ELSEVIER

International Journal of Pharmaceutics 170 (1998) 129–133

international journal of pharmaceutics

Note

Skin permeability data: anomalous results

I. Tuncer Degim [1], W. John Pugh, Jonathan Hadgraft *

The Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF1 3XF, Wales, UK

Received 9 January 1998; received in revised form 2 February 1998; accepted 4 March 1998

Abstract

Analysis of published skin permeation data has shown that a few compounds appear to have anomalous skin permeability coefficients. These include penetrants such as naproxen, atropine and nicotine. The permeabilities of these materials were re-determined together with aspirin, benzoic acid, diclofenac, ibuprofen and methyl nicotinate. The results are discussed in conjunction with published regression analyses and compared with values predicted by estimating the octanol–water partition coefficients using commercial software packages.

Keywords: Skin permeability; Prediction; Transdermal; Skin absorption

1. Introduction

A number of publications have examined structure activity relationships for skin permeability. The data used in these have usually been obtained from one predominant source, that of Flynn (1990). There have also been attempts to consider the in vivo absorption of a series of salicylates and non-steroidal anti-inflammatory agents (Yano et al., 1986; Hadgraft, 1989). Pugh and Hadgraft (1994) and Hadgraft (1989) determined that naproxen did not appear to fit with the published data and it was postulated that there may be an interaction between naproxen and the structured lipids of the stratum corneum thus creating an 'abnormal' permeability barrier. Attempts were made to examine the interaction of naproxen with model structured lipids (Degim et al., 1995). Naproxen did not appear to behave differently from aspirin, diclofenac or ibuprofen in its interaction with dipalmitoylphosphatidylcholine (DPPC) monolayers. These studies were conducted in the light of observed interactions

* Corresponding author. E-mail: Hadgraft@cardiff.ac.uk

[1] Present address: Department of Pharmaceutical Technology, Faculty of Pharmacy, Gazi University, 06330 Etiler, Ankara, Turkey.

0378-5173/98/$19.00
PII S0378-5173(98)00113-6

Table 1
Conditions for HPLC analysis

Chemicals	Column	Mobile phase	Flow rate (ml/min)	UV (nm)	R_t (min)
Aspirin	C_{18} (5 μm), 25 × 0.4 cm	Acetonitrile 137.5 ml; water 112.5 ml; KH_2PO_4 0.689 g; orthophosphoric acid 1 ml	1	245	3.43
Atropine	C_{18} (5 μm), 25 × 0.4 cm	Acetonitrile 40 ml; 0.01 M KH_2PO_4 60 ml; orthophosphoric acid (to adjust pH to 8.4)	0.9	225	6.95
Benzoic acid	C_{18} (5 μm), 25 × 0.4 cm	Acetonitrile 137.5 ml; water 112.5 ml; KH_2PO_4 0.689 g; orthophosphoric acid 1 ml	1	245	3.16
Diclofenac	C_{18} (5 μm) 25 × 0.4 cm	Acetonitrile 137.5 ml; water 112.5 ml; KH_2PO_4 0.689 g orthophosphoric acid 1 ml	1	245	7.07
Ibuprofen	C_{18} (5 μm), 25 × 0.4 cm	Acetonitrile 137.5 ml; water 112.5 ml; KH_2PO_4 0.689 g; orthophosphoric acid 1 ml	1	245	7.55
Methyl nicotinate	C_{18} (5 μm), 25 × 0.4 cm	Acetonitrile 137.5 ml; water 112.5 ml; KH_2PO_4 0.689 g; orthophosphoric acid 1 ml	1	245	3.03
Naproxen	C_{18} (5 μm), 25 × 0.4 cm	Acetonitrile 137.5 ml; water 112.5 ml; KH_2PO_4 0.689 g; orthophosphoric acid 1 ml	1	245	4.42
Nicotine	C_{18} (10 μm), 25 × 0.4 cm	Acetonitrile 50 ml; water 140 ml; octanesulphonic acid 0.55 g; glacial acetic acid 1.25 ml	0.75	254	13.07
Salicylic acid	C_{18} (5 μm), 25 × 0.4 cm	Acetonitrile 137.5 ml; water 112.5 ml; KH_2PO_4 0.689 g; orthophosphoric acid 1 ml	1	245	3.12

between NSAIDs and dietary phospholipids (Kivinen et al., 1994). Therefore alternative explanations were sought.

Pugh and Hadgraft (1994) calculated permeability coefficients of compounds in the data set of Flynn and compared 'ab initio' approaches with the equation of Potts and Guy (1992). A number of compounds were always seen to be outliers: these included naproxen, atropine and nicotine. For this reason it was decided to re-measure the permeability coefficients for these compounds and to compare the results with those published in the literature and those calculated. In addition, five other compounds were chosen to enlarge the database of NSAIDs and simple marker compounds. The additional substances were in aspirin, benzoic acid, diclofenac, ibuprofen and methyl nicotinate.

2. Materials

Aspirin, benzoic acid, disodium hydrogen phosphate, sodium chloride, sodium hydroxide, potassium dihydrogen phosphate, orthophosphoric acid were obtained from BDH Ltd. (Poole, UK). Atropine, naproxen, nicotine and salicylic acid were obtained from Sigma Chemical Co. (Poole, UK). Methyl nicotinate was obtained from Aldrich Chemical Co. (Gillingham, UK). HPLC grade acetonitrile was from Rathburn Chemicals (Scotland).

3. Methods

HPLC analyses were conducted using standard equipment using the conditions given in Table 1. All-glass Franz type diffusion cells were used. The stirred receptor phase ~2.5 ml contained phosphate-buffered saline, pH 7.4, and was thermostatted at 37°C. The donor compartment contained 1.5 ml aqueous solutions of the permeant. The diffusional cross-sectional area was 1 cm^2. Full-thickness female abdominal human skin was obtained from cosmetic surgery. Samples of the receptor phase were removed periodically (2, 4, 6, 8, 12, 24, 30, 48, 60, 72 h). Receptor phase was replenished with fresh solution after each sample. Permeant concentrations were determined by HPLC. Three replicates were conducted. Steady-state fluxes were determined by curve fitting (Ultrafit) and permeability coefficients esti-

Table 2
Permeability coefficients determined for the various permeants ($n = 3 \pm$ S.E.) and comparable values from the literature

Permeant	Log k_p (cm h^{-1})	Log k_p (cm h^{-1}) (Literature)
Aspirin	-2.14 ± 0.43	
Atropine	-4.12 ± 0.15	-5.07 (Flynn, 1990)
Benzoic acid	-1.60 ± 0.01	
Diclofenac	-3.00 ± 0.19	-3.45 (Wilschut et al., 1995)
Ibuprofen	-1.44 ± 0.02	-1.44 (Watkinson et al., 1993)
Methyl nicotinate	-2.41 ± 0.24	-2.49 (Nastruzzi et al., 1993)
Naproxen	-2.54 ± 0.08	-3.40 (Flynn, 1990)
Nicotine	-2.48 ± 0.08	-1.71 (Flynn, 1990)
Salicylic acid	-1.86 ± 0.03	-2.20 (Flynn, 1990)

mated by dividing the steady-state flux by the applied aqueous concentration.

4. Results and discussion

Table 2 shows the permeability coefficients determined for the different permeants compared to those in the literature (when available).

There are some discrepancies, the largest differences observed are, in descending order, atropine, naproxen and nicotine. It is instructive to compare the values to those calculated using the equation of Potts and Guy (1992).

$$\log k_p(\text{cm h}^{-1}) = -2.7 + 0.71 \log K_{oct} - 0.0061\, M_w \quad (1)$$

where K_{oct} is the octanol water partition coefficient and M_w, the molecular weight.

Values of K_{oct} can be predicted using a variety of algorithms available in commercial software. Four different packages were chosen to estimate K_{oct} for the different permeants. These were clog P (Biobyte Corp., USA), log P (Advanced Chemistry Development, Canada), Hyperchem (with ChemPlus Module) (Hypercube, Canada) and Kowwin (Syracuse Research Corporation, USA). Some of these packages also quote measured values of log K_{oct} where available. Table 3 shows the values obtained.

There is rather good agreement between the mean predicted values and the literature values (as obtained through the software packages). The largest discrepancy is for diclofenac.

It is now possible to calculate the permeability coefficient using Eq. (1). The results, either using mean K_{oct} values or literature K_{oct} values are given in Table 4.

The experimental and predicted values of k_p for atropine, naproxen and nicotine are considerably closer than originally thought. Naproxen and nicotine do not appear to be 'outliers' as described in the publication by Pugh and Hadgraft (1994). The new values for k_p for these two compounds are significantly different to those in the 'Flynn' database. There are clear implications

Table 3
Log K_{oct} values obtained from the commercial software packages described in the text

Permeant	ACD	clog P	Hyperchem	Kowwin	Mean ($\pm$ S.D.)	Literature
Aspirin	1.19	1.02	1.24	1.13	1.15 (0.09)	1.19
Atropine	1.53	1.32	1.71	1.91	1.62 (0.25)	1.83
Benzoic acid	1. 89	1.88	1.75	1.87	1.85 (0.07)	1.87
Diclofenac	3.28	3.03	3.97	4.02	3.58 (0.50)	4.40
Ibuprofen	3. 72	3.68	3.83	3.79	3.76 (0.07)	3.50
Methyl nicotinate	0.88	0.77	0.46	0.64	0.69 (0.18)	0.83
Naproxen	3.0 0	2.82	2.99	3.10	2.98 (0.12)	3.34
Nicotine	0.72	1.32	1.02	1.00	1.02 (0.25)	1.17
Salicylic acid	2.06	2.19	1.46	2.24	1.99 (0.36)	2.26

The literature values are derived from a number of sources and are those supplied by the clog P and or Kowwin software as "recommended measured values".

Table 4
Log k_p values calculated using mean predicted K_{oct} values or K_{oct} values from the literature

Permeant	Log k_p (cm/h)		
	Predicted from mean calculated K_{oct}	Predicted from literature experimental K_{oct}	Experimental
Aspirin	−2.99	−2.95	−2.14
Atropine	−3.32	−3.17	−4.12
Benzoic acid	−2.13	−2.12	−1.60
Diclofenac	−1.97	−1.38	−3.00
Ibuprofen	−1.29	−1.47	−1.44
Methyl nicotinate	−3.05	−2.95	−2.41
Naproxen	−1.99	−1.73	−2.54
Nicotine	−2.97	−2.86	−2.48
Salicylic acid	−2.13	−1.94	−1.86

Also included are those obtained experimentally in this work.

concerning the reproducibility of generating k_p values between laboratories. The reasons for this should be explored further.

This problem has also been highlighted in a recent publication by Abraham et al. (1997) in which there appears to be a discrepancy between the published skin permeabilities of a series of steroids.

Using a sub-set of 24 compounds from Flynn's data, Roberts et al. (1995) obtained an r^2 value of 0.803 for the Potts and Guy regression

$$\log k_p = -1.97 + 1.08 \log K_{oct} - 0.0169\, M_w$$
$$N = 24,\ r^2 = 0.803$$

This left 20% of the variation in $\log k_p$ unexplained. A substantial part of this can be accounted for by the hydrogen bonding strengths α and β of the permeants.

$$\log k_p = -1.24 - 2.04\alpha - 3.89\beta + 0.016\, M_w$$
$$N = 24,\ r^2 = 0.882$$

This improved still further by using the molecular volume (V (Å^3))

$$\log k_p = -1.35 - 1.37\alpha - 4.53\beta + 2.05\, V$$
$$N = 24,\ r^2 = 0.933$$

This suggests that if the solvatochromic parameters α and β can be determined, then experimental variability accounts for about 10% of the variation in $\log k_p$. Since Southwell et al. (1984) found a coefficient of variation (standard deviation/mean) of almost 40% for inter-subject variation it seems unlikely that the 10% variation in $\log k_p$ which is not attributable above can be reduced much further.

5. Conclusions

There is a clear requirement in the pharmaceutical, cosmetic and environmental sciences for an equation that can be used reliably to predict skin permeability. The equation generated by Potts and Guy has great utility and was based on a large data set. It is apparent that there are discrepancies in the data set that was originally used. In order to generate an equation that can be used in mechanistic evaluations of skin permeation it is important to obtain reliable data that are validated by different international laboratories.

References

Abraham, M.H., Martins, F., Mitchell, R.C., 1997. Algorithms for skin permeability using hydrogen bond descriptions: the problem of steroids. J. Pharm. Pharmacol. 49, 858–865.

Degim, T., Hadgraft, J., Pugh, W.J., 1995. Modelling the transdermal delivery of non-steroidal anti-inflammatory agents. Proc. Int. Symp. Control. Release Bioact. Mater. 22, 652–653.

Flynn, G.L., 1990. Physicochemical determinants of skin absorption. In: Gerrity, T.R., Henry, C.R. (Eds.), Principles of Route to Route Extrapolation for Risk Assessment. Elsevier, New York, pp. 93–127.

Hadgraft, J., 1989. Formulation of anti-inflammatory agents. In: Lowe, N.J., Hensby, C.N. (Eds.), Nonsteroidal Anti-Inflammatory Drugs. Karger, Basel, pp. 21–43

Kivinen, A., Vikhol, I., Tarpila, S., 1994. A film balance study of the monolayer forming properties of dietary phospholipids and the interaction with NSAIDS on the monolayers. Int. J. Pharm. 108, 109–115.

Nastruzzi, B., Esposito, E., Pastesini, C., Gambari, R., Menegatti, E., 1993. Comparative study on the release kinetics of methyl nicotinate from topical formulations. Int. J. Pharm. 90, 43–50.

Potts, R.O., Guy, R.H., 1992. Predicting skin permeability. Pharm. Res. 9, 663–669.

Pugh, W.J., Hadgraft, J., 1994. Ab-initio prediction of human skin permeability coefficients. Int. J. Pharm. 103, 163–178.

Roberts, M.S., Pugh, W.J., Hadgaft, J., Watkinson, A.C., 1995. Epidermal permeability-penetrant structure relationships: 1. An analysis of methods of predicting penetration of monofunctional solutes from aqueous solution. Int. J. Pharm., 125, 219–233.

Southwell, D., Barry, B.W., Woodford, R., 1984. Variations in permeability of human skin within and between specimens. Int. J. Pharm. 18, 299–309.

Watkinson, A.C., Brain, K.R., Walters, K.A., 1993. The penetration of ibuprofen through human skin in vitro: vehicle, enhancer and pH effects. In: Brain, K.R., James, V.J., Walters, K.A. (Eds.), Prediction of Percutaneous Penetration, vol. 3b. STS Publishing, Cardiff, pp. 335–341.

Wilschut, A., Berge, W.F., Robinson, P.J., McKone, T.E., 1995. Estimating skin permeation, the validation of five mathematical skin permeation models. Chemosphere 30, 1275–1296.

Yano, T., Nakagawa, A., Tsuji, M., Noda, K., 1986. Skin permeability of various non-steroidal anti-inflammatory drugs in man. Life Sci. 39, 1043–1050.