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Research paper

Synergistically enhanced transdermal permeation and topical analgesia of tetracaine gel containing menthol and ethanol in experimental and clinical studies

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Abstract

The aim of this study is to observe the synergistically enhanced percutaneous penetration and skin analgesia of tetracaine gel containing menthol and ethanol through experimental and clinical studies. Four anesthetic gels containing 4% tetracaine in carbomer vehicle named T-gel (containing no menthol or ethanol), 5%M/T-gel (containing 5% menthol), 70%E/T-gel (containing 70% ethanol, an optimal concentration for antiseptic), and 5%M + 70%E/T-gel (containing both 5% menthol and 70% ethanol), respectively, were fabricated. The in vitro mouse skin permeation was investigated using a Franz diffusion cell. The mouse skin morphology was examined by a scanning electron microscope. The in vivo skin analgesic effect in mice was evaluated using the von Frey tests. To determine the efficacy of tetracaine gels for managing the pain in human volunteers, a paralleled, double-blinded, placebo-controlled, randomized controlled trial design combined with verbal pain scores (VPS) was performed. The combination of menthol and ethanol (5%M + 70%E/T-gel) conferred significantly higher tetracaine diffusion across full-thickness mouse skin than 5%M/T-gel, 70%E/T-gel, and T-gel. The ultra structure changes of mouse skin stratum corneum treated with 5%M + 70%E/T-gel were more marked compared with those of any other tetracaine gel. von Frey tests in mice showed a synergistically enhanced effect of menthol and ethanol on the analgesia of tetracaine gel. The mean VPS were significantly lower for volunteers treated with 5%M + 70%E/T-gel than those receiving other gels or the EMLA cream. 5%M + 70%E/T-gel possessed the shortest anesthesia onset time, the longest anesthesia duration and the strongest anesthesia efficacy. Seventy percent ethanol in 5%M + 70%E/T-gel not only improved the analgesic efficacy of the tetracaine gel through synergistically enhanced percutaneous permeation with menthol but also served as an antiseptic agent keeping drug application site from infection. 5%M + 70%E/T-gel is a potential topical anesthesia preparation for clinical use.

Keywords: Menthol; Ethanol; Tetracaine gel; Synergistic effect; Transdermal permeation

1. Introduction

Patients undergoing venipuncture 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 [1]. A topical anesthetic EMLA (eutectic mixture of local anesthetics-lidocaine and prilocaine in a ratio of 1:1 by weight) is available clinically. However, it requires a minimum of 60 min for effective use and its duration of action is only 30–60 min [1–3].

Efficient transdermal permeation is essential to an ideal topical anesthetic preparation, but it is usually compromised by the skin stratum corneum barrier. Tetracaine, a

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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. However, recent clinical studies showed that tetracaine gel is not as effective as anticipated for topical pain relief [4,5], which implied the requirement of further penetration enhancement.

In our previous work [6], menthol was used as a permeation enhancer in the formulation of tetracaine gel. Efficient tetracaine percutaneous delivery and higher anesthetic efficacy than EMLA cream were obtained. However, the anesthetic onset time was still not satisfying. Ethanol, another commonly used permeation enhancer and also an antiseptic agent with optimal concentration of 70% (w/w), has been widely reported in many strategies to achieve better transdermal delivery [7,8]. Its synergistic effect with other permeation enhancers has also been discussed [9–11]. With the expectation of improved anesthetic onset time and better efficacy, ethanol was chosen to combine with menthol in the formulation of tetracaine gel in this study. The synergistically enhanced transdermal permeation and topical analgesia of tetracaine gel containing menthol and ethanol were observed through 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 a group of 10 animals. They 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 to the Care and Use of Laboratory Animals as adopted and promulgated by the Declaration of Helsinki. The allocation of animals to various groups was performed under randomization.

2.2. Tetracaine gel preparation

Two grams of Carbomer-940 (a gift from Hangzhou East Pharmaceutical Company, Zhejiang, China) was dispersed into distilled water under 300 rpm agitation to form homogeneous gel. Four grams of tetracaine (Sigma, St. Louis, MO, USA) mixed with menthol (Shanghai Flavor Inc., Shanghai, China) and/or ethanol (Shanghai Chemical Reagent Corporation, China Medicine Group) was prepared and carefully added to the carbomer gel. The pH of the homogeneous gel was adjusted to 6.1. Distilled water was added to the gel for a final weight of 100 g and the gel was kept under stirring for 6 h in parafilm sealed multi-purpose beakers (Greiner bio-one, Germany). The tetracaine content (4%) of the above carbomer-940 gel remained stable at 4 °C for at least 6 months. The corresponding tetracaine gels were named T-gel (mixed with 0% menthol and ethanol), 5%M/T-gel (mixed with 5% w/w menthol), 70%E/T-gel (mixed with 70% w/w ethanol), or 5%M + 70%E/T-gel (mixed with 5% w/w menthol and 70% w/w ethanol).

2.3. In vitro skin permeation study

Full-thickness abdominal skin was excised from urethane anesthetized mice, whose hair had been previously removed. Subcutaneous fat was carefully removed with a scalpel and washed with saline solution. The excised skin was used as a permeation membrane (2 cm^2) [12]. Briefly, in a Franz diffusion cell that was thermo-regulated 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

After anesthetization with urethane solution, mice were secured, and the hair on the abdominal skin was removed [12]. The skin (2 cm^2) was excised after 6 h exposure to the tetracaine gels (0.5 g), and then washed with phosphate buffered saline (PBS) and prefixed 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, 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 (FEI Company, Hillsboro, OR, USA).

2.5. In vivo evaluation of the skin analgesic effect in mice

To assess the skin analgesic effect in mice, a von Frey filament test was used as previously described [13]. Mice were placed individually in a plastic cage with a wire mesh bottom. They were allowed to habituate for 30 min before the test. After 30-min spread with 0.5 g of tetracaine gels on the hind plantar skin, the gels were removed by wiping with an alcohol swab, and then von Frey tests were performed at predetermined times. Each measurement was repeated three times with at least 3 min interval. A series of von Frey fibers (0.23–3.63 g) (Stoelting, Wood Dale, IL, USA) were applied through the wire mesh onto the plantar surface of both hind paws in ascending order beginning with the finest fiber. A response was considered valid if the mice evaded, brayed, attacked or hind paw completely removed from the platform. For each paw, a von Frey hair was applied five times at 5 s intervals. The pain threshold was determined when valid response was observed in more than three of five applications. The site of every application was changed a little every time and the gel treated and non-gel treated hind paws were stimulated alternately in

case the mice were anaphylactoid. The percentage increases (P) of pain threshold were calculated as following: $P = (\text{pain threshold of gel treated group} - \text{pain threshold of control group})/\text{pain threshold of control group} \times 100\%$.

2.6. In vivo evaluation of the skin analgesic effect in volunteers

After approval of the Medical Ethics Committee, the study was conducted at Shanghai Jiao Tong University, Shanghai, China. Sixteen healthy volunteers (8 male and 8 female, 50–70 kg, age between 19 and 23 years) 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 no history of allergic reactions to local or topical anesthetics or any dermatological conditions. They were withheld from any drugs for 1 week prior to the participation of the study. Gels (1 g) were allocated to volunteers on a paralleled, double-blinded, placebo-controlled, randomized controlled trial design basis. Each volunteer received five different formulations, which were identical in appearance, applied to the selected five ventral surface areas (2-cm diameter) of the forearms. 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 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 was assessed using a verbal pain score (VPS; 0 = no pain, 1 = mild pain, 2 = moderate pain, and 3 = severe pain) [14,15]. Skin reactions such as erythema, itching or swelling, if any, were also recorded.

2.7. Statistical analysis

Analysis of variance (ANOVA) with follow-up LSD tests was used in the study except that Wilcoxon matched-pairs signed-rank tests were used to compare the anesthetic efficacy of treatment versus control in VPS method and Fisher's exact tests to compare the percentage of volunteers anesthetized in the clinical study. Difference between data sets with P values of less than 0.05 was considered significant.

3. Results and discussion

3.1. Effect of penetration enhancers on skin permeation of tetracaine

The cumulative amount of tetracaine increased linearly progressively with time after certain lag time (Fig. 1). With the addition of menthol or ethanol, the permeation of tetracaine was remarkably enhanced compared with that of the control (without menthol or ethanol). The flux was estimated from the slope of the permeation profiles. As shown in Table 1, when menthol (5%) or ethanol (70%) was added to the tetracaine gels, the flux of tetracaine was remarkably increased compared with the control (without menthol or ethanol). Noticeably, the combination of menthol and ethanol (5%M + 70%E/T-gel) dramatically increased the flux of tetracaine, which was about 2.3 ($P < 0.01$) and 2.2 ($P < 0.01$) times as that of 5%M/T-gel and 70%E/T-gel, respectively. Because penetration enhancers need time to diffuse from the vehicle to the skin and also need time for penetration through skin and interact with the stratum corneum, the lag time was increased for the penetration-enhanced tetracaine gels. The phenomenon that penetration enhancers increase lag time while facilitating the penetration of active drug has been reported in other literatures [16,17].

3.2. Effect of penetration enhancers on skin morphology

Morphological changes in the skin surface treated with tetracaine gels containing menthol and/or ethanol were examined by a scanning electron microscope. Fig. 2 shows typical examples of microscopic photographs of the skin surface treated with various tetracaine gels 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 gel treatment (Fig. 2C–F). Compared with T-gel treated group (non-penetration-enhanced gel, Fig. 2C),

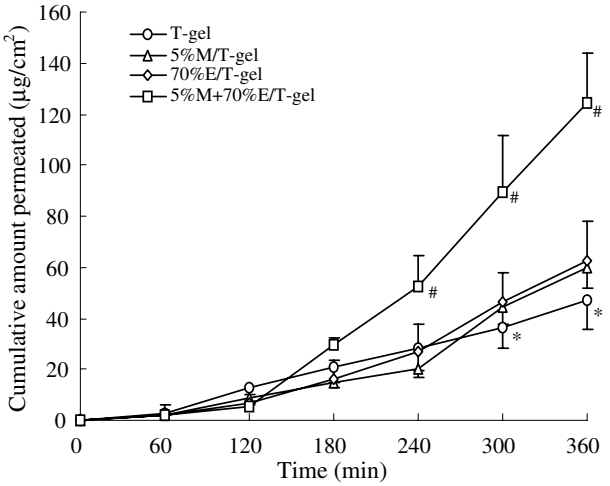

Fig. 1. The cumulative amount permeated curve of tetracaine from tetracaine 4% gels ($n = 5$). Penetration study was performed on the excised mouse skin. The data show the average of five experiments. Statistical significance was indicated as follows: *statistically significant ($P < 0.05$) difference was found between cumulative amount permeated tetracaine from 5%M/T-gel or 70%E/T-gel and from T-gel; #statistical significance ($P < 0.01$) was found between cumulative amount permeated tetracaine from 5%M + 70%E/T-gel and that of other gels (T-gel, 5%M/T-gel, and 70%E/T-gel).

Table 1
The flux and lag time of tetracaine from tetracaine gels through the excised mice abdominal skin ($n = 5$)

Tetracaine gels	T-gel	5%M/T-gel	70%E/T-gel	5%M + 70%E/T-gel
Flux (μg/cm^2/h)	8.36 ± 1.55	11.50 ± 1.65[a]	12.29 ± 2.87[a]	26.59 ± 3.51[b,c]
Lag time (h)	0.70 ± 0.15	1.32 ± 0.13[b]	1.30 ± 0.30[b]	1.58 ± 0.10[b]

[a] $P < 0.05$.
[b] $P < 0.01$ vs T-gel.
[c] $P < 0.01$ vs other groups.

the change was more marked in 5%M/T-gel and 70%E/T-gel treated group (Fig. 2D and E). Moreover, the wrinkles on the skin surface were increased and numerous desquamated cells around the orifice of the hair were fractured and detached. The most marked changes of the skin surface were found with the combination of 5% menthol and 70% ethanol in the tetracaine gel (Fig. 2F). Some areas of stratum corneum were peeled off and wound up as worn-out cotton padding which implied a possible synergistic effect of improving penetration.

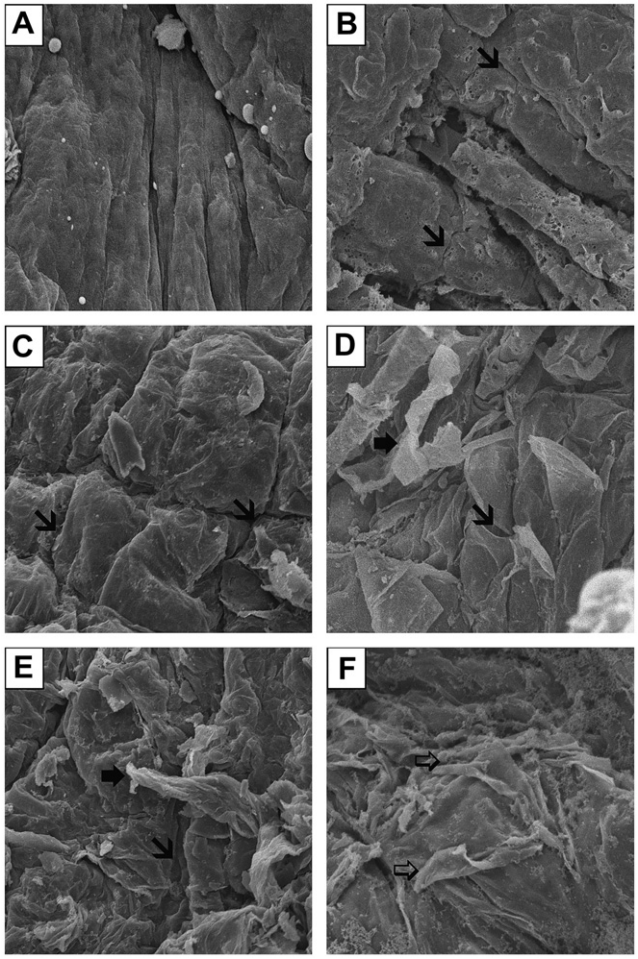

Fig. 2. Scanning electron micrographs of mice abdominal skin treated with tetracaine gel. The skin was excised after 6 h of exposure to the gels and photographed after standard treatment. (A) The intact skin surface showed a rough morphology. (B) After the carbomer gel treatment, the roughness of the skin surface was decreased. The intercellular space (↘) of the stratum corneum in the skin surface was enlarged after tetracaine gel treatment (C–F). Compared with T-gel treated group (non-penetration-enhanced gel) (C), increased skin wrinkles and numerous desquamated cells (➡) were observed in 5%M/T-gel (D) and 70%E/T-gel (E) treated group. Some areas of stratum corneum were peeled off and wound up () as worn-out cotton padding with the combination of 5% menthol and 70% ethanol in the tetracaine gel (F) which implied a possible synergistic effect of improving penetration.

3.3. Synergistically enhanced analgesic effect of tetracaine gel containing menthol and ethanol in mice

Time-dependent topical analgesic effects of tetracaine gels with penetration enhancers in von Frey test are shown in Fig. 3. With the addition of menthol, the percentage increase of pain threshold was markedly enhanced compared with the control (T-gel without any penetration enhancers). The 70% ethanol contained gel could moderately increase the pain threshold in comparison with the control. In contrast to tetracaine gel containing single penetration enhancer (menthol or ethanol), the combination of menthol and ethanol (5%M + 70%E/T-gel) dramatically increased the pain threshold at some time points. After 1-h treatment, the percentage increase in pain threshold of 5%M + 70%E/T-gel reached its peak value (226.7%), which suggested a synergistic effect of menthol and ethanol on the analgesia of tetracaine gel.

3.4. Synergistically enhanced analgesic effect of tetracaine gel containing menthol and ethanol in volunteers

Both the skin analgesic effect of tetracaine gels containing menthol and/or ethanol and that of an active control analgesia cream (EMLA) were evaluated in human volunteers (Table 2). No pronounced skin reactions occurred in any of the 16 volunteers. The EMLA cream (Astrazeneca, Wilmington, DE, USA) achieved moderate pain relieving effect in human volunteers. In contrast, menthol penetration-enhanced tetracaine gel (5%M/T-gel) demonstrated a markedly stronger skin analgesic effect. Although the tetracaine gel containing only ethanol (70%E/T-gel) showed moderate analgesic effect, the combination of menthol and ethanol in tetracaine gel (5%M + 70%E/T-gel) earned the strongest and longest-lasting analgesic effect among all formulations, which displayed a significantly synergistic analgesic effect.

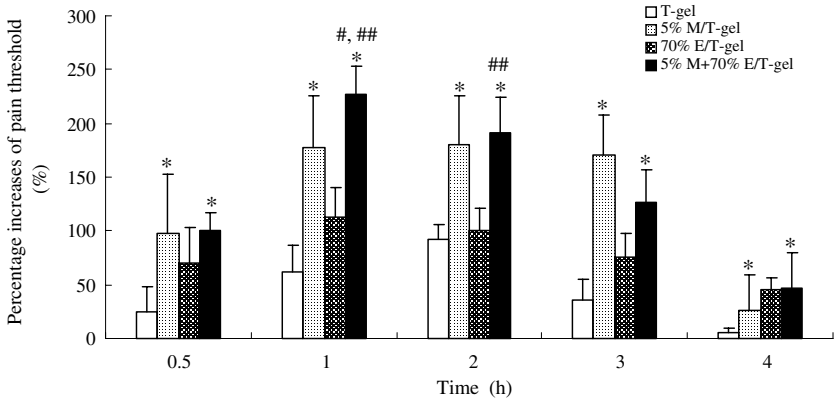

Fig. 3. Time-course of the topical analgesic effects of tetracaine gels with penetration enhancers in von Frey test ($n = 5$) in mice. The data show the average of five experiments. Statistical significance was indicated as follows: *statistical significance ($P < 0.01$) was found between 5%M/T-gel or 5%M + 70%E/T-gel and T-gel; #statistical significance ($P < 0.05$) was found between 5%M + 70%E/T-gel and 5%M/T-gel; ##statistical significance ($P < 0.01$) was found between 5%M + 70%E/T-gel and 70%E/T-gel.

Table 2
Time-dependent analgesic effects of tetracaine gel applied to volunteers ($n = 16$)

Time (h)	Placebo	EMLA	5%M/T-gel	70%E/T-gel	5%M + 70%E/T-gel
0	2.13 ± 0.12	2.23 ± 0.35	2.23 ± 0.48	2.13 ± 0.37	2.22 ± 0.46
1	1.94 ± 0.68	1.56 ± 0.73	1.63 ± 0.72	1.69 ± 0.70	1.13 ± 0.81[a,b]
2	1.81 ± 0.66	1.38 ± 0.89	0.81 ± 0.75	1.50 ± 0.52	0.44 ± 0.73[a,b]
3	1.88 ± 0.50	1.44 ± 0.96	0.69 ± 0.87	1.75 ± 0.68	0.50 ± 0.63[b]
4	2.06 ± 0.57	1.44 ± 1.09	0.69 ± 0.79	1.75 ± 0.77	0.44 ± 0.63[a,b]
5	2.13 ± 0.50	1.31 ± 0.95	0.69 ± 0.79	1.75 ± 0.77	0.63 ± 0.72[b]
6	2.13 ± 0.62	1.31 ± 0.87	0.81 ± 0.66	1.81 ± 0.54	0.75 ± 0.86[b]

[a] Statistically significant ($P < 0.05$) difference was found between 5%M + 70%/T-gel group and 5%M/T-gel group.
[b] Statistically significant ($P < 0.05$) difference was found between 5%M + 70%/T-gel group and 70%E/T-gel group.

The difference between 5%M + 70%E/T-gel and 5%M/T-gel or 70%E/T-gel was statistically significant ($P < 0.05$). Notably, the skin analgesic effect of 5%M + 70%/T-gel is much better than that of the commercially available topical drug EMLA cream ($P < 0.05$).

Fig. 4 shows the time-dependent percentage of volunteers anesthetized in the study. The volunteers whose VPS values were 0 (no pain) or 1 (moderate pain) were clinically considered anesthetized. Fifty minutes after treatment of 5%M + 70%M/T-gel, the percentage of anesthetized volunteers was 88%, while for 5%M/T-gel it was 50% and EMLA 37%. After 70 min, all the volunteers were anesthetized for 5%M + 70%M/T-gel, and this therapeutic result maintained for 190 min. For 5%M/T-gel, the maximum efficacy of 93% was achieved after 120-min treatment and retained for only 60 min. For the active control EMLA cream, the maximum efficacy was only 50%, which was earned after 100-min treatment and lasted for 120 min. The results showed the percentage of volunteers anesthetized and analgesia duration for 5%M + 70%E/T-gel were obviously increased compared with those for EMLA and 5%M/T-gel. Especially, the mean anesthetic onset time was dramatically shortened from 84 min for EMLA and 65 min for 5%M/T-gel to 45 min for 5%M + 70%E/T-gel. However, it should be noted that EMLA might not reach its full anesthetic efficiency after 40 min application in this study in terms of the percentage of volunteers anesthetized and the duration of anesthesia. Further study to compare

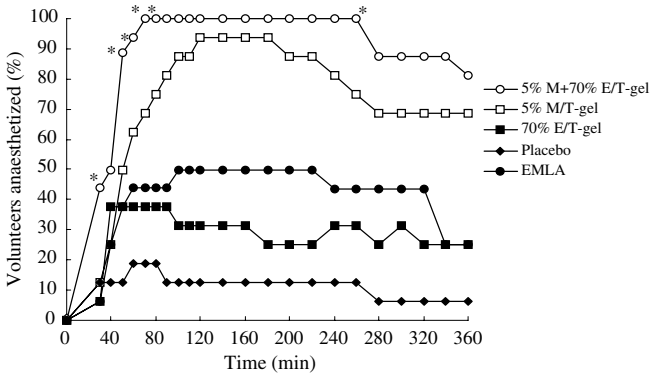

Fig. 4. Time course of the percentage of volunteers anesthetized after application of tetracaine gels and EMLA cream in VPS method. Sixteen healthy volunteers (8 male and 8 female) participated in the study. The volunteers who gave VPS value of 0 or 1 were considered clinically anesthetized, while VPS value of 2 or 3 not anesthetized. Asterisks indicate, at the time points, the percentage of volunteers anesthetized for 5%M + 70%E/T-gel was markedly higher than those of both 5%M/T-gel and 70%E/T-gel ($P < 0.05$), which demonstrated synergistic effect of menthol and ethanol on topical anesthesia of tetracaine gel. Except time point 40 min, the superiority of 5%M + 70%E/T-gel to EMLA was well observed at all other time points ($P < 0.05$).

the anesthetic efficiency of 5%M + 70%E/T-gel with that of EMLA after a longer application period of minimum 60 min would be valuable.

The synergistic effect of menthol and ethanol was well observed in the early and middle-late stage of the application of 5%M + 70%E/T-gel, as shown with asterisks in Fig. 4. This result was consistent with the time-dependent VPS values (Table 2). The synergistic effect of menthol and ethanol conferred the shortest anesthesia onset time, the longest anesthesia duration and the strongest anesthesia efficacy to the 5%M + 70%E/T-gel.

The enhanced permeation and topical anesthesia of tetracaine by menthol have been discussed in our previous work [6]. 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 [6,18,19]. To shorten anesthesia onset time compared with our previous tetracaine–menthol gel, ethanol, another commonly used penetration enhancer in many transdermal formulations was combined with menthol in the tetracaine gel. It is known that ethanol can exert its permeation enhancing activity through mechanisms including alteration of tissue solubility properties to improve drug partitioning in skin, solvent 'drag' function to carry permeant into skin and lipid extraction from the stratum corneum to increase drug flux [20]. As shown in this study, ethanol alone markedly increased the transdermal permeation of tetracaine. As we expected, the combination of ethanol and menthol in tetracaine gel (5%M + 70%E/T-gel) resulted in a highest and synergistically enhanced effect of transdermal penetration and topical anesthesia among all formulations without pronounced reactions and this influence could be closely related to the most marked changes of the epidermis ultra structures (Fig. 2).

4. Conclusions

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 fasting, strong acting, and long lasting. The pilot clinical studies of the tetracaine gel with the combination of 5% menthol and 70% ethanol as penetration enhancers indicated its anesthetic effectiveness was appropriated for most medical applications. Moreover, 70% ethanol (w/w) (an optimal skin sterilizing concentration) in the gel formulation can serve as an antibacterial agent to maintain the application site apinoid against infection, which may outweigh other common topical anesthesia formulations. The 5%M + 70%E/T-gel may be a potential topical preparation for clinical use.

Acknowledgments

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