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Estimating skin permeability from physicochemical characteristics of drugs: A comparison between conventional models and an in vivo-based approach

Sara Farahmand [1], Howard I. Maibach *

Department of Dermatology, School of Medicine, University of California, San Francisco, USA

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ABSTRACT

This study evaluates the correlation of some widely used skin permeability predictive models with a recently proposed empirical model based on human in vivo dermatopharmacokinetic data. Drug fluxes through the skin have been calculated using in vitro- and in vivo-based models, and observed in vivo data, and the values compared.

Most in vitro-based models underestimate the in vivo data by 1–100-fold. The discrepancy between observed data and prediction reaches the maximum (1000–10,000-fold underestimation) for nicotine (with the smallest molecular weight and log K_{oct}), nitroglycerin (with the largest number of hydrogen bond acceptor groups), and for oxybutynin (with the largest molecular weight and log K_{oct}) where there was a 1000-fold flux overestimation. However, most models correlated well with the in vivo data and the in vivo-based model ($p < 0.05$).

The vehicle effect and using non-steady state in vivo data in the flux calculations partly account for the observed discrepancies between predicted and observed values. Nevertheless, these results reveal the need for further refinement of skin permeability predictive equations, using the steady state in vivo data, and consideration of formulation effect.

1. Introduction

The evaluation of percutaneous absorption of molecules is a necessity for optimizing the delivery of chemicals of pharmaceutical and cosmetic concern, for toxicology and risk assessment of these materials and for estimating occupational exposure and similar hazards.

Permeation of drug molecules across the skin occurs by passive diffusion according to the activity gradient. The outer skin layer, stratum corneum, forms a rate-controlling barrier for diffusion of most compounds. The predominant diffusional path for a molecule crossing the stratum corneum appears to be intercellular (Barry, 1983; Walters, 2002; Hadgraft and Guy, 2002). However this path is not exclusive. It is likely that most molecules will pass through the stratum corneum by a combination of intercellular lipid domains, transcellular route and via the appendages (shunt routes) (Williams, 2003). It is difficult to assess the skin permeability of materials, particularly new molecules or novel formulations, using in vivo experiments alone. Consequently, in vitro models are commonly used for percutaneous absorption evaluations. Nevertheless, there has been a reluctance to accept in vitro dermal penetration data due to the limited number of parallel in vitro and in vivo absorption studies in humans or animals, which can be used to confirm the comparability of data. Predictive mathematical models have been developed to statistically relate the experimentally determined percutaneous penetration of a range of exogenous chemicals to known physicochemical parameters (Hadgraft and Guy, 2002; Vecchia and Bunge, 2002; Moss et al., 2002; Yamashita et al., 1994). The rationale for using these models is to choose drugs with more favorable partition and diffusion characteristics which improves the chance of targeted dermal delivery and increased bioavailability. Besides, there are compounds like pesticides, the absorption of which in significant amounts is clearly undesirable. An appropriate mathematical model would allow a reliable risk assessment before conducting in vivo evaluations. Validated mathematical models can be considered as economic alternatives for the assessment of skin permeation, and their use has been recommended before in vitro and in vivo experiments are conducted (Hadgraft and Guy, 2002). However, these models are still being critiqued for their uncertainity due to (1) the limitations of the models in terms of statistical fit, (2) their failure under severe non-linear conditions and (3) the inability to extrapolate their conclusions to other systems, particularly when

* Corresponding author at: University of California, San Francisco, Department of Dermatology, 110 Surge Bldg., 90 Medical Center Way, San Francisco, CA 94143-0989, USA. Tel.: +1 415 476 2468; fax: +1 415 753 5308.
E-mail addresses: farahmand.s@gmail.com (S. Farahmand), Maibachh@derm.ucsf.edu (H.I. Maibach).
[1] Current address: University of Cincinnati, James L. Winkle College of Pharmacy, 3225 Eden Ave., Cincinnati, OH 45267-0004, USA.

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Table 1
Permeability coefficient correlations based on $\log K_{oct}$ and MW[a].

Model no.	Equation source	Chemical class[b]	Permeability correlation (K_p in cm/h)	Data range
1	Abraham et al. (1995)	Misc ($n=43$)	$\log K_p = -2.184 + 0.851 \log K_{oct} - 0.012$ MW	NS[c]
2	Vecchia and Bunge (2002)	Misc ($n=170$)	$\log K_p = -2.44 + 0.514 \log K_{oct} - 0.0050$ MW	$18 < MW < 585$; $-3.1 < \log K_{oct} < 4.6$
3	Bronaugh and Barton (1991)	Flynn database ($n=90$)	$\log K_p = -2.61 + 0.67 \log K_{oct} - 0.0061$ MW	$18 < MW < 765$; $-3 < \log K_{oct} < 5.5$
4	Cleek and Bunge (1993)	Flynn database ($n=90$)	$\log K_p = -2.8 + 0.74 \log K_{oct} - 0.006$ MW	$18 < MW < 765$; $-3 < \log K_{oct} < 6$
5	Flynn and Amidon (Vecchia and Bunge, 2002)	Flynn database ($n=90$)	$\log K_p = -1.44 + 0.79 \log K_{oct} - 1.45 \log MW$	$18 < MW < 765$; $-3 < \log K_{oct} < 6$
6	Kasting et al. (1992)	Flynn database ($n=90$)	$K_p = [(1/P_{lip} + P_{pol}) + (1/P_{aq})]$; $\log P_{lip} = -2.87 + \log K_{oct} - 0.0078$ MW; $P_{pol} = 1 \times 10^{-5}\sqrt{300/MW}$; $P_{aq} = 0.15\sqrt{300/MW}$	$18 < MW < 518$; $-1.4 < \log K_{oct} < 6.3$
7	Potts and Guy (1992)	Flynn database ($n=90$)	$\log K_p = -2.72 + 0.71 \log K_{oct} - 0.0061$ MW	$18 < MW < 765$; $-3 < \log K_{oct} < 6$
8	Wilschut et al. (1995)	Wilschut database ($n=123$)	$\log K_p = \log(0.00284 + 0.000256 \log K_{oct}) - 0.00591$ MW	$18 < MW < 765$; $-3 < \log K_{oct} < 6$
9	Wilschut et al. (1995)	Wilschut database ($n=123$)	$\log K_p = -1.55 + 0.481 \log K_{oct} - 0.143$ MW	$18 < MW < 765$; $-3 < \log K_{oct} < 6$
10	Lien and Gao (1995)	Flynn database ($n=22$)	$\log K_p = 0.84 \log K_{oct} - 0.07(\log K_{oct})^2 - 0.27\, H_b - 1.84 \log MW + 4.39$	$18 < MW < 365$; $-1.4 < \log K_{oct} < 4$
11	Vecchia and Bunge (2002)	Flynn database ($n=84$)	$\log K_p = -2.76 + 0.52 \log K_{oct} - 0.0041$ MW	$18 < MW < 765$; $-3 < \log K_{oct} < 6$
12	Vecchia and Bunge (2002)	Flynn database ($n=84$)	$\log K_p = -2.72 + 0.53 \log K_{oct} - 1.32$ (MW/T[d])	$18 < MW < 765$; $-3 < \log K_{oct} < 6$

[a] Molecular weight and logarithmically transformed octanol–water partition coefficient.
[b] n = total number of data points; the total number of different chemicals may be fewer.
[c] Not specified.
[d] Temperature (K) was considered 300 (32 °C) in our study.

vehicle effects have to be taken into account (Guy and Hadgraft, 1985).

Prediction of the in vivo blood concentration has become increasingly important in the development of transdermal therapeutic systems. Several reports attempt to mathematically correlate or predict the in vivo drug levels from in vitro permeation data based on a diffusion model (Yamashita et al., 1994; Guy and Hadgraft, 1985; Ainbinder and Touitou, 2005) or a convolution technique (Sato et al., 1988). Recently, we evaluated the statistical correlation of maximal plasma concentration (C_{max}) of drugs administered via transdermal delivery systems with their physicochemical properties. Multivariate regression analysis was used to analyze the relationship between C_{max} and molecular weight, hydrogen bonding, and octanol–water partition coefficient (Farahmand and Maibach, 2009).

An empirical model was proposed:

$$C_{max}(\text{ng/ml}) = 8.625\text{E}-7 \times \text{HA} + 8.231\text{E}-7 \times \log K_{oct} - 1.22\text{E}-6 \times \text{HD} - 2.58\text{E}-6 \quad N = 10,\ r = 0.974,\ F = 37.45,\ \text{SD} = 0.82,\ p < 0.001 \tag{1}$$

Where, C_{max} is the mean reported value for a given drug, normalized to dose. HA and HD are the total number of hydrogen bond acceptor and donor groups on the molecule, respectively; $\log K_{oct}$ is the logarithmically transformed octanol–water partition coefficient.

The present study compares the predictability and explores the correlation of our adopted model (in vivo-based model) and some widely used empirical models which were based on in vitro epidermal permeability coefficient data (in vitro-based models).

2. Materials and methods

12 various in vitro-based models and our developed in vivo-based equation were utilized to estimate the fluxes of 10 different transdermally administered drugs (including: scopolamine, clonidine, testosterone, estradiol, norelgestromine, rivastigmine, methyl phenidate, selegiline, nitroglycerin, nicotine). Calculated fluxes were then compared between models as representatives of models predictability. In order to calculate the fluxes, three different approaches were considered based on the following categories:

2.1. In vitro-based predictive models

12 mathematical models (permeability coefficient correlations based on logarithmically transformed K_{oct} ($\log K_{oct}$) and molecular weight (MW), were considered. Model numbers, equations, and references are shown in Table 1. Most models were developed through analysis of Flynn database where in vitro human skin permeabilities of 97 drugs from aqueous solutions are listed (Flynn, 1990), models 8 and 9 were developed through analysis of a more extensive database (Wilschut) and models 1 and 2 were based on the miscellaneous data taken from different resources.

Using MW and $\log K_{oct}$ data (Budavari, 1989; Hansch et al., 1995), the permeability coefficient (K_p) of each drug was calculated using all the predictive equations. (In case of various brands for a drug, permeability coefficient was calculated for the selected brands.) As the Flynn database concerns percutaneous transport from aqueous solution, the maximum achievable flux (J) was estimated by multiplying permeability coefficient (K_p) by the aqueous solubility (S_w):

$$J = K_p \times S_w \tag{2}$$

2.2. In vivo-based predictive model

We assumed:

$$C_{max} = A \times \frac{J}{CL} \tag{3}$$

Where, A is the surface area of the patch (cm^2), J is the flux ($ng/cm^2\,h$), and CL is the transdermal clearance ($CL = CL_{total}$ (total body clearance)/F (absolute bioavailability)) (ml/h) (Hadgraft and Guy, 2002). Flux was estimated in two steps: first, CL was calculated using labeled delivery rate of transdermal systems for the above mentioned drugs ($A \times J$), and corresponding C_{max} values reported for them. The C_{max} values were obtained form a pharmacokinetic database (Farahmand and Maibach, 2009). The second step was to calculate the flux based on C_{max} values predicted by our developed in vivo-based model and the calculated CL (ml/h) in the first step described above. Afterwards, C_{max} was estimated using Eq. (1):

$$C_{max}(\text{ng/ml}) = 8.625\text{E}-7 \times \text{HA} + 8.231\text{E}-7 \times \log K_{oct} - 1.22\text{E}-6 \times \text{HD} - 2.58\text{E}-6$$

Flux ($ng/cm^2\,h$) was then calculated having surface area of the patch, calculated clearance, and predicted C_{max}, by applying Eq. (3).

Table 2
Parameters used for flux calculations.

Drug	Active ingredient	MW[a,b]	log K_{oct}[c,d]	HA[e,f]	HD[f,g]	Expected $A{\cdot}J$ (delivery rate)[h] (ng/h)	$CL^{i}_{transdermal}$ (ml/h)	A^{j} (cm^2)	S_W[k] (ng/ml)	Observed C_{max}[m] (ng/ml)
TD Scop	Scopolamine	303.35	1.24	5	1	5.00E+03	5.00E+04	2.5	2.50E+06	1.00E−01
Oxytrol	Oxybutynin	357	4.3	4	1	1.63E+05	4.28E+04	39	3.90E+07	3.80E+00
Androderm	Testosteone	288.42	3.32	2	1	1.04E+05	1.98E+04	37	3.70E+07[l]	5.25E+00
Exelon	Rivastigmine	250.34	1.98	3	0	3.96E+05	5.82E+04	10	1.00E+07	6.80E+00
Orthoevra-evra	Norelgestromin	327.46	4	3	2	6.25E+03	6.17E+03	20	8.80E+03[l]	1.01E+00
Daytrana	Methylphenidate	233.31	3.65	3	1	3.33E+06	7.17E+04	37.5	3.75E+07	4.65E+01
EMSAM	Selegiline	187.3	2.7	1	0	2.50E+05	1.40E+05	20	2.00E+07	1.79E+00
Estraderm	Estradiol	272.39	4.01	2	2	2.08E+03	4.58E+04	18	3.60E+03[l]	4.55E−02
Estradot	Estradiol	272.39	4.01	2	2	4.17E+03	4.12E+04	10	3.60E+03	1.01E−01
Menorest	Estradiol	272.39	4.01	2	2	4.17E+03	3.60E+04	29	3.60E+03	1.02E−01
Menostar	Estradiol	272.39	4.01	2	2	5.83E+02	2.83E+04	3.25	3.60E+03	2.06E−02
Oesclim	Estradiol	272.39	4.01	2	2	4.17E+03	3.57E+04	44	3.60E+03	1.17E−01
Tradelia	Estradiol	272.39	4.01	2	2	2.08E+03	4.34E+04	18	3.60E+03	4.80E−02
Habitrol	Nicotine	162.23	1.17	2	0	8.75E+05	4.17E+04	30	3.60E+03	21
Nicotine-alza	Nicotine	162.23	1.17	2	0	8.75E+05	4.00E+04	12	3.60E+03	2.19E+01
Nitroderm	Nitroglycerin	227.11	1.62	9	0	4.00E+05	4.00E+06	20	2.00E+07	1.02E−01
Nitro-Dur	Nitroglycerin	227.11	1.62	9	0	4.00E+05	1.04E+06	20	2.00E+07	1.00E−01
Nitro-Dur-2	Nitroglycerin	227.11	1.62	9	0	4.00E+05	8.58E+05	20	2.00E+07	4.66E−01

[a] Molecular weight.
[b] Data obtained from Budavari (1989).
[c] Logarithmically transformed octanol–water partition coefficient.
[d] Data is taken from Hansch et al. (1995).
[e] Number of hydrogen bond acceptor groups on the molecule.
[f] Data compiled from PubChem (2008).
[g] Number of hydrogen bond donor groups on the molecule.
[h] Labeled delivery rates are cited data of PDR (2006).
[i] Transdermal clearance was calculated using labeled delivery rate of transdermal systems and corresponding C_{max} values reported for them.
[j] Patch surface areas obtained from PDR (2006).
[k] Water solubility data are mainly taken from Budavari (1989).
[l] Reported by Miura et al. (2006).
[m] Data obtained from (Farahmand and Maibach, 2009).

2.3. Observed in vivo data

Observed C_{max} values, surface areas and calculated clearance values were used as described earlier by utilizing Eq. (3), to estimate the flux (J) for the observed in vivo data.

Calculations were performed with Microsoft Excel 2003.

2.4. Statistical analysis

Statistical analysis was performed using SPSS software (SPSS 11.5, SPSS Science, Chicago, IL, USA). Normality of distributions was evaluated using Kolmogrov–Smirnov test of normality, and for non-normal distributions, Spearman rank order correlation analysis was performed to evaluate the correlation of in vitro and in vivo models. Significance level was set at 0.05.

3. Results

Tables 2–4 present the parameters used for flux calculation, flux values predicted by different models and correlations of in vitro and in vivo models predictions, respectively. Fig. 1 compares fluxes calculated for the in vivo observed data (J_{exp}), with predictions (J_{pred}) from the in vivo- and in vitro-based mathematical models. As shown in Table 4, all the in vitro-based models except one (model 10) show significant correlations with the observed data and the in vivo-based model ($p < 0.05$).

The grid lines in Fig. 1 distinguish those permeability coefficients that are underestimated or overestimated by 10-fold or more. For the in vivo model, the data is distributed around $J_{exp}/J_{pred} = 1$ (i.e., $\log J_{exp}/J_{pred} = 0$) with the exception of norelgestromin with 40-fold discrepancy between data and prediction.

For the in vitro models, the data is mainly distributed in the range of $0 < \log J_{exp}/J_{pred} < 2$, which means most models underestimate the data 10-fold but sometimes up to 100-fold. For selegiline the flux is overestimated by 10–100-fold by different models. The discrepancy between data and the prediction reaches the maximum (1000–10,000-fold underestimation) for nicotine (with the smallest molecular weight and log K_{oct}), nitroglycerin (with the largest number of hydrogen bond acceptor groups), and for oxybutynin (with the largest molecular weight and log K_{oct}) where the flux was overestimated by around 1000-fold.

4. Discussion

Development of QSPRs (Quantitative Structure Permeability Relationship) has been an issue of interest among skin research scientists in the last 30 years. The potential advantages of applying an appropriate model are great, particularly for the prediction of in vitro percutaneous absorption and formulation development and optimization. However, there are limitations attributed to the models. Many models are produced from data which have been compiled from different sources and laboratories. Although model development was greatly facilitated by using Flynn database, which is based on in vitro human skin permeability data, this data is a compilation from 15 literature sources (Moss et al., 2002). It is inevitable that this data contains a high degree of experimental variation, due to inter-laboratory variability, and variability due to the use of skin from different sources and sites on the body, etc. However, it has been commented that the scale of variance observed is within the expected range of variability in skin delivery experiments, and it has been accepted that most of the equations can adequately predict the available skin permeability coefficient of chemicals (Vecchia and Bunge, 2002; Moss et al., 2002).

In our study, we extended the QSPR models beyond their in vitro boundary by the use of a clinical end point such as C_{max}. Previously we showed that with the exception of fentanyl and clonidine as outliers, the developed in vivo-based model predicted the C_{max} form the log K_{oct}, HA, and HD for drugs administered

Table 3
Predicted fluxes (ng/cm^2 h) by different models.

Drug	Active ingredient	Observed in vivo	In vivo model	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
TD Scop	Scopolamine	1.94E+03	2.13E+03	1.13E+02	3.17E+03	1.55E+03	1.31E+03	5.77E+02	7.32E+02
Oxytrol	Oxybutynin	3.98E+03	3.41E+03	3.11E+05	1.93E+06	2.48E+06	3.09E+06	3.60E+06	6.69E+06
Androderm	Testosteone	2.20E+03	1.76E+E+03	6.06E+01	2.67E+02	2.86E+02	3.20E+02	1.65E+02	5.75E+02
Exelon	Rivastigmine	5.25E+04	5.72E+04	9.05E+04	1.06E+05	7.74E+04	7.22E+04	2.21E+04	7.17E+04
Orthoevra-evra	Norelgestromin	1.30E+02	5.69E+03	1.72E+01	8.38E+01	1.04E+02	1.24E+02	1.04E+02	2.63E+02
Daytrana	Methylphenidate	9.58E+04	1.03E+05	1.33E+03	1.86E+03	2.58E+03	2.67E+03	1.02E+03	5.94E+03
EMSAM	Selegiline	1.25E+04	2.12E+04	3.67E+05	5.13E+05	5.69E+05	5.58E+05	1.25E+05	1.04E+06
Estraderm	Estradiol	5.51E+01	1.79E+02	3.28E+01	6.54E+01	9.36E+01	1.01E+02	5.67E+01	2.25E+02
Estradot	Estradiol	4.12E+02	6.63E+02	3.28E+01	6.54E+01	9.36E+01	1.01E+02	5.67E+01	2.25E+02
Menorest	Estradiol	1.59E+02	2.28E+02	3.28E+01	6.54E+01	9.36E+01	1.01E+02	5.67E+01	2.25E+02
Menostar	Estradiol	1.79E+02	9.81E+01	3.28E+01	6.54E+01	9.36E+01	1.01E+02	5.67E+01	2.25E+02
Oesclim	Estradiol	7.58E+02	1.825E+03	3.28E+01	6.54E+01	9.36E+01	1.01E+02	5.67E+01	2.25E+02
Tradelia	Estradiol	1.16E+02	1.70E+02	3.28E+01	6.54E+01	9.36E+01	1.01E+02	5.67E+01	2.25E+02
Habitrol	Nicotine	2.92E+04	3.15E+04	2.63E+00	8.06E+00	5.50E+00	4.43E+00	6.90E-01	3.93E+00
Nicotine-alza	Nicotine	7.27E+03	7.55E+03	2.63E+00	8.06E+00	5.50E+00	4.43E+00	6.90E-01	3.93E+00
Nitroderm	Nitroglycerin	4.00E+04	4.26E+04	3.68E+02	2.26E+03	1.54E+03	1.35E+03	3.31E+02	1.20E+03
Nitro-Dur	Nitroglycerin	2.00E+04	2.17E+04	3.68E+02	2.26E+03	1.54E+03	1.35E+03	3.31E+02	1.20E+03
Nitro-Dur-2	Nitroglycerin	1.55E+04	1.466E+04	3.68E+02	2.26E+03	1.54E+03	1.35E+03	3.31E+02	1.20E+03

Drug	Active ingredient	Model 7	Model 8	Model 9	Model 10	Model 11	Model 12
TD Scop	Scopolamine	1.35E+03	7.76E+02	2.38E+03	9.07E+01	2.89E+03	1.67E-01
Oxytrol	Oxybutynin	2.86E+06	7.94E+06	1.31E+06	9.16E+08	2.06E+06	9.49E+01
Androderm	Testosteone	3.02E+02	4.25E+02	1.66E+02	4.71E+05	2.43E+02	4.86E+00
Exelon	Rivastigmine	7.21E+04	4.52E+04	6.90E+04	1.80E+08	8.75E+04	6.78E+01
Orthoevra-evra	Norelgestromin	1.17E+02	2.62E+02	5.38E+01	3.95E+04	8.35E+01	3.77E+00
Daytrana	Methylphenidate	2.81E+03	4.79E+03	1.05E+03	1.22E+06	1.52E+03	1.65E+02
EMSAM	Selegiline	5.67E+05	5.13E+05	3.09E+05	2.48E+09	3.76E+05	1.65E+02
Estraderm	Estradiol	1.05E+02	2.32E+02	3.76E+01	4.25E+04	5.82E+01	1.65E+02
Estradot	Estradiol	1.05E+02	2.32E+02	3.76E+01	4.25E+04	5.82E+01	1.65E+02
Menorest	Estradiol	1.05E+02	2.32E+02	3.76E+01	4.25E+04	5.82E+01	1.65E+02
Menostar	Estradiol	1.05E+02	2.32E+02	3.76E+01	4.25E+04	5.82E+01	1.65E+02
Oesclim	Estradiol	1.05E+02	2.32E+02	3.76E+01	4.25E+04	5.82E+01	1.65E+02
Tradelia	Estradiol	1.05E+02	2.32E+02	3.76E+01	4.25E+04	5.82E+01	1.65E+02
Habitrol	Nicotine	4.76E+00	2.62E+00	5.59E+00	1.69E+04	2.64E+00	5.91E-02
Nicotine-alza	Nicotine	4.76E+00	2.62E+00	5.59E+00	1.69E+04	2.64E+00	5.91E-02
Nitroderm	Nitroglycerin	1.39E+03	7.68E+02	1.48E+03	7.92E+04	2.64E+00	5.91E-02
Nitro-Dur	Nitroglycerin	1.39E+03	7.68E+02	1.48E+03	7.92E+04	2.64E+00	5.91E-02
Nitro-Dur-2	Nitroglycerin	1.39E+03	7.68E+02	1.48E+03	7.92E+04	2.64E+00	5.91E-02

through transdermal patches (Farahmand and Maibach, 2009). A possible explanation for the divergence observed with fentanyl and clonidine could be formation of skin reservoirs which had been reported for these drugs (MacGregor et al., 1985; Grond et al., 2000). Despite the small number of drugs analyzed in our model, and the need for a comprehensive explanation of the model in parallel with mechanistic approaches, development of this model begins the challenge of developing empirical models for skin absorption using clinical endpoints and validating the in vitro-based predictive equations.

Table 4
Correlation of models with observed in vivo data, and the in vivo model[a].

Model no.	Correlation with Observed in vivo data		Correlation with in vivo model	
	Spearman rank correlation coefficient	*p*-Value	Spearman rank correlation coefficient	*p*-Value
1	0.686	0.002	0.57	0.013
2	0.581	0.012	0.57	0.012
3	0.628	0.006	0.626	0.006
4	0.668	0.003	0.663	0.004
5	0.628	0.006	0.626	0.006
6	0.651	0.004	0.651	0.004
7	0.644	0.005	0.636	0.005
8	0.593	0.010	0.593	0.01
9	0.581	0.023	0.593	0.01
10	0.293	0.289	0.1	0.48
11	0.596	0.009	0.586	0.01
12	0.593	0.013	0.58	0.015

[a] n (total number of data points) = 18.

The present results show that fluxes predicted by all of the models except one, are correlated with the fluxes calculated through observed in vivo data, and in vivo model. The exception is the model developed by Lien and Gao (model 10), which considered a biphasic effect of $\log K_{oct}$ on skin permeation, and a negative correlation with the number of hydrogen bonds that can be formed by a compound. The largest correlation coefficients were observed with models 1, 4, 6, and 7. A major difference of model 1 (developed by Abraham et al.) with other studied models, is the consideration of a large partial effect for $\log K_{oct}$ (coefficient of $\log K_{oct}$ as predictor is 0.851), which is combined with a large negative coefficient for MW. On the other hand, the Potts and Guy and the Kasting et al. correlations (models 7 and 6) were developed assuming a theoretically expected exponential dependency on molecular size as represented by MW (5). Vecchia and Bunge (2002) developed models 11 and 12, by reanalyzing the Flynn database using $\log K_{oct}$ values recommended by Hansch et al. instead of Flynn's tabulated values (model 11), and incorporating the temperature of the permeability coefficient measurement into analysis (model 12). Although their modifications have been reported to reduce the regression uncertainty and variance and improve the predictability of the in vitro-based models, fluxes predicted by these models, do not show a stronger correlation with the fluxes calculated from observed in vivo data as compared with other models.

As presented in Fig. 1, for the in vitro models, $\log J_{exp}/J_{pred}$ is mainly distributed in the range of 0–2, which implies most of the models underestimate the flux up to 100-fold or two orders of magnitude. This is more obvious for the drugs with the molecular weights in the range of 200–350 g/mol, and $1.5 < \log K_{oct} < 4$.

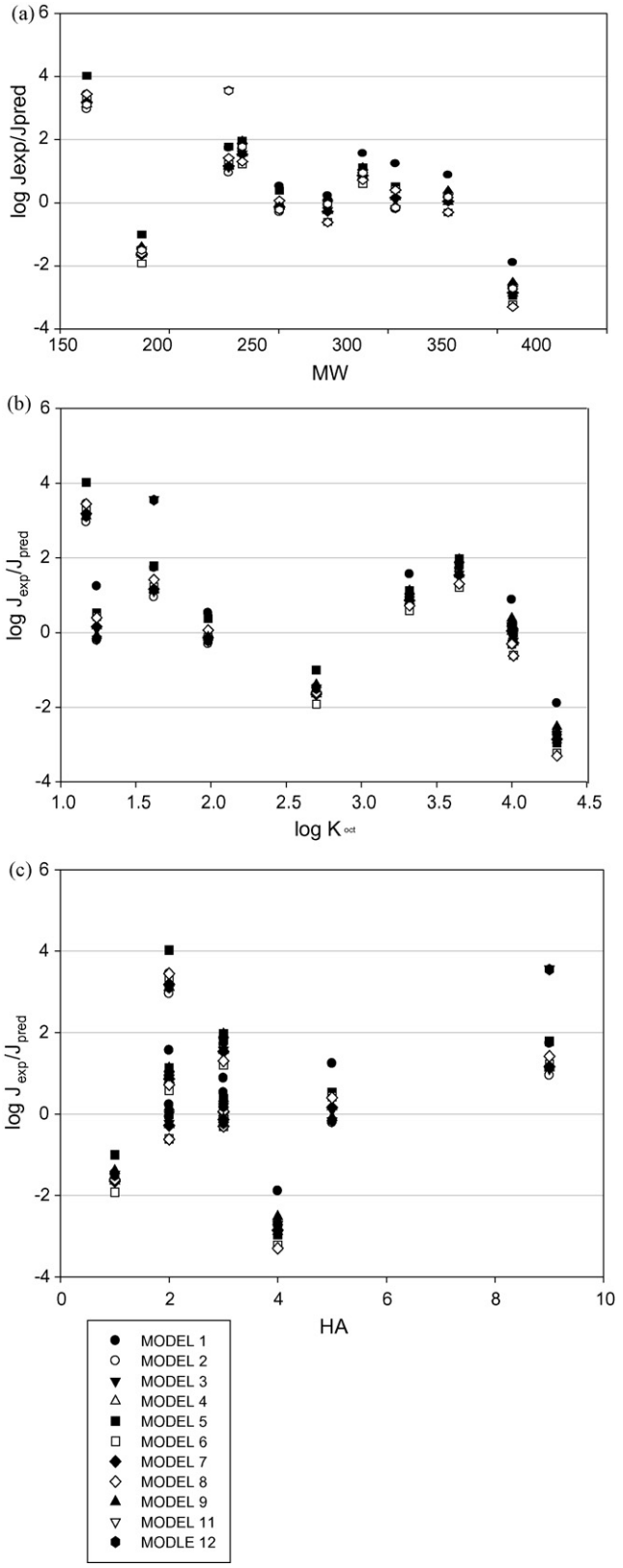

Fig. 1. Comparison of different models predictive power according to the observed in vivo data; correlations with physicochemical parameters (a) molecular weight, (b) logarithmically transformed octanol–water partition coefficient, and (c) number of hydrogen bond acceptor groups on the molecule. Most in vitro-based models underestimate the in vivo data by 1–100-fold. The discrepancy between observed data and prediction reaches the maximum (1000–10,000-fold underestimation) for nicotine (with the smallest molecular weight and $\log K_{oct}$), nitroglycerin (with the largest number of hydrogen bond acceptor groups), and for oxybutynin (with the largest molecular weight and $\log K_{oct}$) where there was a 1000-fold flux overestimation.

Some earlier studies in which human skin was used in vitro to predict absorption in vivo in human volunteers or primates, support this observation as they reported lower absorption in vitro than in vivo (Bronaugh and Maibach, 1985; Bronaugh and Franz, 1986; Guy et al., 1986). Underestimation of in vivo absorption by in vitro models has often been explained by the fact that lipophilic compounds are not sufficiently soluble in the receptor fluid used, thus absorption of these compounds is limited in the in vitro system. The lower solubility of lipophilic chemicals in aqueous receptor fluids can result in the development of a reservoir of test compound in the skin or in the stratum corneum in vitro which may not be formed in comparable in vivo studies (Wilkinson and Williams, 2005). It has been suggested that the receptor fluid and thickness of the skin section used in in vitro studies are critical for certain compounds if absorption in vivo is not to be underestimated in vitro (Wilkinson and Williams, 2005). These facts should be considered when analyzing the Flynn database, or any other in vitro skin permeability coefficient database, where the coefficients could have been measured under different experimental conditions.

Selegiline is the only drug in the range of $-2 < \log J_{exp}/J_{pred} < 2$ for which the flux is overpredicted by the in vitro-based equations. It has the smallest number of hydrogen acceptor groups among the studied molecules, which according to the in vivo-based model will result in lower C_{max} values. This is further supported by the fact that the flux is underestimated by two of the models (models 11 and 12) for nitroglycerin with the highest number of hydrogen bonding acceptor groups. Moreover, as the only evaluated model considering the effect of hydrogen bonding, model 10 highly overestimates selegiline flux, as it assumes a negative correlation between total number of hydrogen bonds and skin absorption (see Tables 1 and 2). Further mechanistic evaluations are needed to elucidate the influence of this molecular feature on blood concentration of drugs after transdermal administration.

Analyzing in vitro-based predictive models, Vecchia and Bunge (2002) suggested that the distinctive differences between equations are in their prediction effect of MW. They observed that nearly all of their studied equations reasonably predict the experimental data for chemicals of MW ~100 with some exceptions, and differences between model equations and between equations and experimental values increase with the MW of the penetrating chemical. This may explain the flux overestimation observed for the oxybutynin, with the highest molecular weight in the studied data set. Besides, it might be concluded that because of its high lipophilicity, the main route for its transdermal absorption, is diffusion through the intercellular pathway, as the highest degree of flux overestimation is made by model 6, which also considers a polar pathway for drug transport. Nevertheless, flux for the smallest molecule with a low $\log K_{oct}$, nicotine, has been largely underestimated by most in vitro-based equations.

Despite all the observed discrepancies, all the models except one (model 10) show good correlations with the observed data and the in vivo model. Several parameters may be involved in the deviation of in vitro and in vivo-based predictions. Our calculations are based on C_{max} instead of C_{ss}, since we assumed transdermal delivery systems provide steady blood concentrations over time and the difference between C_{max} and C_{ss} can be ignored. However, the original form of Eq. (3) assumes the steady state condition, where the drug input rate from its transdermal system is expected to be equal to its output rate, determined by total body clearance multiplied by the therapeutic plasma concentration. The variances between predicted and observed data might be less if C_{max} could be replaced by C_{ss}, which was unavailable for most of the drugs. The variability of the reported data for water solubility and partition coefficient in different resources, particularly for the drugs with low water solubilities, may also cause variations from the real predicted flux

values, since water solubilities are used for calculation of flux for in vitro-based models.

One important cause of variations between studied in vitro-based predictive models and the in vivo-based model, or observed data is the formulation effect. The development of more appropriate models for skin delivery will be required to focus on the effect of formulation type. Permeabilities from aqueous solutions alone are considered for the in vitro models derived form Flynn database. However, the observed data and in vivo model are based on the plasma concentration values reported for transdermal patches. Skin patches can be designed with hydrophilic or hydrophobic matrixes, which provide different drug release rates (Williams, 2003). Moreover, presence of small solvent molecules such as ethanol or propylene glycol, and/or occlusive backing layers will change the partitioning of the drug between the delivery device and the skin (Hadgraft and Guy, 2002). Advanced technologies such as DOT matrix – where the drug is blended into microscopic pockets uniformly dispersed throughout the patch's drug/adhesive layer – (e.g. Estradot and Daytrana) will also affect the flux and the blood concentration of drugs.

Riviere and Brooks (2005, 2007) presented an approach using hybrid QSPR relationships where absorption through porcine skin in flow-through diffusion cells is predicted by individual penetrants, coupled with a mixture factor (MF) that accounts for physicochemical properties of vehicle/mixture components. They showed that the use of the MF in combination with a classic QSPR model based on penetrant properties significantly improved the ability to predict dermal absorption of compounds dosed in complex chemical mixtures. Similar studies are needed using human skin to improve the predictability of currently used in vitro-based models.

Another limitation concerning the developed in vivo-based model is that the in vivo data is obtained from studies using different pharmacokinetic study design, sample size, and analytical techniques with different accuracies and precisions. Hence, same fluxes from in vitro and in vivo-based predictive models cannot be expected. Along with skin–blood transport of the molecule and the kinetic parameters affecting the blood concentration (such as protein binding and elimination), it can be concluded that the drug–vehicle interactions and physiochemical aspects of release are also reflected in the in vivo-based model.

Finally, complexity of physicochemical and physiological conditions of transdermal absorption and numerous other biological, physical and chemical factors may explain our findings.

Despite all the limitations, this is a step in developing empirical predictive models for transdermal drug delivery using clinical endpoints. Further studies, with the emphasis on dermatopharmacokinetic parameters such as AUC or C_{ss}, with consideration of the formulation effect, are needed to support these results. Validation and standardization of existing permeability coefficient data can help in making more appropriate estimations for human skin permeability. Validation of predictive models using the parallel observed in vitro and in vivo data will be useful in feasibility assessment in topical and transdermal delivery, before development of new transdermal drugs and/or vehicles.

5. Conclusion

Passive diffusion of a solute from its vehicle into the skin is determined by the unique molecular and physical properties of the diffusant, the vehicle, and the skin. The properties of these components, along with their interactions, largely determine chemical penetration. Effects of physicochemical factors on percutaneous penetration may extend beyond the permeation process (which involves drug–skin, vehicle–skin, and drug–vehicle interactions). Associations are probably more complex than simple linear numerical relationships.

This study compared the predictability of different in vitro-based mathematical models of skin permeation with the recently proposed in vivo-based model. Most in vitro models correlated well with the in vivo model and observed data; however, there are 0–4 orders of magnitude discrepancies observed in the expected and predicted values, which can be attributed to the end point differences and the vehicle effects. Further studies with the emphasis on dermatopharmacokinetic parameters such as AUC or C_{ss}, with consideration of the formulation effect, are needed to support these findings.

The practical implications of our observations for drug development and toxicology appear clear, and offer stimulus for refinement of our experimental data and mathematical interpretation.

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