In vitro and in vivo Evaluation of New Topical Anaesthetic Cream Formulated with Palm Oil Base

S.N. Khamdiah Khodari¹, Mohamed Ibrahim Noordin²,5, Lucy Chan³ and Zamri Chik¹,4,*

¹Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia; ²Department of Pharmacy, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia; ³Department of Anaesthesiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia; ⁴Department of Clinical Pharmacy, Faculty of Pharmacy, Jazan University, PO Box 114, Postal Code 45142, Jazan, Kingdom of Saudi Arabia; ⁵Institute for Pharmaceutical and Nutratectual Malaysia, Penang, Malaysia

Abstract: Background: Topical local anaesthetic cream was reported to be useful for pain relief for cutaneous procedures such as minor surgery and venipuncture.

Objective: The aim of this study was to evaluate the effectiveness of new formulation of lidocaine topical anaesthetic using palm oil base, HAMIN© and to determine how fast this new formulation produces adequate numbness compared to the currently used EMLA cream, in the University of Malaya Medical Centre (UMMC) set-up.

Method: The skin permeation test was conducted by using Franz type diffusion cell and pain assessment was carried out in healthy subject by using Verbal Rating Score (VRS) and Visual Analogue Score (VAS) evaluation.

Result: Result of permeation test demonstrated that the cumulative amount of lidocaine released from HAMIN© cream was increased with time and slightly higher than EMLA cream. The clinical study showed that HAMIN© single lidocaine cream can produces numbness through venepuncture procedure and comparable with EMLA cream which is a combination therapy for local anaesthetic (lidocaine and prilocaine).

Conclusion: It can be concluded that HAMIN© Lidocaine cream is suitable for cream preparation especially for topical application and it can be regarded as an achievement in palm oil and medical industries.

Keywords: EMLA, Franz diffusion test, HAMIN© base, lidocaine, pain assessment, self emulsifying base, topical anaesthetic cream.

1. INTRODUCTION

Topical anaesthetic cream is commonly used in several medical procedures such as minor surgery and venipuncture by numbing the site of action. A minor procedure like venipuncture will cause pain, discomfort, trauma and sometimes causes needle phobia especially in children [1]. Lidocaine is popularly used as local anaesthetic (LA) and widely chosen for topical preparation [2-4] due to its rapid onset of action, inherent potency, and moderate duration of action [5] which make it suitable for some minor procedures such as venipuncture, etc. [2, 6, 7]. It was the first LA introduced in clinical practices and has remained the most versatile and widely used nowadays.

The eutectic mixture of local anaesthetic (EMLA) cream has been introduced in 1993 and now widely used in clinical practice for local anaesthesia. EMLA cream contains a combination of two local anaesthetic agents which are lidocaine and prilocaine using eutectic mixture technique [8]. Fredrick Broberg has found that using eutectic system, the equal part of both lidocaine and prilocaine was able to produce adequate anaesthetic effect for topical application [9]. EMLA cream is widely used for local anaesthesia procedures for blood collection in paediatric unit in University of Malaya Medical Centre (UMMC). However, the application time recommended for EMLA cream is ~60 minutes prior to venipuncture [10], and this limits the clinical usage and patient acceptance especially for emergency cases.
There are few other new topical anaesthetic agents commercially available such as ELA-Max, Amethocaine and Topicane and all claimed to improve the onset of action [8]. Eidelman et al. 2005 reported that there were three alternative topical anaesthetic to EMLA which are as effective as EMLA, namely, tetracaine, liposome-encapsulated tetracaine and liposome-encapsulated lidocaine [6]. In United States, liposomal lidocaine is commercially available which offers a more rapid onset of action and less expensive compared to EMLA. In addition, a study has been conducted to observe the effectiveness between EMLA and liposomal-encapsulated ropivacaine for topical anaesthesia for palatal mucosa. From the study, the liposomal-encapsulated ropivacaine is effective to give adequate anaesthesia in buccal mucosa but EMLA did not reduce the pain during needle insertion of palatal mucosa [11].

HAMIN® base is a self emulsifying base that was formulated from a mixture of hydrogenated palm oil and palm kernel oil which is more thermal stable and robust to temperature changes. It is a new revolution in drug delivery system for semi solid and suppository which provides thermally stable bases. This base provides a larger range of possible melting points which is between 45°C-60°C and withstand to extreme temperature changes without any problem of polymorphism. These properties eased manufacture during the preparation stage. HAMIN® was also found to be very stable and resistant to oxidation during storage without interfering drug release and pharmacological activity. HAMIN® base, developed purely from the mixture of palm oil and kernel oil, contains various fatty acid with carbon chains ranging of C8, C10, C12, C14, C16, C18:1 and C18:2. HAMIN® has been successfully tested for dermal irritation, acute toxicity, and contact sensitization by Covance Laboratories Ltd, UK and proved to be safe in all the tests performed. All the tests were carried out under OECD Principles on Good Laboratory Practice (GLP).

HAMIN® has proved to be successful in delivering aspirin systemically via suppository formulation [12]. Previous study indicated that this palm kernel oil blend can be used alternatively to theobroma as a suppository base [13]. The developed insulin suppository from HAMIN® base was able to produce hypoglycaemic effect for glucose control in rabbit [14]. This base also was suitable to be used in formulation of testosterone transdermal delivery system (TDDS) which showed successful delivering of testosterone systemically in animal model [15]. Judging from these benefits, this study could greatly contributes to the development of palm oil base in pharmaceutical industry. Thus, this study focused on the evaluation of effectiveness of new formulation of topical anaesthetic using palm oil base and how fast this new formulation produces adequate numbness compared to EMLA cream.

2. MATERIALS AND METHOD

HAMIN® Lidocaine cream was prepared in laboratory of Pharmacology Department, University of Malaya. Lidocaine, prilocaine hydrochloride, benzocaine, sodium phosphate dibasic were purchased from Sigma Aldrich Co (St. Louis, USA). Acetonitrile was supplied by Merck (Darmstadt, Germany) and sodium phosphate monobasic were purchased from Fisher Scientific (New Jersey, USA).

2.1. Preparation of HAMIN® Lidocaine Cream

The preparation of HAMIN® Lidocaine cream involved two main phases which were oil phase and water phase. All the ingredients involved were weighted in % w/w and dissolved in appropriate phase before combining as a cream. This has been accomplished by using mixing by fusion technique with water emulsification in controlled temperature of ~50-60°C.

2.1.1. Oil Phase

HAMIN® 10
Lidocaine 5
Carbomer (Carbopol 741p NF) 0.3

2.1.2. Water Phase

Propylene glycol (PG) 10
Purified water Added to 100
Sodium Hydroxide To adjust the pH at 8 - 10

HAMIN® base was melted on the hot plate at the temperature of ~50-60°C. The lidocaine then was dissolved in molten base followed by addition of carbomer and let it dispersed completely inside the base. Meanwhile, propylene glycol and sodium hydroxide were added into the water and the mixture was heated to temperature at ~50-60°C. After temperature of both phases was maintain at the same degree, the water phase was added into oil phases while stirring using mechanical stirrer with controlled speed started from slower speed at ~400 rpm and gradually increased to ~800 rpm. Once the emulsion was combined completely, the speed was gradually decreased to ~200 rpm to avoid the presence of air bubbles. Lastly, the emulsion was transferred to cream container and left in room temperature for 24 hours to stabilize the whole texture.

2.2. In vitro Diffusion Test

In vitro permeation of lidocaine was tested by using Franz Cell System (PermGear Inc, Hellertown, PA), Strat M™ membrane (Merck, Darmstadt, Germany) which possesses similar characteristics of human skin was used in this experiment. The Strat-M™ membrane demonstrates the similar permeability coefficient with excised human skin and hairless rat skin [16]. This membrane which mimics the human skin was capable to simulate the skin. The surface area of the membrane was fixed on Franz cell system at 0.64 cm². The receiver compartment was filled with 5mL of 0.01M phosphate buffer at pH 6 as the media. The system was performed under controlled temperature at 37°C. Approximately 1 g of sample was loaded into donor compartment and left for 2 hours. 500µL of the media sample was collected every 30 minutes and replaced with the same volume of fresh media. The collected samples then were diluted with six times dilution factor (6 × d5) with 0.01M phosphate buffer at pH 6. The concentration of lidocaine in the media collected was...
analysed by HPLC with UV detection at wavelength 210 nm. The permeation profile was demonstrated using the following formula [17].

\[ Q = \frac{C \cdot V}{A} \]

where:

- \( Q \) = Cumulative amount of drug release
- \( C \) = Concentration of lidocaine in the receiver compartment in \( \mu g/mL \)
- \( V \) = Volume of the receiver compartment
- \( A \) = Surface area of the membrane in cm²

2.3. Analytical Method

A HPLC (Shimadzu, Kyoto, Japan) series LC 20AD was used to analyze the sample. The UV detector (Shimadzu, Kyoto, Japan, SPD-20A) was set at a detection of 210 nm. The column used was Gemini C18 (5 μm, 150×4.6 mm) purchased from Phenomenex, USA. Volumes of 10 μL were injected using an autosampler (Shimadzu, Kyoto, Japan, series SIL-20A HT). 0.01M phosphate buffer at pH 6 and acetonitrile (55:45) was used as the mobile phase. The total flow was set for 1mL/min and total run time was 8 min. The benzocaine was used as an internal standard (IS). The method was validated to presented adequate specificity, linearity, sensitivity, precision and accuracy. The limit of detection (LOD) was 0.1μg/mL and the limit of quantification (LOQ) was 0.2 μg/mL. The standard curve showed good linearity with regression of coefficient (r²) was greater than 0.998. The retention times were 6.50, 3.70 and 4.40 minutes for lidocaine, prilocaine hydrochloride and benzocaine, respectively.

2.4. In vivo Pain Assessment

The efficacy of HAMIN®-Lidocaine cream in delivering LA through human skin and produce numbness on applied skin area was examined by clinical study on healthy subjects. There were two phases of clinical studies performed. Two methods of pain assessment were used which were Verbal Rating Score (VRS) and Visual Analogue Score (VAS). Both systems have been fully validated in the literature [4, 18]. The VRS assessment was designed with pain classification according to the severity of pain. It was provided with a choice of five answers which are no pain, minimal sensation, mild pain, moderate pain and severe pain [1]. The VAS assessment was performed by designing a 100mm of horizontal line which marked with ‘no pain’ at the end line and ‘severe pain’ at the another end line. The subjects were requested to make a vertical cross on the line which relates to intensity of pain experienced during the procedure.

2.5. Study Design and Subject Admission

The first phase of clinical study (study 1) was performed to determine the onset of action of HAMIN®-Lidocaine cream in healthy subjects. It was designed with single blinded and placebo-controlled study in healthy adult subjects with normal skin condition. Twenty healthy subjects were recruited for this study. The second phase of clinical study (study 2) was performed to investigate the effectiveness of new formulation to give anaesthetic effect after application period which was determined from study 1, by using pain assessment method. The study was a single blinded, crossover, randomized, balanced and single dose comparative study of HAMIN®-Lidocaine cream vs. EMLA cream in healthy adult subjects with normal skin condition. Forty healthy subjects were recruited for this study. The study was approved by Medical Ethics Committee, University Malaya Medical Centre (UMMC), Ref. No. 1044.12/1045.39.

2.6. Study Procedures

Heart rate and blood pressure were recorded following entry to the study. A pregnancy test was offered to the female volunteers before starting study procedures. The application of the test compounds to the volunteer and data recording were performed blindly. For study 1, the ventral aspect of the forearm of right and left upper limbs were marked with the area of 10 cm² at three different sites. A 3cc syringe (without needle) was used to take out approximately 2 g of HAMIN®-Lidocaine cream and placebo from the cream container prior application at each site on either hand. The HAMIN®-Lidocaine cream or placebo was applied on either hand for 30, 45 and 60 minutes on site 1, 2 and 3 respectively. All applications were covered by occlusive dressing. After the desired application time, the cream was removed by using cotton swab and the pinprick was performed 5 minutes after cream removal. 20 pinpricks were applied disperedly on the marked site. For study 2, the venepuncture site was marked with the area of 10 cm² at the ventral aspect of the forearm of right or left upper limbs. Following randomisation schedule, a 3cc syringe (without needle) was used to take out approximately 2 g of HAMIN®-Lidocaine cream or EMLA cream prior application at venepuncture site. All applications were covered by occlusive dressing. After the desired application time, the cream was removed by using cotton swab and cannulation was performed 5 minutes after cream removal. VRS and VAS test were used to assess the pain of the cannulation. Subjects were repeated according to the above procedures with another treatment after one day washout period.

2.7. Statistical Data Analysis

Results are presented as the mean of replicates. The data were analyzed using Student’s t-test with p<0.05 as level of significance. For clinical study analysis, the data were analyzed using Wilcoxon Signed Ranks test with p<0.05 as level of significance.

3. RESULTS

3.1. In vitro Study

The ability of cream to release lidocaine from the formulation and to cross the membrane was studied by using Franz diffusion cell system. Fig. (1) showed the amount of lidocaine permeated the membrane area per unit time for HAMIN® formulation and concentration of lidocaine and prilocaine for EMLA cream. For HAMIN® formulation, lidocaine amount that crossed the membrane was increased proportionally with time increase from 30 minutes to 120 minutes indicating the drug was able to cross the membrane effectively. In addition, the concentration of lidocaine across
the membrane for HAMIN® formulation was higher compared to EMLA cream but no significant difference. The concentration of prilocaine of EMLA cream released from HAMIN® formulation was also determined.

Fig. (1). The cumulative amount of LA released from HAMIN® Lidocaine formulation (H-LAS) and EMLA cream obtained from Franz cell diffusion test (n=3, sd≤14.27)(p=NS).

3.2. Pain Assessment

In study 1, the VRS analysis using Wilcoxon Signed Ranks Test presented the number of subject in 5 severity of pain score in three application times of HAMIN®-Lidocaine cream in comparison with placebo. In 30 minutes of cream application, the HAMIN®-Lidocaine cream showed significant decrease in pain response compared to placebo with 9 subjects rated for mild sensation and no pain in total (p<0.05). In 45 and 60 minutes of cream application, the HAMIN®-Lidocaine cream showed significant reduction in pain response compared to placebo with 14 and 20 subjects rated for mild sensation and no pain in total for 45 and 60 minutes of application respectively (p<0.05) (Fig. 2). From VAS analysis, the pattern of pain response was similar to VRS analysis. The distribution of pain scores for HAMIN®-Lidocaine cream was significantly reduced by application time from 30 minutes to 60 minutes (p<0.05) (Fig. 3). HAMIN® Lidocaine cream showed lower pain score compared to placebo for all the application times but the result was great for 60 minutes application which showed significant numbness compared to placebo. Thus, the application time was decided to be set at 60 minutes for study 2 in comparison with EMLA cream for venepuncture treatment.

In study 2, 40 subjects were involved with 60 minutes of application time. From VRS result, the pain response was rated for mild pain, minimal sensation and no pain only. However, the result showed significant difference between both EMLA and HAMIN®-Lidocaine cream (p<0.05) (Fig. 4). There was higher number of subjects which did not experienced pain by the treatment of EMLA cream compared to HAMIN® Lidocaine cream. Contrary, the VAS result showed no significant different between EMLA and HAMIN® Lidocaine cream with median differences was 53.33% (p>0.05, p=NS) (Fig. 5).

4. DISCUSSION

HAMIN® base was functioning as a drug carrier crossing the skin membrane. The permeation study of HAMIN® formulation was observed by comparing cumulative amount of permeated LA of HAMIN® formulation to EMLA cream.
The study was performed to investigate the ability of HAMIN® formulation in delivering the LA and how well it penetrated the membrane compared to EMLA cream. The amount of cream used in the study was not followed the recommended dosage as applied in clinical study due to issues of dosage and surface area for cream application in human subjects. Because of this, the collected sample of permeation study cannot be considered as adequate concentration for producing numbness.

HAMIN® Lidocaine cream was formulated with single LA which contained 5% of lidocaine whereas EMLA cream was formulated with mixture of LA which contained 2.5% of lidocaine and 2.5% of prilocaine that made up to 5% of LA content in total. According to this proportion, the higher concentration of lidocaine release from HAMIN® Lidocaine cream has been estimated. Both lidocaine and prilocaine content of EMLA cream produced the effective anaesthetic effect. However, prilocaine which less soluble in lipid rather than lidocaine probably will affect the anaesthetic potency. The lipid solubility of LA determines their ability to penetrate the neuronal tissue and membrane to reach the site of action [10]. Indeed, the lidocaine amount released from HAMIN® formulation was higher than EMLA. However, the involvement of prilocaine to give enough anaesthetic effect should be considered as well since the concentration of prilocaine for EMLA is higher than lidocaine in HAMIN® formulation. So, the clinical trial was performed to study this further and clarify the effectiveness issue for the new formulation.

In VRS analysis for pain assessment, score of no pain, mild sensation and mild pain were taken to represent successful anaesthesia whereas moderate and severe pain were taken to represent unsuccessful anaesthesia. In study 1, three application times were tested to determine the onset of action of HAMIN® Lidocaine cream to produce numbness. In comparison with placebo, HAMIN® Lidocaine cream showed an effective numbness in three application times. Among the three application times, the application for 60 minutes has shown the best score in pain assessment. Thus, the onset of action for HAMIN® Lidocaine cream was identified for 60 minutes of application which was used in effectiveness evaluation in study 2. Study 1 was performed not only to determine the onset of action of HAMIN® Lidocaine cream, but it also can be considered as pre-study or initial study to evaluate the effectiveness of the cream and verified the technique used for study 2. Many aspects have been identified during study 1 in order to minimize the erratum. The using of pain assessment as study evaluation was a complicated method to be used. It was subjective and can be interpreted differently by individual because of involvement of emotion and feeling.

Study 2 was carried out by involving 40 healthy subjects for pain assessment. The sample size of 40 subjects was decided to be used based on previous study done by Franz-Montan et al. In their study, they were using 40 subjects in the study of effectiveness of liposome-encapsulated ropivacaine for topical anaesthetics of palatal mucosa [11]. In comparison with EMLA cream, HAMIN® Lidocaine cream showed slightly less effective with percentage of median difference which was about 50%. The pain score in VRS assessment indicated that the higher number of subject rated HAMIN® Lidocaine cream with mild sensation and mild pain compared to EMLA but in no pain score, the HAMIN® Lidocaine cream scored lower number of subject.

According to permeation study, the concentration of lidocaine release from EMLA cream was much lower than HAMIN® Lidocaine cream but the concentration of prilocaine released from EMLA cream was higher than lidocaine released from HAMIN® Lidocaine cream even though there was only 2.5% prilocaine used in the formulation. It can be suggested that the combination of lidocaine and prilocaine in the topical anaesthetic formulation as EMLA cream produced more effective numbness compared to single LA formulation. Even though the HAMIN® Lidocaine formulation was less effective in 60 minutes of application, it was comparable with EMLA cream. In the future, the HAMIN® Lidocaine formulation can be improved and further study should be done to enhance the onset of action.

CONCLUSION

HAMIN® Lidocaine cream is the first of its kind formulated using palm oil base. This will be a revolution in palm oil research especially in medical and pharmaceutical industries. In comparison with EMLA cream, HAMIN® Lidocaine cream showed potential alternative as topical anaesthetic cream. The formulation of HAMIN® Lidocaine cream could be improved in further study to accelerate the onset of action and defeat the effect of EMLA cream.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We would like to acknowledge University of Malaya Research grant, UMRG389-11HTM for funding this study.

PATIENT CONSENT

Declared none.
In vitro and in vivo Evaluation of New Topical Anaesthetic Cream Formulated

REFERENCES


