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Lidocaine Hydrochloride Absorption from a Subcutaneous Site

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Abstract □ Subcutaneous disappearance of lidocaine hydrochloride was followed as a function of time using a specially designed "closed" subcutaneous absorption cell affixed to anesthetized rats. Unbuffered, stirred lidocaine hydrochloride solutions in cells open to the atmosphere were previously shown to increase in pH with time because of carbon dioxide loss. The closed cell was designed to prevent this loss, but pH shifts still occurred, making the derivation of a simple pharmacokinetic absorption model impossible. Because the pH of the solution shifted to higher pH values, the data suggest that precipitation of lidocaine base may have occurred in some experiments.

Keyphrases □ Lidocaine hydrochloride—absorption from subcutaneous site, design of closed subcutaneous absorption cell □ Absorption—lidocaine hydrochloride from subcutaneous site, design of closed subcutaneous absorption cell, effect of pH changes □ Parenteral dosage forms—lidocaine hydrochloride absorption from subcutaneous site, design of closed subcutaneous absorption cell

Since the publication of Schou's (1) review of drug absorption from subcutaneous connective tissue, there has been an increasing interest in quantitatively measuring the absorption rates of drugs in aqueous solution from the subcutaneous site (2–8). Quantitative measurements of drug absorption rates should result in a better understanding of which of the many possible pharmacokinetic models are appropriate for describing subcutaneous drug absorption of various drugs and of what mechanisms are involved in subcutaneous drug absorption. To date, there has not been a study where different commercial products containing the same drug have been compared in their absorption behavior from the subcutaneous region under conditions where the drug was sampled periodically at the subcutaneous absorption site, where the solution was continuously stirred, and where the surface area for absorption was held constant.

The purposes of this report are to develop and discuss the strengths and weaknesses of experimental methods that might be useful in comparing the subcutaneous absorption behavior of commercially prepared parenteral dosage forms of lidocaine hydrochloride. This drug might be considered a model compound for this purpose, because many parenteral drugs are water-soluble salts of weak organic bases and the unionized base often has limited water solubility.

EXPERIMENTAL

Animals—Female Sprague–Dawley rats were used. The anesthesia and the method used for surgically exposing the subcutaneous tissue were described previously (3).

Reagents—Lidocaine hydrochloride was obtained from two manufacturers[1,2]. According to the label claims, each preparation contained (per milliliter) 10 mg of lidocaine hydrochloride, 7 mg of sodium chloride, 1 mg of methylparaben, and sodium hydroxide to adjust the pH. Both preparations conformed to the standards for lidocaine hydrochloride injection USP without epinephrine (9). Cyclizine hydrochloride[3] (10, 11) was used as an internal standard for the GC analysis at a concentration equivalent to 243 mg of cyclizine base/liter in 0.1 *N* HCl. The *n*-hexane used as the extraction solvent in the procedure was of spectrographic grade[4]. All water was double-distilled from dissolved potassium permanganate. All glassware except the microsyringe[5] was initially cleaned in concentrated nitric acid.

Subcutaneous Absorption Cell—The design of the absorption cell used in this study differed somewhat from that used in previous work (3). The glass cell was nearly hemispheric in shape, where the maximum distance from one inside point of the open end to another inside point was 19 mm and the distance between the top inside point and the plane across the open end of the cell was 10 mm. Two holes were made in the top region of the cell. One hole, used for the stirrer, was at the top center of the cell when the cell was placed flat on a horizontal surface. A glass tube was fused to the glass surrounding this hole, and the tube extended 3 mm up

[1] Lot L 107770, Astra Pharmaceutical Products, Worcester, Mass.
[2] Lot 2016923C, Invenex Pharmaceuticals, Grand Island, N.Y.
[3] Marezine, Burroughs Wellcome & Co., Research Triangle Park, N.C.
[4] Spectroquality reagent, Matheson, Coleman and Bell, East Rutherford, N.J.
[5] Hamilton Co., Whittier, Calif.

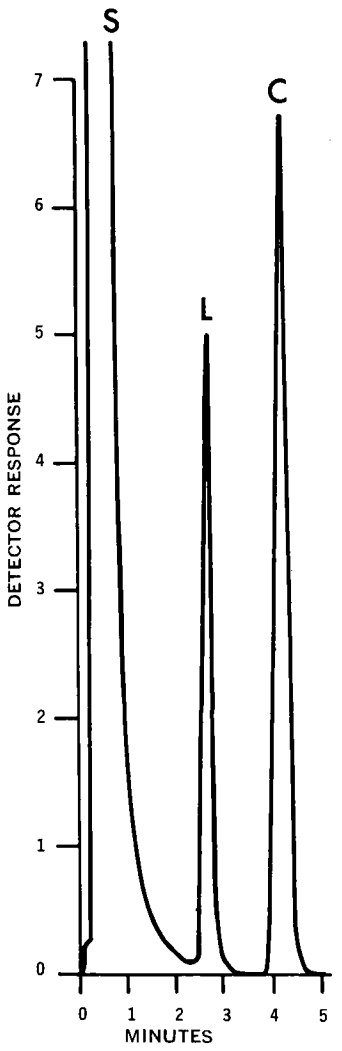

Figure 1—*Typical gas chromatogram of lidocaine (L) and cyclizine (C) internal standard in* n*-hexane solvent (S). The retention time of lidocaine is 2.7 min, and that for cyclizine is 4.2 min.*

from the top outside of the cell. The glass tube had an inside diameter large enough so that a polyethylene tube[6] could just pass through it snugly.

The second hole was used for sampling the cell's contents. The center of the second hole was about 7 mm from the center of the first hole and its inside diameter was 4 mm. It was ground flat so that it could be closed between sampling times by pressing a small piece of paraffin film[7] over it. The glass absorption cell was affixed to the moist subcutaneous tissue with a medical silicone adhesive, which was repackaged into an ophthalmic ointment tube as described previously (3).

The stirring of the cell's contents was accomplished by placing a 2-cm piece of polyethylene 100 tubing into the first hole so that one end was flush with the top inside region of the cell and the remaining portion passed through the glass tube and extended beyond it. The polyethylene 100 tube was affixed to the cell with a medical silicone adhesive in such a way that no hole existed between the glass and the polyethylene 100 tubing so as to prevent possible gas exchange between the inside and outside of the cell. A 30-cm piece of polyethylene 10 tubing was passed partially through the polyethylene 100 tubing. The end of the polyethylene 10 tubing nearest the inside of the absorption cell was softened by carefully heating it and was pressed flat with a pair of pliers to form a short paddle. The second end of the polyethylene 10 tubing was passed through 21 cm of glass tubing having an outside diameter of about 7 mm and then was placed into a chuck attached to a variable-speed motor[8].

After the absorption cell was attached to the subcutaneous tissue, the glass tubing was aligned and clamped in a straight line between the top of the absorption cell and the chuck. The glass tubing tended to prevent the polyethylene 10 tubing from kinking while it was being rotated. The amount of polyethylene 10 tubing remaining outside the cell was adjusted so that when the chuck attached to the variable-speed motor was rotated at about 14–16 rpm, the paddle inside the absorption cell would be free to rotate and not touch either the tissue or the cell. After the appropriate distances were established, a small amount of silicone stopcock grease[9] was placed at the region where the polyethylene 10 tubing was in contact with the external end of the polyethylene 100 tubing. In addition to its obvious lubrication function, the grease was used to maintain a nearly airtight seal between the two polyethylene tubes. Care was taken to keep the grease from entering the absorption cell.

GC—A gas chromatograph[10] equipped with dual flame-ionization detectors and a recorder[11] were employed. The chromatographic column consisted of glass tubing, 1.83 m long × 0.318 cm i.d., and a silicone stationary phase[12] on a 100–120-mesh solid support[13]. Conditioning was carried out by injecting a silylation reagent[14] and by maintaining the column at 250° for 24 hr with low nitrogen flow.

Operating temperatures for the lidocaine analysis were: column, 230°; injection port, 250°; and detector, 250°. The carrier gas was nitrogen at a flow rate of 30 ml/min. The hydrogen gas flow rate was 30 ml/min, and the air flow rate was 300 ml/min. The recorder speed was 50.8 cm/hr. An integrator[15] measured the area under the detector response *versus* time curve on recorder paper.

Assay—A 1.2-g sample of 1% lidocaine hydrochloride was transferred from the commercial 20-ml[1] or 30-ml[2] multiple-dose vial to the absorption cell through the second or side hole by means of a 2-ml syringe. The filled syringe was weighed on an analytical balance prior to adding the solution to the cell and then was reweighed just after the addition. Individual values for the weights added are listed in Table I. The mean weight added was 1.225 (±0.020) g, where the value in parentheses represents ±1 *SD* about the mean weight added to all cells.

Immediately after the 1.2-g sample of solution was added, a small piece of paraffin film was placed over the hole to prevent gas exchange. The 20-μl zero time sample was taken directly from the contents of the multiple-dose vial using a 25-μl syringe[5]. At other sampling times, 20-μl samples were withdrawn from the absorption cell. The accuracy of the sampling technique was checked. The syringe containing the aliquot was weighed on an analytical balance and reweighed after the sample was transferred to a 5-ml glass-stoppered tube[16]. In all cases the difference between the two weights corresponding to the volume of sample delivered (1 μl = 1 mg) agreed with that estimated from the syringe barrel markings within ±1%. One milliliter of the stock solution of cyclizine hydrochloride (internal standard) was added to rinse the drug sample down from the sides of the flask. The samples were then frozen and stored for future assay.

At the time of assay, the samples were allowed to reach ambient temperature and then 4 ml of *n*-hexane and 0.5 ml of 5 *N* NaOH were added to each. The solutions were shaken mechanically for 1 hr. About 2 ml of the *n*-hexane layer was decanted into another test tube. About 10 mg of anhydrous sodium sulfate was added to the *n*-hexane solution, which was allowed to stand for several hours to remove trace amounts of water from the organic solvent. A 1.2-μl sample was removed from each tube, using a 5-μl microsyringe[5], and injected into the gas chromatograph. Ten standard solutions of lidocaine hydrochloride were prepared, starting at 0.1% and increasing at 0.1% concentration increments up to 1.0%. Twenty-microliter samples were removed from the standard solutions and treated according to the procedures previously described.

[6] PE 100, Clay-Adams, Inc., New York, N.Y.
[7] Parafilm "M," American Can Co., Neenah, Wis.
[8] Electronic controller GT 21 laboratory mixer, Gerald K. Heller Co., Las Vegas, Nev.

[9] Stopcock grease, Dow Corning Corp., Midland, Mich.
[10] Varian Aerograph model 2100, Varian Instrument Division, Palo Alto, Calif.
[11] Varian model 20 recorder, Varian Instrument Division, Palo Alto, Calif.
[12] OV-17, Varian Instrument Division, Palo Alto, Calif.
[13] Gas Chrom Q, Applied Science Laboratories, State College, Pa.
[14] Silyl-8, Varian Instrument Division, Palo Alto, Calif.
[15] Disc Instruments, Santa Ana, Calif.
[16] Catalog Number 20908-040, Van Waters and Rogers, Brisbane, Calif.

Table I—Percent Unabsorbed Lidocaine Hydrochloride from Subcutaneous Absorption Cell as a Function of Time

Sampling Time, hr	Animal[a] (g)						Animal[b] (g)					
	A[c] (279)	B[c] (254)	C[c] (256)	D[c] (261)	E[c] (258)	F[c] (269)	G[c] (208)	H[c] (218)	I[c] (210)	J[c] (206)	K[c] (214)	L[c] (296)
0.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
0.5	59.0	98.1	87.9	80.5	92.0	74.0	63.4	77.0	68.2	91.6	95.4	95.6
1.0	56.4	87.1	75.0	90.0	77.4	63.3	68.4	72.0	54.1	77.6	70.0	58.0
1.5	38.0	97.1	70.5	77.1	54.6	50.7	50.7	53.0	45.7	61.0	52.4	55.7
2.0	23.8	66.7	57.8	37.8	47.4	37.2	37.6	31.2	41.6	46.0	38.0	29.4
2.5	15.6	63.0	44.8	24.2	36.0	33.1	36.6	23.1	37.8	27.7	24.8	24.3
3.0	11.8	44.5	39.8	14.2	24.7	22.1	34.5	19.2	34.5	15.4	15.2	11.4
3.5	4.7	35.2	24.4	9.3	13.3	14.5	23.4	11.0	26.1	2.9	13.6	5.8
4.0	3.7	31.8	23.2	1.1	15.9	12.4	13.6	7.4	17.7		10.5	2.5

[a] Lot 2016923C, Invenex. Initial cell pH for Animals A–F was 6.18. Final cell pH's for Animals A–F were 8.49, 7.89, 7.69, 7.99, 7.69, and 7.72, respectively. [b] Lot L 107770, Astra. Initial cell pH for Animals G–L was 6.15. Final cell pH's for Animals G–L were 7.40, 7.48, 7.48, 8.15, 7.92, and 7.84, respectively. [c] Solution weights in grams for Animals A–L were 1.218, 1.179, 1.222, 1.221, 1.227, 1.219, 1.249, 1.231, 1.221, 1.230, 1.220, and 1.261, respectively.

After GC analysis, the areas under the lidocaine curve, A_L, and under the cyclizine curve, A_C, were measured for each injected sample. A plot of the ratio, A_L/A_C, *versus* drug concentration was made. The standard curve was linear over the 0.1–1.0% lidocaine hydrochloride concentration range. Figure 1 shows a detector response *versus* time curve for the *n*-hexane extract of the sample taken from Animal E at the 0.5-hr time point. A pH meter[17] equipped with a miniature glass and reference electrode[18] system was used to determine the initial and final pH values of the solution in the absorption cell.

RESULTS AND DISCUSSION

Several points should be noted about the results shown in Table I. First, the pH of the solution measured before the drug solution was placed in the subcutaneous absorption cell was lower than the pH of the cell's contents measured after the experiment was concluded. Second, there was generally a decline in the percent of drug remaining in the subcutaneous cell as time increased; however, in several instances the percent of drug at a particular time point appeared to *increase* over that found at earlier time points. This apparent increase was then followed by another drop in the percent of drug remaining at later times. Third, if one plots the logarithm of the percent remaining in the subcutaneous absorption cell *versus* the time at which the samples were taken, there does not seem to be any convenient way to analyze the resulting curve in terms of either apparent mono- or biexponential loss of drug from the subcutaneous absorption cell.

It is not too surprising that the pH of the solution initially slightly greater than pH 6 should shift to higher pH values as the experiment progressed. According to the label statements, the commercial solutions have no added buffers. The pH of rat arterial blood (12) is 7.35 (range 7.26–7.44), and this system is known to be buffered. Thus, a small volume of a nonbuffered solution in the subcutaneous absorption cell adjacent to a larger volume of a buffered rat blood should eventually reach a pH value closely approximating that of the buffered system.

However, in a subcutaneous absorption cell open to the atmosphere, the pH often rose well above the expected maximum value of about 7.4. Levy and Rowland (8) noted that the pH of a nonbuffered isotonic saline solution in an open subcutaneous absorption cell rose from an initial value of 5.75 to about 8.2, lasting from 3 to 7 hr later. A major reason why the pH of the system open to the atmosphere rose substantially above pH 7.4 was that bicarbonate ion migrated into the subcutaneous absorption cell. Appropriate shifts in cations, such as K^+ and Na^+, and other anions, such as Cl^-, also occurred and electroneutrality in the subcutaneous cell was maintained. The loss of carbon dioxide from the bicarbonate-ion solution and the concomitant increase in the carbonate-ion concentration resulted in a cell pH greater than 7.4.

The nearly closed hemispherical subcutaneous absorption cell used in this work was designed to minimize gas exchange between the cell and the atmosphere. It can be seen from the data presented in Table I that there was considerable variation in the final pH of the solutions found in various subcutaneous cells. The exact cause for this pH variation is not known, but it is probably related to how freely gases could exchange between the cell and the atmosphere. With regard to the first point, therefore, it appears that considerably more work is needed to design an absorption cell that will allow the solution contained in it to be stirred, that will allow samples of solution to be removed periodically for analysis, and that will not allow any gases likely to influence the pH of the subcutaneous cell's contents away from the physiological range to be exchanged between the cell and the atmosphere.

The second point mentioned was that there was generally a decline in the percent lidocaine hydrochloride remaining in the subcutaneous absorption cell with respect to time (Table I). However, in some cases the apparent percent remaining at one time point *exceeded* that for a previous time point. For Animals B, D, and G, this phenomenon was observed somewhere between the 0.5- and 1.5-hr time points. The possibility that gross analytical or procedural errors had occurred was investigated, but no evidence of such errors could be found.

The most reasonable explanation for this phenomenon was that the shift in subcutaneous cell pH to increasing values caused some lidocaine base to precipitate. Since the contents of the cell were continuously stirred, fine crystals would have the tendency to stay suspended for some time. If a 20-μl sample removed from the subcutaneous cell contained crystals of lidocaine base, then that sample would give a higher reading for lidocaine content than a similar sample not containing suspended crystals. The pH at which the base just begins to precipitate, pH_p, is defined by Eq. 1, which is appropriate to physiological pH ranges (13, 14):

$$pH_p = pKa + \log_{10} \frac{[B]_{sol}}{[A^0] - [B]_{sol}} \qquad \text{(Eq. 1)}$$

where pKa is the negative logarithm of the ionization constant K_a for the protonated species of the base, $[B]_{sol}$ is the molar aqueous solubility of lidocaine base, and $[A^0]$ is the molar aqueous concentration of the protonated species in solution. At 24°, Levy (7) found that $[B]_{sol}$ was 9.184×10^{-4} *M* and that the pKa was 7.86. If these values are substituted into Eq. 1 and the values for $[A^0]$ are systematically varied, then the information contained in Table II can be obtained.

In Table II, one notes that, to avoid possible precipitation of lidocaine base, the pH of a 1% lidocaine hydrochloride solution must be pH 6.268 or less, which is approximately the pH of commercial 1% lidocaine hydrochloride products prior to their injection. If the pH of the system is increased to pH 7.4, for example, then the lidocaine hydrochloride concentration in the solution would have to be reduced according to Eq. 1 to about 0.097% to prevent the formation of a precipitate. Thus, if the pH in the subcutaneous absorption cell increased rapidly to about pH 7.4 during the experiment, then conditions were favorable for drug precipitation at the absorption site. Drug precipitation might not occur if supersaturation occurred or if drug interactions occurred with the proteins present in the biological fluids in the subcutaneous absorption cell (15), which might reduce the drug concentration below the point at which precipitation would occur.

The third point was that, by using the data shown in Table I,

[17] No. 7664, Leeds and Northrup, North Wales, Pa.
[18] No. 124138, Leeds and Northrup, North Wales, Pa.

Table II—Calculated pH values from Eq. 1 at which Precipitation of Lidocaine Base Will Just Begin to Occur from Various Concentrations of Lidocaine Hydrochloride at 24°

pH_p	Percent (w/v)	Molar
6.246	1.05	38.77×10^{-3}
6.268	1.00	36.93
6.367	0.80	29.54
6.497	0.60	22.16
6.683	0.40	14.77
7.013	0.20	7.39
7.399	0.097	3.58
7.515	0.08	2.95
7.711	0.06	2.22
8.077	0.04	1.48

plotting the logarithm of the percent of lidocaine present in the subcutaneous absorption cell *versus* sampling time resulted in plots that could not be resolved conveniently into a pharmacokinetic model that could be described by mono- or biexponential loss of drug from the cell. In contrast, Levy (7) found that the disappearance of lidocaine hydrochloride from an open subcutaneous cell *versus* time could be described by an apparent biexponential equation. The initial drug concentration used was 7×10^{-4} *M* and the aqueous isotonic system was buffered at pH 7.95, so that drug precipitation at the absorption site would not occur. The disadvantage of adding a buffer is that the commercial solution under study is not precisely the same as that formulated by the manufacturer. Also, the added buffer may cause initial precipitation of the free base.

When the subcutaneous absorption cell technique has been used (3, 7), absorption rate differences among animals have been attributed in part to surgical techniques resulting in differences in the thickness of the subcutaneous membrane through which the drug must pass and in part to microcirculation changes in the subcutaneous region as the experiment progresses, resulting from such factors as trauma, inflammation, anesthesia, and drug effects. These and other factors represent a limitation to the method when the results of this experimental design are related to actual subcutaneous drug administration, where most of these factors are absent or greatly reduced.

An "internal standard" method has been used (7); in a given experiment, it is the ratio of the half-life of the rate-limiting step for drug absorption divided by the half-life of the rate-limiting step for the internal standard. This ratio should be reasonably constant from experiment to experiment. A reason for not using an internal standard method in this study is that the solution under consideration would not be precisely the same as that formulated by the manufacturer. Also, it must be shown that neither the drug nor the internal standard will influence the absorption of the other.

In conclusion, the use of the subcutaneous absorption cell technique in evaluating drug absorption is most useful in studying salts of poorly soluble organic weak bases, such as lidocaine hydrochloride, when the drug base will not precipitate over the pH range studied and where some control of cell pH is maintained. The technique yields less satisfactory results even in a nearly closed cell when the solution administered has no added buffers and when the drug salt concentration is sufficiently high so that precipitation of uncharged drug is likely to occur if the pH shifts from that in the original solution to a more alkaline pH value.

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