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Assessment of drug permeability distributions in two different model skins

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Abstract

Past in vitro studies with human skin have indicated that drug permeability coefficient (K_p) distributions do not always follow a Gaussian-normal pattern. This has major statistical implications, exemplified by the fact that use of *t*-tests to evaluate significance is limited to normally distributed populations. Percutaneous absorption research often involves using animal or synthetic skins to simulate less readily available human skin. However, negligible work has been performed on assessing the permeability variabilities of these model membranes. This paper aims to fill this gap. To this end, four studies were undertaken representing two different drugs (caffeine and testosterone) with each drug penetrating through two different model skins (silicone membrane and pig skin). It was determined that in the silicone membrane studies, both compounds' K_p distributions could be fitted to a normal pattern. In contrast, in the pig skin studies, there were notable differences between each drug. While the testosterone K_p values could be fitted to a normal distribution, this was not possible with the caffeine K_p data, which could be fitted to a log-normal distribution. There is some evidence from the literature as well as physicochemical considerations that these outcomes may reflect general trends that are dependent upon both membrane and penetrant properties.

Keywords: Testosterone; Caffeine; Skin; Permeability coefficient; Variability; Gaussian distribution

1. Introduction

Previous in vitro studies with human skin have indicated that drug permeability coefficient (K_p) distributions do not generally follow a simple Gaussian-normal configuration but rather tend to follow other patterns. Over a decade ago (Williams et al., 1992; Cornell and Barry, 1995), Barry and co-workers studied the penetration kinetics of both 5-fluorouracil (a hydrophilic drug) and oestradiol (a lipophilic drug) through human epidermis samples. They found that by in large, the K_p values for both compounds could be more closely fitted to log-normal frequency distributions than to normal frequency distributions. Using results derived from

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seven donors and 539 samples, another group (Kasting et al., 1992) measured water permeation through split-thickness human cadaver skin. It was found that the distribution of K_p values exhibited a strong positive skew, suggestive of a non-normal pattern. More recently, analysis of K_p databases relating to tritiated water transport through human epidermis again provided evidence for non-normal distributions (Watkinson et al., 1998; Roper et al., 2000; Fasano et al., 2002).

The phenomenon of non-normal K_p behaviour has widespread implications with regards to the statistical analysis of transdermal drug penetration data. For example, use of the *t*-test to evaluate statistical significance assumes that the population exhibits Gaussian-normality. If this is not the case for K_p values then, strictly speaking, alternative non-parametric tests such as Wilcoxan's signed rank test or the Mann–Whitney *U*-test should be applied. Another option is to establish that the data exhibits Gaussian-normality following some appropriate mathematical transformation. For instance, if it is proven that the database fits a log-normal distribution, the mean and standard deviation of the logarithmically transformed data may be used for a valid *t*-test.

Although several groups have analysed drug K_p distributions obtained from in vitro human skin penetration experiments, negligible work has been published with respect to other model skin membranes. Porcine skin, which is similar to human skin in its permeability properties (Simon and Maibach, 2000), is frequently used as a substitute for human skin in drug delivery studies (Medi and Singh, 2003; Jarvis et al., 2004; Sekkat et al., 2004). Additionally, readily available synthetic membranes can be employed to simulate the drug partitioning properties of the human skin barrier (Smith and Irwin, 2000). The aim of the current study is to characterise the K_p distributions in both of these model skin membranes. Out of the different types of available synthetic membranes, poly(dimethylsiloxane) (PDMS) membranes were selected for this study as these have been used quite extensively in recent years in transdermal modeling studies (Cronin et al., 1998; Du Plessis et al., 2002; Dias et al., 2003; Gu et al., 2004). Caffeine ($\log P = -0.07$) was used as a model hydrophilic drug and testosterone ($\log P = 3.32$) was used as a model lipophilic drug. Thus, four studies were undertaken, representing two different drugs with each penetrating through two different model skins.

2. Materials and methods

2.1. Chemicals

Caffeine, testosterone, HPLC-grade acetonitrile and phosphate buffer saline (PBS) tablets (pH 7.4) were purchased from the Sigma–Aldrich (Poole, UK). HPLC grade glacial acetic acid was obtained from Fisons Scientific Equipment (Loughborough, UK) while absolute ethanol was purchased from Bamford Labs (Rochdale, UK). [1,2,6,7-^{3}H]-testosterone (1 mCi/ml; 95 Ci/mmol in toluene:ethanol, 9:1) was bought from Amersham Biosciences (Little Chalfont, UK). The liquid scintillation cocktail and scintillation vials were purchased from Fisher Scientific (Loughborough, UK) and Packard Instrument Co. (Meriden, CT), respectively. Translucent poly(dimethylsiloxane) membranes of nominal thickness 300 μm were obtained from Samco Silicone Products Ltd. (Nuneaton, UK).

2.2. Preparation of membranes

For the synthetic membrane studies, PDMS membranes were soaked overnight in receptor solution before use the following day in the permeation studies. For the skin membrane studies, porcine ears (Landrace species) were obtained immediately after slaughter from a local abattoir and cleaned under cold running water. These were sectioned by scalpel to yield whole skin samples, of area ~8 cm^2. The skin sections were then checked for integrity before subsequent storage in a frozen state (−80 °C) for a maximum of 2 months before use. The samples were thawed at room temperature before their use in the transport studies. Each study of 60 or 63 replicates involved experiments performed on skin sections derived from 3 different donator pigs, i.e. there were 20 or 21 skin samples derived from the same donator animal.

2.3. Transport studies

The test membranes (either silicone or whole porcine skin) were mounted in Franz diffusion cells (PermeGear, Bethlehem, PA), exhibiting a diffusion-available surface area of 0.64 cm^2 and a receptor compartment volume of 5.3 ml. For the caffeine studies, the receptor solution consisted of PBS (pH

7.4) that had been degassed by sonication for 5 min (Camlab Transsonic T310, Cambridge, UK). For the testosterone studies, the receptor solution consisted of 10% (v/v) ethanol in degassed PBS (pH 7.4). The receptor fluids were stirred at 600 rpm and maintained at 37 ± 0.5 °C by the use of a thermostatic water pump (Haake DC10, Karlsruhe, Germany) that circulated water through each chamber jacket. The membranes were initially left in the Franz cells for 1 h in order to facilitate hydration. Subsequently, 0.5 ml of donor solution was deposited on to each membrane surface. The donor solution consisted of either 0.5% (w/v) caffeine in PBS (pH 7.4) or 10% (w/v) testosterone in a 20:80% (v/v) mixture of ethanol:PBS (pH 7.4). In the case of testosterone, added radiolabelled drug resulted in each 0.5 ml volume exhibiting 0.5 μCi of activity. Each donor compartment was covered with a taught layer of Parafilm® in order to minimise evaporation. At selected time intervals (0, 1, 2, 3, 4, 5, 6, 20, 22 and 24 h) a 100 μl aliquot of receiver solution was withdrawn from each receiver solution and replaced with the same volume of blank PBS solution. Permeant amounts in the withdrawn solutions were determined by either HPLC or liquid scintillation counting. The caffeine-PDMS membrane study consisted of 60 replicates but each of the other three studies consisted of 63 replicates.

2.4. Quantitative assays

For the caffeine experiments, analysis of samples was performed on a HPLC system (Finnigan SpectraSystem®, ThermoElectron Corp.) equipped with a solvent degasser (Model SCM1000), an autosampler (Model AS3000) and UV photodiode array detector (Model 6000). Samples were eluted on a C_{18}-Phenomenex® column (150 mm × 4.6 mm; 5 μm) using a mobile phase consisting of consisting of 0.05 M acetic acid:acetonitrile (85:15). The elution parameters were a flow rate of 1 ml/min and an injection volume of 20 μl. The detection wavelength was 272 nm. The analytical parameters for this assay were as follows: retention time was 3.4 min, limit of detection was 0.5 μg/ml and reproducibility relative standard deviation was 2%.

For the testosterone studies, each 100 aliquot was vortexed with 3 ml of scintillation cocktail and then placed in a liquid scintillation counter (Packard, TriCarb™ 1600TR). The emitted activity of each aliquot was converted to a testosterone concentration value.

2.5. Data analysis

For both drugs, the derived concentration values were corrected for progressive dilution using the equation:

$$M_t(n) = V_r \times C_n + V_s \times \sum C_m$$

where $M_t(n)$ is the current cumulative mass of drug transport across the skin at time t, C_n the current concentration in the receiver medium, $\sum C_m$ the summed total of the previous measured concentrations [$m = 1$ to $(n-1)$], V_r the volume of the receiver medium and V_s corresponds to the volume of the sample removed for analysis.

Linear regression analysis was used to determine the gradient of the steady-state segment of each permeation experiment, thus yielding a permeability coefficient (K_p) value for each individual replicate. All the replicate K_p values for each study were pooled together without the omission of any outliers. Each resulting distribution was tested for Gaussian-normality by applying the Kolmogorov–Smirnov test (Miller and Miller, 1986). However, since the true mean and standard deviation of each entire population remain unknown, we could not employ the pure form of the Kolmogorov–Smirnov test. Hence, we used a modified version of the test known as "Dallal and Wilkinson's approximation to Lilliefors' method" (Dallal and Wilkinson, 1986). This method estimates the population mean and population standard deviation values from the inputted sample. This statistical analysis was performed by suitable IBM-compatible software—Prism Version 2 (GraphPad Software, San Diego, CA).

3. Results

3.1. Drug penetration–time plots

Figs. 1 and 2 present the pooled penetration–time plots for caffeine and testosterone, respectively. It can be seen that linear steady-state segments were obtained

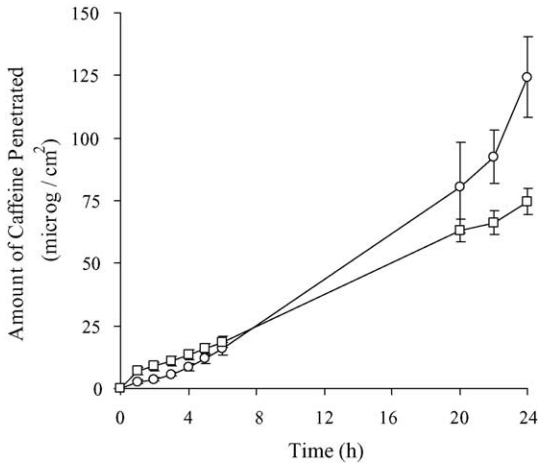

Fig. 1. Penetration of caffeine through both silicone and porcine skin membranes as a function of time. Squares and circles denote silicone membrane and pig skin data, respectively. Error bars represent S.E.M. values with $n \geq 60$.

in all four studies. As explained above, we actually selected a steady-state segment from each individual replicate penetration–time plot. Importantly, approximately 90% of all selected steady-state segments exhibited excellent linearity ($0.99 \leq r^2 \leq 1.00$) while the rest exhibited a reasonable degree of linear correlation ($0.91 \leq r^2 < 0.99$). From Figs. 1 and 2, it is also possible to calculate that the maximal receiver

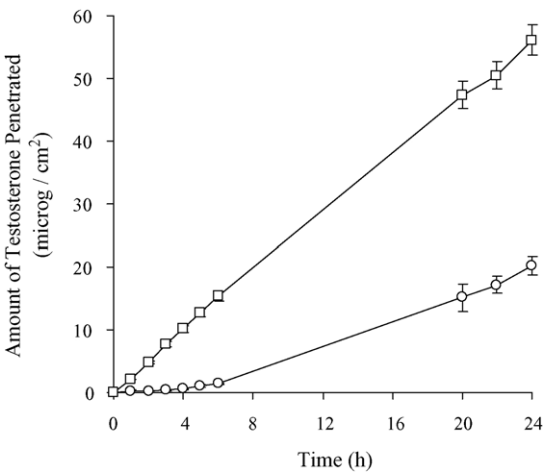

Fig. 2. Penetration of testosterone through both silicone and porcine skin membranes as a function of time. Squares and circles denote silicone membrane and pig skin data, respectively. Error bars represent S.E.M. values with $n \geq 60$.

concentrations of caffeine and testosterone were 15 and 6.8 μg/ml, respectively. Since caffeine solubility in water is 21,880 μg/ml (Akomeah et al., 2004) and testosterone solubility in 10% aqueous ethanol is 130 μg/ml (Kim et al., 2000), our solute concentration values never exceeded 10% of maximal solubility. In other words, we can validate that the experimental systems permitted free diffusion of both candidate drugs.

3.2. Silicone membrane data

Fig. 3 presents the two K_p frequency distributions that describe caffeine and testosterone transport through silicone membranes. Table 1 lists the derived statistical parameters describing each K_p distribution. One of these parameters is the KS distance. If each permeant's K_p values followed an exact Gaussian

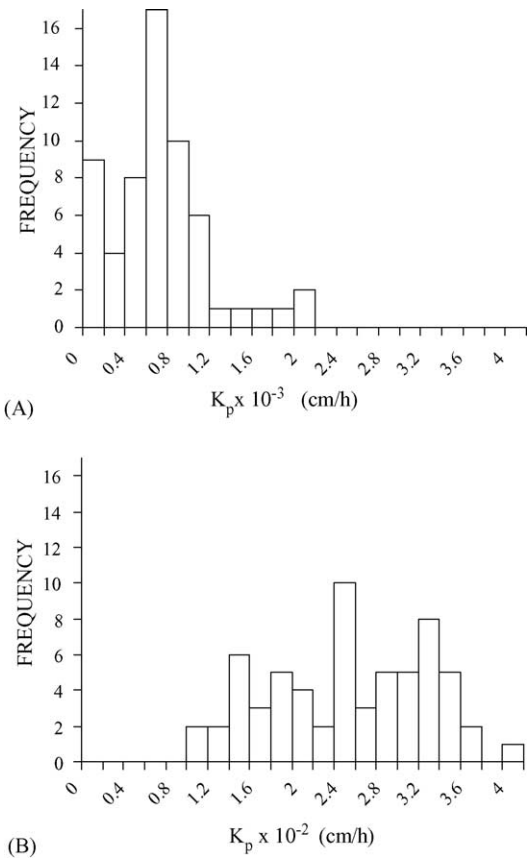

Fig. 3. Frequency distributions of: (A) caffeine and (B) testosterone permeability coefficients as derived from silicone membrane experiments.

Table 1
A statistical analysis of each permeability coefficient database

Membrane	Permeant	$K_p \times 10^{-3}$ (mean ± S.D.)	KS distance	*P* value	Passed normality test (* = 0.05)?
Silicone	Caffeine	0.74 ± 0.48	0.146	>0.10	Yes
Silicone	Testosterone	23.47 ± 7.58	0.094	>0.10	Yes
Porcine skin	Caffeine	1.36 ± 1.74	0.243	0.001	No
Porcine skin	Testosterone	11.33 ± 7.19	0.119	>0.10	Yes

distribution, the KS distance would equal zero. Larger values of the KS distance correspond to larger deviations from an ideal Gaussian distribution. By taking into account the KS distance and the sample size, it was possible to calculate a *P* value that represented the chance that the sample was derived from a normally distributed population. As Table 1 shows, our results indicated that the distribution of both caffeine and testosterone K_p values closely fitted a Gaussian-normal pattern.

3.3. Porcine skin data

Fig. 4 shows the two K_p frequency distributions describing caffeine and testosterone penetration through full-thickness pig skin. Subjective visual observation suggests that the caffeine data is highly asymmetric, exhibiting extensive tailing in the direction of higher permeability values. The testosterone distribution also appears to exhibit some positive skew, although to a much lesser extent than the caffeine. Again, the calculated statistical parameters are presented in Table 1. It can be seen that the caffeine K_p data clearly did not pass the Gaussian-normality test. However, the testosterone K_p data did pass the test for normality despite the apparent asymmetry. Since others have shown that transdermal K_p data often follows a log-normal distribution, we logarithmically transformed the caffeine data. Fig. 5 shows the resulting frequency distribution. Statistical analysis of the logged caffeine data showed that it could be fitted to a normal distribution (KS distance = 0.126; $P > 0.10$) thus indicating that the original K_p database could be fitted to a log-normal distribution.

4. Discussion

Our results showed that for porcine skin permeation, K_p values for the lipophilic testosterone could be fitted to a normal distribution. In contrast, the K_p data for the more hydrophilic molecule, caffeine, could not be fitted to a normal distribution but were compatible with a log-normal distribution. Although it could be argued that the slight differences in donor and receiver solution compositions confound our data, this pattern correlates well with the findings published by others who also employed biological skins. For example, it was reported that flux variability in human skin was dependent upon the nature of the selected test penetrant (Liu et al., 1991). The flux data were symmetrically distributed for neutral molecules but was positively skewed for polar or ionic compounds. Another group

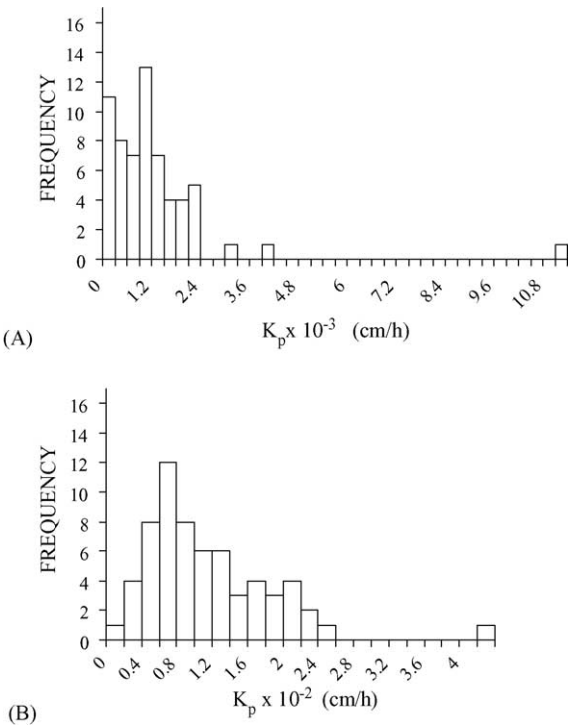

Fig. 4. Frequency distributions of: (A) caffeine and (B) testosterone permeability coefficients as derived from porcine skin experiments.

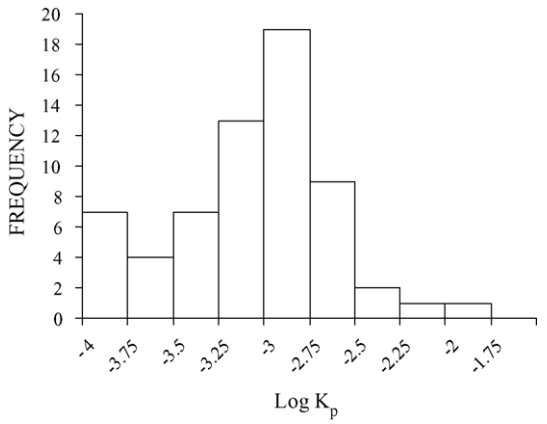

Fig. 5. Frequency distributions of logarithmically transformed caffeine permeability coefficients as derived from porcine skin experiments.

found that the variation in water permeability of split-thickness human skin could not be fitted to a normal distribution but could be fitted to a log-normal distribution (Kasting et al., 1992). Water permeabilities through rat epidermis were also not normally distributed but could be incorporated into a log-normal model (Fasano et al., 2002). Barry's group (Cornell and Barry, 1995), using human epidermis, tested 5-fluorouracil ($\log P = -0.95$) as a model hydrophilic drug and oestradiol as a model lipophilic drug ($\log P = 2.3$). They determined that K_p values for 5-flouoruracil could not be fitted to a normal distribution but that they could be fitted to a log-normal distribution. This contrasted with the K_p values for the lipophilic drug, which could be fitted to a normal distribution. Although this latter result somewhat contradicted the group's previous oestradiol results where non-normal behaviour combined with a possible bimodal tendency was identified (Williams et al., 1992).

In our current study, apart from characterising the dispersion profile, it is also important to address the data dispersion magnitude. In each tested membrane, the coefficient of variation (CV) was higher for caffeine than for testosterone. In PDMS studies, the respective CVs were 64.9% versus 32.3%, while in porcine skin studies, the respective CVs were 127.9% versus 63.4%. It is also important to consider the mean K_p values. In each membrane type, these were much lower for caffeine than for testosterone. This probably reflects the greater partitioning and higher diffusion rate of the more lipophilic testosterone through either the stratum corneum or PDMS matrix. By combining these findings together, we can postulate that the presence of imperfections such as abrasions, defects or hair follicles (in skin) will have a bigger impact on the flux of hydrophilic, slowly penetrating molecules than on the flux of lipophilic, more rapidly penetrating molecules. This would explain why the caffeine data is more widely dispersed and more skewed than the testosterone data. Such defects are much rarer in synthetic membranes, which is why when both solutes permeate through silicone membrane, both K_p databases can be fitted to Gaussian-normal distributions.

It is intriguing to consider why hydrophilic solute–biological skin systems should yield K_p distributions that are log-normally distributed as opposed to some other pattern. This question is difficult to answer as very little quantitative research has been conducted on variability in percutaneous delivery systems. Actually, very little is known about the underlying principles of membrane permeation variability right across the sciences (Limpert et al., 2001). The few literature reports describing other membrane transport systems have yielded mixed results. Variability in carboxyfluorescein permeation through corneal epithelium (McCarey and Reaves, 1995) could be fitted to a Gaussian distribution as could the transport of both glucose and a small zwitterion through synthetic lipid bilayers (Clerc and Thompson, 1995). However, measurements of water and organic salt mobility through lipophilic plant cuticles indicated that the coefficients could not be fitted to a Gaussian distribution (Baur, 1997). It has been proposed that transport processes can be either additive or multiplicative in nature, thus leading to either normal or log-normal distributions, respectively (Limpert et al., 2001).

In conclusion, we have presented data showing that in silicone membranes, the K_p values for both caffeine and testosterone could be fitted to normal distributions. Contrastingly, in the porcine skin studies, there were notable differences between each drug. While the testosterone K_p values could be matched to a normal distribution, this was not possible with the caffeine K_p data, which fitted a log-normal distribution. There is some evidence from the literature reports that our caffeine results may be typical for most hydrophilic drug–biological skin systems. If that is indeed the case, then such systems may be evaluated by parametric tests

such as the *t*-test only after logarithmic transformation of the data. Clearly, this field requires further research. In particular, extended studies involving a broader range of test drugs and other membranes must be performed before really firm conclusions can be reached.

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