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Variation of human skin permeation in vitro: Ionic vs neutral compounds

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Summary

The variation of skin permeation data has been investigated for ionic vs neutral permeants through human cadaver skin. In contrast to neutral ones, ionic permeants produced highly variable flux data with a positively skewed asymmetrical distribution. This permeant-dependent flux variation may suggest that different mechanisms are involved in the in vitro skin transport for ionic and neutral compounds.

Introduction

The outermost layer of skin, the stratum corneum, has been identified as the rate-limiting barrier to permeants. This layer, composed of dead, keratinized cells imbedded in a multilamellar lipid matrix, is heterogeneous and lipophilic in nature (Scheuplein and Blank, 1971; Scheuplein and Bronaugh, 1983). Depending on the physicochemical nature of the barrier and the permeant, the permeant molecule prefers to follow the path of least diffusional resistance (Flynn, 1989). It has been hypothesized in recent permeation and spectroscopic studies that the lipid bilayer and keratinized protein components of stratum corneum constitute the barrier function for lipophilic and polar permeants, respectively (Knutson et al., 1990).

Ionized permeants are of practical interest for transdermal delivery since many pharmaceutical compounds are ionized at physiologic pH. Scheuplein and Bronaugh (1983) have suggested that shunt diffusion through skin appendages may play a significant role for ionic permeants. Unfortunately, ionic permeants applied from aqueous solution do not readily penetrate the stratum corneum. Compared to neutral permeants, transdermal delivery of ionic compounds has not been commercially successful. Although several investigators have begun to examine this area (Wahlberg, 1968; Nishihata et al., 1989; Green et al., 1989; Kasting, 1990; Kurihara-Bergstrom et al., 1990; Padmanabhan et al., 1990; Kurihara-

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Bergstrom and Liu, 1991), the literature is insufficient to provide a base for understanding ionic permeability through skin. Recently, Kasting (1990) reported a large variation in the permeation of ionic triprolidine through human skin in vitro. Ionic hydromorphone also exhibited a large variation of pre-iontophoretic, passive flux through pig skin in vitro (Padmanabhan et al., 1990). While this variation is known, a description of the variation has not been reported and the cause of the variation remains unclear.

In the present study, in vitro permeation data through human cadaver skin were analyzed for two ionic compounds, terbutaline sulfate and sodium diclofenac. These were compared to three neutral permeants, estradiol, ethanol, and isopropanol.

Materials and Methods

Terbutaline sulfate (pK_a 8.7, 10.0, and 11.0; free base Mol. Wt 225; Sigma Chemical Co., St. Louis, MO), sodium diclofenac (pK_a 4.0; free acid Mol. Wt 296; Ciba-Geigy Pharmaceuticals, Summit, NJ), β-estradiol (Mol. Wt 272; Diosynth, The Netherlands), ethanol (Mol. Wt 46; Quantum Chemical Corp., Tuscola, IL), and isopropanol (Mol. Wt 60; Fisher Scientific, Fair Lawn, NJ) were used as received. Dermatomed human cadaver skin (about 400 μm thick) harvested from the dorsal region (Ohio Valley Tissue and Skin Center, Cincinnati, OH) was used for permeation experiments.

In vitro skin permeation studies were conducted at 32°C using either single-permeant or two-permeant methodologies. The latter involves the simultaneous determination of two permeants through the same human skin specimen. The single-permeant donor solutions included (a) saturated terbutaline sulfate in water (pH 3.8), (b) 0.048 M sodium diclofenac in water (pH 7.5), and (c) saturated estradiol in water, while the two-permeant donor solutions were (a) saturated terbutaline sulfate in 20% aqueous isopropanol and (b) saturated estradiol in 20% aqueous ethanol. Two-chamber glass diffusion cells were used with deionized distilled water containing 0.01% (w/v) gentamicin sulfate in the receiver chamber. The experimental procedures were similar to those reported previously (Kurihara-Bergstrom et al., 1990). Steady-state flux (μg cm^{-2} h^{-1}) through the skin was determined with an 'infinite dose' in the donor and sink conditions in the receiver for each permeant.

Terbutaline, diclofenac, and estradiol were assayed by HPLC, while ethanol and isopropanol were assayed by GC. Assay methods have been described for terbutaline (Kurihara-Bergstrom and Liu, 1991), estradiol (Liu et al., 1991), ethanol (Kurihara-Bergstrom et al., 1990), and isopropanol (Kurihara-Bergstrom and Liu, 1991). For diclofenac, the HPLC method utilized a Model 715 UltraWISP with Model 600E Fluid Handing Unit (Waters, Milford, MA), a Spectroflow 980 UV detector (Applied Biosystems, Bristol, CT) at 280 nm, a 4.6 mm i.d. $\times$ 15 cm NovaPak C-18 column (Waters, Milford, MA), and a mobile phase of 0.02 M aqueous sodium acetate/methanol/acetonitrile (55:25:20). The retention time for diclofenac was 2.7 min using a flow rate of 1.5 ml/min.

At steady state, the concentrations in the receiver chamber were precisely determined for all permeants except for the single-permeant (terbutaline) experiment where about 10% of the data points were below the detectable limit.

Results and Discussion

In vitro skin permeation results are summarized as histograms in Figs 1–3. The flux of terbutaline ranged from 0.02 to 22.9 μg cm^{-2} h^{-1} from aqueous donor solution (Fig. 1a). Similarly, another ionic compound, sodium diclofenac showed flux ranging from 0.6 to 144 μg cm^{-2} h^{-1} ($n = 21$). In contrast, the flux data for estradiol were not highly variable, ranging from 0.018 to 0.061 μg cm^{-2} h^{-1} from aqueous donor solution (Fig. 1b). The cotransported two-permeant experiments (Figs 2 and 3) further demonstrated the difference in the flux variations between ionic and neutral permeants. In the terbutaline/isopropanol experiment, the flux of terbutaline varied from 0.02 to 21.99 μg cm^{-2} h^{-1} while that

for isopropanol ranged from 400 to 850 $\mu g\ cm^{-2}\ h^{-1}$ (Fig. 2). In the estradiol/ethanol experiment, the fluxes were 0.11–0.30 and 372–750 $\mu g\ cm^{-2}\ h^{-1}$ for estradiol and ethanol, respectively (Fig. 3). The similar shape of data distributions for both estradiol (Fig. 1b vs Fig. 3a) and terbutaline (Fig. 1a vs Fig. 2a) was observed with water and 20% of the alcohols. Although multiple skin donors with approximately equal number of skin pieces for each donor were used to construct the histograms, the flux range was overlapped for different skin donors. For the flux data of neutral permeants such as isopropanol, the skin donor-to-donor variations were reasonably small with the overall coefficient of variation (C.V.) being

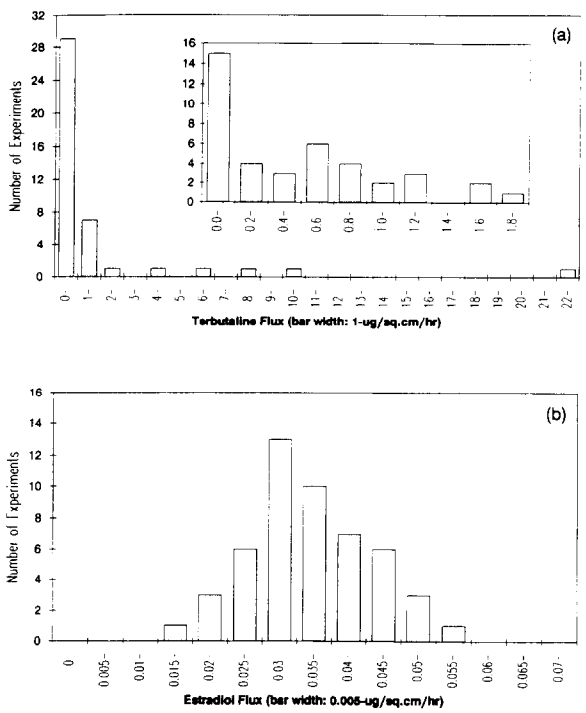

Fig. 1. Histogram of frequency distribution for the in vitro skin flux data for single-permeant experiments: (a) Terbutaline flux data ($n = 42$ experiments with different skin specimens from four skin donors) with saturated terbutaline sulfate in water (1.0 M, pH 3.8). Inset contains data between 0 and 2 $\mu g\ cm^{-2}\ h^{-1}$ (bar width: 0.2 $\mu g\ cm^{-2}\ h^{-1}$). Five data points were below the detection limits (0.015 $\mu g\ cm^{-2}\ h^{-1}$). (b) Estradiol flux data ($n = 49$ experiments with different skin specimens from four skin donors) with saturated estradiol in water (0.01 μM).

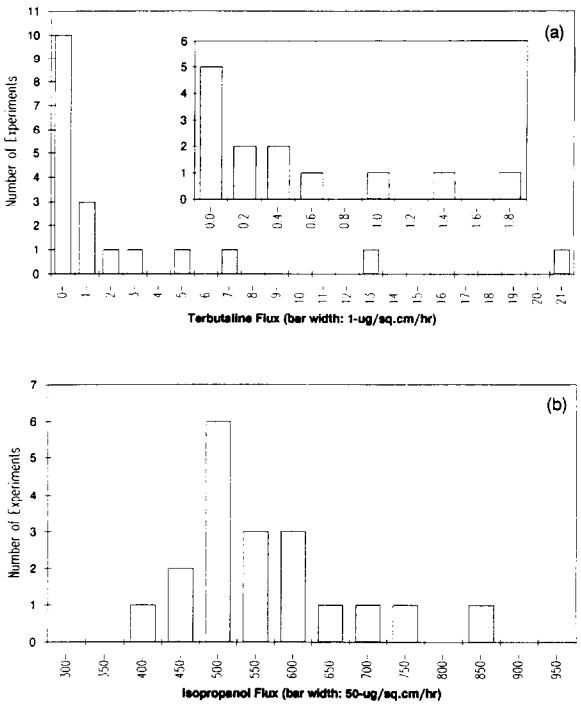

Fig. 2. Histogram of frequency distribution for the in vitro skin flux data for cotransported two-permeant (terbutaline/isopropanol) experiments ($n = 19$ experiments with different skin specimens from three skin donors) with 20% isopropanol aqueous solution saturated with terbutaline sulfate (0.87 M, pH 4.1): (a) terbutaline flux data. Inset contains data between 0 and 2 $\mu g\ cm^{-2}\ h^{-1}$ (bar width: 0.2 $\mu g\ cm^{-2}\ h^{-1}$). (b) Isopropanol flux data.

19% (see Table 1). However, the terbutaline flux data which was simultaneously determined showed large skin piece-to-piece variations, even for the same skin donor.

The general shape of the flux data distribution is also presented in the histograms (Figs 1–3). The distribution of terbutaline data was highly asymmetrical and positively skewed. The highest frequency (mode) in the flux data occurred in the lower class, around 0.02 $\mu g\ cm^{-2}\ h^{-1}$. The number of observations for each class then declined quickly toward zero. In contrast, the data distribution for ethanol and isopropanol were much more symmetrical and that for estradiol was even more so. Due to the presence of a relatively small number of high flux values, which might be considered outliers, the arithmetic mean with stan-

dard deviation is a poor description for the terbutaline data. For example, ~ 85% of experiments produced terbutaline fluxes below the arithmetic mean (1.87 μg cm^{-2} h^{-1}) and only ~ 15% above it (Fig. 1a). The arithmetic mean and its standard deviation are only appropriate for groups in which the data distribution is symmetrical or nearly symmetrical (Woolson, 1987; Bolton, 1990). The distribution profile and other parameters expressing central tendency, such as median, mode, or geometric mean should be used for asymmetrical data distributions (Woolson, 1987). The advantage of using these descriptive parameters is that they are unaffected or slightly affected by extreme values.

Table 1 compares these data descriptors with the arithmetic mean for the compounds studied. The values of all parameters are indistinguishable for estradiol, suggesting that the data is symmetrically distributed and that the arithmetic mean and its standard deviation accurately describe the data. For ethanol and isopropanol, the parameter values are similar. However, the parameter values for the ionic permeants are very different, especially when the arithmetic mean is compared to the mode, median and geometric mean. The Shapiro-Wilks normality test (SAS User's Guide, 1985) was used to statistically confirm the non-normal distribution for ionic terbutaline and diclofenac (Table 1). A large data set and/or prior knowledge of the asymmetric distribution model is needed in order to know the central tendency and the variance of data. For example, a lognormal distribution may be applied in an empirical way (Crow and Shimizu, 1988; Bolton, 1990). The antilog of the arithmetic mean with standard deviation of the logarithmically transformed data set is the geometric mean with an unequal variance. The parameter estimates resulting from the inverse transformation may, however, be biased; the reader is referred to theory of the lognormal distribution (Crow and Shimizu, 1988).

Another interesting feature from the histograms of the cotransport experiments (Figs 2 and 3) is that the variability of data is more dependent on the identity of the permeant than on the skin specimen. Only terbutaline flux data exhibited the large variations which show no correlation with isopropanol flux data. These permeant-dependent flux variations may suggest that different mechanisms are involved for transport of ionic and neutral compounds through human cadaver skin in vitro, where different skin specimens have very different barrier functionality to ionic permeants.

In summary, the variation of in vitro skin permeation data has been investigated for ionic (terbutaline and diclofenac) vs neutral (estradiol,

TABLE 1

In vitro skin flux data: descriptive parameters and Shapiro-Wilks normality test

Permeant	Descriptive parameter (μg/cm^2/hr)				Shapiro-Wilks test W value (p value)
	Mode	Median	Geometric mean	Arithmetic mean ± S.D. (C.V.) [f]	
Terbutaline [a]	~ 0.02	0.55	0.34	1.87 ± 4.27 (228%)	0.470 (0.0001)
Terbutaline [b]	~ 0.1	0.70	0.77	3.42 ± 5.73 (168%)	0.645 (0.0001)
Isopropanol [b]	~ 500	551	571	580 ± 108 (19%)	0.958 (0.540)
Diclofenac [c]	~ 1.5	2.80	4.12	12.2 ± 26.2 (215%)	0.462 (0.0001)
Estradiol [d]	~ 0.033	0.036	0.035	0.037 ± 0.009 (24%)	0.983 (0.832)
Estradiol [e]	~ 0.185	0.193	0.192	0.199 ± 0.052 (26%)	0.988 (0.986)
Ethanol [e]	~ 430	491	498	508 ± 105 (21%)	0.932 (0.179)

[a] Fig. 1a data.
[b] Fig. 2 data.
[c] n = 21 experiments with different skin specimens from three skin donors. Donor chamber: sodium diclofenac in water (0.048 M, pH 7.5).
[d] Fig. 1b data.
[e] Fig. 3 data.
[f] S.D., standard deviation; C.V., coefficient of variation.

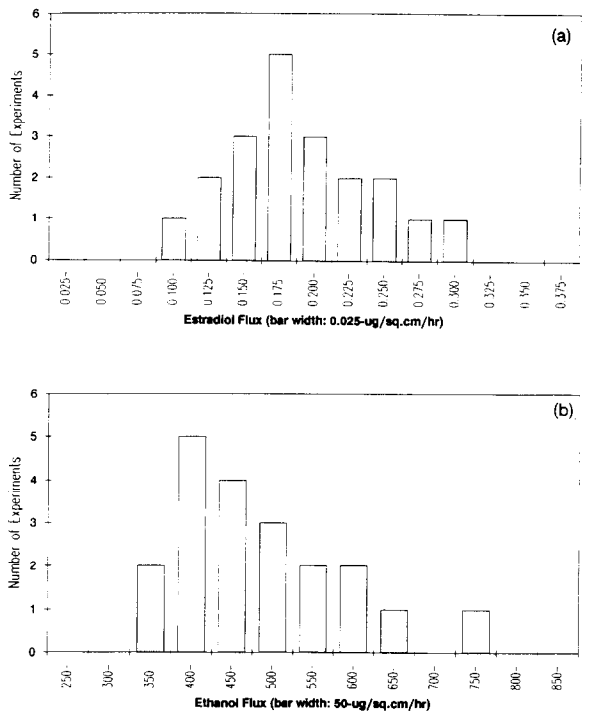

Fig. 3. Histogram of frequency distribution for the in vitro skin flux data from cotransported two-permeant (estradiol/ethanol) experiments ($n = 20$ experiments with different skin specimens from two skin donors) with 20% ethanol aqueous solution saturated with estradiol (0.13 μM): (a) estradiol flux data; (b) ethanol flux data.

ethanol, and isopropanol) permeants through human cadaver skin. In contrast to the neutral permeants, the flux data for the ionic permeants were highly variable. Two conclusions can be drawn from this result. Firstly, the large variation for the ionic permeants illustrates a positively skewed asymmetric distribution, which the arithmetic mean with standard deviation poorly describes. It is recommended that the distribution profile and other descriptive parameters such as median, mode, or geometric mean be used to present the permeation data for ionic compounds. Secondly, the permeant-dependent flux variations may suggest that the different mechanisms exist for the in vitro transport of ionic and neutral compounds through human cadaver skin. More studies are needed to determine the causes of the variation and the basis for the different mechanisms.

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