used, polyoxyethylene 2-oleyl ether showed the highest enhancing effects on drug permeation through skin. The analgesic activity was examined using a tail-flick analgesimeter. In the AUEC (area under the efficacy curve) of the rat-tail flick test, tetracaine gel containing polyoxyethylene 2-oleyl ether showed about 3.2-fold increase in analgesic activity compared with the control. The results of this study support that the tetracaine gels with efficient anesthetic effects could be developed using HPMC and poloxamer with combination of enhancer and vasoconstrictor.

Key Words: Local anesthetics; Hydroxypropyl methylcellulose; Tetracaine; Gels; Enhancer; Tail flick.

INTRODUCTION

In relieving local pains, local anesthetics such as lidocaine, procaine, and tetracaine have been extensively used. Since they have short half-lives after parenteral injection,[1,2] an alternative route to achieve the substantially sustained analgesic effects while avoiding any side effects needs to be considered.

Of many drug delivery systems, percutaneous drug delivery has been widely used as advanced drug

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delivery systems and has some advantages in the controlled and sustained delivery of various locally active drugs.[3] In their percutaneous applications, the local anesthetics must remain in the skin surface as its uncharged, lipophilic form for a substantial period of time, so that it penetrates the stratum corneum and desensitizes the underlying pain receptors within skin.[4,5] It is a little difficult to administer the drug by ointments or creams, since wetting, movement, or contact easily removes them. Therefore, percutaneous delivery formulations that have suitable bioadhesion and enhanced analgesic efficacy are required.

The systemic absorption of various topical formulations is affected by the presence of enhancers and delivery system used. The effect of various classes of enhancers, such as bile salts, surfactants, glycols, and chelators, on percutaneous permeation of model drugs has been studied.[6–15] The results of these studies show that the enhancers greatly influence cutaneous barrier impairment.[3,14] The increase in permeation rate of a drug at low surfactant concentration is normally attributed to the ability of the surfactant molecules to penetrate skin.[15]

The present work aimed to develop a locally active anesthetic formulation with enhanced efficacy and suitable bioadhesive property. Bioadhesive gels for local anesthetics containing penetration enhancers and a vasoconstrictor were formulated. The effects of temperature of surrounding solutions, the loading concentration of drug, and gel compositions on drug release were evaluated. The anesthetic effects of gels were evaluated by the tail flick analgesic test. This study provides insight into the mechanisms of permeation of locally applied anesthetics and lead to the development of a bioadhesive gel that has good analgesic activities.

MATERIALS AND METHODS

Materials

Hydroxypropyl methylcellulose (HPMC) such as K4M, K15M, and K100M were obtained from Dow Chemical Co. (Midland, MI), and Poloxamer 407 was from BASF Co. (Germany). Tetracaine hydrochloride was gifted from Daihan Pharm. Co. Ltd. (South Korea). Polyoxyethylene 2-stearyl ether, polyoxyethylene 2-oleyl ether, polyoxyethylene 23-lauryl ether, tetraethylene glycol (TEG), diethylene glycol (DEG), sodium taurodeoxycholate, sodium deoxycholate, propylene glycol (PPG), and tetrahydrozoline (THZ) were purchased from Sigma Chemical Co. (St. Louis, MO). All reagents of analytical grade were used without further purification.

Preparation of HPMC-Poloxamer 407 Gels Containing Drug and Enhancer

HPMC (0.2 g), poloxamer 407 (2 g) were dissolved in water (about 5 mL) with gentle stirring. The solution was left in a refrigerator overnight to complete polymer dissolution. Three mL of 10% drug solution and enhancer were added to the above polymer solution with vigorous stirring, and water was added to make a final volume of 10 mL.

Measurement of Bioadhesive Forces

To evaluate the bioadhesive forces of polymer, intestines of rats were used. Adhesive forces were determined by measuring the maximum detachment force using Auto Peeling Tester (C.K. Trading Co., South Korea). Cyanoacrylate adhesive was used to fix the intestine mucosa of rats to both the upper and lower supports. The HPMC-poloxamer gels were placed on the mucosa attached to the lower supports (contact surface area, 0.5 cm^2). After applying the gels to intestine mucosa, the force (contact pressure, 50 gram force) was applied for 5 minutes. The detachment procedure was carried out at a speed of 150 mm/min until the complete detachment of the components was achieved. The force required to completely separate two compartments was recorded as adhesion forces, which was designated as gram force (gf).

In Vitro Drug Release

Tetracaine gel was used for release test with 40% PPG/PBS solution using the modified Keshary-Chien diffusion cells. The diameter of the cell was 1.5 cm, providing 1.77 cm^2 effective constant area and the volume of receptor chamber was 7 mL. The synthetic cellulose membrane (Spectra/Por) was mounted on top of the receptor compartment of diffusion cells. The gel (0.5 g) was placed in intimate contact with the cellulose membrane and the donor cap was covered with a parafilm and clamped. The sampling port was sealed with a parafilm to prevent the evaporation of the receptor medium. The receptor compartment was filled with the receptor solution, which was stirred by a magnetic stirring bar and maintained at 37°C by a circulating water bath. The donor compartment was maintained at an ambient temperature of 25° ± 1°C. The effect of drug concentration on its release from the gels was studied according to drug concentrations of 1.5%, 2%, 2.5%, 3%, and 3.5% (w/w). The temperature effects on drug release was studied at 28°, 32°, 37°, and 42°C.

The total samples from the receptor compartment were withdrawn at 1-hour intervals for 6 hours and immediately replaced by the same volume of fresh medium to maintain a sink condition. The sample withdrawn from the receptor compartment was then analyzed by UV spectrophotometer at 310 nm. Each data point represents the average of three determinations.

Permeation Studies

A male mouse (ICR strain) was sacrificed by snapping the spinal cord at the neck. 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 fats and other visceral debris in the skin were carefully removed from the undersurface with tweezers and the excised skin was used immediately. The prepared dermal tissue was mounted on the diffusion cell with a diffusion area of 1.33 cm^2. Five hundred mg of gels containing drug and enhancer, such as the glycols, the nonionic surfactants, or the bile salts were loaded on the diffusion cell. The diffusion studies were undertaken at $37° \pm 0.5°C$. Samples were taken from the receptor side at a predetermined interval, refilled with the same amount of fresh buffer solution, and subsequently analyzed using a UV spectrophotometer. Since using the various permeation enhancers could increase the permeation rate of drug from the HPMC-poloxamer 407 gels, the effects of enhancers on the permeation of drug through skin also were investigated using a similar method. Each data point represents the average of three determinations.

Tail-Flick Analgesic Test

The bioadhesive tetracaine gels containing polyoxyethylene 2-oleyl ether as a penetration enhancer and tetrahydrozoline as a vasoconstrictor were formulated and the anesthetic effect of the gels were evaluated by the tail-flick analgesic test. The rat was fixed on a tail-flick analgesimeter (Tail Flick Analgesimeter, Harvard, MA) with the portion of the tail, 10 cm from its tip, exposed to heat from a projector lamp. A single control switch simultaneously activated the light and a timer. The timer stops automatically when the exposed rat's tail flicks. The time interval between switching on the light and flick of the tail was recorded. A 30-second cut-off time was used to avoid thermal injury. Drug gel (50 mg) was covered at the root of the tail on midline. The tail-flick test started 5 min after a local application of gels, and the test was conducted every 5 min until duration time fell to that of the control gels. Each data point represents the average of three determinations with their standard deviation. An unpaired Student's t-test was utilized to determine significance.

RESULTS AND DISCUSSION

Effects of Kinds of HPMC on the Bioadhesive Forces

To select the appropriate HPMC, the bioadhesive forces of three kinds of HPMC such as K4M, K15M, and K100M at 2% concentration were studied (Fig. 1). The bioadhesive force of 2% HPMC K4M, K15M, and K100M gels was 80, 108, and 128 gf (gf=gram force), respectively. Among three kinds of HPMC, HPMC-K100M gel showed the best bioadhesive force. HPMC-K100M was chosen at 2% as an optimal concentration of gels and used for the rest of studies.

Effects of Loading Dose on Drug Release

It is important to formulate optimal drug concentration for the development of drug product. The high drug concentration induces better efficacy in general. However, when the drug reaches a certain optimal

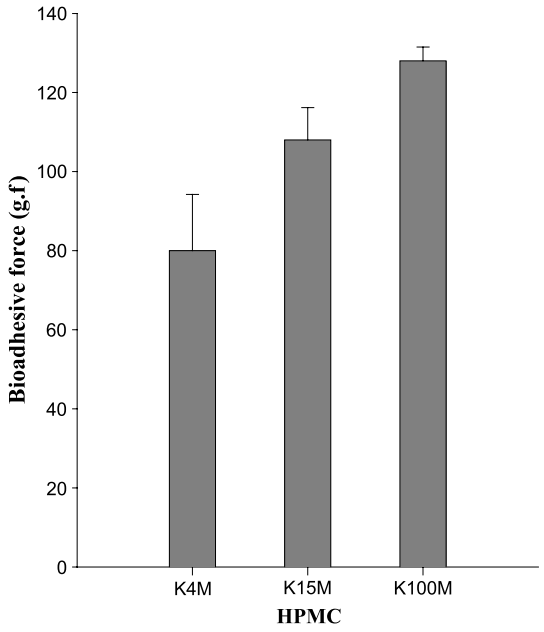

Figure 1. Bioadhesive forces of various kinds of HPMC at 2% concentration.

Table 1. Effects of drug concentration on the flux of tetracaine through cellulose membrane from the poloxamer-HPMC gels.

Tetracaine concentration (w/w%)	Tetracaine gels (n=3) Flux (ug/cm^2/h)
1.5	158.03±7.8
2	250.78±12.3
2.5	334.11±15.4
3	444.98±19.2
3.5	452.39±20.3

concentration, there can be no differences in efficacy for certain drugs, even those treated with much higher concentration. Therefore, the optimal concentration should be decided to have good efficacy for each drug. For this, the effects of loading dose on drug release were studied from the prepared HPMC-poloxamer gels at 37°±0.5°C (Table 1). The effects of loading dose on drug release across synthetic cellulose membrane (Spectra/Por MW 12–14,000) was studied from the prepared HPMC-poloxamer gels at 37°±0.5°C. The drug concentrations tested were 1.5%, 2%, 2.5%, 3%, and 3.5% (Table 1). As the drug concentration in the gels increased up to about 3%, the drug release increased, thereafter slightly increased, but not significantly. Therefore, we could formulate the drug concentration at 3%.

Effects of Temperature on Drug Release

The effects of temperature on drug release from the 3% tetracaine gels was evaluated at temperatures of 28°, 32°, 37°, and 42°C. As the temperature of system increases, the apparent permeation coefficient increases. The relationship between the permeation coefficient and the temperature is as follows:

$$P = P_o e^{-Ea/RT} \quad (1)$$

$$\ln P = \ln P_o - (E_a/1000\ R)(1000/T) \quad (2)$$

The logarithm of permeation coefficient was plotted as a function of the reciprocal of temperature (Fig. 2) and the slope calculated from the linear portion of plot was used to calculate the activation energy. The activation energy for tetracaine permeation was 4.47 kcal/mol. The observation indicates clearly that the release of drug from the gels is an energy-linked process.[16] The increased release with increasing temperature suggests that release characteristics of drug from the gels would change over the body temperature range. These findings indicate that special precautions should be taken with regard to monitoring body temperature in practical applications.

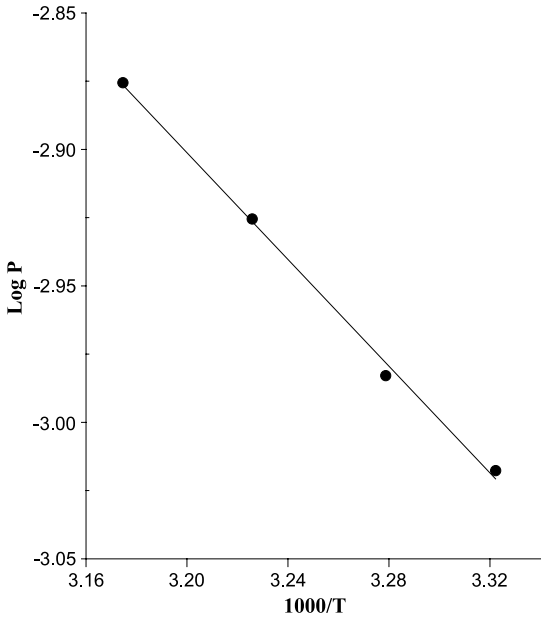

Figure 2. Permeation coefficient of tetracaine as a function of temperature (K).

Effects of Permeation Enhancers on Drug Permeation Through Skin

The effects of various permeation enhancers on permeation of drug through skin were investigated (Table 2). Since local anesthetics have short half-lives after parenteral injection, such as 40–50 minutes for tetracaine, the sustained and enhanced delivery for analgesic drugs is needed. The enhancers such as bile salts, glycols, and nonionic surfactants were used at 5% concentration. The cumulative amount of the permeated drug through skin was plotted as a function of time.

$$EF = (\text{flux of gels containing enhancers})/(\text{flux of control sample})$$

The effects of penetration enhancers on the permeation rate of model drug were determined by comparing the flux of drugs in the presence or the absence of enhancers.[8] It was defined as the enhancement factor (EF). Among the permeation enhancers tested, polyoxyethylene 2-oleyl ether showed the most enhancing effects.

Table 2. Enhancing effects of enhancer on tetracaine permeation through rat skins from the HPMC-poloxamer gels.

	Tetracaine gels (n=3)	
Enhancer	Flux (ug/cm^2/h)	Enhancement factor
Control	4.17±0.3	1.00
Sod. taurodeoxycholate	5.96±0.5	1.43
Sod. deoxycholate	9.21±0.8	2.21
Polyoxyethylene 23-lauryl ether	8.56±0.7	2.05
Polyoxyethylene 2-stearyl ether	6.15±0.5	1.48
Polyoxyethylene 2-oleyl ether	11.68±1.1	2.80
Tetraethylene glycol	4.42±0.4	1.06
Diethylene glycol	11.18±1.2	2.68

Tail-Flick Analgesic Test of Tetracaine Gels Containing Enhancer

Tetracaine gels containing polyoxyethylene 2-oleyl ether that showed the best enhancing effects in percutaneous permeation studies were used for the rat tail-flick analgesic test. The $AUEC_{0\rightarrow 50\ min}$ of the rat-tail flick test for tetracaine gels is shown in Table 3. The value of AUEC of tetracaine gel containing polyoxyethylene 2-oleyl ether was 295.68±59.81 sec·min, while that without enhancer was 92.53±1.91 sec·min. The value of AUEC of the gel containing polyoxyethylene 2-oleyl ether and tetrahydrozoline was 287.71±56.31 sec·min. The efficacy of tetracaine gel containing polyoxyethylene 2-oleyl ether is about 3.20-fold greater than that without polyoxyethylene 2-oleyl ether.

Table 3. The comparison of $AUEC_{0\rightarrow 50\ min}$ from the rat tail flick test for the tetracaine gels with enhancer.

	Tetracaine gels (n=3)	
Tetracaine gels	AUEC (sec·min)	Efficacy factor
Tetracaine gels	92.53±1.91	1
Tetracaine gels containing polyoxyethylene 2-oleyl ether	295.68±59.81	3.20
Tetracaine gels containing polyoxyethylene 2-oleyl ether and THZ	287.71±56.31	3.11

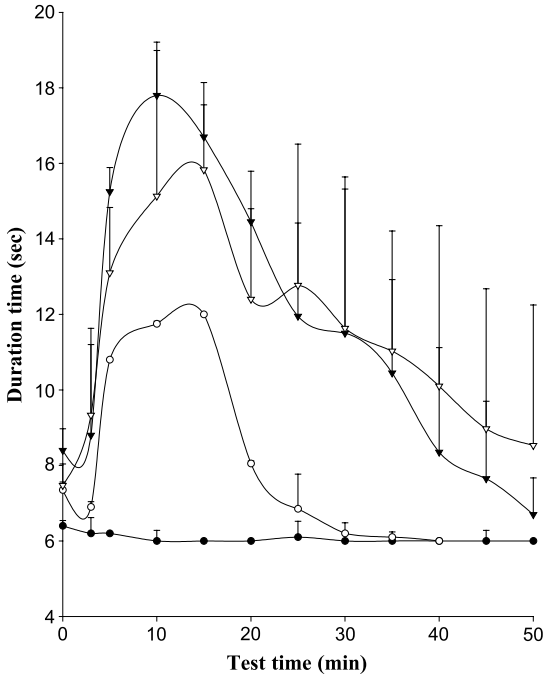

Figure 3. Tail flick test of 3% tetracaine gels (n=3). Key: ●, control gels without tetracaine and additives; ○, tetracaine gels without additives; ▼, tetracaine gels containing polyoxyethylene 2-oleyl ether; ▽, tetracaine gels containing polyoxyethylene 2-oleyl ether and tetrahydrozoline.

In the rat-tail flick test, 3% tetracaine gels containing polyoxyethylene 2-oleyl ether showed the most prolonged analgesic effects. The highest analgesic effects of tetracaine gels containing polyoxyethylene 2-oleyl ether were shown at 10 minutes. In elucidating the analgesic activity of a vasoconstrictor (i.e., tetrahydrozoline) added in analgesic gels, the tetracaine gels containing both polyoxyethylene 2-oleyl ether and tetrahydrozoline showed similar efficacy to those containing polyoxyethylene 2-oleyl ether, but the former showed a little prolonged analgesic effects, not significantly, than the latter (Fig. 3). This indicates that the addition of a vasoconstrictor further enhanced the efficacy of the local anesthetics.

CONCLUSIONS

As the drug concentration in the gels and the temperature of surrounding solutions increased, drug release increased. Among the enhancers used, polyoxyethylene 2-oleyl ether showed the most enhancing

effects on drug permeation through skin. In the AUEC of the rat-tail flick test, tetracaine gels containing polyoxyethylene 2-oleyl ether showed about a 3.2-fold increase in analgesic activity compared with the control. The results of this study support that the tetracaine gels with efficient anesthetic effects could be developed using HPMC with a combination of enhancer and vasoconstrictor.

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REFERENCES

1. Ben-David, B.; DeMeo, P.J.; Lucyk, C.; Solosko, D.A. Comparison of minidose lidocaine-fentanyl spinal anesthesia and local anesthesia/propofol infusion for outpatient knee arthroscopy. Anesth. Analg. **2001**, *93*, 319–325.
2. Strichartz, G.R.; Richie, J.M. The action of local anesthetics on I: ion channels of excitable tissues. In *Local Anesthetics Handbook of Experimental Pharmacology*; Strichartz, G.R., Ed.; Springer Verlag: Berlin, 1987; Vol. 81, 21–53.
3. Morganti, P.; Ruocco, E.; Wolf, R.; Ruocco, V. Percutaneous absorption and delivery systems (3). Clin. Dermatol. **2001**, *19*, 489–501.
4. Hou, S.M.; Yu, H.Y. Comparison of absorption of aqueous lidocaine and liposome lidocaine following topical application on rabbit vessels. J. Orthop. Res. **1994**, *12*, 294–298.
5. Hou, S.M.; Yu, H.Y. Comparison of systemic absorption of aqueous lidocaine and liposome lidocaine following intra-articular injection in rabbits. J. Formos. Med. Assoc. **1997**, *96*, 141–143.
6. Evrard, D.; Touitou, E.; Kolusheva, S.; Fishov, Y.; Jelinek, R. A new colorimetric assay for studying and rapid screening of membrane penetration enhancers. Pharm. Res. **2001**, *18*, 943–949.
7. Angust, B.J.; Rogers, N.Y. Comparison of the effects of various transmucosal absorption promoters on buccal insulin delivery. Int. J. Pharm. **1989**, *53*, 227–235.
8. Shin, S.C.; Lee, H.J. Enhanced transdermal delivery of triprolidine from the ethylene-vinyl acetate matrix. Eur. J. Pharm. Biopharm. **2002**, *54*, 325–328.
9. Shin, S.C.; Cho, C.W.; Oh, I.J. Effects of non-ionic surfactants as permeation enhancers towards piroxicam from the poloxamer gel through rat skins. Int. J. Pharm. **2001**, *222*, 199–203.
10. Shin, S.C.; Kim, J.; Yoon, M.K.; Oh, I.J.; Choi, J.S. Transdermal delivery of triprolidine using TPX polymer membrane. Int. J. Pharm. **2002**, *235*, 141–147.
11. Shin, S.C.; Cho, C.W.; Oh, I.J. Enhanced efficacy by percutaneous absorption of piroxicam from the poloxamer gel in rats. Int. J. Pharm. **2002**, *193*, 213–218.
12. Shin, S.C.; Shin, E.Y.; Cho, C.W. Enhancing effects of fatty acids on piroxicam permeation through rat skins. Drug Dev. Ind. Pharm. **2000**, *26*, 563–566.
13. Shin, S.C.; Cho, C.W.; Choi, H.K. Permeation of piroxicam from the poloxamer gels. Drug Dev. Ind. Pharm. **1999**, *25*, 273–279.
14. Ishida, M.; Machida, Y.; Nambu, N.; Nagai, T. New mucosal dosage form of insulin. Chem. Pharm. Bull. **1981**, *209*, 810–816.
15. Shokri, J.; Nokhodchi, A.; Dashbolaghi, A.; Hassan-Zadeh, D.; Ghafourian, T.; Barzegar, J.M. The effect of surfactants on the skin penetration of diazepam. Int. J. Pharm. **2001**, *228*, 99–107.
16. Miyazaki, S.; Ishii, K.; Takada, M. Controlled release of anticancer agents through ethylene-vinyl acetate copolymer membranes. Yakuzaigaku **1983**, *42*, 259.