THE EFFECTS OF SOME PERMEABILITY ENHANCERS ON THE PERCUTANEOUS ABSORPTION OF LIDOCAINE

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ABSTRACT

Local anesthesia of the intact skin is difficult because of the barrier properties of skin to epicutaneous penetration of local anesthetic drugs. Using local anesthetics with combination of penetration enhancers could overcome this problem. The main objective of this study was to assess the effects of some permeability enhancers on the percutaneous permeation of lidocaine. The effect of polysorbate 80, polysorbate 20, dimethylsulfoxide (DMSO), tert-butyl cyclohexanol (TBCH), and α-terpinol in different concentrations and various ratios of lidocaine to enhancers was evaluated. The results showed that polysorbate 80 and polysorbate 20 has no detectable penetration enhancing effects in guinea pig skin mounted to diffusion cells. The same results were obtained to water/oil ratio and the type of oil phase (liquid paraffin vs. castor oil). Addition of DMSO to the previous formulations had a considerable enhancing effect. According to the data, the extent of lidocaine permeation was proportional to the concentration of DMSO in these formulations. The best results belonged to the addition of terpenes but interestingly there wasn't any linear relationship between the concentrations of α- terpinol/ or TBCH and the duration of antinociceptive effects of lidocaine. Based on the results of this study the ratio of 1: 4 from α- terpinol or TBCH to lidocaine results in a better antinociceptive effect and α- terpinol was the best one among of these compounds. This effect was proven with in vivo tail-immersion test to assess the antinociceptive effect of formulations which have shown more penetration.

Keywords: Lidocaine, terpene, DMSO, surfactants, permeability enhancer.

INTRODUCTION

It is a well known fact that one must first tolerate a considerable amount of discomfort prior to the administration of a local anesthetic agent. This displeasure is a result of the necessity of a needle insertion prior to the administration of the anesthetic agent. In the past 40 years, many attempts have been made to develop a suitable topical anesthetic that would be capable of eliminating the associated pain during minor dermal procedures such as venipuncture, intravenous cannulation, vaccination, circumcision, punch biopsy and other surgical incisions. EMLA cream (Eutectic mixture of local anesthetics by Astera Zeneka), consisting of lidocaine 2.5% and prilocaine 2.5% in an emulsified cream, is the only effective topical anesthetic product currently marketed world-wide for use on the intact skin (Kang *et al*., 2001). Lidocaine itself has low skin permeability and conventional products of this local anesthetic agent can not penetrate adequately to the intact skin.

The ideal topical anesthetic agent is one that provides 100% anesthesia in a short period of time, be effective on the intact skin without systemic side effects, and invokes neither pain nor discomfort. The attempt to formulate such an agent continues today. The difficulty in achieving such an agent partly lies in the inadequate diffusion and delivery of lidocaine through the skin (Lener *et al*., 1997).

Although eutectic mixture of lidocaine 2.5% and prilocaine 2.5%has proven to be an effective analgesic and is useful in a variety of clinical situations, there have been reports of adverse reactions. The most typical of these reactions is blanching or redness at the site of application due to peripheral vasoconstriction (Juhlin and Evers, 1990). Methemoglobinemia has been the most serious side effect reported to date after eutectic mixture of lidocaine and prilocaine use (Jakobson and Nilsson, 1985; Juhlin and Evers, 1990).

The improved efficacy of eutectic mixture of lidocaine and prilocaine, as compared to the conventional topical formulation was attributed to the high drug concentration in its oil phase (Gajraj *et al*., 1994; Kang *et al*., 2000; Lener *et al*., 1997; Nortier *et al*., 1995). In this study the effect of low oil phase and combination effects of polysorbate 20 or 80 with DMSO and some terpenes in low concentration as permeability enhancers was evaluated (Barry, 2001a; Barry, 2001b; Brechner *et al*., 1967; Evrard *et al*., 2001; Lopez *et al*., 2000).

MATERIALS AND METHODS

Materials

The following chemicals and solvents were used;

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lidocaine (Darou-Pakhsh, Iran), carbomer 940 (BF Goodrich, USA.), castor oil (Dineh, Iran), polysorbate 20, polysorbate 80, liquid paraffin, dimethylsulfoxide (DMSO), chloroform, hydrochloric acid, 4-tert-butyl cyclohexanol (TBCH), α-terpinol and sodium hydroxide (Merck, Germany). All the other chemicals used were analytical grade.

Preparation of topical lidocaine cream

Creams were prepared by heating both oil phase and aqueous phase to 60°C separately. The aqueous phase was added to the oil phase under mechanical stirring. In all formulations lidocaine was added to the oil phase. The compositions of different formulations are presented in tables 1 and 2. The creams were neutralized by drop-wise adding of the 1 % NaOH solution during the stirring until the creams became viscose. The creams were allowed to cool down quickly in an ice bath under constant stirring and finally each formulation was packaged in aluminum tube.

In vitro permeation study

Permeation studies were carried out through abdominal skin of guinea pig using the modified Franz Diffusion cell. The animals were sacrificed by an overdose of chloroform inhalation. The hairs of the dorsal side of animal skin were removed with using scissors. The shaven part of the skin was separated from the animal and the hypodermis including blood vessels and fat tissues were surgically removed using a surgical blade (No. 22). The skin surface area exposed to receptor phase was 4.9 cm^2. In all the permeation studies degassed 0.1 N hydrochloric acid solution was used as receiver phase. From each various cream formulations 500 mg of cream was applied onto the prepared guinea pig skin facing on the diffusion cells. An aliquot of 5 ml of samples was withdrawn at suitable time intervals and replaced with the same amount of medium to maintain the receptor phase volume at 40 ml. The samples were alkalized with 0.15 ml NaOH 2N and then extracted by 5 ml chloroform in three steps. After combining the chloroformic phases, the chloroform was evaporated under nitrogen gas and the residue was dissolved in 5 ml HCl 0.1 N and finally the samples were quantified by UV spectrophotometer (Cecile 9050 UK) at 263 nm.

Mathematical data treatment

The first law of Fick can be written as:

$$\frac{dM}{dt} = \frac{DSK(C_d - C_r)}{h} \quad (1)$$

Where M is the amount of drug penetrating through a cross section, S, of skin in unit of time, t, and K is partition coefficient of drug between the stratum corneum and vehicle, C_d and C_r are the concentrations of the drug in donor and receiver compartments, respectively and finally h, represents the thickness of the skin. If the sink condition was held, then C_r would be negligible in comparison with C_d and then the Eq. (1) could be simplified to the Eq. (2):

$$\frac{dM}{dt} = \frac{DSKC_d}{h} \quad (2)$$

If the permeability coefficient, P, is substituted in Eq. (2), then the Eq. (3) would be derived:

$$\frac{dM}{dt} = PSC_d \quad (3)$$

Therefore the permeability coefficient, P, could be obtained from the slope of a linear plot of the M versus t. In this regard, P would be the slope of the depicted line divided by SC_d. In all the cases permeability coefficients for different formulations were evaluated according to Eq. (3)

In vivo studies

Tail-flick test was carried out in the present study following the previous report (Sewell and Spencer, 1976). The rat was held in a cloth restrainer during the experiment. This method of restraining is a less stressful method of animal holding during tail-immersion test and has been shown to be able to reduce variability in response latencies, compared with commercial restrainers (Owen *et al*., 1984). The end of the tail (5 cm) was placed in a 50±2°C water bath. The reaction time was considered as the time taken by the rats to deflect their tails. The first reading was discarded and the reaction time was taken as a mean of the next two readings. The latent period of the tail-flick response was taken as the index of antinociception and was determined at 30, 45 and 60 min after the application of relevant cream formulations. The cut off time was 20 s. In this study 10 different groups of rats, each of which consisting of 5 animals were used and each of the formulations in table 3 was assessed with each group.

STATISTICAL ANALYSIS

The results of tail flick test are shown as mean ± standard deviation (SD). The differences between the control and experimental groups were determined by one way ANOVA and a $P < 0.05$ was considered as significant differences between samples and control groups.

RESULTS AND DISCUSSIONS

According to tables 1 and 2, the different formulations maybe categorized into three different types including formulations containing polysorbate 20 or 80 alone, polysorbate 20 or 80 with DMSO and polysorbate 20 with terpenes including TBCH or α-terpinol as permeability enhancers. All of these formulations had semisolid consistency and because of carbomer 940 nature, they were thixotropic formulations and the physical stability and homogeneity of each formulation was considered at room temperature during 1 month of preparation, but in

permeation studies freshly formulated creams was used one day before the experiments. The permeability data showed that formulations F1-F5 and F7 have minimum permeability through the skin and no detectable amounts of lidocaine were found in receiver phase. Formulations F1 and F2 contained liquid paraffin as oil phase while formulation F3-F5 and F7 had castor oil. Based on the permeability data, it seems that the type of oil phase doesn't have any detectable effect on the permeability of the lidocaine across the guinea pig skin. Similarly, it suggests that oil/water ratios also has no considerable effect on the lidocaine penetration. These formulations also contained polysorbate 80, although there are several reports that emphasize the permeability enhancing behavior of surfactants (Nokhodchi *et al*., 2003; Tezel *et al*., 2002; Walker and Smith, 1996). However according to our results, no considerable effect on the lidocaine penetration across the skin was detected with respect to polysorbate 80 alone in these formulations except in formulation 6 which showed a detectable concentrations

Table 1: Composition of the different cream formulations containing DMSO and polysorbates as permeability enhancer, each formulation contain 0.5% carbopol.

No.	Lidocaine	liquid paraffin	Castor oil	Polysorbate 80	Polysorbate 20	Water	DMSO
F1	5	30	-	2.5	-	62.0	-
F2	5	20	-	2.5	-	72.0	-
F3	5	-	10	1	-	83.5	-
F4	10	-	20	1	-	68.5	-
F5	10	-	20	2.5	-	67.0	-
F6	10	-	20	5	-	64.5	-
F7	10	-	20	-	1	69.5	-
F8	5	-	10	1	-	69.5	5
F9	10	-	20	1	-	64.5	5
F10	10	-	20	1	-	59.5	10
F11	10	-	20	-	1	64.5	5
F12	10	-	20	-	2	58.5	10
F13	10	-	10	-	1	68.5	10

All of these materials were used as %w/w.

Table 2: Composition of the different cream formulations containing tert-butyl cyclohexanol and α-terpinol as permeability enhancer, each formulation contain 0.5 % carbopol

No.	Lidocaine	TBCH*	Castor oil	α-Terpinol	Polysorbate 20	Water
F14	10	-	10	-	1	78.5
F15	10	2.5	10	-	1	66.0
F16	10	-	10	2.5	1	66.0

*TBCH: tert-butyl cyclohexanol
All of these materials were used as %w/w

Table 3: Composition of the different cream formulations containing α- terpinol and TBCH and control formulations for tail-flick test.

C.F.	Lidocaine %	α-terpinol %	TBCH %	Water %
α-T1	5	1	-	82.5
α-T2	5	2.5	-	81.0
α-T3	-	5	-	78.5
α-T4	10	2.5	-	76.0
TBCH1	5	-	1	82.5
TBCH2	5	-	2.5	81.0
TBCH3	5	-	5	78.5
TBCH4	10	-	2.5	76.0
C1	-	-	-	88.5
C2	10	-	-	78.5

C. F. (code of formulation), All of these formulations also contain 10 % castor oil, 1 % polysorbate 20 and 0.5 % carbopol 934.

of lidocaine in receiver phase. The solubility studies showed that castor oil is a better solvent for lidocaine than liquid paraffin (data not shown) and then in other formulations castor oil was considered as oil phase. Formulations F3 and F4 contain different concentrations of lidocaine but none of them showed detectable concentrations of lidocaine in receiver phase. In formulations F8-F10, DMSO at different concentrations was introduced and according to fig. 1 permeability of lidocaine was considerably increased in these formulations and which was proportional with the concentrations of DMSO, that is an effective penetration enhancer that promotes penetration via reducing skin resistance to drug molecules or by inducing of drug partitioning from the dosage form (Liu *et al*., 2006; Tezel *et al*., 2002). Some other researchers propose that DMSO denatures the intercellular structural proteins of the horny layer of the skin or promotes lipid fluidity by disruption of the ordered structure of the lipid chains (Walker and Smith, 1996). In addition, DMSO may alter the physical structure of the skin by extraction of lipids, lipoproteins and nucleoproteins of the stratum corneum (Barry, 1987; Embery and Dugard, 1971). In formulations F11-F13, polysorbate 20 was used instead of polysorbate 80. Based on the results presented in fig. 2, these formulations showed better permeability results which may contribute to the synergistic action between DMSO and polysorbate 20 as a binary mixture of chemical penetration enhancers (Karande *et al*., 2006). The effects of terpenes and terpene-like molecules such as TBCH were shown in fig. 3. According to Nokhodchi and his coworkers (Nokhodchi *et al*., 2007), terpenes in 2.5 % have profound effects on the permeability of diclofenac sodium. In this regard, same concentrations from α-terpinol and TBCH were used to assess the permeability of lidocaine. Based on the data in fig. 3, it is evident that these formulations have better permeability behaviors than all other formulations and F16 was the best one among them. Both mono- and sesquiterpenes are known to increase precutaneous absorption of drugs by increasing diffusivity of the compounds in stratum corneum (Cornwella and Barry, 1993; Lim *et al*., 2006; Walker and Smith, 1996). Terpenes may disrupt the intercellular lipid barrier and some studies indicate that terpenes could increase electrical conductivity of tissues thereby opening polar pathways through the stratum corneum (Scott *et al*., 1991). Some other investigators believe that terpenes are able to produce eutectic mixtures with lidocaine and then could increase thermodynamic activity of the lidocaine in the relevant formulations (Kang *et al*., 2001; Kang *et al*., 2000). To confirm the effects of terpenes concentrations on the permeability of lidocaine, tail-immersion test was performed. In this in vivo test, 3 different concentrations of terpenes (1, 2.5 and 5%) were evaluated. According to table 4, of the different concentrations of terpenes, 2.5 % concentration proved the most effective. Based on the physicochemical interactions between the skin and penetration enhancers it seems that higher concentration of terpenes should result in more penetration, but the results of the present study showed that 5% concentration of α-terpinol or TBCH were not better chance to promote more permeation. It seems that at a suitable proportions eutectic mixture of lidocaine and terpenes were probably formed. This study reveals that these formulations have better permeability when the concentration is 2.5% from α-terpinol or TBCH. Probably higher concentration of these compounds can reduce the thermodynamic activity of lidocaine at these formulations because they can dilute the oil phase and then with the same concentration of lidocaine in these formulations, thermodynamic activity would be decreased. Along with increment in lidocaine concentrations in formulation with the same amounts of terpenes, the animal responses to pain also were increased. In this regard, it seems that thermodynamic activity of the lidocaine itself has a significant effect on the percutaneous penetration of the drug.

Tail-immersion test is a simple and rapid test to evaluate the animal response to painful condition. Increment in animal response to flicking of the tail after exposure to heat is the base of this test (Kang *et al*., 2001). After application of control formulations (cream base and creams containing 10% lidocaine without any permeability enhancer) and test formulation containing α-terpinol and TBCH in different concentration (table 4), the latency times was assessed. Although all the formulations containing α-terpinol and TBCH have significant differences in response time, the most prolonged response belonged to the formulations containing 2.5% permeability enhancer and 10% lidocaine. These data are in agreement with formulations F15 and F16 in fig. 3 and the best one was F16 (α-T2). The best response time was 30 min in F15 and F16, but in other formulations the best response time was 60 min after the application of drug to the tail of the animals.

Table 4: The effect of various permeability enhancers on tail-immersion test in rat.

Code of formulation	Time after application of cream (min)		
	30	45	60
α- T1	9.01±1.05	11.95±1.02	14.75±1.05
α- T2	9.05±1.71	11.76±1.78	15.95±1.97
α- T3	9.82±1.49	9.93±1.57	10.13±1.51
α- T4	16.02±1.43	12.00±1.04	13.15±1.14
TBCH1	7.99±0.92	12.12±2.88	13.67±2.04
TBCH2	8.26±1.26	11.38±1.02	13.40±1.74
TBCH3	9.04±1.06	12.14±2.08	12.84±2.51
TBCH4	13.12±1.29	10.58±2.02	10.34±2.34
C1	4.21±0.71	3.69±1.94	4.45±1.03
C2	6.86±0.92	9.07±1.45	10.00±1.89

Values are expressed as mean of tail withdrawing time (seconds) ± S.D, N=5, * P<0.05. compared with C1 and C2 control

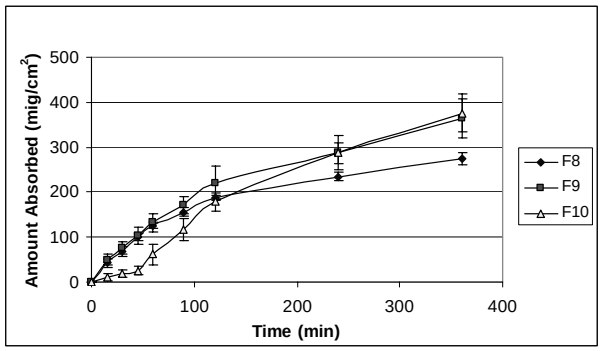

Fig. 1: The effect of different DMSO concentrations on the skin penetration of lidocaine from formulation containing polysorbate 80.

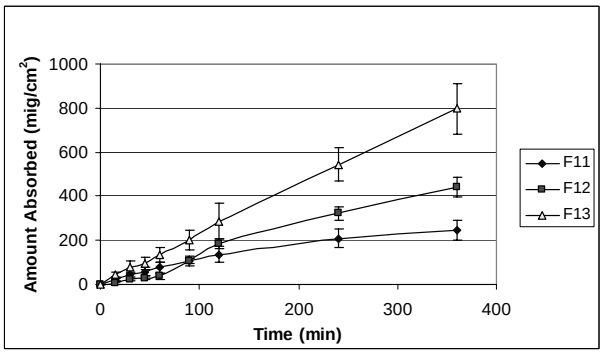

Fig. 2: The effect of different DMSO concentrations on the skin penetration of lidocaine from formulation containing polysorbate 20.

Based on the data presented in table 5, the most outstanding penetration enhancer was α-terpinol, providing an almost 7-fold increase in permeability of lidocaine, followed by TBCH in the same concentrations of enhancer and lidocaine.

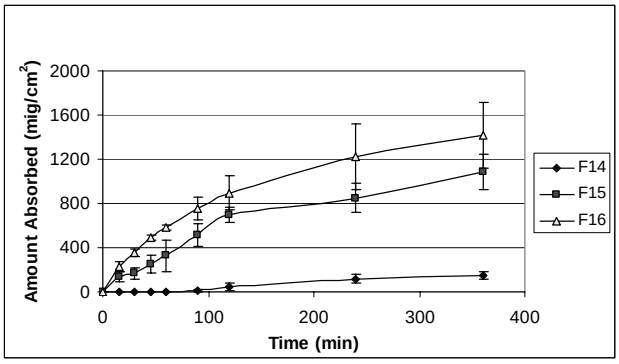

Fig. 3: The effect of terpenes on the permeability of lidocaine across the guinea pig skin.

Table 5: Permeability coefficient of lidocaine in different formulations

Code of formulation	P (cm/sec)x10^{-6}	Sdev	ER
F8	13.47	0.67	2.1
F9	10.00	1.25	1.6
F10	13.67	1.52	2.2
F11	8.37	0.70	1.3
F12	16.73	1.86	2.6
F13	28.16	4.02	4.5
F14	6.33	2.11	1.0
F15	35.10	5.01	5.5
F16	42.65	4.74	6.7

CONCLUSION

Although there are many reports showing that surfactants may act as permeability enhancers (Evrard *et al*., 2001; Lopez *et al*., 2000; Nokhodchi *et al*., 2003), in this study no detectable effect was observed for polysorbate 20 and polysorbate 80, while to be seems related to the physicochemical nature of the penetrant. There are also a lot of reports that suggest different mechanisms for penetration enhancing effects of terpenes (Jakobson and Nilsson, 1985; Kang *et al*., 2001; Walker and Smith, 1996), so it is over simplification if one considers a unique and simple mechanism for these compounds. Some authors propose that limited solubility of lidocaine in oil phase results in low concentrations of drug in the site of application (Gajraj *et al*., 1994; Jakobson and Nilsson, 1985), but lidocaine approximately is soluble in castor oil in 1:1 ratio and then solubility of the lidocaine in the oil phase is not an important factor in the permeability of lidocaine through the intact skin. The same results have been shown for diclofenac sodium (Nokhodchi *et al*., 2007). According to the results of the present study, α-terpinol has the best permeability enhancing effects on the lidocaine penetration though the skin. Since terpenes are relatively safe compounds, their incorporation in low concentrations into local anesthetic cream formulations could be recommended. Also, it was revealed that the permeability of lidocaine was increased with the increment of DMSO concentration in various formulations, but α-terpinol and TBCH have the best effects at 2.5% concentration. The same results were reported by Nokhodchi and co-worker for other terpenes (Nokhodchi *et al*., 2007). We can conclude that the effects of terpenes on the integrity of the cells membrane are more reversible than DMSO and may serve as safer permeability enhancers than DMSO.

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