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Menthol facilitates the skin analgesic effect of tetracaine gel

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Abstract

The aim of this study is to observe the effect of menthol on the percutaneous penetration and skin analgesic action of tetracaine gel (T-gel). Anesthetic gels containing 4% tetracaine in carbomer vehicle with and without menthol were prepared. The menthol penetration-enhanced gel conferred significantly higher diffusion of tetracaine across full-thickness mouse skin than non-penetration-enhanced gel, in a dose-dependent manner. The inter-cellular spaces of the stratum corneum in skin treated with menthol penetration-enhanced gel became extended as compared with those in non-penetration-enhanced gel. This may suggest that menthol's action was related to the changes of the epidermis ultra structures. An enlarged inter-cellular space, per se, would allow a better passage to tetracaine. To determine the efficacy of menthol penetration-enhanced tetracaine gel in the management of pain, a double-blind, placebo-controlled, randomized controlled trial (RCT) design was used. The mean verbal pain scores (VPS) were significantly lower in volunteers treated with penetration-enhanced tetracaine gel than those in volunteers receiving non-penetration-enhanced tetracaine gel or placebo. Menthol improved the analgesic efficacy of the tetracaine 4% gel in part through enhanced percutaneous permeation.

Keywords: Menthol; Tetracaine gel; Percutaneous permeation

1. Introduction

Patients undergoing venepuncture or intravenous catheterization often experience pain. Therefore, a fast acting and long lasting topical anesthetic formulation would be of considerable clinical benefit in reducing pain associated with invasive medical procedures (Wong, 2003). Efficient percutaneous penetration is essential to an ideal topical anesthetic preparation. One major element to the skin barrier function was the hydrophobic nature of the stratum corneum. A topical anesthetic EMLA cream (eutectic mixture of

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local anesthetics, a cream containing 2.5% lidocaine and 2.5% prilocaine) is available clinically. However, it requires a minimum anesthesia onset time of 1 h, while its anesthetic effect is short lived, lasting only 30–60 min (Wong, 2003; Friedman et al., 2001; Rogers and Ostrow, 2004). Tetracaine, a known surface anesthetic agent, is more lipophilic and may penetrate through stratum corneum more easily than the active components of EMLA cream, lidocaine and prilocaine. Hence, tetracaine gel (T-gel) would offer an improved local anesthesia (Romsing et al., 1999). However, recent clinical studies have disappointed investigators that the tetracaine gel is not as effective as anticipated for topical pain relief (Aaron et al., 2003; Ballantyne et al., 2003). A percutaneous penetration enhancer might be needed to improve its anesthetic effect.

Menthol was the primary component of the essential oil of peppermint (Umezu and Morita, 2003). It acts as a penetration enhancer either due to its vasodilatation property or it may reversibly change the strong barrier property of the stratum corneum (Yosipovitch et al., 1996; Fujii et al., 2003). There have been several strategies using menthol to achieve better transdermal delivery of some substances such as nicardipine, propranolol, and ketoprofen (Krishnaiah et al., 2002; Zahir et al., 1998; Wu et al., 2001). Menthol also has local anesthetic property, demonstrated in combination products for relief of minor pain (Green and McAuliffe, 2000; Galeotti et al., 2002). In view of an improved efficacy in skin local anesthesia, we have formulated menthol penetration-enhanced tetracaine gel, and conducted both experimental and clinical studies for the treatment of procedural pain.

2. Materials and methods

2.1. Animals

Female Kunming mice, weight 18–20 g (Shanghai Exp. Animals, Shanghai, China), were purchased and housed in groups of 10 animals. The animals were kept in a room maintained at 18–22 °C with free access to a standard laboratory diet and tap water. All experiments were conducted in accordance with Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the Declaration of Helsinki. The allocation of animals to various groups was done under randomization.

2.2. Tetracaine gel preparation

Two grams of carbomer-940 (a gift from Hangzhou East Pharmaceutical Company, Zhejiang, China) were dispersed into distilled water under 300 rpm agitation to form a homogeneous gel. Four grams of tetracaine (Sigma, St. Louis, MO, USA) mixed with menthol (Shanghai Flavor Inc., Shanghai, China) was prepared and carefully added to the carbomer gel. The pH of the homogeneous gel was adjusted to 6.1 by triethylamine (Sigma, St. Louis, MO, USA). Distilled water was added to the gel for a final weight of 100 g and the gel was kept under stirring for 6 h. The corresponding tetracaine gels were named T-gel (mixed with 0% menthol), 1% M/T-gel (mixed with 1%, w/w menthol), or 5% M/T-gel (mixed with 5%, w/w menthol). The tetracaine content (4%) of the carbomer-940 gel containing menthol (0%, w/w, 1%, w/w, or 5%, w/w) remained stable at 4 °C for 6 months.

2.3. In vitro skin permeation study

Full-thickness abdominal skin, whose hair had been previously removed, was excised from anesthetized mice. Subcutaneous fat was carefully removed with a scalpel and washed with saline solution. The excised skin was used as a permeation membrane (Li et al., 2001). A Franz diffusion cell was used and thermoregulated at 37 °C. The receiver side was filled with saline solution and the donor side was filled with test gel (0.5 g) under occlusive conditions. At appropriate times, an aliquot of the receiver fluid was withdrawn and the same volume of saline solution was supplied to the receiver side. The concentration of filtered tetracaine in the filtrate was analyzed spectrophotometrically at 310 nm wavelength.

2.4. Morphological study

Under urethane anesthesia, mice were secured, and the hair on the abdominal skin was removed (Li et al., 2001). The skin was excised after 6 h exposure

to the tetracaine gels (0.5 g), and the skin surface was washed with phosphate buffered saline (PBS) and pre-fixed with a 2% glutaraldehyde buffer solution for 2 h. The skin was fixed with a 1% osmic acid buffer solution (pH 7.3) for 2 h. The skin was dehydrated in a graded ethanol series (50–100%) and dried in carbon dioxide. The skin surface was coated with gold-palladium and examined under the QUANTA-200 scanning electron microscope (SEM) (FEI Company, Hillsboro, OR, USA).

2.5. In vivo evaluation of the skin analgesic effect

After approval of the medical ethics committee, the study was conducted at Shanghai Second Medical University, Shanghai, China. Sixteen healthy volunteers (6 male and 10 female, 50–70 kg, age between 19 and 22 years old) participated in the study. The nature and purpose of the study were fully explained to them. An informed written consent was obtained from every volunteer. The recruited volunteers had neither a history of allergic reactions to local or topical anesthetics nor any dermatological conditions. They were withheld from any drugs for 1 week prior to the participation of the study. Gels were allocated to volunteers on a Latin Square Design basis. Each volunteer received three different formulations, identical in appearance, applied to the selected three ventral surface area (2 cm diameter) of the forearm. A standard dressing was placed over the gel for 40 min. The dressing and gel were removed by wiping with an alcohol swab. For noxious stimulation, von Frey hair type needles (Stoelting, Wood Dale, IL, USA) were applied as the loading force (gram weight) strong enough to evoke moderate to severe pain before gel exposure. The corresponding von Frey hair was applied to each volunteer at indicated time. The pain intensity and quality were assessed using a verbal pain score (VPS; 0 = no pain, 1 = mild pain, 2 = moderate pain, and 3 = severe pain) (Carr and Horton, 2001; Alexander et al., 2002). Skin reactions such as erythema, itching, or swelling, if any, were also recorded.

2.6. Statistical analysis

The Student's *t*-test was used throughout the study. Difference between data sets with P values less than 0.05 was considered significant.

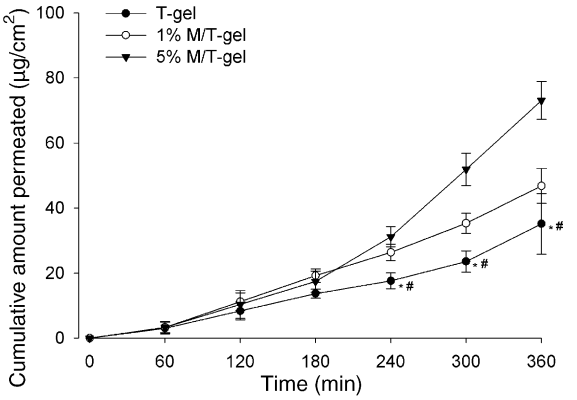

Fig. 1. The cumulative amount permeated curve of tetracaine from tetracaine 4% gels. Penetration study was performed on the excised mouse skin. Note that menthol markedly enhanced the permeation of tetracaine. The data show the average of five experiments. Statistical significance was indicated as follows: (*) statistically significant ($P < 0.05$) difference was found between cumulative amount permeated tetracaine from 5% M/T-gel and from T-gel; (#) statistically significant ($P < 0.05$) difference was found between cumulative amount permeated tetracaine of 5% M/T-gel and that of 1% M/T-gel.

3. Results

3.1. Effect of menthol on skin permeation of tetracaine

The cumulative amount of tetracaine increased linearly with an increase of time after certain lag time (Fig. 1). With the addition of menthol, the permeation of tetracaine was markedly enhanced compared with the control (without menthol). The flux was estimated from the slope of the permeation profiles. As shown in Table 1, when menthol (1 or 5%) was added to the tetracaine gels, the flux of tetracaine was significantly increased compared with the control (without menthol).

Table 1
The flux and lag time of tetracaine for tetracaine gels through the excised mice abdominal skin

Tetracaine gels	T-gel	1% M/T-gel	5% M/T-gel
Flux ($\mu g/cm^2/h$)	5.99 ± 1.97	8.49 ± 0.55	$13.91 \pm 0.72^{*,**}$
Lag time (h)	0.67 ± 0.01	0.71 ± 0.01	$1.26 \pm 0.12^{*,**}$

[*] Statistically significant ($P < 0.05$) difference was found between 5% M/T-gel group and T-gel group.
[**] Statistically significant ($P < 0.05$) difference was found between 5% M/T-gel group and from 1% M/T-gel group.

3.2. Effect of menthol on skin morphology

Morphological changes in the skin surface treated with tetracaine gels containing menthol were examined by a scanning electron microscope. Fig. 2 showed typical examples of microscopic photographs of the skin surface treated with tetracaine gels containing menthol (0 or 5%) for 6 h. The intact skin surface (without gel treatment) showed rough and irregular morphology (Fig. 2A), and the roughness of the skin surface decreased after placebo (carbomer-940 gel) treatment (Fig. 2B). The intercellular space of the stratum corneum in the skin surface was enlarged after tetracaine gels treatment. Compared with T-gel treated group (non-penetration-enhanced gel, Fig. 2C), the change was more marked in 5% M/T-gel treated group (menthol penetration-enhanced gel, Fig. 2D).

3.3. Effect of menthol on skin analgesic effect of tetracaine gel

Both the skin analgesic effects of tetracaine gels containing menthol and an active control analgesia cream (EMLA) were evaluated in human volunteers (Table 2). No skin reactions occurred in any of the 16 volunteers. The EMLA cream (Astrazeneca, Wilmington, DE, USA) and the non-penetration-enhanced tetracaine gel achieved short moderate pain relieving effect in human volunteers with a duration of 30–60 min. In contrast, menthol penetration-enhanced tetracaine gel (5% M/T-gel) demonstrated a long-lasting, strong, skin analgesic effect. The difference between T-gel and 5% M/T-gel was statistically significant ($P < 0.05$). Notably, the skin analgesic effect of 5% M/T-gel is much better than the effect of the commercially available topical drug EMLA cream.

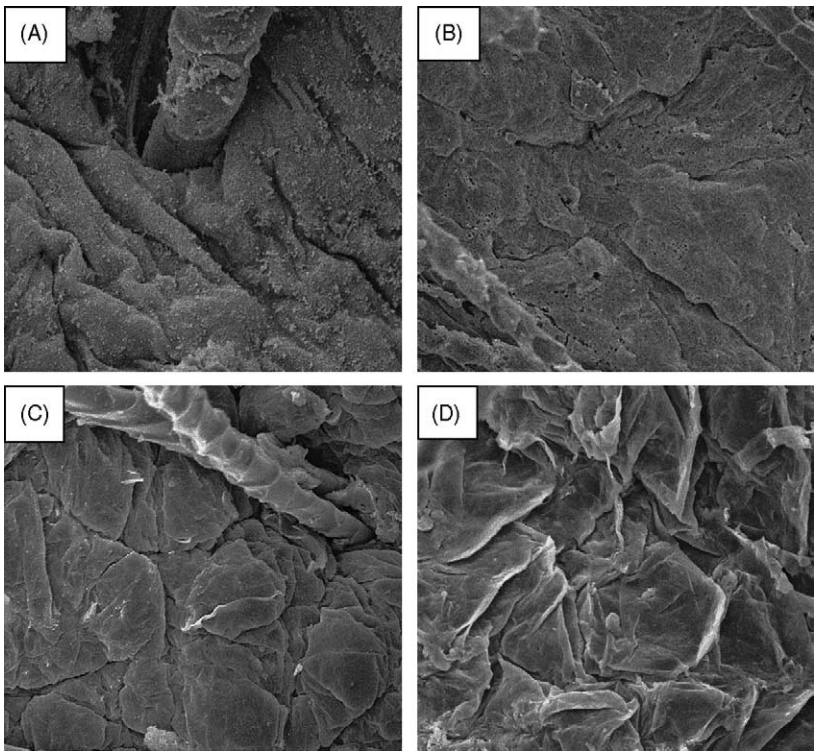

Fig. 2. Electron micrographs of mice abdominal skin treated with tetracaine gels. The skin was excised after 6 h of exposure to the gels and photographed after standard treatment. (A) The intact skin surface showed a rough morphology. (B) After the carbomer gel treatment, the roughness of the skin surface was decreased. Compared to non-penetration-enhanced gel (C), marked changes in the skin surface were observed with 5% menthol penetration-enhanced gel (D).

Table 2
Time-dependent analgesic effects confirm the improvement of skin analgesic effect of tetracaine gels by menthol

Time (h)	Treatment			
	Vehicle	EMLA	T-gel	5% M/T-gel
0	2.33 ± 0.58	2.25 ± 0.45	2.17 ± 0.58	2.25 ± 0.62
1	1.92 ± 0.67	1.58 ± 0.79	1.67 ± 0.78	1.58 ± 0.79
2	1.75 ± 1.33	1.33 ± 0.89	0.92 ± 0.51	0.58 ± 0.67*
3	1.75 ± 0.45	1.50 ± 1.00	1.00 ± 0.43	0.25 ± 0.45*,**
4	2.08 ± 0.67	1.50 ± 1.17	0.92 ± 0.67	0.33 ± 0.49*
5	2.17 ± 0.58	1.33 ± 0.98	0.92 ± 0.67	0.50 ± 0.80*
6	2.25 ± 0.62	1.33 ± 0.89	1.42 ± 0.51	0.67 ± 0.49*,**

* Statistically significant ($P < 0.05$) difference was found between 5% M/T-gel group and vehicle treated group.
** Statistically significant ($P < 0.05$) difference was found between 5% M/T-gel group and T-gel group.

4. Discussion

Menthol was classified by the U.S. Food and Drug Administration as a topical analgesic (Anonymous, 1983). In pharmacy, it was part of topical antipruritic, antiseptic and cooling formulations. Applied topically, menthol causes tingling sensation and a feeling of coolness due to the stimulation of "cold" receptors, achieving a short-term antinociceptive effect. Moreover, menthol increases percutaneous permeation, when mixed with local anesthetic agents, as shown in this study. The mechanism might be attributed to the preferential hydrogen bonding of menthol with ceramide head groups, thereby breaking the lateral/transverse hydrogen bond network of stratum corneum lipid bilayer (Jain et al., 2002; Narishetty and Panchagnula, 2004). Menthol needs time to diffuse from the vehicle to the skin and also needs time to penetrate through skin and interact with the stratum corneum lipids. With the increase of menthol in tetracaine gel, the lag time became longer as observed in this study. The phenomenon that penetration enhancers increase lag time while facilitating the penetration of active drug has also been reported in other literatures (Vaddi et al., 2002; Nokhodchi et al., 2003).

In case of percutaneous medical procedures, such as punctures and catheterizations, the suppression of pain sensation is most desirable. An ideal local pain suppressor would be fast acting and long lasting. The pilot clinical studies of the tetracaine–menthol gel indicated its anesthetic effectiveness was appropriate for most medical applications. Since the enhancement of penetration by menthol is due probably to a nonspecific physic-chemical action, we think that menthol might also be useful for improving skin analgesic effect of other topical anesthetics, including EMLA cream, ELA-Max cream (a 4% lidocaine cream), or ELA-Max5 cream (a 5% lidocaine cream) (Friedman et al., 2001; Goldman, 2004).

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