Modelling dermal drug distribution after topical application in man

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Abstract

Purpose: To model and interpret drug distribution in the dermis and underlying tissues after topical application which is relevant to the treatment of local conditions. Methods: We created a new physiological pharmacokinetic model to describe the effect of blood flow, blood protein binding and dermal binding on the rate and depth of penetration of topical drugs into the underlying skin. We used this model to interpret literature in vivo human biopsy data on dermal drug concentration at various depths in the dermis after topical application of 6 substances. This interpretation was facilitated by our in vitro human dermal penetration studies in which dermal diffusion coefficient and binding were estimated. Results: The model shows that dermal diffusion alone cannot explain the in vivo data and blood and/or lymphatic transport to deep tissues must be present for almost all of the drugs tested. Conclusion: Topical drug delivery systems for deeper tissue delivery should recognise that blood/ lymphatic transport may dominate over dermal diffusion for certain compounds.
Introduction

Drugs and other solutes are frequently applied to the skin for a range of purposes including the treatment of local dermatological disorders, systemic delivery (e.g., nicotine, nitroglycerin, fentanyl patches), supportive treatment of local muscle injuries, cosmetic, UV protection and insect repellents. This interest in topical applications of pharmaceutical and cosmetic products generated significant research effort into measurement, modelling and prediction of the rate of solutes penetration through the skin. The research effort into understanding the distribution of topically applied solutes in underlying tissues was relatively modest, confounded by inability to recreate in-vivo conditions using in vitro experiments (1) and the invasiveness associated with the collection of such data using biopsy. Most prominent in this regard is the work of Schaefer and colleagues who obtained human tissue concentration-depth profiles of various drugs in-vivo after topical application (1-5).

As studies on skin solute concentration-tissue depths profiles after topical application in man are, in general, limited due to the invasiveness associated with the collection of such data, mathematical modelling and prediction of concentration-depths profiles for different drugs is of importance in dermatology. Singh and Roberts (6, 7) have used compartmental pharmacokinetic model assuming first-order diffusional mass transfer between the dermis and underlying tissues compartments with its concurrent elimination due to blood flow to model salicylic acid distribution in rat after topical application. Later Gupta et al (8) successfully modelled spatial distribution of 2',3'-dideoxyinosine in the dermis of a rat using a distributed elimination model. Cross & Roberts (9) used a tissue diffusion-dermal blood flow clearance model similar to the model of Gupta et al (8) but taking into account declining concentration in the donor phase to describe drug distribution kinetics in wound tissue after topical application. Recently, the data of Singh and Roberts (7) was reanalysed with a similar distributed diffusion-clearance model by Kretsos et al (10) and found to be consistent with this model. Kretsos and Kasting (11) also developed a new model to describe the dermal capillary clearance process based on assumed periodic microscopic distribution of dermal capillaries in three dimensional space. This model yields localised concentration in the dermis, but so far only applies to the steady state case.

In this work, we developed a new model that takes into account transport to deeper tissues by blood and/or lymphatics. The model also includes the potential contribution to transport processes by
dermal diffusion and vascular wall permeability. We use a combination of human biopsy data generated by Schaefer and colleagues (1-5) and our own human in-vitro dermis penetration experiments to obtain two critically important parameters of the distributed model: dermis diffusion/dispersion coefficient and dermal blood clearance rate for six solutes - Desoximetasone (Des), Econazole (Ec), Hydrocortisone (Hyd), 8-Methoxypsoralen (Met), Retinoic acid (RA), Triamcinolone acetonide (TA) (molecular weight, solubility and other physicochemical properties of this solutes are presented in Table 1).

Theory

In vivo dermal distribution model

The distributed elimination model (8, 9, 12) is often used to describe dermis concentration-depth profiles and is similar to the model previously introduced for peritoneum by Dedrick et al. (13). These previous studies have assumed all solute transport to deeper layers occurs by molecular diffusion. The distributed elimination model defines concentration in the dermis by the diffusion equation with elimination:

$$\frac{\partial C_d}{\partial t} = D \frac{\partial^2 C_d}{\partial x^2} - k_e C_d$$

where $C_d(x,t)$ is the concentration of the solute in the layer of the dermis at depth $x$ at time $t$, $D$ is the effective molecular diffusion coefficient in the dermis and $k_e$ is an assumed elimination rate from the dermis. An alternative to the molecular diffusion of the solute is that $D$ is a dispersion term used widely in chemical engineering (14) and in organ pharmacokinetics (15) and defines transport by both blood and diffusion in the dermis. Partitioning of solute into blood capillaries of the dermis and its subsequent convective transport and partitioning back into the tissue could also significantly contribute to the spatial transport of the solute. Blood in the tissue capillaries can flow in all possible directions, it can be argued that this repartitioning and convective transport of the solute will be similar to a random walk process. The lymphatic flow could also be a contributing factor to the solute tissue transport. In order to recognise these facts $D$ in equation 1 needs to be replaced by $D_t$:

$$D_t = D_v + D$$

where $D_t$ is the contribution to the transport from blood and/or lymphatics. If the repartitioning and convective transport or lymphatic transport do contribute significantly to the transport of solute, $D_t$ will be much greater than molecular diffusion coefficient ($D$). If the transport is dominated by
molecular diffusion, then $D_t \approx D$. For the solute that is bound in the dermis, and only unbound solute is diffusing, the effective diffusion coefficient is defined as:

$$D = D_u f_{u_d}$$

where $D_u$ is the diffusion rate of unbound solute.

In equation 1, it is implicitly assumed that the elimination rate ($k_e$) is due to blood flow clearance, but hitherto no connections to physiological parameters made it difficult to analyse physiological meaning of this parameter. In order to relate this elimination rate to such physiological parameters as blood flow in the dermis and permeability surface area of blood capillaries in the dermis we consider a simple two compartmental approach to diffusion/dispersion in the dermis and elimination by blood flow in the small volume of the dermis ($V_d$) at depth $x$ (see Fig 1). Equations which describe the diffusion/dispersion in the dermis compartment and partitioning into the adjacent blood compartment with subsequent elimination are:

$$V_d \frac{\partial C_d}{\partial t} = V_d D_t \frac{\partial^2 C_d}{\partial x^2} - PS(C_d f_{u_d} - C_b f_{u_b})$$  

$$V_b \frac{dC_b}{dt} = -Q_b C_b + PS(C_d f_{u_d} - C_b f_{u_b})$$

where $V_b$ is the blood volume adjacent to the dermis, $Q_b$ is the blood flow rate in the volume $V_d$, and $PS$ is the permeability surface area product (PS, sometimes abbreviated as PA) which is a standard term in pharmacokinetics that consists of the permeability coefficient ($P$) often used to describe the transport of various solutes across the capillary wall multiplied by the surface area of the wall ($S$) in the volume $V_d$. Further, $C_b$ is the concentration of solute in blood compartment, $f_{u_d}$ and $f_{u_b}$ are fractions of solute unbound in the dermis and blood. In equation 5 it was assumed that fresh arterial blood reaching the skin does so without any solute present ($C_{b0}=0$). The boundary conditions for $C_d(x,t)$ are:

$$-D_t \frac{\partial C_d}{\partial x} \bigg|_{x=0} = J_0$$

$$\lim_{x \to +\infty} C_d(x,t) = 0$$

and represent the flux ($J_0$) through the epidermal/dermal juncture (equation 6) and diminishing concentration in the deeper layers (equation 7).

It is important to emphasise that equations 4 and 5 do not take into account the complex physiology of blood circulation in the skin (see Fig 1A). The model that has a more detailed consideration of
the capillaries in dermal clearance was developed by Kretsos and Kasting (11), but only applies to
the steady state case. The presented model can be viewed as a simplification of the approach of
Kretsos and Kasting (11), which allows to model transient kinetics.

Partial differential equations 4 and 5 with boundary conditions 6 and 7 could be solved in Laplace
domain to yield:

\[
\hat{C}_d(x,s) = \frac{J_0}{s \sqrt{(s + g(s))}} \exp\left( -x \sqrt{(s + g(s))/D_t} \right)
\]

where \( \hat{C}_d(x,s) \) is the Laplace transform of \( C_d(x,t) \) and \( s \) is the Laplace variable. Further, the
function \( g(s) \) in equation 8 is defined as:

\[
g(s) = \frac{fu_bq_bps + psfu_dv_b}{q_b + fu_bps + v_b}
\]

where \( q_b \), \( ps \) and \( v_b \) are the blood flow rate, the permeability surface area product for blood
capillaries and blood capillaries volume per unit volume of dermis, respectively. In equation 8 the
flux through epidermis (\( J_0 \)) is assumed constant, but replacing \( J_0 \) with \( J_0(t) \) in equation 6 and then \( J_0 \)
with \( \hat{J}_0(s) \) in equation 8 allows the analysis for the case of the transient flux through epidermis.

For the steady state case the time derivatives in eqs 4 and 5 are zero and \( C_b \) can be expressed in
terms of \( C_d \) using equation 5, these can be substituted into equation 4 to yield:

\[
D_t \frac{\partial^2 C_d}{\partial x^2} - \frac{fu_dQ_bPS}{(Q_b + fu_bPS)W_d} C_d = 0
\]

Hence, at steady state, the form of the derived two compartmental dermal distribution model is
identical with the distributed elimination model (equation 1), except that the elimination rate can
now be defined in terms of physiological parameters as follows:

\[
k_e = \frac{fu_dQ_bPS}{(Q_b + fu_bPS)W_d} = \frac{fu_dq_bps}{q_b + fu_bps}
\]

Kretsos et al (12) arrived at a similar expression for \( k_e \) but did not recognise the effect that solute
binding may have on the elimination rate.

It follows from equation 10 that when penetration of the solute into the blood capillary is the rate
limiting process, that is \( fu_b ps \ll q_b \), \( k_e \) will be determined by \( ps \) so that equation 10 simplifies to \( k_e \)
being dependent on permeability surface area for blood capillaries \( ps \):
\[ k_e = f u_d ps \]  

On the other hand, when blood flow \( q_b \) is rate limiting, \((f u_d ps >> q_b)\), so that now equation 10 simplifies to \( k_e \) being dependent on tissue blood flow \( q_b \):

\[ k_e = q_b f u_d / f u_b \]  

Solving equation 9 with boundary conditions 6 and 7 yields the concentration of solute in the dermis for the steady state:

\[ C_{ss}(x) = C_0 \exp(-k_d x) = \frac{J_0}{\sqrt{k_d D_t}} \exp(-x \sqrt{k_e / D_t}) \]  

We note that equation 13 can also be obtained by taking a limit \( C_{ss}(x) = \lim_{s \to 0} \hat{C}_d(x, s) \) where \( \hat{C}_d(x, s) \) is defined in equation 8. Using equation 10 the distribution parameter \( k_d \) can be defined in terms of physiological parameters of the model:

\[ k_d = \frac{f u_d q_b ps}{D_t(q_b + f u_b ps)} \]  

**Methods**

**Materials** All chemicals (Des, Ec, Hyd, Met, RA, TA) were purchased from Sigma-Aldrich Chemie Gmbh (Steinheim, Germany).

**Dermis preparation technique** Female abdominal skin was obtained following abdominoplasty from two donors. Extraneous fat and subcutaneous tissue from the underside of the skin was removed by blunt dissection. Dermal membranes were prepared by removing epidermis using heat separation (immersion in water at 60°C for 1 min) from full-thickness skin.

**In vitro penetration studies** All studies were carried out in horizontal static Franz-type diffusion cells (receptor volume approximately 3.5 ml, surface area approximately 1.3 cm²). Receptor compartments were filled with phosphate-buffered saline, pH 7.4, containing 4% bovine serum albumin (further referred to as receptor solution), maintained at 36°C in a water bath and continuously stirred with magnetic flasks. In this work, 4% bovine serum albumin was used to mimic typical albumin concentrations present in the blood, with identical concentrations being used on both sides of the dermis to avoid creating an albumin concentration gradient that could potentially lead to an osmotic flow of water through the dermis. Dermal membranes were
equilibrated overnight at room temperature with receptor solution on both sides of the membrane before the start of experiments. Saturated solutions of chemicals in the receptor solution were used as donor solutions and were produced by stirring in orbital shaker excess amount of the chemical for at least 10h. One ml of donor solution was applied at the start of the penetration experiment and aliquots of 200μl were taken from the receptor and replaced with fresh solution. Samples were then analysed by HPLC. This data were analysed by fitting the steady state penetration equation to the steady state section of the cumulative amount penetrated \( Q(t) \) vs. time experimental curve (17):

\[
Q(t) = k_p C_0 A(t - t_{\text{lag}})
\]

where \( C_0 \) is the concentration of solute in the donor solution, \( A \) is the area of the dermis, \( k_p \) is the dermal permeability coefficient and \( t_{\text{lag}} \) is the lag time. The linear regression of the steady state portion of the curve yielded two parameters \( k_p \) and \( t_{\text{lag}} \). These parameters are, in turn, defined by the partition coefficient between donor solution and the dermis \( (K_m) \), the thickness of the dermis \( (h) \) and effective diffusion coefficient in the dermis \( (D) \) as: (17)

\[
k_p = \frac{K_m D}{h}
\]

\[
t_{\text{lag}} = \frac{h^2}{6D}
\]

At the end of the penetration experiment, diffusion cells were dismantled and the thickness of a dermal membrane \( (h) \) was determined by placing them between glass slides and measuring with callipers. Sections of the membrane exposed to the donor and receptor solutions was cut, weighed and put into 10ml of fresh receptor solution. This solution was sampled after at least 16 hours, its concentration measured with HPLC and amount of drug in the dermis determined. The partition coefficient between donor solution and the dermis \( (K_m) \) was determined from the amount of solute in the dermis \( (A m_{\text{derm}}) \) of mass \( m_{\text{derm}} \) after the penetration experiment assuming linear concentration gradient in the dermis (17):

\[
K_m = \frac{2A m_{\text{derm}} \rho_{\text{derm}}}{C_0 m_{\text{derm}}}
\]

where \( \rho_{\text{derm}} \) is the density of the dermis assumed to be 1g/ml. Using eqs (16-18) diffusion coefficient was determined from both \( k_p \) and \( t_{\text{lag}} \). We note that factor 2 in (18) arises from the fact that concentration in the dermis reaches steady state linear profile with concentration in contact with the donor being \( K_m C_0 \) and zero concentration at the contact with the receptor (sink condition).
**Solubility measurements** For receptor solution solubility ($S_r$) measurements, saturated donor solutions produced by stirring in orbital shaker excess amount of the chemical for at least 10h were filtered (Millipore Millex Syringe Driven Filter, 0.22 μm) diluted 5 times with receptor solution and measured with HPLC. Solubilities in human plasma ($S_p$) were measured similarly. To estimate solubility in human blood ($S_b$), partitioning between plasma and red blood cells (RBC) was measured: 1ml of plasma with known drug concentration was mixed with 1ml of RBC and incubated for at least 12 h and then plasma concentration was measured. The total amount and concentration in RBC was then calculated and solubility in blood approximated assuming hematocrit of 0.5. The fraction unbound in the dermis ($f_{ud}$) and blood ($f_{ub}$) were estimated as the ratio of solubility in the water at pH=7.4 ($S_{w\ pH=7.4}$) to solubility in the dermis ($S_d=K_mS_r$) and solubility in the blood ($S_b$) respectively.

**Analysis of published in vivo study data.** Data was extracted from the published literature using Data Thief III ([http://www.datathief.org/](http://www.datathief.org/)). In accordance with equation 13, data was expressed as a log-linear relationship of log of concentration vs. distance profiles for all solutes with the distribution parameter $k_d$ determined as the negative value of the slope of the resulting linear relationship for steady state data. Nonlinear regressions of data were performed using the program SCIENTIST (MicroMath Scientific software, Salt Lake City) with weighting of $1/y_{\text{observed}}$.

**HPLC analysis** BSA in 200μl receptor samples was precipitated using 300μl of ACN with 50 μg/ml of internal standard (TA for Des, Met and Hyd; Des for Ec and TA; Benzyl Salicylate for RA). Samples were then centrifuged (Clements Orbital 100, Clements Medical Equipment Pty Ltd, Rydalmere, NSW, Australia) for 6 min at high speed and supernatant analysed by HPLC system (Shimadzu, Kyoto, Japan). The flow rate of 1ml per minute was always used through a reverse phase C18 column (Waters® Symmetry C18, 5 μm, 150×3.9 mm) including a guard column (Phenomenex® C18, 4×3 mm) and 20μl of sample was injected. Mobile phase was 40%ACN 60% Phosphate buffer (20mM KH$_2$PO$_4$ at pH=3 adjusted with H$_3$PO$_4$) for Ec, 35%ACN 65%Water for Hyd, 80%ACN 20% Acetate buffer (5mM NaC$_2$H$_3$O$_2$, pH=2.7 adjusted with acetic acid) for RA and 40%ACN 60%Water for other solutes. Retention times were: 4.5, 6.6, 2.7, 4.1, 7.5 and 3.5 minutes for Des, Ec, Hyd, Met, RA and TA respectively. Detection wavelengths were for Ec 230nm, Hyd 254nm, RA 354nm and 245nm for other solutes.
Results

In Fig 2, human literature data for concentration-depth profiles of 6 solutes are presented together with the results of the regression with the steady state model (equation 13 with fitting parameters $C_0$ and $k_d$, solid line). Values for $k_d$ from the regressions using the steady state model are presented in Table 2. The regression with the steady state model yields satisfactory fits for most solutes, although the quality of the regression varies: there is lower regression quality for Desoximetasone (Coefficient of determination: $cd = 0.54$), Retinoic acid ($cd = 0.33$), Triamcinolone acetonide ($cd = 0.39$) (poor regression quality is apparently due to larger variability of experimental data for these solutes) and is better for Ec, Hyd and Met, with coefficients of determination 0.84, 0.91, 0.94 respectively. It follows from the analysis of the new model (see equation 14) that $k_d$ depends on different physiological parameters, such as $D_t$, $ps$, $fu_b$, $fu_d$ and $q_b$ and for further investigation some of these parameters should be determined independently. This underpinned the necessity of investigating physicochemical properties of solutes together with human dermis in vitro experiments. In Table 1 physicochemical properties of solutes obtained from PhysProp database (Syracuse Research Corporation) and in our experiments are presented. It can be seen that the solutes cover a reasonably large range in terms of solute molecular weight (MW), logarithm of the solute’s octanol/water partition coefficient (LogP) and solute solubility in water ($S_w$). We have measured solubility in the receptor/donor solution ($S_r$) in blood plasma ($S_p$) and in blood ($S_b$). Two solutes are partially ionised at the experimental conditions (pH=7.4): Ec is a base, and RA is an acid, and therefore their effective solubility in the buffer at pH=7.4 will be higher than that in water, especially for the acid RA.

It can be seen from Table 1 that the solubility in plasma for all solutes with the exception of Ec is higher but close to that in the receptor phase, which is consistent with receptor phase bovine serum albumin concentration being similar to that of blood. The much higher solubility in plasma and blood for Ec is probably due to its strong binding to some proteins other than albumin (which is present in the receptor phase).

Dermal partition, permeability and diffusion coefficients are derived from our in vitro dermal penetration studies and presented in Table 2. It should be noted that $K_m$ is the partitioning coefficient between dermis and the donor solution that contains 4% bovine serum albumin and that this partitioning coefficient can differ from $K_{dermis/water}$, especially for strongly protein bound solutes. Diffusion coefficients were derived from both lag time and permeability coefficient and
were found to be similar with the exception of Met, for which there is about a factor of two difference between the two values. The fraction unbound in the dermis and blood were estimated as the ratio of solubility in the water at pH=7.4 (Table 1) to solubility in the dermis \( (S_d = K_m S_t) \) and solubility in the blood \( (S_b) \) respectively. Two of the solutes in Table 2 (Hyd and Met) were previously analysed by Kretso et al (12), using the same human data ((3) for Hyd and (5) for Met). They reported similar values for \( k_d \) (referred in (12) as decay parameter \( E \)) to what we estimated in our analysis (i.e. \( k_d \) values of 43cm\(^{-1}\) vs 48 cm\(^{-1}\) for Hyd and 21cm\(^{-1}\) vs 24cm\(^{-1}\) for Met).

Fig 3 shows dermal diffusion coefficients \( (D) \) plotted versus fractions of solute unbound in the dermis \( (f_{ud}) \). It can be seen that, with the exception of Des, all the solutes roughly fall onto the straight line in agreement with equation 3 with unbound diffusion coefficients being about the same for all solutes \( (D_u \approx 10^{-6} \text{cm}^2\text{s}^{-1}) \). This is expected as \( D_u \) can be approximated by the solute diffusion in water which is expected to be proportional to \( MW^{-\frac{1}{2}} \) (18) (in (18) it is stated that diffusion coefficient is proportional to \( MV^{-0.6} \), where MV is Molecular Volume; for simplicity we used an approximation \( MW^{-\frac{1}{2}} \) here). Further, as the square root of molecular weight of solutes does not change significantly, \( D_u \) is expected to be roughly the same for all solutes. The deviation of diffusion coefficient for Des from the trend could be explained by its binding to some component of the skin capable of diffusing. This binding would decrease Des’s fractions unbound in the dermis \( (f_{ud}=0.054, \text{see Table 2}) \), but not reduce its mobility/diffusion coefficient to the extent predicted by equation 3, as this equation assumes that only unbound solute is diffusing.

In Fig 2, it was assumed that the steady state was achieved at the time of data collection. This will only be true, if \( D_t \) is large enough. In previous studies (8), (9, 10), it was assumed that the transport in the dermis is solely due to solute diffusion \( (D_t=D) \). To test this assumption we substituted \( D_t=D \) in equation 8 and applied this transient model to the human biopsy data. The results of these regressions are shown in Fig 4. During the regressions parameters \( D, f_{ub} \) and \( f_{ud} \) for solutes were fixed to the values taken from Table 2 and \( q_b \) and \( v_b \) were fixed to their physiological values: \( q_b =0.0005\text{s}^{-1}, v_b = 0.1. \) (19) Parameters \( J_0 \) and \( p_s \) were obtained by fitting the data. It can be seen in Fig 4 that the transient model fits were not satisfactory for four solutes (Ec, Met, RA and TA) for which application times are relatively short (about 100 minutes). In all these unsatisfactory regressions the transient model fails to fit concentration in the deeper layers of the dermis. This failure is most pronounced for RA. The transient model regression for Des and Hyd produced
straight lines identical to the steady state model, which is expected as the application time was very long for these solutes (1000 minutes). The unsatisfactory regression of the transient model for deeper layers is due to the assumption of the slow diffusional transport to deeper layers \(D_t = D\) and could not be resolved by varying parameters \(q_b\) and \(v_b\) (fits not shown). We are consequently forced to assume that the dermal transport to deeper layers is faster than molecular diffusion alone and therefore \(D_t \gg D\). The assumption of fast dispersion transport leads to the conclusion that the solutes reach the steady state relatively early (before 100 minutes) and indeed, as discussed above, provides satisfactory fits for most solutes (Fig 2).

In Figs 5A and 5B the data for parameter \(k_d\) (Table 2) was analysed using equation 14, where it was assumed that permeability-surface area per unit volume of dermis is proportional to the octanol/water partitioning, that is \(p_s = A \times 10^{\log P} = A \times P\), where \(A\) is a fitting parameter. In Fig 5A another fitting parameter was \(q_b\), while \(D_t\) was fixed to the molecular diffusion coefficient. It can be seen that the quality of regression is very poor, confirming the conclusion of the above analysis that transport cannot be explained by molecular diffusion alone. In Fig 5B similar regression was performed, but \(D_t\) was given a chance to be higher, corresponding to \(D_t\) determined by blood/lymphatic transport, or equal to diffusion coefficient. In order to reduce the number of fitting parameters it was assumed that the dispersion coefficient is the same for all solutes. The regression was significantly better compared to the case of \(D_t = D\) and resulted in all but one solute (Hyd) \(D_t\) determined by the dispersion transport with \(D_{disp} = 5 \times 10^{-6}\) cm\(^2\) s\(^{-1}\). Other fitting parameters were: \(A = 1\) and \(q_b = 0.0014\) ml s\(^{-1}\) per ml of dermis, which indicates that for all solutes \(p_s f_{ab} \gg q_b\), that is clearance is blood flow limited as described by equation 12. Some deviation of data in Fig 5B is expected; given that the simplifying assumptions of equal blood flow in all the experiments (which were conducted in different subjects) was used to fit the data. The blood flow rate obtained in the regression \((q_b = 0.0014\) ml s\(^{-1}\) per ml of dermis\) is in reasonable agreement with literature data, given the variability of dermal blood flow depending on subject and experimental conditions. (19)

Discussion

This work shows that, for the compounds studied here in human dermis in vivo, the transport of the compounds into deeper tissues after topical application must involve transport into those tissues via the blood and/or lymphatics as well as by diffusion and that this transport cannot be described by dermal diffusion alone. In order for convective blood flow transport to make a significant contribution to transport to deeper tissues, there must be sufficient binding to plasma proteins and
blood flow as the surface area of blood vessels is much less than that for the dermal matrix through which diffusion transport will occur. Hence, the contribution of dermal blood flow transport is likely to be markedly reduced when there is vasoconstriction. Others have previously assumed that only molecular diffusion in the dermis have contributed to penetration to deeper layers of dermis, and therefore used the simplified model (as defined in equation 1) to model tissue concentration-distance profiles (8-10). In general, molecular diffusion was assumed a priori, in order to enable modelling in the steady state. This assumption, in turn, leads to a strong correlation between diffusion/dispersion parameter and the elimination parameter. Resolution of the actual value for either parameter is only possible if one of the parameters, dispersion or elimination, is defined and fixed. In this work, we used the experimentally obtained in vitro dermal diffusion coefficient as the independently determined parameter.

The modelling dilemma addressed here is similar one that reported in the hepatic elimination literature several decades ago. (20) Here, hepatic extraction data was controversially attempted to be fitted by either a well-stirred model or a tube model. An important outcome was that the intrinsic clearances obtained for highly extracted drugs estimated by the well stirred model was several orders of magnitude different to physiologically expected values. When the actual enzymatic clearances and blood flow patterns were taken into account, a more realistic convection – dispersion model was derived. (15, 21) The analysis in this paper is somewhat analogous, in that our in vitro diffusion constants and realistic values of dermal blood flow were used to uncouple inter-dependencies between parameters. This was possible due to elimination rate ($k_e$ in equation 1) in our modelling was defined for the first time in terms of physiologically based parameters such as the blood flow rate ($q_b$), the permeability surface area of blood capillaries ($p_s$), fractions of solute unbound in the dermis ($f_{ud}$) and blood ($f_{ub}$). This allowed us to analyse dependence of elimination rate and diffusion/dispersion coefficient on the physiologically based parameters. It was concluded that the previously published in vivo human experimental data are not consistent with the assumption of transport in the dermis due to molecular diffusion only and the dispersion coefficient, which is about an order of magnitude larger, have to be used instead. Our analysis suggests that transport occurs mostly by blood/lymphatic flow rather than molecular diffusion explaining the transport of solutes to deeper tissues, as assumed previously. (8-10)

We note that the model, as formulated in this work, is somewhat a simplification of the physiological dermal transport and clearance. Most significantly it was assumed that the blood flow rate per unit volume of dermis ($q_b$) is a constant parameter. Recent work on spatial distribution of
dermal circulation (22) suggests that $q_b$ decreases quasi-exponentially to a certain depth and is site and skin condition dependent. As this spatial dependence will prohibit the simple analysis of data with our model, it was considered beyond the scope of this paper. It has to be noted that this simplification will not affect the main conclusion of this paper that transport to deeper tissues is not by diffusion alone, as blood flow rate ($q_b$) only contributes to clearance ($k_c$) but not to diffusion coefficient ($D$). Further simplification is that, similar to the modelling of liver clearance (15), dispersion was assumed to be a constant parameter independent of other physiological parameters of the model. We have also previously related the dispersion coefficient used to describe liver elimination and transport directly to the morphology of the system and physiologically related parameters (23). The model described here for dermal transport and clearance could, in due course, be further developed in more precisely to be related to the detailed morphology and physiology of the dermis and its blood vessels. However, the present model does give a global view of transport of solutes in the dermis in vivo being potentially either limited by dermal blood flow or by molecular diffusion in the dermis.

The proposed global model for dermal transport could be further complicated by including the concurrent transport by lymphatic flow. The extent of the contribution to the dispersion parameter from the lymphatic transport can be gained by simple analysis of data for the transport and clearance of isothiocyanate-dextran (MW=150kDa) in human skin. (24) Figure 2 in this work shows, as a consequence of lymphatic dispersion, isothiocyanate-dextran spreads radially in the dermis a distance $\Delta d \approx 0.3$ cm (as approximated from the figure) after $t=24$ hours following intra-dermal injection. Accordingly, when a simple random walk/diffusion/dispersion process is assumed, $\Delta d \approx \sqrt{D_{\text{lymphatic}}t}$, yielding a lymphatic dispersion estimate of: $D_{\text{lymphatic}} \approx 10^{-6}$ cm$^2$s$^{-1}$. If this dermal dispersion is indicative also of the axial lymphatic transport to deeper tissues, it is more than a factor of two higher than the fastest dermal diffusion coefficient measured in this work ($D = 4.3 \times 10^{-7}$ cm$^2$s$^{-1}$ for hydrocortisone, see Table 2). Such a difference in dispersion would be consistent with a lower variation in dermal blood flows and vessel dimensions than found for lymphatic vessels.

In this work, we have limited our analysis of dermal distribution to data obtained from human experiments after a topical application to solute concentration profiles in the dermis obtained from skin biopsy. We felt that biopsy represents a “gold standard” in measuring dermal distribution of solutes. Human dermal distribution data is also available from microdialysis experiments and we
have analysed this data separately, with similar findings. The nature of cutaneous microdialysis is such that there are additional substantial experimental and data interpretation considerations are beyond the scope and the focus of this paper. Hence, we are seeking to analyse this in a separate paper. A limitation of the present analysis is the assumption of no contribution of topically absorbed drug into the systemic circulation contributing to the underlying tissue concentrations on recirculation. Our previous work has suggested that this only occurs at long times and then with most substantial contribution to tissue concentrations deep below the applied site (25), relative to the superficial tissues close to the topical application site, as studied here.

Conclusions

In this work, dermal disposition of solutes after topical application was investigated. A new two compartment dermal clearance model that includes both transport by dermal blood vessels and by dermal diffusion was introduced to better relate dermal transport to the known dermal morphology and physiology in vivo. A key outcome of our analysis is that the molecular diffusion of solutes in the dermis is insufficient to alone explain solute transport to the deeper layers of dermis in vivo. When the contribution of dermal transport to the deeper layers due to blood or lymphatic transport is included, consistency is obtained between observed and previously described in vivo literature data.

Acknowledgements

We are grateful to the financial support of the National Health & Medical Research Council of Australia and the Queensland and New South Wales Lions Medical Research Foundation. We also acknowledge Prof Hans Schaefer’s advice in relation to experimental data in his work.

References

**Figure legends**

Fig 1. Skin (A) and its schematic representation in the model (B).

Fig 2. Experimental data for dermal distribution of Desoximetasone (from (5), fig 5 after 1000min), Econazole (from (1), fig 2 application thigh after 90min), Hydrocortisone (from (3), fig 1 after 1000min), 8-Methoxypsoralen (from (5), fig 6 after 100min), Retinoic acid (from (2) fig 1 after 100min), Triamcinolone acetonide (from (6) fig 1 after 100min) and regression curves with the steady state model (equation 13). Coefficients of determination (cd) for each of the solute's regression and number of data points are: Desoximetasone cd=0.54, n=40 ; Econazole cd=0.84, n=28; Hydrocortisone cd=0.91, n=20; 8-Methoxypsoralen cd=0.94 , n=33; Retinoic acid cd=0.33, n=22; Triamcinolone acetonide cd= 0.39, n= 28.

Fig 3. Experimental diffusion coefficients versus fraction unbound in the dermis data. Solid line represents a regression with equation 3 where $D_u$ is constant.

Fig 4. The experimental data as in Fig 2 with regression curves using the transient model (equation 8 with $D_t = D$, dashed line). For Desoximetasone and Hydrocortisone the transient and steady state models produced identically result as for these solutes application time is very long (1000min). Coefficients of determination (cd) for each of the solute's regression are: Desoximetasone cd=0.54; Econazole cd=0.77; Hydrocortisone cd=0.91; 8-Methoxypsoralen cd=0.89; Retinoic acid cd=0.30; Triamcinolone acetonide cd=0.39.

Fig 5. Experimental $k_d$ versus theoretically predicted $k_d$ using equation 14 for cases of $D_t = D$ (A) and $D_t = D_{disp}$ (B).

**Tables**

Table 1. Physicochemical properties of solutes

Table 2. Solute model parameters for transport in the dermis