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Report

Optimization of Topical Therapy: Partitioning of Drugs into Stratum Corneum

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To optimize a topical formulation for therapeutic effect generally implies that the flux of drug into the skin be maximized. This requirement means that the product of drug concentration in the vehicle (C_v) and drug partition coefficient (PC) between stratum corneum (SC) and vehicle be as large as possible. While C_v is a formulation variable which can be easily manipulated up to the drug's saturation solubility, PC is a parameter that is difficult to predict a priori. However, there is no question that an ability to evaluate PC would greatly facilitate the efficient screening of drugs and formulations. We have measured the SC/water and SC/isopropylmyristate (a model lipophilic vehicle) PCs of seven drugs: acitretin, progesterone, testosterone, diazepam, estradiol, hydrocortisone, and caffeine. SC/water PCs were determined as a function of the following variables: (i) initial drug concentration in the vehicle, (ii) length of equilibrium, (iii) SC source and preparation technique, and (iv) SC delipidization. The data obtained were reproducible and physicochemically consistent, and they show that useful partitioning information from both aqueous and nonaqueous vehicles can be obtained with the biological tissue of greatest relevance. The SC/water PCs of the steroids were in reasonable agreement with previous measurements. A facile approach to an integral determinant of formulation optimization is suggested, therefore, by these observations.

KEY WORDS: partition coefficient; steroids; stratum corneum; skin; percutaneous absorption.

INTRODUCTION

The stratum corneum (SC), which is the outermost layer of mammalian skin, typically provides the major barrier to transdermal drug absorption. The SC is a thin, heterogeneous structure comprised of stacked layers of terminally differentiated and keratinized epidermal cells distributed in a complex, lamellar, intercellular lipid domain (1,2). The key determinants of transport through this "brick-and-mortar" structure are the apparent diffusivity of the penetrant and its solubility in the SC relative to the applied delivery system (the vehicle). The literature (3–7) reveals that the latter parameter, the SC/vehicle partition coefficient (PC) of the permeant, is most sensitive to changes in drug structure and properties. However, relatively few measurements of PC have been reported (5,8–10) and simple relationships between this coefficient and more easily measured PC (oil/water) values have been infrequently established (10–12). Nevertheless, the ability to evaluate PC routinely might facilitate the efficient screening of drugs and their proposed formulations.

The objectives of this study, therefore, were to develop and to validate a simple approach for the measurement of solute partition coefficient between SC and a vehicle. In this regard, the effect of the following variables on the value of PC were examined: drug concentration, equilibration time, vehicle lipophilicity/hydrophilicity,and SC delipidization.

MATERIALS AND METHODS

Skin Preparation

Dermatomed human skin (0.5 mm, Dermatome Model B; Padgett, Kansas City, MO) was obtained from cadavers at autopsy (School of Medicine, University of California, San Francisco). The skin samples were stored for up to a maximum of 3 days in phosphate-buffered saline at 4°C prior to isolation of the SC. Before this separation, one of two techniques was utilized to loosen the dermal–epidermal junction:

(a) The tissue was submerged in phosphate-buffered saline (pH 7.2) for 45 sec at 50°C, or

(b) The tissue was sandwiched in aluminum foil, which was pressed on a slide warmer for 45 sec at 50°C.

After these treatments, the SC/epidermis layer was peeled from the dermis with dissection forceps. The thin sheets of SC/epidermis were then placed dermal side down

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on a filter paper soaked with 0.0001% trypsin for 24 hr at 25°C. After digestion of the epidermal layer, the SC was gently rinsed and then dried at 37°C in an incubator. Dry SC was stored in a desiccator.

Delipidization. Dry, preweighed SC samples were placed in a glass beaker containing 100 cm^3 2:1 chloroform/methanol and were gently agitated for 24 hr at 25°C. The delipidized SC samples were then removed, rinsed twice with fresh chloroform/methanol, and dried. Lipid content was determined by the change in weight of the SC after solvent extraction.

SC/Vehicle Partition Coefficient Determination

As model hydrophilic and lipophilic vehicles, water and isopropylmyristate (IPM), respectively, were chosen. The partitioning of drug between the SC and the vehicle was determined by measuring the disappearance of radiolabeled compound from the vehicle and the concomitant increase in radioisotope in the SC. The SC/vehicle partition coefficient was defined (8) as

$$PC = C_{sc}/C_v \quad (1)$$

where C_{sc} was the drug concentration in 1000 mg SC and C_v was the drug concentration in 1000 mg vehicle. In certain cases, two alternative calculation methods were used. (a) It was assumed that the decrease in drug concentration in the vehicle exactly equaled the uptake of drug into the SC (10). Hence,

$$PC = (C_i - C_v)/C_v \quad (2)$$

where C_i was the initial concentration of the drug in the vehicle and C_v is defined above. (b) The amount of radioactivity measured in the SC at the end of the equilibrium period was corrected for the bulk solvent taken up by the SC during the partitioning process:

$$PC = (C_{sc} - C_b)/C_v \quad (3)$$

Where C_b is the fraction of radioactivity in the SC attributable to bulk solvent uptake into the membrane. It was found that these different methods of calculation resulted in insignificantly different values of PCs. The data presented, therefore, have been determined using the simple formula given as Eq. (1).

In a typical experiment, 500 μl of vehicle solution and an accurately weighed, dry SC sample (3–8 mg) were placed in a screw-cap borosilicate glass vial, which was capped with a Teflon septum. The vial contents were equilibrated, with occasional gentle agitation, for various time intervals at 25°C. The SC sample was then removed, gently blotted on filter paper, and immediately dissolved in Soluene 350 (Packard Instrument Company, Downers Grove, IL). An aliquot of the vehicle (300–400 μl) was removed from the vial.

Concentrations of radiolabeled compound in the vehicle and in the dissolved SC sample were then determined by liquid scintillation counting (Tri Carb, Model 1500; Packard Instrument Company, Downers Grove, IL). All experiments were performed in quintuplicate.

All octanol/water and IPM/water partition coefficients of the solutes were obtained from the literature (13,14).

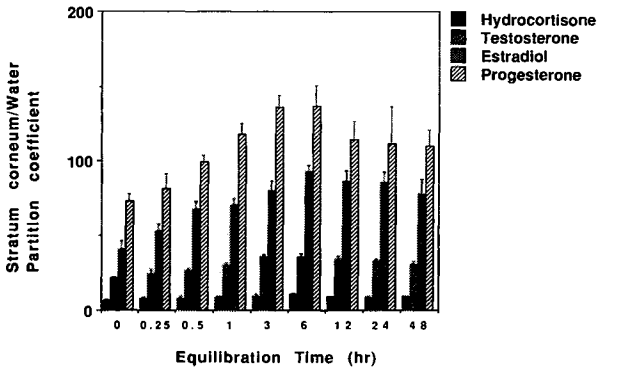

Fig. 1. Stratum corneum/water partition coefficients (mean ± SD; n = 5) of four steroids determined as a function of equilibration time.

Compounds

The steroids were obtained from Research Products International Corp. (Mount Prospect, IL): [4-^{14}C]progesterone, [4-^{14}C]estradiol, [4-^{14}C]testosterone, and [4-^{14}C]-hydrocortisone all had a specific activity of 56 mCi/mmol. [*Methyl*-^{14}C]caffeine and [*methyl*-^{3}H]diazepam were obtained from NEN Research Products (Wilmington, DE). Their specific activities were 56 mCi/mmol and 60 Ci/mmol, respectively. [7-^{14}C]Acitretin, a retinoid, was a gift from Roche Dermatologics (Nutley, NJ). The specific activity was 58.2 mCi/mmol. The radiochemical purity determined by TLC was ≥98% for all compounds.

RESULTS AND DISCUSSION

Figures 1 and 2 show the effect of incubation time on the SC/water partitioning of the drugs studied. In the experiments involving an equilibration time of "0 hr," the SC was in contact with the vehicle phase for approximately 3 min. For the steroids, caffeine and diazepam, equilibrium was attained within 6 hr; the PC of acitretin, however, appeared to continuously increase with incubation time up to 24 hr. In liquid–liquid partitioning processes, it is often advisable to allow the equilibration time to be as long as possible. When one phase is a biological membrane, though, one must be cautious about the use of very long incubation periods be-

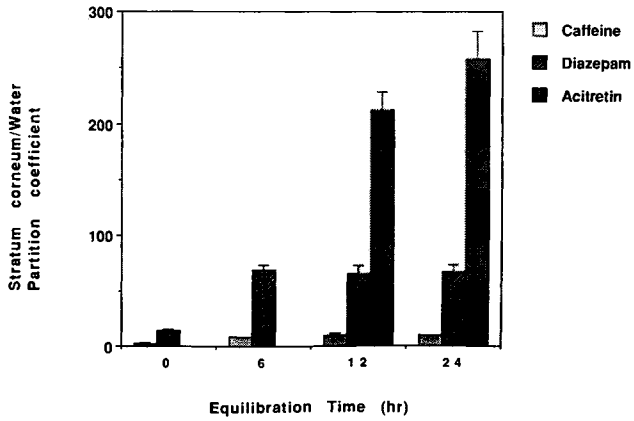

Fig. 2. Stratum corneum/water partition coefficients (mean ± SD; n = 5) of caffeine, diazepam, and acitretin determined as a function of equilibration time.

Table I. Effect of Initial Aqueous Phase Drug Concentration (C_i) ($\mu g\ cm^{-3}$) on Stratum Corneum[a]/Water Partition Coefficient (PC) (Mean ± SD; $n = 5$)

Hydrocortisone		Testosterone		Estradiol		Progesterone	
C_i	PC	C_i	PC	C_i	PC	C_i	PC
0.3	9.5 ± 1.1	0.4	44 ± 3.4	0.2	133 ± 10	0.4	211 ± 23
0.8	8.7 ± 1.0	2.0	41 ± 4.2	0.4	117 ± 16	0.6	178 ± 23
6.8	8.3 ± 1.2	7.3	37 ± 7.2	2.8[b]	103 ± 12	11.9[b]	165 ± 16
391[b]	8.6 ± 1.0	24[b]	37 ± 7.0				

[a] The stratum corneum was obtained from the thigh of a 32-year-old male Caucasian. SC was separated by the first of the two procedures described under Materials and Methods. Equilibration time was 24 hr.
[b] Saturation solubility.

cause changes in the membrane may occur that alter, in turn, the "real" PC. The 6-hr "optimal" equilibration time, observed for all but one of the drugs considered, probably reflects the period for complete hydration of the SC (15). The reason that the PC of acitretin continues to rise is not revealed by these data. Although it was the most lipophilic drug studied, it is possible that its rate of partitioning into the SC intercellular lipids is slow. Alternatively, there may be a progressive water-induced alteration of SC with time to which this hydrophobic solute is particularly sensitive.

Table I shows the effect of varying initial aqueous phase drug concentration (C_i) on the SC/water PC of four steroids. Not unexpectedly, there is a tendency for the PC values to decrease with increasing C_i. This type of behavior has been previously reported for other compounds (e.g., scopolamine) and has been interpreted using a "dual sorption model" (16). This explanation postulates the existence of "bound" and "freely diffusible" molecules within the SC. As the number of molecules available for partitioning into the SC increases, the immobilized fraction becomes saturated while the unbound compound can continue to rise. Hence, the measured PC may not be constant with increasing concentration until the "binding sites" in the SC are saturated. The PC results in Table I were determined after an incubation period of 24 hr. The SC used was taken from the thigh of a 32-year-old male Caucasian and was isolated using the first of the two procedures described under Materials and Methods.

In Table II, the effects on steroid SC/water PC of (a) SC source and (b) SC isolation procedure are examined. The methods, by which the SC was obtained, resulted in no significant ($P \leq 0.05$, Student's t test for unpaired data) impact on PC values for any of the four steroids. Relatively benign separation techniques (8,17) were employed and care was taken to ensure that the SC temperature did not exceed 50°C. Thermal transitions in the intercellular lipids of the SC are known to occur above 60°C (18–20), and these structural reorganizations have been associated with altered SC permeability. From a practical standpoint, we found that the second method of SC separation was the simpler and the quicker of the two employed. "Intersubject" PC variability (i.e., coefficient of variation) was 10–20%, a range rather modest compared to typical skin permeability measurements (21). The rankings of the compounds between subjects was totally consistent. Taken together, the data represent, therefore, reliable estimates of the steroid PC values. The results are in reasonable agreement with previously published figures (9,10).

Table III demonstrates the impact of SC delipidization on the SC/water PC of the four steroids and acitretin. These measurements may be relevant to drug partitioning into damaged or diseased skin. For all compounds except progesterone, delipidization significantly ($P \leq 0.001$, Student's t test for paired data) increased the PC. SC was obtained from the thigh of a 46-year-old black male and was separated by the second procedure described under Materials and Methods. A two-phase model of the SC (the "mortar-and-brick" idea), in which the terminally differentiated, and completely keratinized, corneocytes reside within, and are surrounded by, a lamellar intercellular lipid domain, is now generally accepted (2,22,23). The importance of the lipids with respect to permeation (i.e., that the intercellular pathway is dominant) has been demonstrated (23–25). It is somewhat surprising, there-

Table II. Stratum Corneum (SC)/Water Partition Coefficients (Mean ± SD; $n = 5$) of Four Steroids:[a] Influence of SC Source and SC Preparation Technique

		Partition coefficient			
SC source[b]	SC preparation[c]	Hydrocortisone	Testosterone	Estradiol	Progesterone
47/M/C	Water/heat	8.6 ± 0.9	33 ± 1.9	86 ± 7.2	112 ± 24
45/M/C	Water/heat	9.4 ± 0.1	42 ± 4.5	105 ± 6.4	169 ± 7.4
32/M/C	Water/heat	8.6 ± 1.0	40 ± 4.2	117 ± 16	178 ± 23
40/M/C	Water/heat	7.5 ± 0.5	36 ± 2.2	92 ± 4.4	137 ± 14
Average		8.5 ± 0.8	38 ± 4.1	100 ± 14	149 ± 30
57/M/C	Metal/heat	7.9 ± 0.8	39 ± 4.5	111 ± 5.6	190 ± 19
33/M/C	Metal/heat	10.3 ± 1.8	35 ± 3.2	87 ± 7.9	181 ± 20
78/M/C	Metal/heat	10.6 ± 0.4	44 ± 2.9	94 ± 10	158 ± 12
46/M/B	Metal/heat	7.1 ± 0.5	49 ± 3.4	113 ± 14	209 ± 27
Average		9.0 ± 1.7	42 ± 5.9	101 ± 13	184 ± 21

[a] Equilibration time was 24 hr.
[b] Age (in years), sex (M = male), race (C = Caucasian, B = Black).
[c] See Materials and Methods.

Table III. Stratum Corneum (SC)/Water Partition Coefficients (Mean ± SD; $n = 5$) of Four Steroids and Acitretin:[a] Influence of Delipidization

Stratum corneum	Hydrocortisone	Testosterone	Estradiol	Progesterone	Acitretin
Intact	7.1 ± 0.5	49 ± 3.4	113 ± 14	209 ± 27	189 ± 28
Delipidized	16.0 ± 0.4	74 ± 1.9	203 ± 13	214 ± 15	512 ± 18

[a] The stratum corneum (SC) was obtained from the thigh of a 46-year-old black male. SC was separated by the second procedure described under Materials and Methods. Equilibration time was 24 hr.

fore, that lipid removal enhances (in most of the cases) drug partitioning. This result is inconsistent with the recently published data of Raykar *et al.* (10), who indicated that the partitioning of hydrocortisone esters into the SC could be considered as the summation of partitioning into three structurally distinct domains: the extractable lipid, the protein, and the solvent domain. It follows from this hypothesis that removal of one of the structural components must lead to a decrease (as opposed to the increase observed in our work) in the measured PC. The results presented in Table III do not reveal an explanation for the discrepancy between our findings and those of Raykar *et al.* (10). The SC delipidization procedure followed, and described under Materials and Methods, was similar to the published technique (10). A possible explanation, and one that, in our opinion, warrants careful evaluation, is that delipidization exposes regions of SC, to which the drug is denied access when the membrane is intact and unperturbed. The PCs into delipidized SC then assume relevance only for the damaged barrier and cannot be used to deduce features of the partitioning process into the complete membrane. In other words, the SC, as a complex biological membrane, cannot be considered a simple two-phase structure ("red blocks and blue blocks") into which solute partitioning proceeds as two independent and additive processes (i.e., partitioning into the total membrane = partitioning into the red blocks + partitioning into the blue blocks). Parenthetically, it should be noted that other researchers have also reported the "anomalous" increase in solute PC following delipidization (26–28).

Finally, in Table IV, the measured SC/water and SC/IPM partition coefficients are compared and are presented with the available literature values of drug PCs between octanol and water and between IPM and water (13–15). IPM was chosen as the model lipid phase because it is a frequently used vehicle constituent in various dermatological formulations. As expected, there is an inverse relationship between PC (SC/water) and PC (SC/IPM) and there are direct correlations between PC (SC/water) and both PC (octanol/water) and PC (IPM/water) (Fig. 3). The absolute values of the partition coefficients suggest that IPM discriminates between the solutes considered in a fashion much closer to the SC than does octanol. It may also be noted that the theoretical equality

$$\log [K(\text{SC/IPM})] = \log K\,[\text{SC/water}] - \log K\,[\text{IPM/water}]$$

is reasonably supported (with the exception of hydrocortisone) by the measured data. While the SC/IPM partitioning results presented here are relatively few compared to the SC/water measurement (in that the effect of the different variables examined was not determined using IPM as the vehicle), the usefulness of the techniques described for the evaluation of nonaqueous vehicles has been demonstrated and may be properly investigated in further work.

In conclusion, the procedures described permit measurement of solute PCs between the SC and a vehicle. The data show that reproducible information can be obtained using human SC, the tissue of greatest relevance for percutaneous absorption. On the whole, the pattern of behavior observed, in experiments utilizing intact SC, aqueous and organic vehicles, and seven model compounds, is compatible with physicochemical expectations. We suggest that the ability to evaluate SC/vehicle PC values should enable improved predictions of percutaneous drug flux and should facilitate the rational optimization of topical formulations. Further work relating PC to *in vitro* and *in vivo* skin absorption kinetics is required to substantiate these hypotheses.

Table IV. Partition Coefficients (PC) of Seven Drugs

Drug	log[*P*][a]	log *K*[IPM/water][b]	log *K*[SC/water][c]	log *K*[SC/IPM][d]
Acitretin	6.4[e]	NA[f]	2.4	−0.62
Progesterone	3.9	2.6	2.3	−0.34
Testosterone	3.3	2.0	1.6	−0.22
Diazepam	2.8	NA	1.8	NA
Estradiol	2.7	2.3	2.1	0.04
Hydrocortisone	1.5	−0.2	0.98	0.54
Caffeine	−0.02	NA	0.96	NA

[a] Octanol/water PC from Ref. 14.
[b] Experimentally determined IPM/water PC from Ref. 13.
[c] SC/water PC.
[d] SC/IPM PC.
[e] Dr. G. Gross, Hoffmann-La Roche Inc., Basel, personal communication.
[f] Not available.

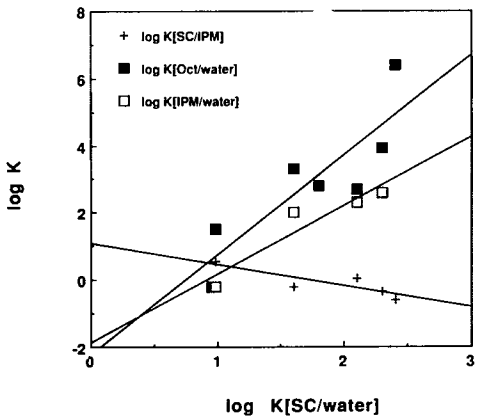

Fig. 3. Correlation of drug SC/water PC (log K[SC/water]) and (a) drug SC/IPM PC (log K[SC/IPM]), (b) drug octanol/water PC (log [P]), and (c) drug IPM/water PC (log K[IPM/water]). Simple linear regression of the data yielded the following correlations: (a) $y = 1.08 - 0.64x$, $r = 0.85$; (b) $y = -2.11 + 2.91x$, $r = 0.86$; (c) $y = -1.89 + 2.04x$, $r = 0.94$.

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