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SYNTHESIS AND SKIN PERMEATION STUDY OF LIDOCAINE ORGANIC SALTS

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ABSTRACT

Four organic quarternary ammonium salts of lidocaine, lidocaine adipate, lidocaine maleate, lidocaine malonate and lidocaine tosylate were prepared in this study. These compounds were regarded as prodrugs of lidocaine and were expected to enhance skin permeability by ion-pair approach. *In vitro* permeation study through pig skin and determination of partition coefficients were carried out. The skin permeation of lidocaine adipate and lidocaine malonate after 12 hours of application was significantly higher than lidocaine hydrochloride but was not significantly different from lidocaine ($\alpha < 0.05$). The increase in permeation of organic salts from lidocaine hydrochloride and the different profile of permeation from lidocaine may be the result of prodrug structure contributing ion-pair transportation beside normal route of transportation.

INTRODUCTION

Since the discovery of licocaine (I), in 1943, only two forms of lidocaine, base and hydrochloride, are available for preparation in dosage forms. Structural modification and formulation development

were therefore attempted to improve the efficacy of I. As structural manipulation, lidocaine analogs with permanent cationic charge[1,2] were found to have appreciable local anesthetic action. However, the covalent quartenary ammonium group affected the poor penetration across membrane. The relationship between potency and lipid solubility of lidocaine analogs was also studied.[3-6] It was found that lipid solubility was the important factor governing the drug potency.[4,5] Nevertheless other factors namely, pk_a, physiological pH and receptor specificity were also important and had to be taken into account.[3,6] As formulation development, transdermal anesthetic patch[7,8] and cream[9-11] were introduced with good effectiveness, and adequate duration of action but the onset of action was not satisfactory[11]. Iontophoresis, the other drug delivery system by aid of electrical current, was found to enhance the penetration of lidocaine hydrochloride across membrane[12,13] Besides iontophoresis, the ionized drug can be delivered by ion-pair transportation.[12,13] Ion-pair between cation of ionized drug and anion of organic acid or fatty acid was formed, an increase in lipophilicity was account for the enhancement.[1]

In this study, a series of organic salt of lidocaine was designed to promote skin permeability. The proposed adipate, maleate, malonate and tosylate salts which are easily ionized organic salts are regarded as prodrugs of lidocaine. The skin permeability of lidocaine salt may be enhanced by ion-pair approach. In addition to the syntheses, determination of skin permeability and apparent partition coefficients were also carried out.

MATERIALS AND METHODS

Melting points of the compounds were determined on a Buchi Capillary Melting Point Apparatus and uncorrected. The proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained with a Joel FX 90Q (90 MHz). Chemical shifts were reported in ppm related to the internal standard, tetramethylsilane. Infrared (IR) spectra were recorded as potassium bromide disc on a Shimadzu IR-400. Mass (Joel FX 3000 double focusing) and ultraviolet (Hitachi U-3200) spectra were determined for all compounds. Analytical results from

elemental analyzer (Perkin Elmer 240C) obtained for all compounds were within ± 0.4% of the theoretical value. All substances and solvents used were of analytical grade and were used as received.

Synthesis of Organic Salts of Lidocaine

The following procedures are representative of the synthesis of lidocaine (I), lidocaine adipate (I-A), lidocaine maleate (I-B) lidocaine molonate (I-C) and lidocaine tosylate (I-D).

Lidocaine adipate (I-A). A solution of 0.937 g (4 mmole) of lidocaine in 25 mL of anhydrous ethyl ether was prepared. Then, a solution of 0.585 g (4 mmole) of adipic acid in 15 mL of acetone was added and the mixture was stirred for 10 minutes and cool at 4^{0}C, the precipitated solid was removed by filtration, washed with anhydrous ethyl ether and then dried. The resulting solid was collected and recrystallized with ethyl acetate to give lidocaine adipate as white crystals (1.22 g ; 80% yield), m.p. 119-120^{0}C. IR (KBr) ν: 3450 (O-H), 3200 (N-H), 3050 (aromatic C-H), 2950-2900 (aliphatic C-H), 2650-2500 (N^+-H), 1690 (C=O of acid and amide), 1550 (aromatic C=C), 1480-1460 (N-C=O), 1260-1220 (C-O), 1200 (aliphatic C-N) , 780, 730 (3-adjacent protons of aromatic) cm^{-1} λ_{max} (isotonic phosphate buffer pH 7.4) : 262.5 (ε 457) nm. ^{1}H NMR (DMSO-d_6+$CDCl_3$) : 1.15 (t, J 7.18 Hz, 6H, -CH_2CH_3), 1.62 (m, 4H, adipic β -CH_2), 2.20 (10H, overlap of Ph-CH_3 and adipic α-CH_2), 2.65 (q, 4H, -CH_2CH_3), 3.20 (s, 2H, CO-CH_2-N), 7.06 (s, 3H, aromatic protons), 7.65 ($CHCl_3$), 8.99 (bs, 1H, exchangeable with D_2O, CO-NH), 10.40 (bs, 2H, exchangeable with D_2O, COOH). MS : M/E 234 (56.9%).

Anal. Calcd. for $C_{20}H_{32}N_2O_5$: Calculated C = 63.14 ; H = 8.48 ; N = 7.36. Found : C = 63.29 ; H = 8.73 ; N =7.35

Lidocaine tosylate (I-D). A solution of 0.937 g (4 mmole) of lidocaine in 5 mL of anhydrous ethyl ether was prepared. Then, a solution of 0.761 g (4 mmole) of p-toluenesulfonic acid(monohydrate) in 30 mL of anhydrous ethyl ether was added and the mixture was stirred. White precipitates were formed immediately. After standing overnight, the precipitated solid was removed by filtration, washed

with anhydrous ethyl ether and then dried. The resulting solid was collected and recrystallized with ethyl acetate to give lidocaine tosylate as white crystals, (1.10 g, 67% yield), m.p. 149-151°C. IR (KBr) ν: 3250 (N-H), 3050 (aromatic C-H), 2950-2850 (aliphatic C-H), 2850 (N^+ H), 1690 (C=O), 1600, 1545 (aromatic C=C), 1470 (N-C=O), 1210, 1030, 1010 (S=O), 1180 (C-N), 680 (S-O) : 1470 (NH), 820 (2-adjacent protons of aromatic), 770 (3-adjacent protons of aromatic) cm^{-1}. λ_{max} (isotonic phosphate buffer, pH 7.4) : 261.4 (ε 792) nm. ^1H NMR ($CDCl_3$) :1.28 (t, J 7.18 Hz, 6H, -CH_2CH_3), 2.09 (s, 6H, NH-Ph-CH_3), 2.30 (s, 3H, SO_3-Ph-CH_3), 3.34 (m, -CH_2CH_3), 4.36 (d, 2H CO-CH_2), 6.95 (s, 3H, aromatic protons of lidocaine), 7.05 (d, J 8.2 Hz, 2H, aromatic protons of tosylate), 7.26 ($CHCl_3$), 7.64 (d, J 8.2 Hz, 2H, aromatic protons of tosylate, meta to CH_3), 9.47 (bs, 1H, exchangeable with D_2O, N^+H), 9.83 (bs, 1H, exchangeable with D_2O, CO-NH) MS : M/E 234 (39.6%).

Anal. Calcd. for $C_{21}H_{30}N_2O_4S$: C = 62.04 ; H = 7.44 ; N= 6.89. Found C = 62.18 ; H = 7.71 ; N = 6.89

Determination of Solubilities

Approximate solubilities of the compounds were determined at 32°C by dissolving 100.0 mg of the test compound in 0.05 ml of water, ethanol, chloroform and ether individually. The mixtures were placed in ultrasonic bath for half and hour and observed for clarity. If the true solution was not obtained, a further 0.05 ml of the solvent was gradually added, and sonicated. The procedure was repeated until the true solution was obtained. Discriptive terms according to USP XXII were used to express approximate solubilities of the compounds.

Analytical Methods

Partition coefficient studies. In the partition coefficient studies the UV absorption of the compounds was examined at 262.5 nm for I, I-A, I-B and I-C and 261.4 nm for I-D.

In vitro skin permeation studies. First derivative UV spectrophotometry (D_1= dA/dt) is a technique selected for the determination of the content of test compounds in receptor cell to minimize matrix interferences. The wavelengths of maximum D_1 values of all compounds in isotonic phosphate buffer pH 7.4 were determined at 272.9 nm. Least-squares regression analysis of D_1 values vs concentrations indicated linear over the range of 0.004-0.400 mg/mL (R^2 = 0.9999 to 1.0000).

In Vitro Skin Permeation Studies

All permeation experiments were performed with full-thickness pig skin which were excised from side of male pigs. The age of the pigs was 1 day and the weight was about 1 kilogram. The permeation cells were modified from Franz diffusion apparatus and the apparatus in Hadgraft's study[15]. The permeation cell consisted of two compartments. The capacity of the receptor cell was 25 mL and the cross-sectional area of the donor cell which was effective permeation area was 3.8 cm^2. A circular sheet of pig skin of approximately 4 cm^2in area was placed dermal side down between the donor and receptor phase of the permeation cell. The receptor phase was isotonic phosphate buffer pH 7.4, maintained at 32^{0}C and stirred with stirring rate maintained at 600 rpm. The skin was kept in contact with the receptor phase for 48 hours prior to the application of the donor phase. The receptor phase was changed every 12 hours during 48-hour preapplication leach period. Aftet this period, a 1.0 mL aliquot of the test solution, equivalent to 10 mg of test compound was applied to the donor side of the skin. The receptor phase was stirred at stirring rate maintained at 600 rpm. Samples (5.0 mL) were taken from the receptor phase every 12 hours during 48 hour period after application. The volume taken was replaced by fresh isotonic phosphate buffer pH 7.4. The samples were assayed by first derivative UV spectrophotometry. Four determinations of skin permeation tests were performed and two pigs were used for each test compounds.

Control tests were carried out by application of the vehicle, 1.0 mL propylene glycol, instead of the test compound solutions. D_1

values of control tests were used for correction of D_1 values from samples in order to eliminate matrix interferences. Percent recovery of D_1 and the corrected D_1 were 102.6 and 100.6 respectively when the control test solutions were spiked with the test compounds.

Concentrations of test compound in the samples taken at various time intervals were calculated by substitution corrected D_1 values measured at 272.9 nm in the regression equations. The flux (J) of each test compound permeated through pig skin was determined. The J values were plotted as a function of time as displayed in Figure 1. The comparison of J values between the test compounds at observed time interval were evaluated by analysis of variance, Duncan multiple range test at the significant level of α 0.05.

Determination of Apparent Partition Coefficients.

The apparent partition coefficients (P) and log P of the test compounds were determined in octanol-isotonic phosphate buffer pH 7.4 at 32°C. The octanol-buffer mixtures were shaken for 12 hours to reach a distribution equilibria. The mixtures were transferred to separatory funnels and stood to separate for at least two hours. The aqueous phase was separated and quantitated by spectrophotometry. Four determinations were performed for each test compound.

RESULTS AND DISCUSSION

The syntheses of organic salts of lidocaine were accomplished in this study. They were lidocaine adipate (I-A), lidocaine maleate (I-B), lidocaine malonate (I-C) and lidocaine tosylate (I-D). The determination of skin permeability, approximate solubility, apparent partition coefficients were therefore performed to evaluate the assumption of the enhanced skin permeability of these compounds by ion-pair transportantion.

Synthesis

Four organic salts of lidocaine (I) were prepared by the reaction of lidocaine and organic acids namely adipic acid, maleic acid,

malonic acid and p-toluenesulfonic acid, respectively. Physical properties of the prepared salts were listed in Table 1. As salts, the products obtained have higher melting points than lidocaine and lidocaine hydrochloride.

The ^1H NMR spectra of the products showed characteristic proton peaks of lidocaine and organic acid in the molecule (Table 2). NMR spectrum of lidocaine adipate (I-A) showed peaks of and CH_2 at 1.62 and 2.20 and peak of dicarboxylic proton of adipic acid at 10.40 ppm, exchangeable with D_2O. For lidocaine maleate (I-B), peaks of olefinic proton (=C-H) appeared at 6.29 ppm. Only one proton of carboxylic of maleic acid was found as very broad peak at 13.10 ppm, exchangeable with D_2O. Dicarboxylic protons of malonic acid in lidocaine malonate (I-C) showed D_2O exchange peak at 11.87 ppm. A broad peak, exchangeable with D_2O at 9.47 ppm of lidocaine tosylate (I-D) indicated quarternary ammonium proton (N^+-H) in the compound which was due to strong acidity (low pK_a) of p-toluenesulfonic acid. Acidic proton of p-toluenesulfonic acid was more capable to donate and attach to N-amine of lidocaine. The NMR spectrum of I-D confirmed that this salt was more stable in form of ion-pair. The chemical shifts of protons on C-atoms attached to both sides of N-atom of amine concluded that strength of N^+H bond between lidocaine and salt of I-D was the strongest. The chemical shifts of N-CH_2-CH_3 and CO-CH_2-N of I-B (3.33 and 4.25 ppm) and I-C (3.19 and 4.14 ppm) were in downfield region when compared with 2.65 ppm for N-$CH_2CH_3$and 3.22 ppm for CO-CH_2-N of lidocaine. These resulted from quarternary ammonium proton as existing in the molecule in the same pattern as I-D. Peaks of dicarboxylic protons of I-A, I-B and I-C were found to vary from 10.40 ppm of I-A to 11.87 ppm of I-C and to 13.10 ppm for I-B. This was due to variation in pK_a of the acids. The lower in pK_a of the acid indicated that the acid was able to donate proton more easily, such as maleic acid when compared with malonic acid and adipic acid. Thus, the acidic proton of I-B and I-D had more dishielding effect on N-amine and proton of C-atom attaching to N-atom than acidic protons of I-A and I-C. Hence, the organic acid which formed salt with lidocaine had effect on the chemical shift of protons of N-CH_2-CH_3 and CO-CH_2-N of lidocaine. The values of chemical shift related to the pK_a value of the acid part, the lower in pK_a of acid, the more deshielding effect on the protons.

TABLE 1
Physical properties of lidocaine and its salts.

Test Compoun d[a]	Molecular Formular	Recrystallization Solvent	m.p. °C	%yield
I	$C_{14}H_{22}N_2O$	n-Hexane	68-69	96
I-A	$C_{20}H_{32}N_2O_5$	EtoAc	119-120	80
I-B	$C_{18}H_{26}N_2O_5$	EtoAc	93-94	80
I-C	$C_{17}H_{26}N_2O_5$	EtoAc	136-137	72
I-D	$C_{21}H_{30}N_2O_4S$	EtoAc	149-150	67

[a]I = lidocaine, I- HCl = lidocaine hydrochloride,
I-A = lidocaine adipate, I-B = lidocaine maleate,
I-C = lidocaine malonate, I-D = lidocaine tosylate.

TABLE 2
Characteristic ^1H NMR data of lidocaine and its salts.

Test Compound[a]	Solvent	Chemical Shift of Proton(ppm)							
		CH_2CH_3	ph-CH_3	N-CH_2CH_3	CO-CH_2-N	ar-H	N^+H	CO-NH	COOH
I	$CDCl_3$	1.14	2.23	2.65	3.22	7.08		8.93	
I. HCl	$CDCl_3$	1.42	2.21	2.60	3.22	7.02		10.24	
I-A	$DMSOd_{6+}$ $CDCl_3$	1.15	2.20	2.65	3.20	7.06		8.99	10.40
I-B	$CDCl_3$	1.33	2.18	3.33	4.25	7.04		9.89	13.10
I-C	$CDCl_3$	1.26	2.16	3.19	4..14	7.03		9.84	11.87
		1.28	2.09	3.34	4.36	6.95	9.47	9.83	
						7.05			
						7.64			

[a]I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate,
IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate

The infrared absorption spectra of the synthesized products showed N^+-H stretching at about 2850-2500 cm^{-1}which confirmed the formation of quarternary ammonium salt.

Approximate Solubilities

Approximate solubilities of the synthesized compounds (Table 3) showed that they were easily dissolved in water but very slightly

TABLE 3
Approximate solubility of lidocaine and its salts

Test Compound[a]	Approximate Solubility			
	Water	Ethanol	Chloroform	Ether
I	slightly soluble	very solube	very soluble	very soluble
I. HCl	freely	sparingly	slightly soluble	insoluble
IV-A	freely soluble	sparingly soluble	slightly soluble	very slightly soluble
IV-B	very soluble	very soluble	soluble	insoluble
IV-C	very soluble	freely soluble	soluble	very slightly soluble
IV-D	freely soluble	freely soluble	soluble	insoluble

[a]I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate

soluble or insoluble in ether. The solubility property was more similar to lidocaine hydrochloride than lidocaine. However, there were still more differences of solubilities among these four salts. The salts were classified into 2 subgroups by solubility differences. The subgroup of lidocaine maleate and lidocaine tosylate were insoluble in ether which resulted from the low dissociation constant (pK_a) of acidic part of these compounds (Table 5). Another subgroup of lidocaine adipate and lidocaine malonate, the increase in solubility of these compounds in non-polar solvent such as ether was found. It was due to lipophilic alkyl chain in acidic part of them.

Apparent Partition Coefficients

The results of apparent partition coefficient (P) and log P in Table 4 indicated that the more lipophilicity of the molecules together with the higher value of pK_a of I-A and I-C made them partitioned into the oil phase (octanol) better than I-B and I-D.

Skin Permeation Studies

In vitro skin permeation study was performed by using a system consisting of a permeation cell and pig skin. The four synthesized compounds, lidocaine and lidocaine hydrochloride were prepared as

TABLE 4
Apparent partition coefficients of lidocaine and its salts

Test Compound[a]	p[b]	log P
I	1918.90	3.28
I.HCl	64.85	1.81
I-A	52.63	1.80
I-B	25.96	1.41
I-C	58.85	1.77
I-D	1.28	0.11

[a]I = lidocaine, I.HCl = lidocaine hydrochloride,
I-A = lidocaine adipate, I-B = lidocaine maleate,
I-C = lidocaine malonate, I-D = lidocaine tosylate.
[b]Average of four determinations.

1% W/V solution in propylene glycol. The test solution was applied to donor cell at 48 hours after preapplication leach period. After application of test compound, the sample from receptor cell was taken and replaced by fresh buffer at the time 12, 24, 36 and 48 hours. The first derivative UV spectrophotometry was used to determine the amount of test compounds and was found to minimize the interferences from endogeneous substances from pig skin. Ultraviolet absorption spectrum showed that interferences from pig skin gave high value of absorbance in the wavelength range of analysis. Thus, the use of normal mode of ultraviolet spectrophotometry for analysis in this study was impossible according to large and unstable value of the interferences. Besides, the absorbance value of interferences was more than ten times the value of the test compound permeated through pig skin to receptor phase. D_1 spectrum of skin interferences showed low and stable value but the D_1 value of skin interferences was not zero. However, the percent recovery of the analysis by D_1 was acceptable, within 5% of the added amount. Percent recovery were improved to be less than 2% off the added value by using corrected D_1. The corrected D_1 was used in the analysis of permeated test compound in this study.

The permeation results in Figure 1 and Table 5 showed that at 12 hours after application, I-A and I-C were not significantly different

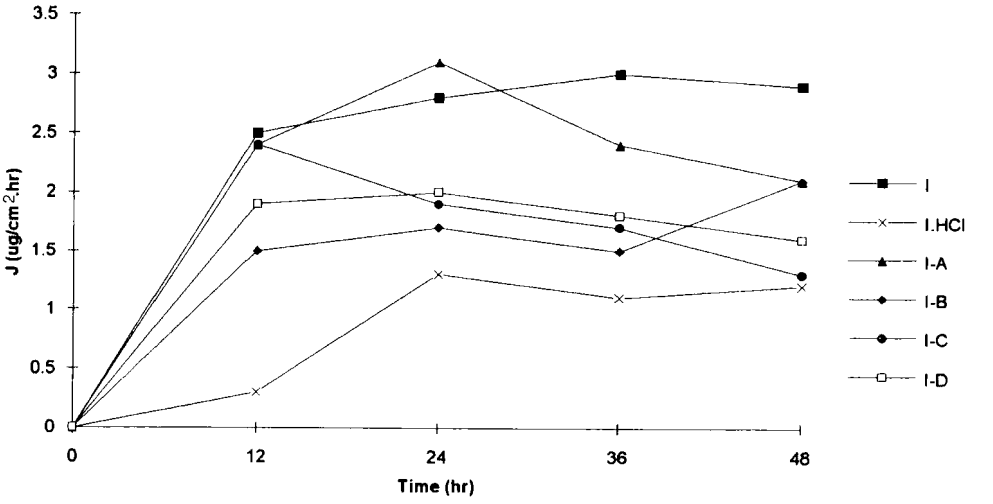
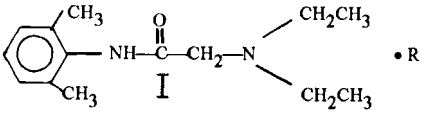


FIGURE 1
Flux (J) of Test Compounds versus Time.

TABLE 5
Permeation data of lidocaine and its salts



Test Compound[a]	R	Permeability(J)		log P	Aquous Solubility	^{1}H NMR(ppm)		pK_a of R
		12 hr.	24 hr.			N-CH_2-CH_3	CO-CH_2-N	
I	-	2.5	2.8	3.28	slightly soluble	2.65	3.22	7.7
I.HCl	HCl	0.3	1.3	1.81	freely soluble	2.60	3.22	-7.0
I-A	$(CH_2CH_2COOH)_2$	2.4[b]	3.1[b]	1.80	freely soluble	2.65	3.20	4.41, 4.43
I-B	$(CHCOOH)_2$	1.5	1.7	1.41	very soluble	3.33	4.25	1.83, 6.07
I-C	$CH_2(COOH)_2$	2.4[b]	1.9	1.77	very soluble	3.19	4.14	2.83 5.69
I-D	$CH_3C_6H_4SO_3H$	1.9	2.0	0.11	freely soluble	3.34	4.36	-1.34

[a]I = lidocaine, I.HCl = lidocaine hydrochloride, I-A = lidocaine adipate, I-B = lidocaine maleate, I-C = lidocaine malonate, I-D = lidocaine tosylate
[b]significantly different ($\alpha < 0.05$) when compared with I.HCl.

from I but they gave higher value of J than I-HCl, I-B and I-C (significantly different, $\alpha < 0.05$). After 24 hours application, J value of I were the highest whereas I-A and I-C lay midway in the range. I-HCl, I-B and I-D gave low J value. The permeability profile of synthesized organic salts differed from base by showing rapid transportation at 12 hours. The J of salts declined after 24 hours. The permeability profiles of the salts may be resulted from mechanism of transportation other than passive diffusion. The transportation of the salt may be in ion-pair fashion as proposed which resulted in the improvement of permeability at the first 12 hours after application. As organic salt prodrug, the permeability was enhanced from I-HCl. Nevertheless the permeability was still below I.

The results of permeation study were in agreement with apparent partition coefficient, lidocaine with the highest partition coefficient penetrated through pig skin better than other test compounds. When the length of alkyl chain of organic acid was considered in case of I-A and I-C, it was found that the longer alkyl chain, the higher lipophilicity of molecule and the higher skin permeation occured. The study also showed that lidocaine organic salts with lower values of P, I-B and I-D, gave satisfactory skin permeability. It was due to the low values of both pK_a and chemical shifts (ppm) of protons of N-CH_2-$CH_3$and CO-CH_2-N (Table 5). These values were related to the ability to form ion-pair. For this reason, more stable ion-pairs of I-B and I-D were formed and skin permeability was found to be enhanced, in spite of poor lipophilicity. It can be concluded that both lipophilicity and pK_a of acid part contribute the important roles in the transportation of lidocaine organic salts via ion-pair mechanism.

CONCLUSION

1. Four organic salts of lidocaine ; lidocaine adipate, lidocaine maleate, lidocaine malonate and lidocaine tosylate were synthesized. The structures were confirmed by ^1H, NMR, IR, mass spectroscopy and elemental analysis. Approximate solubility of the products was also determined. The synthesized compounds were regarded as prodrugs of lidocaine and were expected to enhance skin permeability by ion pairing.

2. *In vitro* skin permeation study of synthesized compounds were accomplished in comparison with lidocaine and lidocaine hydrochloride. Lidocaine adipate and lidocaine malonate gave satisfactory results. The skin permeabilities after 12 hours of application were of the same order as lidocaine, not significantly different at $\alpha < 0.05$. In addition, the permeabilities at this time interval were significantly higher than lidocaine hydrochloride. The skin permeabilities at 24 hours after application of all synthesized compounds were lower than lidocaine, the permeability of lidocaine adipate was still greater than lidocaine hydrochloride and other salts. For lidocaine maleate and lidocaine tosylate, the skin permeation were not significantly different from lidocaine hydrochloride at any observed time intervals. The synthesized compounds also showed different profile of permeation. The transportation was rapid at the beginning and declined after 24 hours of application.

3. Apparent partition coefficients (P) of the synthesized compounds were determined and compared with P of lidocaine and lidocaine hydrochloride. The rank order of the compounds ranged from the highest value of P to lowest was lidocaine, lidocaine hydrochloride, lidocaine adipate, lidocaine malonate, lidocaine maleate and lidocaine tosylate. The results indicated that the increase in permeability of proposed prodrugs from lidocaine hydrochloride may relate to the chemical structrue and the apparent partition coefficient of the synthesized salts contributing ion-pair of transportation.

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