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Mechanistic and empirical modeling of skin permeation of drugs

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Abstract

The skin forms a barrier to the external environment, maintaining body fluids within our system and excluding harmful substances, while the skin is a site of administration of drugs for topical and systemic chemotherapy. It is an important issue to predict the rate at which drugs or other xenobiotics penetrate the skin. In this article, we review modeling approaches for predicting skin permeation of compounds, including both mechanistic and empirical approaches. Mechanistic approaches can give us much information on understanding of skin permeation of the compounds, such as structure–permeability relationship, contribution of each barrier step, mechanism of penetration enhancers, and in vivo–in vitro relationship. On the other hand, empirical modeling can overcome any inaccuracies of mechanistic models caused by the existence of uncertainties and, therefore, give us better predictions from the practical point of view. Artificial neural networks are being available for empirical modeling of complex skin transport phenomenon.

Keywords: Mechanistic modeling; Empirical modeling; Skin permeability; Diffusion model; Structure–permeability relationship; Transport pathways; Artificial neural networks

Contents

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1. Introduction: mechanistic and empirical approaches

The skin forms a barrier to the external environment, maintaining body fluids within our system and excluding harmful substances. On the other hand, the skin is a site of administration of drugs for topical and systemic chemotherapy. In the pharmaceutical, cosmetic and agrochemical fields, therefore, it is an important issue to predict the rate at which substances penetrate the skin.

The transport of a substance across biological membranes such as skin is a complex phenomenon comprising physical, chemical, and biological interactions, many of which are essentially nonlinear. Nevertheless, data-based stochastic models are not so many as rule-based mechanistic models in this research field. This anomaly would be due to the successful history of physical science over the last century. Modeling in deterministic terms has permeated scientific endeavor and led to a pattern of scientific investigation that is reductionistic in nature. Such deterministic reductionism is based on a belief that physical systems can be described, if not exactly, by deterministic mathematical equations based on well-known scientific laws. With respect to skin transport of a solute, a Fick's diffusion law has generally been accepted, where diffusion is assumed to be a process of mass transfer of individual molecules, brought about by random molecular motion. The distribution law is adopted to define the rate of transport against a measurable donor concentration, not the concentration at the membrane. Thus, the rate of transport is expressed as,

$$\frac{dM}{dt} = \frac{KD}{L} C_0$$

where C_0 is the donor concentration; K is the partition coefficient; D is the diffusion coefficient; and L is the thickness of the barrier. Such representation led us to link skin transport of a solute with their physicochemical properties.

These mechanistic models are basically extensions of our mental models and perceptions of the real phenomenon rather than necessarily accurate representations of the real phenomenon itself. Although these models provide insights into mechanistic relevance of an observed phenomenon, they frequently sacrifice the precision of the representations due to overlooked existence of uncertainties. On the other hand, empiricism is a philosophical approach that the only valid form of knowledge is that which is gathered by use of the senses; explanations are based on actual observations, rather than theoretical statements. Empirical models are able to overcome any inaccuracies of mechanistic models caused by the existence of uncertainties. To discuss superiority either of mechanistic models or of data-based empirical models is a philosophical issue towards perceptions of the real phenomenon. Not only the modelers but the users would give different answers, according to a diversity of their intellect or interest. If a mechanistic model with a reasonable accuracy of prediction is available, it will be no reason to discard it in favor of a data-based one.

The aim of this article is to review mechanistic and empirical modeling of skin transport. Mechanistic models can help us to understand barrier characteristics of skin; in particular, diffusion models are useful for optimizing the design of drugs and their formulations from the physicochemical point of view. On the other hand, data-based empirical models, including artificial neural networks, are also available to predict skin permeability of structurally diverse compounds with reasonable accuracy of prediction.

2. Mechanistic analysis of skin permeation of drugs

2.1. Processes of percutaneous absorption of drugs

The skin is composed of three layers; epidermis, dermis, and subcutaneous tissues. The stratum corneum, the outermost layer of epidermis, is the primary barrier against permeation of topically applied drugs. The interior of the cornified cells consists of the cross-linked keratin filaments, while the intercellular space is filled with lipid materials arranged in multilamellar bilayers. The lipids possess a highly ordered structure at physiological temperature and, therefore, are considered to play an important role in barrier function of the skin.

Two general diffusional routes exist for a molecule to penetrate the skin: one is the appendageal route, and the other is the epidermal route. While the appendages, however, have a limited available surface area (approximately 0.1% of the total), there have been reports that have implicated the 'shunt' pathway as the major contributor to the initial phase of skin permeation [1]. For ions and polar compounds, contribution of the appendageal route has been considered to be relatively high [2,3]. Studies in afollicular animals have suggested a significant role for the transfollicular route [4].

The majority of drugs permeate across the bulk of the epidermis. The route of penetration across the stratum corneum can be geometrically subdivided into transcellular and intercellular routes [5,6]. However, the tortuous intercellular pathway has been widely believed to provide the principal route for drug permeation [1,3,7]. Berner and Cooper [8] modeled the permeation through three pathways (i.e., a continuous polar pathway, a continuous lipophilic pathway, and an lipid–water multilaminate pathway) and indicated that the steady-state skin transport is insensitive to the existence of the lipid–water multilaminate pathway. To visualize penetration pathways, Menon and Elias [9] applied hydrophilic and hydrophobic tracers in vivo to murine skin, which can be precipitated in situ with osmium vapor and colorized by microwave postfixation methods. Regardless of polarity of the tracers or methods of permeation enhancement, they invariably localized to discrete lacunar domains embedded within extracellular lamellar membrane system. Taking together with that the lacunar domains appear to gain structural continuity with permeation enhancement, they concluded that extracellular lacunar domains comprise a pore pathway for penetration of polar and nonpolar molecules across the stratum corneum [9].

Following penetration across the stratum corneum, drugs diffuse across the viable epidermis and dermis and transported away by the cutaneous microvasculature. The blood supply is very rich, with a flow rate of 0.05 ml min^{-1} per cm^3 of skin, and reaches to within 0.2 mm of the skin surface [1], since it needs to regulate temperature and pressure of the skin, deliver nutrients to the skin, and remove waste products. This generous blood volume usually functions as a 'sink' with respect to the diffusing molecules which reach it during the process of percutaneous absorption [10].

2.2. Analysis based on simple diffusion models

2.2.1. Fick's diffusion law

As described in the Introduction, the simplest way of modeling the process of skin transport is to assume that Fick's diffusion law is applicable. Fick's first law is to assume that the rate of transfer of diffusing substance through unit area of a section is proportional to the concentration gradient:

$$J = -D\frac{\partial C}{\partial x}$$

where J is the rate of transfer per unit area, C is the concentration of diffusing substance, x is the space coordinate, and D is the diffusion coefficient. As such the law is not readily usable, Fick's second law is employed. The second law is a mass-balance equation that can be derived from Fick's first law:

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}\left(D\frac{\partial C}{\partial x}\right) = D\frac{\partial^2 C}{\partial x^2}$$

According to the experimental design, appropriate boundary conditions of the diffusion problem are given to solve the differential equation. The simplest boundary conditions are:

$$C = KC_0 \quad \text{at} \quad x = 0$$

$$C = 0 \quad \text{at} \quad x = L$$

where K is the skin–vehicle partition coefficient; C_0 is the concentration of the solute in the vehicle and constant over time; and L is thickness of the skin. Taking together with an assumption that $C(0 < x < L)$ equals zero at $t = 0$, the amount of the solute permeating the skin (M) is derived as,

$$M = KLC_0\left(\frac{D}{L^2}t - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(-\frac{D}{L^2}n^2\pi^2 t\right)\right)$$

This expression approaches the straight line with time:

$$M = KLC_0\left(\frac{D}{L^2}t - \frac{1}{6}\right)$$

Therefore, the steady-state flux (dM/dt) and lag time (LT) are:

$$\frac{dM}{dt} = \frac{KD}{L}C_0$$

$$LT = \frac{L^2}{6D}$$

2.2.2. Quantitative structure–permeability relationship

Quantitative structure–activity relationship (QSAR) methods, or more specifically quantitative structure–permeability relationship, attempt to statistically relate the skin permeation of compounds to their structural descriptors or physicochemical parameters. Attempts to relate the skin permeability of compounds to their physicochemical properties have been made for at least 30 years. The majority of these studies was based on the analysis of homologous, or closely related, series of compounds [11–15], until Flynn [16] published a data set of human skin permeability for 94 compounds. Many of these studies revealed a linear relationship between skin permeability and lipophilicity. However, analyzing homologous, or closely related, series may result in co-linearity between descriptors; that is, lipophilicity and molecular size would be collinear for a congeneric series so that it is not possible to discriminate the effect of hydrophobicity from molecular size on the permeability of large lipophilic molecules [17].

Flynn [16] compiled 97 human skin permeability coefficients from 15 different literature sources. Although some investigators pointed out that these data contain a high degree of experimental errors due to inter-laboratory variability [18–20], the publication by Flynn made progress in QSAR studies of skin permeability. In the original research of Flynn [16], he proposed a simple algorithm of predicting skin permeability based on their octanol–water partition coefficient and molecular weight (Table 1). Potts and Guy [21] analyzed a Flynn's data set of skin permeability based on a solubility-diffusion model, in combination with a free-volume theory [22–24], and proposed the following equation:

$$\log K_p = -6.3 + 0.71 \cdot \log P_{oct/w} - 0.0061 \cdot MW \cdot (r^2 = 0.67, n = 93)$$

where $\log K_p$ is the human skin permeability coefficient; $\log P_{oct/w}$ is the octanol–water partition coefficient; and MW is the molecular weight.

El Tayar et al. [25] examined various data sets of skin permeability and found that skin permeability was inversely correlated with the parameter $\Delta\log P_{oct\text{-}hep}$ (i.e., $\log P_{octanol}$ minus $\log P_{heptane}$), which is mainly a measure of the hydrogen-bond donor acidity of the solutes. They explained that hydrogen-bond acceptor groups (ester linkage, phosphate groups, etc.) are abundant in lipidic phases in the stratum corneum, and protic compounds may tend to form comparatively stable hydrogen bonds

Table 1
Algorithms for calculating permeability coefficient (K_p), obtained from Flynn [16]

	Low molecular weight compounds (MW<150)	High molecular weight compounds (MW>150)
$\log P_{oct/w} < 0.5$	$\log K_p = -3$	$\log K_p = -5$
$0.5 \le \log P_{oct/w} \le 3.0$	$\log K_p = \log P_{oct/w} - 3.5$	
$0.5 \le \log P_{oct/w} \le 3.5$		$\log K_p = \log P_{oct/w} - 5.5$
$\log P_{oct/w} > 3.0$	$\log K_p = -0.5$	
$\log P_{oct/w} > 3.5$		$\log K_p = -1.5$

with lipids in media of low polarity, resulting in their retarded diffusion. In addition, they suggested that molecular size was not important in the prediction of skin permeability [25]. However, it should be noted that $\Delta\log P_{\text{oct-hep}}$ is not just a measure of solute hydrogen-bond acidity but is a composite parameter involving lipophilicity, basicity and dipolarity, as well as acidity [26].

In a more physicochemically rigorous fashion, a linear solvation energy equation has been applied [19,26,27]. Abraham et al. [26] obtained the following equation using skin permeability coefficients (K_p) of 47 compounds:

$$\log K_p = -5.241 + 0.437R_2 - 0.410\pi_2^H - 1.631\sum\alpha_2^H - 3.286\sum\beta_2^H + 2.012V_x$$

$$(n = 47, r^2 = 0.9567, \text{S.D.} = 0.197, F = 181)$$

where R_2 is an excess molar refraction, π_2^H is the dipolarity–polarizability, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ are the overall or effective hydrogen-bond acidity and basicity, and V_x is the McGowan characteristic volume. Although the absolute values of the coefficients associated with the compound's properties varied among the studies [19,26,27], these models showed the structural features in the compound that influence permeability: if the compound is dipolar, or is a hydrogen-bond acid, then $\log K_p$ is slightly reduced; if the compound is a hydrogen-bond base, the $\log K_p$ is greatly reduced; and as the molecular size increases, the $\log K_p$ increases. The positive coefficient with the parameters for molecular size is a reflection of the correlation of the parameters with hydrophobicity. Considering that molecular size, on the other hand, correlates negatively with diffusivity in skin, it is confirmed that partitioning effects dominate compared with diffusion effects [27].

2.3. Analysis based on heterogeneous diffusion models

2.3.1. Development of heterogeneous diffusion models

When a wide range of compounds, especially including highly hydrophilic compounds, is concerned, structure–permeability relationship is not so simple because of the heterogeneity of skin structure. Thus, it would be necessary to consider the processes of drug absorption through skin.

From existing skin transport data [1,16,28,29], it is reasonable to assume that there are at least two parallel pathways for diffusion. The permeability coefficient of polar compounds is independent of their oil–water partition coefficient. This pathway is often referred to as the polar or aqueous pathway. As the polarity is decreased, the permeability coefficient becomes a function of partition coefficient, and this is referred to as the nonpolar or lipophilic pathway. On the other hand, for highly lipophilic drugs, the permeability coefficient is almost constant regardless of lipophilicity, since their permeation rate is limited by the viable layer underlying the stratum corneum which is an aqueous tissue. Therefore, skin permeation of a wide range of compounds varying in their lipophilicity would be explained comprehensively, when the skin is composed of the two layers, i.e., the stratum corneum and aqueous viable layer, and polar and nonpolar pathways exist in the former layer.

For the models that assume heterogeneity of skin, however, their mass-balance equation cannot be analytically solved from Fick's diffusion law. Therefore, only the steady-state portion of the permeation profile is analyzed due to mathematical simplicity [14,30–33].

We have solved this problem by using a non-linear least-squares method combined with a numerical integration method [34–39]: the Laplace-transformed equation representing skin permeation was obtained and then the corresponding real time course was generated by the fast inverse Laplace transform (FILT) algorithm [40]. We obtained Laplace-transformed equations based on various skin diffusion models [36–39], even including a metabolic process [34,35]. For example, the Laplace transform of the amount of drug permeated ($\overline{Q}$) at a finite dosing based on a two-layer diffusion model with polar and nonpolar pathways [37] is,

$$\overline{Q} = Z_d X_0 (Z_{np}\sinh d_p + Z_p \sinh d_{np})/s/k(s)$$

where,

$$d_p = L_s(s/D_p)^{1/2},\ d_{np} = L_s(s/D_{np})^{1/2},\ d_d = L_s(s/D_d)^{1/2}$$

$$Z_p = K_p V_p/d_p,\ Z_{np} = K_{np}V_{np}/d_{np},\ Z_d = K_d V_d/d_d$$

$$k(s) = V_v(Z_p\cosh d_p \sinh d_{np}\sinh d_d + Z_{np}\sinh d_p \cosh d_{np}\sinh d_d$$

$$+Z_d \sinh d_p \sinh d_{np} \cosh d_d)$$
$$+Z_p(Z_p \sinh d_p \sinh d_{np} \sinh d_d$$
$$+Z_{np} \sinh d_d(\cosh d_p \cosh d_{np}-1)$$
$$+Z_d \cosh d_p \sinh d_{np} \cosh d_d)$$
$$+Z_{np}(Z_{np} \sinh d_p \sinh d_{np} \sinh d_d$$
$$+Z_p \sinh d_d(\cosh d_p \cosh d_{np}-1)$$
$$+Z_d \sinh d_p \cosh d_{np} \cosh d_d)$$

where V_v is the volume of vehicle and L_s and L_d are diffusion length of the stratum corneum and second layer, respectively; D_i, K_i and V_i (i=p, np, or d) are the diffusion coefficient in the i domain, the partition coefficient between the i domain and vehicle, and the effective volume of the i domain for diffusion; s is the Laplace operator (Fig. 1).

2.3.2. Prediction of skin permeation and its enhancement

In one of our earlier studies, Yamashita et al. [37] analyzed the effect of penetration enhancers on skin permeation of drugs with different lipophilicities. 1-Geranylazacycloheptan-2-one (GACH) is one of azacycloalkanone derivatives, similar in chemical structure to 1-dodecylazacycloheptan-2-one (Azone), and possesses an enhancement effect comparable to and less toxicity than Azone [41,42]. The enhancement effect of GACH varied according to the drugs, where the maximal effect was observed with drugs with intermediate lipophilicity [37,41]. The effect of the penetration enhancers is considered to be determined by the primary action site of the enhancers and the rate-limiting step of transport of the drugs. Contribution of each pathway to overall skin permeation depends on lipophilicity of the drugs, while the penetration enhancers also distribute to their preferential site according to their physicochemical properties. Therefore, combination between the primary action site of penetration enhancers and lipophilicity of the drugs is important to determine the effect of the enhancers. When penetration profiles of drugs through the skin treated with GACH were analyzed based on a two-layer diffusion model with polar and nonpolar pathways, it was found that the penetration enhancer primarily increased partition coefficients of the drugs towards the nonpolar pathway in the stratum corneum [37]. Difference in enhancement effect was summarized as follows: GACH was ineffective for highly hydrophilic drugs, since they mainly penetrate the polar pathway; GACH was also ineffective for lipophilic drugs, since the drugs readily penetrate the nonpolar pathway and are rate-limited by penetration through the aqueous viable layer; therefore, intermediate drugs are most responsive to the action of GACH. This diffusion model could also explain that permeation of a highly lipophilic drug at a finite dose was reduced by GACH.

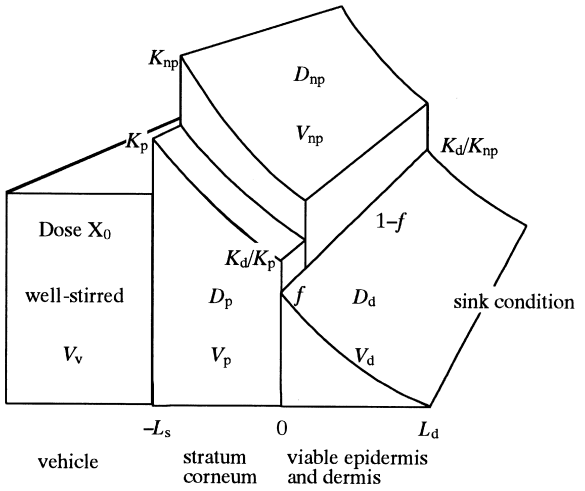

Fig. 1. Two-layer skin diffusion models with polar and nonpolar pathways in stratum corneum layer. Well-stirred and sink conditions are assumed in the vehicle and the receiver fluids, respectively. Note that each partition coefficient is defined against the vehicle solvent. *K*, partition coefficient; *D*, diffusion coefficient; *V*, volume; *L*, distance; *f*, area fraction; v, vehicle; p, polar domain; np, nonpolar domain; d, viable epidermis and dermis. Taken from Ref. [37].

Based on this analysis, Bando et al. [43,44] investigated the optimal design of a combinatorial approach of prodrug and penetration enhancer. Although skin permeation for the hydrophilic drug, e.g., acyclovir, was not enhanced by GACH, it was assumed that when a prodrug of acyclovir with an appropriate lipophilicity was developed, skin permeation of the prodrug would be enhanced by GACH [43,44]. In fact, the permeation of acyclovir butyrate through excised rat skin was 12.3-times increased by 25.5 μmol/3.14 cm^2 of GACH, while

the permeation of acyclovir was only 3.4-times increased by the same dose of the enhancer [43]. Among the prodrugs of acyclovir investigated (from acetate to hexanoate), their skin permeability in the presence of the penetration enhancer was predicted well based on a two-layer diffusion model with polar and nonpolar pathways in the stratum corneum (Fig. 2) [44]. Thus, the model considering the pathways of permeation would be useful for predicting skin permeation of drugs and designing their formulations rationally.

2.3.3. Analysis of skin metabolism

The skin exhibits a high metabolic activity towards xenobiotics that get into the body, in addition to its nature of diffusional barriers. It has been reported that many of topically administered drugs are metabolized during skin diffusion [45,46]. In this sense, evaluation of metabolic activity of the skin is an important issue in developing transdermal drug formulations.

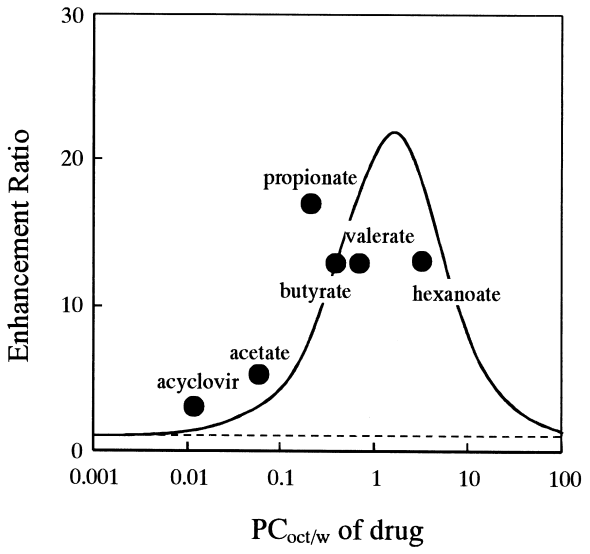

Fig. 2. Simulation of penetration enhancement by 1-geranylazacycloheptan-2-one (GACH) treatment based on a two-layer diffusion model with polar and nonpolar pathways. The penetration enhancement ratio was defined as the ratio of permeability coefficient between GACH treatment and non-treatment. To obtain the theoretical curve, the following parameters were used: $D_p/L_s^2 = 60$ (h^{-1}); $D_{np}/L_s^2 = 1.5$ (h^{-1}); $D_d/L_d^2 = 0.08$ (h^{-1}); $K_pV_p = 0.00007$ (cm^3); $K_dV_d = 3.5$ (cm^3); $\log K_{np}V_{np}$ (cm^3) $= 1.3 \cdot \log PC_{oct/w} - 3.0$ (non-treatment); $\log K_{np}V_{np}$ (cm^3) $= 1.1 \cdot \log PC_{oct/w} - 0.9$ (25.5 μmol GACH). The observed enhancement ratios for acyclovir prodrugs were also shown in this figure. Taken from Ref. [44].

Susceptibility of drugs to cutaneous metabolic enzymes has often been evaluated by in vitro experiments using skin homogenates. However, it would be unclear how accurately the metabolic rate estimated by this experiment reflects the cutaneous metabolism during transport. An uptake-metabolism experiment is one of the methods that allow us to evaluate cutaneous metabolism with the structure of skin kept intact [47,48]. In this experiment, the drugs are loaded into the skin from the dermis side and their metabolic rate is estimated from the back flux of the metabolites considering their diffusion coefficients. However, there are some problems associated with the uptake-metabolism experiment: drug loading to the skin is in an opposite direction to real drug administration; and this method is only applicable to the in vitro excised skin. Therefore, it would be more promising approach to analyze penetration profiles of drug and metabolites simultaneously [35,49–51].

Bando et al. [34,35] developed a two-layer diffusion model with polar and nonpolar pathways where cutaneous metabolism in the viable layer is considered, and obtained Laplace transformed equations representing skin permeation of drug and its metabolite. The in vitro permeation of eight acyclovir prodrugs through rat skin was analyzed [35]. Diffusion and partition parameters and metabolic rate constant were estimated by fitting the model-based equation to the time courses of prodrug and regenerated acyclovir appearing in the receiver fluid. According to an increase in lipophilicity of prodrugs, their partition coefficient in the nonpolar pathway was larger. In addition, the metabolic rate constant estimated by curve-fitting was larger for prodrugs with longer acyl chains; among the structural isomers, acyclovir valerate gave a highest metabolic rate constant, followed by isovalerate and pivarate. The rank order was in good agreement with that in the metabolic experiments using rat skin homogenates (Fig. 3) [35]. When effect of GACH on the in vitro permeation of the prodrugs were analyzed, it was found that the enhancer mainly increased partitioning of prodrugs into the nonpolar pathway and decreased their metabolic rate constant.

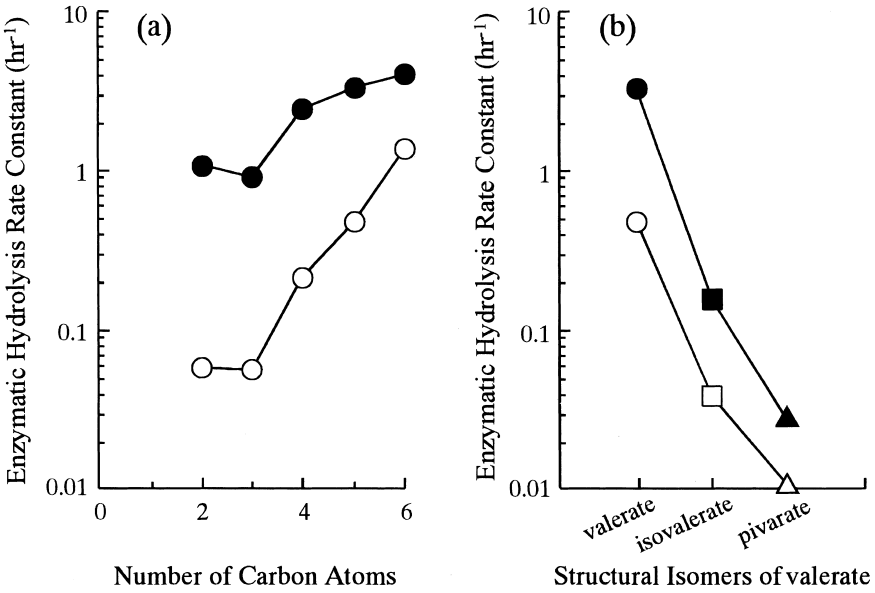

Fig. 3. Effect of substituent of acyclovir prodrugs on their enzymatic hydrolysis rate constant in the viable epidermis and dermis. (a) Prodrugs varying in acyl chain length and (b) structural isomers of acyclovir valerate. Enzymatic hydrolysis rate constants determined by the diffusion-model analysis of in vitro penetration profiles (closed symbol) and by in vitro homogenate experiment (opened symbol) are compared. Taken from Ref. [35].

2.3.4. Analysis of in vitro–in vivo correlation

In most cases, transdermal drug absorption studies are usually carried out in vitro, because of the ease of experiments and efficiency of screening. Since a goal of in vitro studies is the prediction of in vivo absorption, some attempts to predict it from in vitro data have been carried out based on a diffusion model [52–54] or a convolution technique [55]. However, physicochemical and physiological conditions of excised skin differ from in vivo situations. In addition, the existence of microcirculation in vivo might influence the total absorption rate. There have been comparative in vitro–in vivo studies, indicating that in vitro and in vivo permeation is positively correlated but largely different in amount [56–59].

Yamashita et al. [36] analyzed in vitro–in vivo difference in skin permeation by dividing it into each elemental process based on a two-layer diffusion model with polar and nonpolar pathways. In vivo absorption profiles of compounds obtained by a deconvolution method were compared with the corresponding in vitro permeation profiles, indicating that skin permeation of mannitol (a hydrophilic compound) was smaller in vivo but that of butylparaben (a lipophilic compound) was greater. The analysis based on the diffusion model suggested that the diffusion length of a viable layer was shorter in vivo, probably due to the washout by microcirculation, and that the effective area of the polar pathway was larger in vitro, probably due to the hydration effect. As well as in vitro, GACH increased the partition coefficients of drugs into the nonpolar pathway in vivo. Besides, GACH increased the effective area of the polar pathway in vivo presumably due to acceleration of skin hydration; this effect was not observed in vitro since the stratum corneum was fully hydrated even without the enhancer treatment. Several investigators have pointed out that hydration of skin increases in vitro skin permeability [60–67].

In vitro–in vivo difference in metabolism of ester type prodrugs was also investigated [34]. This detailed in vivo analysis was enabled by the novel deconvolution technique that can discriminate local and systemic metabolisms [34,68,69]. In vivo transdermal studies of acyclovir prodrugs (i.e., valerate, isovalerate, and pivarate) revealed that the in vivo total flux of prodrug and parent drug forms were

almost the same among the structural isoforms, but the ratio of bioconversion was largest with valerate followed by isovalerate and pivarate [34]. These results suggested that all of the prodrugs possess similar permeability across the stratum corneum because of their similar lipophilicity while the susceptibility towards cutaneous esterases is different according to branched structures of acyl chain. The analysis using the diffusion–bioconversion model revealed that the metabolic rate constants of isovalerate and pivarate were 2.98 and 0.583 h^{-1}, respectively (the metabolic rate constant of valerate was too high to be estimated). When compared with the corresponding in vitro value [35], the in vivo metabolic rate constant was approximately 20–30 times higher [34]. This may be attributed to leakage of enzymes from excised skin into receiver fluid [70] or inactivation of enzymes during the in vitro experiments [71]. Thus, it should be noted that in vitro skin permeation studies might underestimate metabolic rate of drugs.

3. Empirical analysis of skin permeation of drugs

Empirical modeling for prediction of skin permeability, especially based on molecular structures, has also been conducted. These approaches include the selection of molecular or structural descriptors without mechanistic consideration and/or (non-linear) empirical modeling of the permeability–descriptor relationship. Artificial neural network is a powerful tool for non-linear modeling of complex causal-effect relationships.

3.1. Linear empirical modeling for prediction of skin permeability

Pugh and Hadgraft [72] proposed a fragment approach based on various molecular substructures and features. Two sets of predictors were suggested through analyzing the Flynn's data set [16]: one is based on a SMILES method of molecular structure description and the other is based on an 11-descriptor set of empirically determined functional groups. An adjusted correlation coefficient for the 11-descriptor model ($r^2 = 0.676$) was comparable to, or slightly higher than, that of the prediction model based on $\log P_{oct}$ and MW proposed by Potts and Guy ($r^2 = 0.636$) [21]. They stressed that, although this model lacks mechanistic simplicity and utility of the Potts and Guy method, it has an obvious use in the approximate prediction of permeability in the absence of experimental values.

Wilschut et al. [73] extended the Flynn's data set to 123 measured permeability coefficients of 99 different chemicals, and evaluated several prediction models that had been proposed [21,74–77]. When the reliability of the models was evaluated by testing variation of regression coefficients and of residual variance for subsets of data selected randomly, they found the McKone and Howd model [76], the Potts and Guy model [21], and the Robinson model [77] are significant. In addition, they revealed that as for the Potts and Guy model and the Robinson model, square root of MW as independent variable provided a better fit than MW in the original models; especially, the revised Robinson model, which was based on a two-layer model with parallel polar and nonpolar pathways in the first layer, resulted in the smallest residual variance.

Dearden et al. [78] utilized a total of 81 physicochemical and topological descriptors for QSAR modeling of the Flynn's data set. A highly predictive, though mechanistically complex, model was reported based on six parameters:

$$\log K_p = -0.626 \cdot \sum Ca - 23.8 \cdot \sum (Q+)/\alpha - 0.289 \cdot \mathrm{SsssCH} - 0.0357 \cdot \mathrm{SsOH} - 0.482 \cdot I_B + 0.0405 \cdot B_R + 0.834$$

$$(n = 91,\ r^2 = 0.832,\ s = 0.563,\ F = 69.2)$$

where ΣCa is the HYBOT-PLUS hydrogen-bond acceptor free energy factor; $\Sigma (Q+)/\alpha$ is the HYBOT-PLUS positive charge per unit volume; SsssCH is the electrotopological atom-type index for singly bonded CH; SsOH is the electrotopological atom-type index for OH; I_B is the Balaban index; and B_R is the number of rotatable bonds.

3.2. Neural network modeling for prediction of skin permeability

An artificial neural network (ANN) is an information processing paradigm that is inspired by the

way biological nervous systems, such as the brain, process information. ANNs represent a promising modeling technique especially for data sets having the kind of non-linear relationships. Neural networks, such as feed-forward neural networks, are able to detect complex relationships between explanatory and objective variables and all possible interactions without complicated equations. Therefore, ANNs have been widely used in the pharmaceutical fields [79], where complex phenomena are encountered. Application of ANNs include structure–activity relationship analysis [80,81], structure–property relationship analysis [82–89], pharmacokinetic modeling [90–99], and the design of formulation [100–106].

3.2.1. Feed-forward artificial neural network topology

A feed-forward ANN is the most popular one that can simulate causal and effect relationships. Topologically, a feed-forward ANN consists of an input layer, an output layer, and any number of intermediate layers called hidden layers. The units in the input layer are processing units that receive inputs from the outside world. One or more layers of hidden processing units receive the outputs of a previous layer of units process them in parallel. The set of processing units that represents the final result of the neural network computation is designated as the output units. Each processing unit combines all of the input signals propagating into the unit along with a threshold value. This total input signal is then passed through an activation function to determine the actual output of the processing unit, which in turn becomes the input to another layer of units in a multi-layer network. The most typical activation function used in neural networks is the S-shaped or sigmoid (also called the logistic) function.

Back propagation is a general purpose learning algorithm. The basic back propagation algorithm consists of three steps. The input pattern is presented to the input layer of the network. These inputs are propagated through the network until they reach the output units. This forward pass produces the actual or predicted output pattern. Since back propagation is a supervised learning algorithm, the desired outputs are given as part of the training vector. The actual network outputs are subtracted from the desired outputs and an error signal is produced. This error signal is then the basis for the back propagation step, whereby the errors are passed back through the neural network by computing the contribution of each hidden processing unit and deriving the corresponding adjustment needed to produce the correct output. The connection weights are then adjusted and the neural network has just learned from an experience.

3.2.2. Application of artificial neural networks

Using an ANN, Agatonovic-Kustrin et al. [107] developed a quantitative structure–permeability relationship of penetration across polydimethylsiloxane membranes, which were expected to be the model of skin permeation [108–111]. A set of 254 compounds and their maximum steady state flux was collected from the literature [110,112]. Twelve of 42 molecular descriptors were selected for ANN modeling of maximum steady-state flux by the use of genetic algorithm, that include molecular shape and size, inter-molecular interactions, hydrogen-bonding capacity of drugs, and conformational stability. For the 12-descriptor neural network model, the training set RMS error was 0.36 and the testing set RMS error was 0.59. When the prediction power was evaluated using an external prediction set, an RMS error was 0.60, indicating that the quality of the model would be ensured.

Lim et al. [113] proposed a method for predicting the human skin permeability ($\log K_p$) of compounds from three-dimensional molecular structure using a combination of molecular orbital (MO) calculations and ANN. For 92 compounds that was listed in the Flynn's data [16], their molecular descriptors, such as dipole moment, polarizability, sum of charges of nitrogen and oxygen atoms (sum(N,O)), and sum of charges of hydrogen atoms bonding to nitrogen or oxygen atoms (sum(H)), were calculated from MO-calculations (Fig. 4). The correlation between these molecular descriptors and $\log K_p$ was examined using a feed-forward back-propagation neural network. To improve the generalization capability of a neural network, the network was trained with input patterns given 5% random noise [114]. The neural network model with a configuration of 4-4-1 for input, hidden, and output layers was much superior

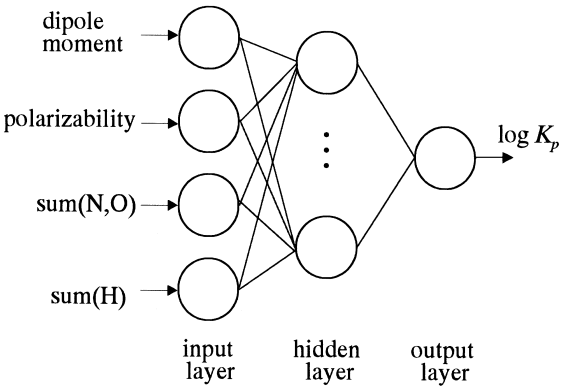

Fig. 4. Schematic representation of a three-layered feed-forward neural network. Taken from Ref. [113].

to the conventional multiple linear regression model in terms of root mean square (RMS) errors (0.528 vs. 0.930) (Fig. 5). Moreover, a 'leave-one-out' cross-validation revealed that the neural network model could predict skin permeability with a reasonable accuracy (predictive RMS error of 0.669). Fu et al. [115] have also performed ANN modeling of skin permeability for 45 compounds based on MO-calculated descriptors. When external validation was conducted for eight compounds, the ANN model gave a mean prediction error of 2.6%, whereas the prediction error of the multiple linear regression model with the same descriptors was 32.09%.

Degim et al. [116] analyzed skin permeability of 40 compounds by an ANN and compared its predictability with the multiple linear regression model obtained by Pugh et al. [117]. According to the linear model of Pugh et al., the partial charges of the penetrants, their molecular weight, and their calculated octanol–water partition coefficient ($\log P_{oct/w}$) were used as molecular descriptors. While the linear equation gave a regression coefficient (r^2) of 0.672, the ANN produced $\log K_p$ values that correlated well with the experimental ones ($r^2 = 0.997$). In addition, they experimentally determined human skin permeability for some compounds that have not been previously investigated, and found that their experimental data can be predicted well from the ANN model developed.

4. Conclusion

In this article, we reviewed modeling approaches for prediction of skin permeation of drugs. Mechanistic models can give us much information on understanding of skin permeation of drugs, while empirical models can give us superior predictions. Depending upon their demands, the modelers and

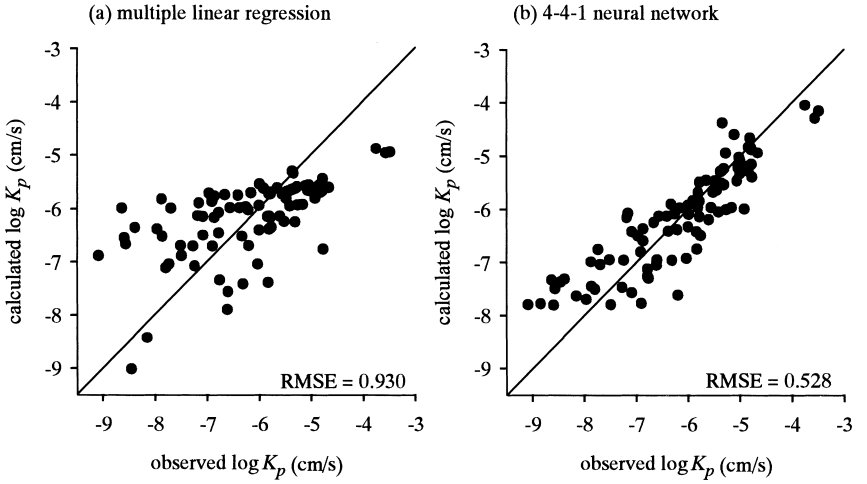

Fig. 5. Relationship between experimental and calculated skin permeability coefficients. (a,b) Based on the multiple linear regression analysis and 4-4-1 neural network analysis, respectively. Taken from Ref. [113].

users will give different answers to the question: which is superior, mechanistic model or empirical model? However, these two approaches should not be exclusive but compensative in nature. Ideally speaking, empirical models should be used to compensate for uncertainties that mechanistic models overlook and yet-to-be-known relationships that mechanistic models cannot deal with.

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