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Influence of pH on lidocaine penetration through human and hairless mouse skin in vitro

Gregory P. Kushla and Joel L. Zatz

Department of Pharmaceutics, Rutgers College of Pharmacy, Piscataway, NJ 08855-0789 (U.S.A.)

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Summary

The permeation of radiolabeled lidocaine through full-thickness hairless mouse skin and dermatomed human epidermis was studied under infinite-dose conditions using a flow-through diffusion apparatus. The donors consisted of 5% suspensions in 40% propylene glycol gelled with 1% hydroxypropyl cellulose and preserved with 0.25% chlorobutanol. The pH was adjusted to several values and the solubility in each donor was measured. Steady-state flux values were subjected to mathematical treatment to estimate the permeability coefficients (P) of the ionized and unionized forms of lidocaine. The permeability coefficient for unionized lidocaine through hairless mouse skin was no more than 15 times greater than for the ionized form; a 50-fold difference was obtained with human epidermis. The permeability differences indicate that hairless mouse skin is not an adequate in vitro substitute for human skin when considering lidocaine penetration.

Introduction

Weak acids and bases are subject to pH-dependent ionization. Small changes in pH may greatly affect the relative amounts of ionized and unionized species in solution depending on the pK_a of the compound. Charged species are presumed to penetrate the skin poorly, if at all, compared to nonelectrolytes (Scheuplein and Blank, 1971), but it has become accepted that the penetration of the ionic form of some weak acids and bases may contribute a significant portion of the total flux of the compound (Michaels et al., 1975; Wallace and Barrett, 1978; Fleeker et al., 1989). In other cases, while the permeability of the ionic species is measurable, the contribution of the ion to the total flux is so small as to be negligible (Michaels et al., 1975; Swarbrick et al., 1984).

The permeability coefficients of the ionized and unionized forms of the same compound may differ greatly. Altering the pH of the donor vehicle may have a profound effect on the observed penetration characteristics as the relative amounts of the two species change. Exploration of the penetration

Correspondence (present address): G.P. Kushla, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., 170 Tabor Road, Morris Plains, NJ 07950, U.S.A.

properties of a compound is not complete until consideration is given to the effect of ionization.

The present study serves to compare the permeability coefficients of both ionized and unionized lidocaine from a saturated propylene glycol-water mixture through both hairless mouse skin and human epidermis, calculated via a linear regression approach.

Materials and Methods

Hairless mouse skin

Female hairless mice, 8 weeks old, strain SkH : HR-1 (Skin and Cancer Hospital, Philadelphia, PA), were killed by cervical dislocation. Skin from the dorsal and ventral regions was removed, lightly trimmed of subcutaneous fat and connective tissue, and stored by wrapping in plastic wrap, overwrapping in aluminum foil, and holding in a freezer at $-14.5\,^{\circ}C$ until needed.

Human skin

Leg skin was obtained from an amputated limb within 24 h of separation from the body of a female Caucasian, age 70. Subcutaneous fat and muscle were removed and the skin dermatomed to approx. 150 μm (Padgett Electrodermatome, Kansas City, MO). The epidermis was stored in the same manner as mouse skin. Skin stored under these conditions showed no change in penetration characteristics compared to fresh skin (Kushla and Zatz, 1989).

Chemicals

[*carbonyl*-^{14}C]Lidocaine hydrochloride (New England Nuclear, Boston, MA) was mixed with cold lidocaine hydrochloride (Astra Pharmaceuticals, Worcester, MA), converted to the base form by adding an excess of sodium hydroxide (J.T. Baker, Phillipsburg, NJ), and diluted with cold lidocaine base (Sigma, St. Louis, MO) to a final activity of approx. 1 nCi/mg. All other chemicals were used as received.

Solubility

The solubility of lidocaine in 40.0% w/w propylene glycol was determined at several pH values: 4.0, 6.0, 7.8, 10.0, and 12.0. Excess [^{14}C]lidocaine base was added to aliquots of the vehicle and the pH adjusted to selected values with either 1 or 0.1 N sodium hydroxide in 40.0% w/w propylene glycol or 1 or 0.1 N hydrochloric acid (J.T. Baker, Phillipsburg, NJ) in 40.0% w/w propylene glycol. The samples were held in a water bath at $37\,^{\circ}C$ for 1 week to allow the lidocaine to reach equilibrium concentration. The pH of the samples was checked daily, adjusting with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid as necessary. The suspensions were filtered and the concentration of lidocaine determined by liquid scintillation counting.

pK_a determination

The pK_a of lidocaine in 40.0% w/w propylene glycol was determined titrimetrically according to the method of Albert and Serjeant (1962). Samples of base or hydrochloride salt were dissolved in 50.00 ml of 40.0% w/w propylene glycol prepared with CO_2-free water. The titrations were performed at $25\,^{\circ}C$. Standardized 0.1 N hydrochloric acid in 40.0% w/w propylene glycol or 0.1 N sodium hydroxide in 40.0% w/w propylene glycol was added incrementally from a 10.00 ml microburet. The pH was measured with a Beckman Zeromatic pH meter and glass electrode.

Formulations

[^{14}C]Lidocaine suspensions (5.0% w/w) were prepared in 40.0% w/w propylene glycol (J.T. Baker, Phillipsburg, NJ), gelled with 1.0% w/w hydroxypropyl cellulose (Klucel LF®, Hercules, Wilmington, DE), and preserved with 0.25% w/w chlorobutanol (Eastman Kodak, Rochester, NY). The final pH of the donors was adjusted as for the solubility samples.

Permeability experiments

Details of the experimental set-up have been published previously (Kushla and Zatz, 1989). Briefly, thawed hairless mouse skin or human epidermis was mounted in flow-through diffusion cells with an available surface area for diffusion of 0.64 cm^2. Normal saline preserved with 0.25% chlorobutanol, pumped through the receptor compartment at approx. 1.5 ml/h, was used as the

TABLE 1

Lidocaine solubility in 40% propylene glycol at different pH values

pH	Solubility (mg/ml)
4.0	437.3 ±86.8 [a]
6.0	406.5 ±62.0
7.8	33.71 ± 0.86
10.0	19.58 ± 1.04
12.0	8.71 ± 0.15

[a] Mean ± standard deviation ($n = 5$).

receptor fluid. [^{14}C]Lidocaine suspension (0.75 ml) was applied to the stratum corneum side of the skin and the donor chamber sealed. The effluent was collected directly into scintillation vials.

Analysis

Lidocaine analysis was performed by liquid scintillation counting as described previously (Kushla and Zatz, 1989).

Results and Discussion

Solubility

The solubility of lidocaine in 40.0% w/w propylene glycol at various pH values is given in Table 1. As expected, the solubility increased as the pH decreased. The solubility at pH 12.0, at which ionization is negligible, is considered the intrinsic solubility of the base form and is assumed to be the concentration of the base in solution when excess solid lidocaine is present and in equilibrium with the dissolved material. Fig. 1 illustrates the pH-solubility profile for ionized and unionized lidocaine calculated via the Henderson-Hasselbalch equation using the total and intrinsic solubility values, the measured pH, and the pK_a in 40% propylene glycol.

pK_a

The pK_a of lidocaine in 40% propylene glycol was estimated as 7.47 ± 0.06, a composite of results from the titration of the base itself ($pK_a = 7.40 \pm 0.02$) and the hydrochloride salt ($pK_a = 7.54 \pm 0.03$). The aqueous pK_a of lidocaine is reported as 7.86 (Truant and Takman, 1959). This correlates with reported depression of pK_a values of other bases in hydroalcoholic mixtures (Bates et al., 1963). Lidocaine is a weak monoprotic base, the ionization of which does not involve a hydroxyl group. Its pK_a is insensitive to ionic strength (Levy and Rowland, 1972). It is important to recognize that pH measurements in semiaqueous solvents reflect the activity of the hydrogen ion in that solvent. Such measurements are not directly comparable to aqueous pH values without knowledge of the delta value for that solvent, a quantity based on the medium effect, the change in solvation energy in transferring the hydrogen ion from water to another solvent (Bates et al., 1963). This quantity is unknown for 40% w/w propylene glycol.

Flux

The steady-state fluxes of lidocaine from these vehicles are given in Table 2. The flux through hairless mouse skin is much greater than through human epidermis at all pH values.

The solubility of lidocaine at pH 4.0 and 6.0 was much greater than 5%, the concentration of lidocaine used in the suspensions at pH 7.8 and pH 10.0. Flux normalized to saturation, J^*, de-

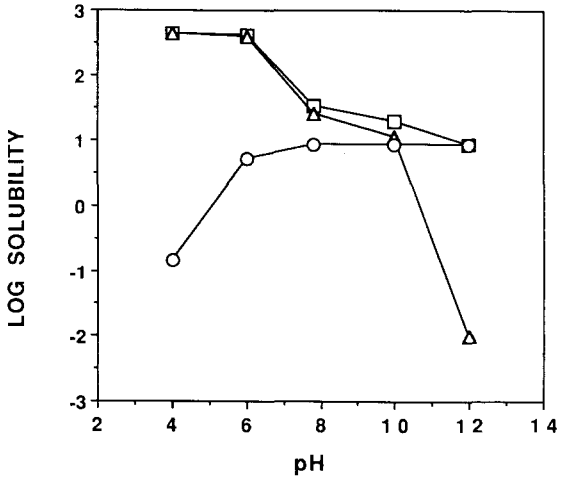

Fig. 1. The pH-solubility profile for lidocaine (□) in 40% propylene glycol. The contributions of the base (○) and ionized form (△) are calculated from the total solubility, the intrinsic solubility, and the pK_a in 40% w/w propylene glycol.

fined by Eqn 1, was calculated at these two pH values:

$$J^* = \frac{JC_s}{C} \quad (1)$$

where J is the experimental flux, C is the permeant concentration, and C_s is the solubility. J^* represents the expected flux of a saturated system; it is assumed that flux is a linear function of concentration in performing this calculation, and that dissolution of the dispersed drug is not rate limiting (Theeuwes et al., 1976). By using J^* values for the solutions as a basis for comparison, differences in solute thermodynamic activity are compensated. For suspensions, $J = J^*$. The values for J^* are also presented in Table 2.

Steady-state flux is a function of both the permeability coefficient and the drug concentration, or solubility in the case of suspensions. Ionization is expected to decrease the permeability coefficient while increasing the solubility. The net effect may be little change in the total flux.

The pH values selected for investigation are not expected to alter the barrier characteristics of the stratum corneum. Between pH 4 and 10, there appears to be no change in hydration, swelling, or permeability characteristics of hairless mouse skin (Matoltsy et al., 1968) or human skin (Barry, 1983). Thus, any changes in lidocaine flux result from changes in the relative amounts of the two species present in solution, not from any direct effect on the stratum corneum itself.

Propylene glycol is hygroscopic and known to be drying to the skin in high concentrations (Barry, 1983). In these formulations, the propylene glycol is present to increase the solubility of lidocaine above that in pure water (7.21 ± 0.10 mg/ml at pH 7.8). Under these experimental conditions, where the skin is bathed in receptor fluid and is hydrated extensively to nonphysiologic levels, the propylene glycol probably exerts no great influence on the barrier properties of the skin.

The steady-state fluxes through hairless mouse skin at pH 4.0 and 6.0 (15.2 and 12.3% of saturation, respectively) were comparable to fluxes from suspensions at pH 7.8 and 10.0. It appears that lidocaine flux through hairless mouse skin is largely independent of pH. Normalizing these fluxes to solubility values yields very high J^* values (Table 2) at pH 4.0 and 6.0, where ionization is 99.97 and 96.72%, respectively, using the pK_a in 40% propylene glycol of 7.47.

Human epidermis showed very low but measurable steady-state lidocaine flux from solutions at pH 4.0 and 6.0. Similar results were obtained by Siddiqui et al. (1985) using human stratum corneum and purely aqueous vehicles. Normalization increased these values (Table 2), but they are still lower than the fluxes at the higher pH values. The J^* values for human skin are almost independent of pH.

TABLE 2

Steady-state flux and lag time of lidocaine through hairless mouse skin and human epidermis from 40% propylene glycol as a function of pH

pH	Hairless mouse skin			Dermatomed human skin		
	Flux ($\mu g/cm^2$ per h)	J^* ($\mu g/cm^2$ per h)	Lag time (h)	Flux ($\mu g/cm^2$ per h)	J^* ($\mu g/cm^2$ per h)	Lag time (h)
4.0	589 [a]	3890 [a]	3.64 [a]	22.7 [a]	150 [a]	1.31 [a]
	(70)	(460)	(0.47)	(5.1)	(34)	(0.97)
6.0	878	7140	0.77	19.4	158	0.69
	(151)	(1230)	(0.90)	(0.4)	(4)	(0.72)
7.8	616	616	1.03	238	238	0.96
	(103)	(103)	(0.39)	(56)	(56)	(0.22)
10.0	511	511	0.74	208	208	0.89
	(106)	(106)	(0.36)	(29)	(29)	(0.05)

[a] Mean (standard deviation); $n = 3$.

The lag times for lidocaine penetration exhibit no clear pattern with respect to the pH of the donor vehicle, but the lag times through human and hairless mouse skin are much greater at pH 4. Ionized lidocaine may be binding to skin components; saturation of the binding sites may delay the establishment of steady-state (Michaels et al., 1975).

Calculation of the permeability coefficients

The mathematical treatment used to calculate the permeability coefficients of the ionized and unionized forms of lidocaine assumes the total flux of the permeant through the skin is the sum of the fluxes of the individual species, with each species permeating independently.

For lidocaine, a weak base, the total flux can be represented by:

$$J_{TOT} = J_L + J_{LH^+} \tag{2}$$

where J_L is the flux of unionized lidocaine and J_{LH^+} is that for the ionized form.

Through Fick's law, flux is given as:

$$J = PC \tag{3}$$

where P is the permeability coefficient and C is the concentration in the vehicle. The total flux, therefore, is:

$$J_{TOT} = P_L C_L + P_{LH^+} C_{LH^+} \tag{4}$$

Swarbrick and co-workers (1984) considered the case of a weak acid, combining the total flux equation with the equilibrium expression for the ionization of a weak acid to obtain an equation that allows estimation of the permeability coefficients of both species. Similarly, by considering the equilibrium expression for the ionization of a weak base:

$$K_b = \frac{C_{LH^+} C_{OH^-}}{C_L} \tag{5}$$

TABLE 3

Permeability coefficients for ionized and unionized lidocaine through hairless mouse skin and human epidermis

Species	P_L (cm/h)($\times 10^3$)	P_{LH^+} (cm/h)($\times 10^3$)	P_L/P_{LH^+}
Hairless mouse	133 ± 5 [a]	8.84 ± 0.31 [a]	15
Human	17.7 ± 0.5	0.336 ± 0.024	52

[a] Mean $\pm$ standard deviation.

together with the autoprotolysis expression for water, Eqns 4 and 5 can be combined to yield:

$$\frac{J_{TOT}}{C_L} = P_L + \frac{P_{LH^+} C_{H^+}}{K_a} \tag{6}$$

Plotting (J_{TOT}/C_L) vs C_{H^+} should give a straight line. Estimation of P_{LH^+} can be made from the slope and of P_L from the intercept. Use of this mathematical treatment demands extensive experimentation; linear regression requires data at several pH values.

Permeability coefficients

Eqn 6 was used to estimate the permeability coefficients of ionized and unionized lidocaine. The regression equation obtained for hairless mouse skin was $J_{TOT}/C_L = 1.33 \times 10^{-1} + 2.61 \times 10^5$ (C_{H^+}) $(r^2 = 0.9873)$; for human skin, $J_{TOT}/C_L = 1.77 \times 10^{-2} + 9.92 \times 10^3$ (C_{H^+}) $(r^2 = 0.9536)$. Permeability coefficient estimates are presented in Table 3.

The permeability coefficients for both lidocaine species through hairless mouse skin differ by approx. 15 times; those for human skin differ by a factor of 50. Comparing the individual lidocaine species permeabilities through mouse and human skin, it is interesting to note that the permeability coefficient for lidocaine base through hairless mouse skin is only about 7 times greater than through human epidermis, while that for ionized lidocaine is 24-fold greater. The large difference in flux, then, must be due to the contribution of the ionized form.

The relative values of the permeability coefficients of both lidocaine species are similar to

previously reported results for other ionigenic compounds. The ratio of permeability coefficients (unionized/ionized) of a series of chromone acids from aqueous solution through human skin was approx. 10^4 (Swarbrick et al., 1984); 10^2 for clonidine from aqueous solution through snakeskin (Fleeker et al., 1989); 4–20 for oxycodone from aqueous solutions through mouse, rat, and rabbit skin (Kuo et al., 1989); 20 for ephedrine and scopolamine and 275 for chlorpheniramine through human skin (Michaels et al., 1975). Working with the data of Inagi and co-workers (1981), the permeability coefficient of unionized indomethacin can be estimated as 10 times greater than for ionized indomethacin from 50% ethanol solutions through guinea pig skin.

The relatively high permeability of hairless mouse skin to ionized lidocaine may be due partly to the structure of its stratum corneum, which consists of 1–3 cell layers with a total thickness of 4–10 μm (Lee and Parlicharla, 1986). In contrast, human stratum corneum has 15–20 cell layers with a total thickness of 10–15 μm (Holbrook and Odlund, 1974). Hairless mouse stratum corneum is thus a more permeable barrier overall (Marzulli et al., 1969). An additional consideration is the resistance of the viable tissue layers (primarily dermis) of hairless mouse skin to lidocaine (Kushla and Zatz, 1989). The aqueous dermis is expected to be more of a barrier to unionized lidocaine than to the ionized form. Together these factors would serve to increase the passage of ionized lidocaine and retard the passage of unionized lidocaine through full-thickness hairless mouse skin, reflected in permeability coefficients of similar magnitude.

Conclusions

The ionized form of lidocaine penetrates both human and hairless mouse skin from 40% propylene glycol vehicles in vitro. The permeability coefficient of unionized lidocaine through hairless mouse skin is 15 times greater than the permeability coefficient of ionized lidocaine. For human epidermis, the permeability coefficient for unionized lidocaine is 50 times greater. This indicates that pH is less important in modifying the flux of lidocaine through hairless mouse skin than through human epidermis. Data from hairless mouse skin, therefore, can be misleading when studying the penetration of an ionigenic compound such as lidocaine. In this case, selection of an inappropriate animal model suggests that in vitro penetration at lower pH is adequate; however, under these conditions, activity in vivo with human subjects is likely to be poor.

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