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Investigation of drug partition property in artificial sebum

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Abstract

Targeted delivery of a therapeutic agent into the hair and sebaceous follicles greatly depends on the extent of drug partitioning/diffusion in the sebum. The objective of the present research was to develop a method to determine the sebum partition coefficient in order to facilitate the selection of sebum-targeted drug candidates. Partition coefficients of model drugs with different chemical structures and 4-hydroxybenzoate series compounds were measured in artificial sebum/water (K_{sebum}) and human stratum corneum/water (K_{sc}) at 37 °C. The relationship was evaluated between $\log K_{sebum}$, $\log K_{sc}$ and clog P. The results of the partition coefficient studies indicate that the K_{sebum} of some drugs was significantly higher than the K_{sc}, whereas some drugs showed lower or similar K_{sebum} when compared with K_{sc}. Overall, a relatively poor correlation was observed between $\log K_{sebum}$, $\log K_{sc}$ and clog P. However, a linear relationship exists between $\log K_{sebum}$ and clog P in the 4-hydroxybenzoate series compounds, indicating that K_{sebum} depends on the lipophilicity and chemical structure of the compounds. The results of the present study demonstrate that K_{sebum} is different from K_{sc} and calculated P and is likely to be a critical parameter reflecting drug delivery into hair and sebaceous follicles.

Keywords: Artificial sebum; Partition coefficient; Sebaceous gland; Hair follicle; Targeted delivery; Human stratum corneum

1. Introduction

The stratum corneum is acknowledged not only as the main barrier to skin penetration but also as a major permeation pathway from topical and transdermal delivery systems. The stratum corneum is made up of tightly packed, semicrystalline intercellular lipid domains and its extremely compact corneocytes create a barrier highly resistant to percutaneous transport (Elias, 1983; Downing, 1992). The lipid layer of the stratum corneum has been described as a possible pathway for transport of hydrophobic substances, whereas the polar heads of lipids represent a relatively hydrophilic pathway (Forsind, 1994). The proposed transport pathways in the stratum corneum have been well defined. However, the existence of appendage pathways for percutaneous absorption remains unclear and is often disregarded. Literature reports suggest that the pilosebaceous unit (hair follicle, hair shaft and sebaceous gland) may contribute significantly to topical and transdermal delivery in addition to the transepidermal route (Meidan et al., 2005; Motwani et al., 2004; Grams and Bouwstra, 2002). Although hair follicle openings occupy only about 0.1% of the human skin surface area, they are invaginations of the epidermis extending deep into the dermis, providing greater actual area for potential absorption below the skin surface (Otberg et al., 2004). In addition, the follicle orifice is estimated to be 1.3% of the skin surface on the forehead (Strauss et al., 1976), indicating that follicular delivery may play a particularly important role for drugs or cosmetics applied on the scalp and facial sites because of the high density of vellus hair follicles in these areas. Targeted drug delivery to the pilosebaceous unit could help for the treatment of skin conditions like acne, androgenic alopecia, oily skin and potentially some skin cancers. Sebaceous glands produce an oily sebum which is primarily composed of waxes, triglycerides, and free fatty acids (Rosenthal, 1964; Greene et al., 1970; Nordstrom et al., 1986; Wertz, 2001). Hence, efficient drug delivery into the sebum filled hair follicle and sebaceous gland is a function of the drug–vehicle, drug–sebum, and vehicle–sebum interaction. The belief is that vehicles miscible with sebum or portions of it will be effective in preferentially delivering drugs to the hair follicle and sebaceous glands (Motwani et al., 2001, 2002, 2004). Additionally, a drug must be present at the skin/vehicle interface and have sufficient solubility in the sebum. This would

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allow for effective transport and diffusion of the molecule into the hair follicle. Interestingly, there were no reports available in the literature on drug partition properties into the sebum. In this publication, we have investigated the drug partition in artificial sebum–water (K_{sebum}) and human stratum corneum–water (K_{sc}), and its relationship with the calculated octanol–water partition coefficient ($P_{o/w}$). The overall objective of this study is to develop a method to determine the sebum partition coefficient in order to facilitate the selection of sebum-targeted drug candidates. It is expected that K_{sebum} as opposed to conventional $P_{o/w}$ values is a more meaningful parameter for predicting follicular drug delivery.

2. Materials and methods

2.1. Materials

Salicylic acid (purity 99%), cholesterol, octyl-4-hydroxybenzoate, methyl-4-hydroxybenzoate, ethyl-4-hydroxybenzoate, butyl-4-hydroxybenzoate and heptyl-4-hydroxybenzoate were obtained from Lancaster Synthesis, Inc. (Pelham, NH). 3,4-Dihydroxybenzoic acid (purity 98%), tretinoin, oxalic acid (purity >99%), *o*-phosphoric acid, paraffin wax (58–62 °C), oleic acid and 4-hydroxybenzoic acid were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). Ketoconazole, minoxidil, cotton seed oil, palmitoleic acid, squalene and octanol were obtained from M.P. Biomedical, LLC (Aurora, OH), lidocaine base (purity >98%), tetracycline base, tetracycline hydrochloride and trifluroacetic acid, propyl-4-hydroxybenzoate were obtained from Sigma Chemical Company, Inc. (St. Louis, MO). Olive oil and palmitic acid were obtained from EMD Chemicals (Gibbstown, NJ). Spermaceti wax and cholesterol oleate were obtained from Sargent-Welch (Buffalo, IL) and Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan), respectively. Amyl-4-hydroxybenzoate, hexyl-4-hydroxybenzoate, phenyl-4-hydroxybenzoate and ethyhexyl-4-hydrxybenzoate were obtained from TCI America (Portland, OR). Acetonitrile, methanol, water for HPLC, tetrahydrofuran, acetic acid, potassium dihydrogen phosphate, sodium dihydrogen phosphate, disodium hydrogen phosphate and ammonium hydroxide were obtained from Mallinckrodt Baker, Inc. (Philipsburg, NJ). 0.1% adapalene solution (Differin®) was used for partition coefficient studies.

2.2. Calculated log P (clog P)

The calculated clog *P* was determined using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris.

2.3. Instruments

Equipment used consisted of an 1100 series high-pressure liquid chromatography (HPLC) instrument with an Agilent 1100 series autosampler and a Diode Array detector model 785A (Agilent Technologies, Inc., Palo Alto, CA), an Innova® 4000 series incubator and shaker (New Brunswick Scientific Co., Inc., Edison, NJ), Hermle Z360K centrifuge (Maschinenfabrik Berthold Hermle, Gosheim, Germany) and a Q1000 DSC (TA instruments, New Castle, DE).

2.4. Preparation of artificial sebum

The lipids from the skin surface are derived mainly from two sources, the sebaceous glands and the epidermis (Strauss et al., 1976). The surface lipids from the gland-rich areas naturally contain a higher proportion of sebaceous lipid. In gland deficient areas such as the arms and legs, there is a greater proportion of epidermally derived lipid. Skin lipids from the face are derived in large part from the sebum. The main components of the sebum are triglycerides, wax esters, squalene, cholesterol and cholesterol esters (Strauss et al., 1976). As illustrated in Table 1, the

Table 1
Chemical composition of the human sebum and artificial sebum

Composition	Human sebum-I (Wertz, 2001) (lumen/surface)	Human sebum-II (Rosenthal, 1964)	Human sebum-III (Greene et al., 1970)	Human sebum-IV (Nordstrom et al., 1986)	Artificial sebum
Squalene	15/15	13	12	19.9	15
Wax esters	25/25	26	26	25.3	
Paraffin wax					10
Spermaceti wax					15
Triglycerides	57/42	32	57.5	16.1	
Olive oil (C16–18)					10
Cotton seed oil (C16–18)					25
Coconut oil (C12–16)					10
Fatty acids	0/15 (C16)	23	–	33.0	
Oleic acid					1.4
Palmitic acid					5
Palmitoleic acid					5
Cholesterol	1/1	1.6	1.5	3.8	1.2
Cholesteryl esters	2/2	3.5	2.0	2.0	
Cholesterol oleate					2.4

Table 2
HPLC Condition for the analysis of tested compounds in the mediums

Compound	Mobile phase	Flow rate (mL/min)	Detection (nm)
Ketoconazole	50:50 of CH_3CN and 0.05 M disodium hydrogen phosphate	1.5	240
Minoxidil	60:40 of CH_3CN and 0.01 M NaH_2PO_4 with 2.5 mM sodium dodecyl sulphate	0.8	281
Salicylic acid	40:60 of CH_3CN and 1% acetic acid	1.0	237
2,5-Dihydroxybenzoic acid	40:60 of CH_3CN and 1% acetic acid	1.0	237
Tretinoin	80:5:15 of CH_3CN, and 1% acetic acid of acetic acid, tetrahydrofurone	1.5	350
Adapalene	45:20:35 of CH_3CN, 1% trifluoroacetic acid and tetrahydrofurone	1.0	270
Lidocaine base	12:88 of CH_3CN and 15 mM KH_2PO_4, pH adjusted to 3.0 with H_3PO_4	1.0	205
Lidocaine HCl	12:88 of CH_3CN and 15 mM KH_2PO_4, pH adjusted to 3.0 with H_3PO_4	1.0	205
Tetracycline base	15:85 of CH_3CN and 0.01 M oxalic acid, pH adjusted to 3.0 with NH_3OH	1.0	355
Tetracycline HCl	15:85 of CH_3CN and 0.01 M oxalic acid, pH adjusted to 3.0 with NH_3OH	1.0	355
4-Hydroxybenzoic acid	70:30 of CH_3CN and 1% acetic acid	0.9	254
Methyl-4-hydroxybenzoate	60:40 of CH_3CN and 1% acetic acid	1.2	254
Ethyl-4-hydroxybenzoate	60:40 of CH_3CN and 1% acetic acid	1.2	254
Propyl-4-hydroxybenzoate	60:40 of CH_3CN and 1% acetic acid	1.2	254
Butyl-4-hydroxybenzoate	80:20 of CH_3CN and 1% acetic acid	1.2	254
Amyl-4-hydroxybenzoate	80:20 of CH_3CN and 1% acetic acid	1.2	254
Hexyl-4-hydroxybenzoate	80:20 of CH_3CN and 1% acetic acid	1.2	254
Heptyl-4-hydroxybenzoate	90:10 of CH_3CN and 1% acetic acid	2.0	254
Octyl-4-hydroxybenzoate	90:10 of CH_3CN and 1% acetic acid	2.0	254
Ethyhexyl-4-hydroxybenzoate	90:10 of CH_3CN and 1% acetic acid	2.0	254
Phenyl-4-hydoxy benzoate	80:20 of CH_3CN and 1% acetic acid	1.2	254

chemical composition of the artificial sebum utilized in the study was based on the human sebum chemical composition reported in the literature (Rosenthal, 1964; Greene et al., 1970; Nordstrom et al., 1986; Wertz, 2001). The components of artificial sebum were obtained from commercially available sources. The ingredients were weighed out (% w/w) in a glass beaker and heated at 60 °C with intermittent stirring until all the solids became liquid (10 min). This was done to ensure uniform mixing of the model sebum lipids. The mixture was allowed to cool at room temperature and was used for the drug partitioning studies.

2.5. DSC analysis of artificial sebum

A small portion of artificial sebum was withdrawn and put into a pre-weighed DSC pan. The DSC pans were weighed again to determine the accurate weight of each sample. The samples were then analyzed in triplicate using the DSC Q1000 and run from −40 to 60 °C at a ramp rate of 5 °C/min.

2.6. Preparation of human stratum corneum (SC)

Dermatomed human cadaver skin (300–400 μm) was obtained from the Ohio Valley Tissue and Skin Center (Cincinnati, OH). The frozen skin was thawed in lukewarm, isotonic saline. The thawed skin was incubated with the SC side facing up in Petri dishes over filter paper soaked with 0.1% (w/v) trypsin in 0.5% (w/v) sodium bicarbonate at 37 °C for 6 h. The SC membranes were separated and rinsed with water, and dried in a vacuum desiccator for 2 days.

2.7. Determination of human stratum corneum/water (K_{sc})

The partitioning studies were carried out with weighed pieces (4–8 mg) of dried human stratum corneum placed in a 1 mL aqueous drug solution, and kept in a shaker for 15 h at 37 °C. At the end of the study, the SC was extracted with the addition of 1 mL of acetonitrile, sonicated for 5 min and kept in a shaker for 6 h at 37 °C. The drug content in the aqueous solution and the SC extract were analyzed by HPLC using the HPLC conditions given in Table 2. The partition coefficient values were expressed as the concentration of drug in 1 g of SC divided by the concentration of drug in 1 g of aqueous solution.

2.8. Determination of artificial sebum and water partition coefficient (K_{sebum})

The partitioning studies were carried out with a weighed amount (1–20 mg) of artificial sebum placed in a vial containing 1 mL of aqueous drug solution (2–20 μg/mL), and kept in a shaker for 15 h at 37 °C. The vials were centrifuged at 8000 rpm for 15 min and the clear aqueous solution was withdrawn and analyzed by HPLC for drug content using the conditions given in Table 2. The amount of drug partitioned into the artificial sebum was measured by subtracting the amount of drug concentration present in the aqueous solution from the initial drug concentration in the aqueous solution. The partition coefficient values were expressed as the concentration of drug in 1 g of artificial sebum divided by the concentration of drug in 1 g of aqueous solution.

2.9. Data analysis

Statistical analysis of the drug partition coefficient parameters in artificial sebum and human stratum corneum were computed with a one-way ANOVA followed by Tukey's post hoc analysis using SIGMASTAT (SPSS, Chicago, IL).

3. Results and discussion

Sebaceous glands reach to depths between 0.2 and 0.5 mm in the skin surface. They are functionally connected to hair follicles. Sebum produced in the sebaceous glands vents through the upper third of the hair follicle in order to get to the skin surface. For the drug to be effectively transported into the hair follicle and sebaceous glands, the drug should be capable of favorable, differential partitioning and diffusion in sebum. The following equations are based on the assumptions that drug transport through the skin is primarily by parallel transepidermal (SC is primary barrier) and follicular pathways (Flynn, 1996):

$$J_{\text{total}} = J_{\text{sebum}} + J_{\text{sc}} = (\text{APC})_{\text{sebum}} + (\text{APC})_{\text{sc}} \quad (1)$$

where J_{total} is the total flux and J_{sebum} and J_{sc} are the fluxes through independent pathways (sebum/hair follicles and stratum corneum). A is the total area of application and C is the concentration of drug in the application. It follows that

$$J_{\text{total}} = \left[A_{\text{sebum}} \frac{D_{\text{sebum}} K_{\text{sebum}} C}{h_{\text{sebum}}}\right] + \left[A_{\text{sc}} \frac{D_{\text{sc}} K_{\text{sc}} C}{h_{\text{sc}}}\right] \quad (2)$$

In these equations, f_{sebum} and f_{sc} are the fractional areas of the transfollicular and transepidermal routes, respectively, and A_{sebum} and A_{sc} are the actual areas of the sebum and stratum corneum routes. D_{sebum} and D_{sc} are the diffusion coefficients for the drug in question through sebum and the stratum corneum, while K_{sebum} and K_{sc} are the drug's partition coefficients in sebum/water and stratum corneum/water, respectively. The terms, h_{sebum} and h_{sc}, refer to the functional thicknesses of the sebum and stratum corneum, respectively. In these equations, the partition coefficients exhibit the greatest variability between compounds within a family and, thus, are the parameters most likely to differentiate the transport mechanism (Surber et al., 1990; Flynn, 1996). Therefore, when $K_{\text{sebum}} \gg K_{\text{sc}}$, drug molecules will be mainly transported through sebaceous and hair follicles. When the reverse is true, that is, when $K_{\text{sebum}} \ll K_{\text{sc}}$, the principal pathway for diffusion and accumulation will be through the stratum corneum (the transepidermal pathway). The ratios of $K_{\text{sebum}}/K_{\text{sc}}$ and $D_{\text{sebum}}/D_{\text{sc}}$ reflect the potential for follicular drug delivery.

As shown in Table 1, the composition of human sebum is variable, particularly the composition of triglycerides and fatty acids because of the degradation of triglycerides during sebum secretion. Therefore, the artificial sebum (Table 1) was formulated based on the average composition of human sebum. Paraffin wax and spermaceti wax were the substitutes of the wax esters. Olive oil, cotton seed oil and coconut oil provided the triglycerides in the artificial sebum because they have the carbon chain lengths of the fatty esters that are similar to those found in the chemical composition of human sebum. The percentage of fatty acids in human sebum samples varies greatly and depends on the degree of triglyceride degradation which occurs when sebum secretes from the sebaceous glands (no fatty acids) to the skin surface (up to 45% fatty acids). Considering the sebum of interest is in the upper duct of hair follicles, a relatively low percentage of fatty acid (~11%) was added in the artificial sebum.

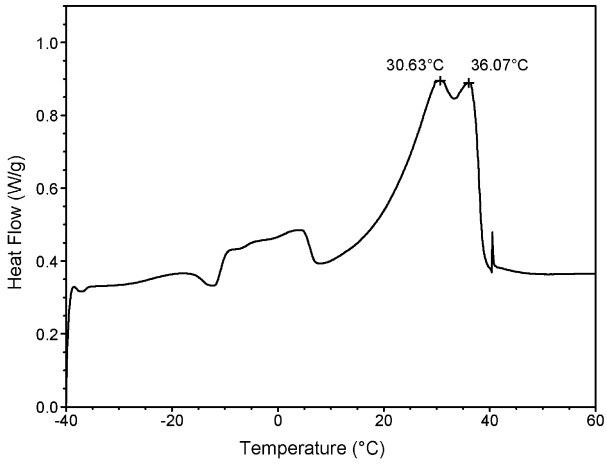

Fig. 1. Typical thermogram of a model sebum.

We have confirmed the similarity of the prepared artificial sebum to the collected human sebum samples using DSC and NMR methods (unpublished data). Furthermore, the partition and diffusion studies were carried out using three model compounds in artificial sebum and human sebum. These studies indicated that the partition/diffusion property of the artificial sebum is closely correlated to the human sebum.

The melting property of artificial sebum is critical to determine the sebum partition coefficient at body temperature (37 °C). Human sebum liquefies around 37 °C and changes to a waxy form at lower temperatures. The composition of the artificial sebum was adjusted to achieve an appropriate melting profile. Fig. 1 shows the representative thermogram of the artificial sebum. Two major transitions were observed at 30.63 and 36.07 °C. The peaks below 5 °C were relatively small and flat. Compared to human sebum, the artificial sebum used in this study appears to demonstrate an acceptable melting profile. The thermogram reflects the melting behavior of the primary chemicals in the sebum samples. Motwani et al. (2002) reported that there were four phase transitions from the model sebum they used. Those transition peaks were assigned as the added fatty acids, triglycerides, and wax ester. It is obvious that the thermograms may be different if the artificial sebum samples contain different excipients.

In order to optimize the K_{sebum} studies, we conducted a study using lidocaine as a model drug by equilibrating varying ratios of artificial sebum and drug solutions for the different time intervals. Fig. 2 shows that lidocaine equilibrium was reached at 15–24 h irrespective of the quantity of artificial sebum used. However, after 24 h, the lidocaine K_{sebum} decreased as the equilibrium time increased and this led to an underestimation of K_{sebum}. This observed decrease may be due to the hydrolysis of lipids present in the artificial sebum, which could alter the polarity and pH of the medium. If studies are conducted at more than 24 h, a deviation of K_{sebum} may result. Therefore, the K_{sebum} studies were limited to 15 h at 37 °C.

Table 3 shows the measured partition coefficients of 11 model compounds in artificial sebum and stratum corneum, the calculated log P (octanol/water), and the ratio of partition in artificial

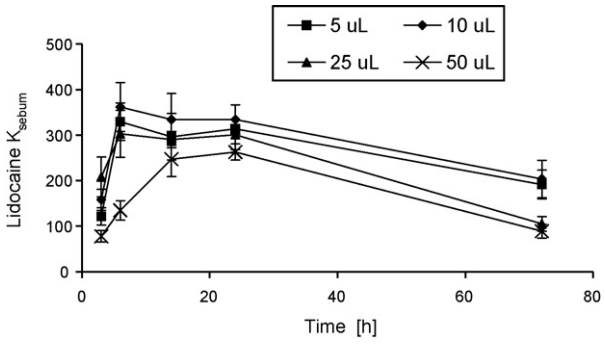

Fig. 2. Profiles of lidocaine K_{sebum} at various time intervals with varying quantities of artificial sebum ($n = 6$).

sebum versus stratum corneum. Ketoconazole exhibited the highest K_{sebum} (9111 ± 1156) in the tested compounds, followed by ethylhexyl-4-hydroxybenzoate and tretinoin (5636 ± 913.1 and 2504 ± 343.9, respectively). Minoxidil, salicylic acid, tetracycline, tetracycline hydrochloride, and 2,5-dihydroxybenzoic acid showed lower K_{sebum} ranging from 2.11 ± 0.35 to 5.17 ± 0.46, whereas adapalene, lidocaine, and phenyl-4-hydroxybenzates showed moderate K_{sebum}. In the case of K_{sc}, adapalene, tretinoin, and lidocaine had a higher mean K_{sc} (132.8 ± 30.2, 117.4 ± 30.5, 165.3 ± 8.41, respectively) than that of the other model compounds. However, the K_{sc} of tetracycline (4.40 ± 0.89) was not significantly ($p > 0.05$) different from that of tetracycline hydrochloride (4.68 ± 1.88). Table 3 shows that the K_{sebum} value of ketoconazole, tretinoin, adapalene, lidocaine, and ethylhexyl-4-hydroxybenzoate was significantly higher ($p < 0.001$) than K_{sc}. This indicates that these drugs are more likely to transport through the hair follicle and sebaceous gland than through the stratum corneum, compared to other tested compounds. In contrast, the K_{sebum} of minoxidil, 2,5-dihydroxybenzoic acid and phenyl-4-hydroxybenzoate was significantly lower ($p < 0.001$) than K_{sc}, indicating that these drugs are less sebum friendly but more likely to selectively transport through the stratum corneum than through hair follicles and sebaceous glands. However, there was no significant difference ($p > 0.05$) in the K_{sebum} of tetracycline, tetracycline HCl and salicylic acid when compared with K_{sc}. In the case of

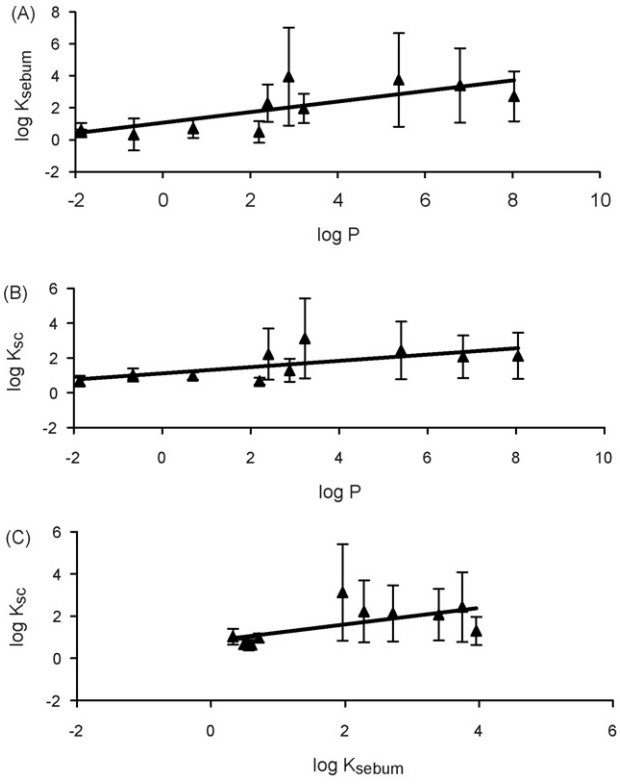

Fig. 3. Plot of correlation for model compounds with different chemical structures (A) clog P vs. log K_{sebum} ($r = 0.5899$), (B) clog P vs. K_{sc} ($r = 0.4738$), and (C) log K_{sc} vs. log K_{sebum} ($r = 0.3901$). Each data point represents the mean of the data ($n = 3$ for K_{sc} and $n = 6$ for K_{sebum}).

tetracycline HCl, the K_{sebum} and K_{sc} were not significantly different ($p > 0.05$) from that of the tetracycline base because of the equivalency in the ionization state in the pH range of 6–8 (pK_a 4.5).

Interestingly, the partition of these tested compounds in artificial sebum is different from that in octanol. The plot of Fig. 3A shows the poor linear relationship between the clog P and log K_{sebum} ($r = 0.5899$), though there is a trend between the clog P and log K_{sebum} with a variety of chemical structures, which indicates clog P is not a good parameter for the partition

Table 3
Mean (±S.D.) partition coefficient values of compounds with different chemical structures in artificial sebum/water (K_{sebum}) and human stratum corneum (K_{sc})

Compounds	Molecular weight	clog P[a]	K_{sebum}[b]	K_{sc}[c]	K_{sebum}/K_{sc}
Ketoconazole	531.43	2.88	9111.20 ± 1156.14	19.73 ± 1.36	461.78
Minoxidil	209.25	0.69	5.17 ± 0.46	9.15 ± 0.78	0.57
Salicylic acid	138.12	2.20	3.10 ± 0.78	4.68 ± 1.2	0.66
2,5-Dihydroxybenzoic acid	154.12	−0.67	2.11 ± 0.35	10.50 ± 0.22	0.20
Tretinoin	300.44	6.80	2504.10 ± 343.92	117.74 ± 30.47	21.27
Adapalene	412.52	8.04	520.83 ± 57.08	132.75 ± 30.22	3.92
Lidocaine	234.34	2.40	192.43 ± 5.27	165.30 ± 8.41	1.16
Tetracycline	444.43	−1.87	4.01 ± 0.32	4.40 ± 0.89	0.91
Tetracycline HCl	480.90	−1.87	3.64 ± 1.13	4.68 ± 1.88	0.78
Ethylhexyl-4-hydroxybenzoate	250.34	3.75	5635.59 ± 913.11	1329.03 ± 201.83	4.24
Phenyl-4-hydroxybenzoate	214.22	1.96	91.93 ± 13.74	271.06 ± 28.40	0.34

[a] Calculated clog P values.
[b] $n = 6$.
[c] $n = 3$.

property of these compounds in sebum. Because its chemical composition is similar to that of human sebum, artificial sebum certainly serves as a better vehicle for the investigation of the thermodynamic/kinetic properties of drugs designed to target the follicle. For example, isopropyl myristate (IPM) has been selected as a substitute for octanol for partition studies because IPM is often used as an excipient in topical formulations (Surber et al., 1990). Therefore, the partition coefficient between IPM and water provides more relevant information for the development of topical formulations, such as creams and lotions. Similarly, it is more meaningful to use artificial sebum as an octanol substitute to determine the partition coefficient for the sebum-targeted drug delivery studies. As expected, a poor correlation ($r = 0.4738$ and 0.3901) was also observed between clog P and log K_{sc} (Fig. 3B), log K_{sebum} and log K_{sc} (Fig. 3C). The partition coefficient between the stratum corneum and water has been considered a sensitive parameter to structural changes of topically applied compounds, and suggested as a critical parameter for drug and formulation screening. In the present study, we show that the partition coefficient of drugs between artificial sebum and water more sensitively discriminates between sebum-targeted compounds than does the partition coefficient between octanol and water, and that the ratio of K_{sebum} and K_{sc} may be a good parameter to use in selecting sebum-targeted molecules and formulations even when the number of tested samples is very small.

In order to further understand the effect of molecular structure on the sebum partition, we have selected a series of compounds with varying the carbon side chain length and conducted partition studies using the artificial sebum and the stratum corneum. Table 4 shows the K_{sebum} values of a group of 4-hydroxybenzoate compounds. By increasing the length of carbon side chain from $n = 0$ to 8, the clog P and K_{sebum} increased from 1.42 to 5.58 and from 2.38 ± 0.25 to 11078 ± 1599, respectively. In contrast, the K_{sc} of 4-hydroxybenzoates series compounds did not change significantly ($p > 0.05$) with an increase in the lipophilicity of the compounds between clog P 1.47 and 2.93. However, it substantially increased ($p < 0.001$) with further increase in the lipophilicity, clog P 3.46 to 5.58.

The results from Table 4 and Fig. 4A indicate that increasing the side chain length facilitated the partitioning of the molecules into the sebum. In addition, a good correlation between the clog P and log K_{sebum} of the 4-hydroxybenzoate compounds was observed ($r = 0.9981$). These results indicate that clog P could be a conditional surrogate for log K_{sebum}, for example, in a series of compounds with a simple structural change. In other words, it appears that for drugs with a similar structure, clog P or log P may be a useful parameter in selecting an appropriate molecule for follicular delivery (Grams and Bouwstra, 2002). However, further investigations are needed to better understand the relationship between clog P and log K_{sebum}, including whether the acidic nature of the components in the sebum plays a role in the discrimination of $P_{o/w}$ and K_{sebum}.

As shown in Table 4 and mentioned previously, K_{sc} did not increase from 4-hydroxybenzoic acid to propyl-4-hydroxybenzoate ($n = 0$–3), but it increased with adding the length of carbon side chain after $n > 3$. The correlation

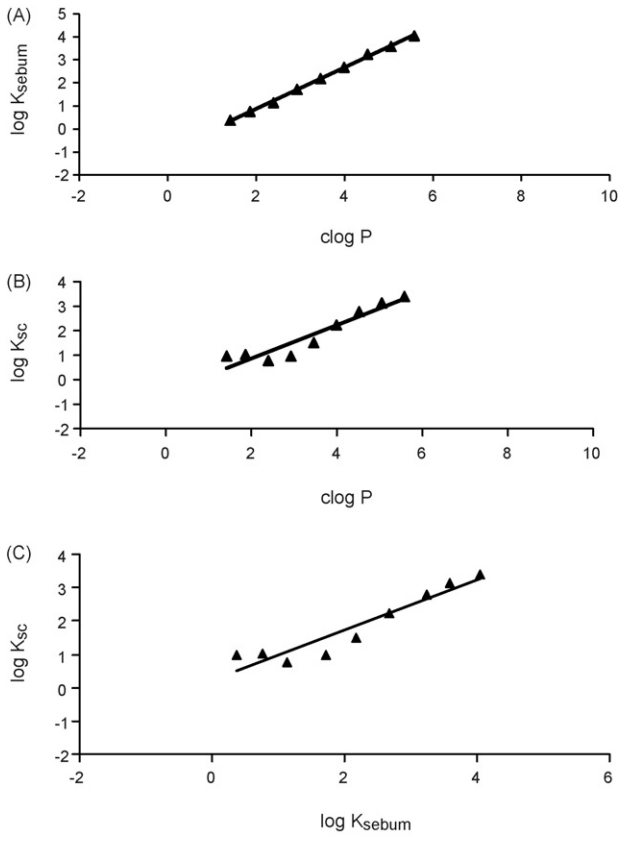

Fig. 4. Plot of correlation for 4-hydroxybenzoate series compounds (straight chain) with increase in the carbon side chain (A) clog P vs. log K_{sebum} ($r = 0.9981$), (B) clog P vs. K_{sc} ($r = 0.8916$), and (C) log K_{sc} vs. log K_{sebum} ($r = 0.8996$). Each data point represents the mean of the data ($n = 3$ for K_{sc} and $n = 6$ for K_{sebum}).

($r = 0.8916$ and 0.8996) between clog P and log K_{sc} (Fig. 4B) and between log K_{sebum} and log K_{sc} (Fig. 4C) is lower when compared to clog P and log K_{sebum} ($r = 0.9981$). The compounds with the highest polarity, 4-hydroxybenzoic acid and methyl-4-hydroxybenzoate, had relatively higher partitioning in SC than ethyl-4-hydroybenzoate and propyl-4-hydroxybenzoate, as shown in Table 4. The stratum corneum is known as a heterogeneous membrane, which may consist of various domains, such as lipid, protein and solvent domains (Raykar et al., 1998). Therefore, the partitioning of a compound into the SC is a function of partitioning in the individual domains. It is likely that the polar compounds tested predominately reside in the polar domain of SC, such as the protein and solvent matrix, while the more lipophilic compounds partition primarily in the lipid domain. The nonlinear relationships between log K_{sc} and log K_{sebum} or clog P reflect the complexity of the partition or distribution of a compound in various domains. This may indicate that K_{sebum} is a function of not only the lipophilicity but also the structure of the molecule. In addition, the ratio of K_{sebum}/K_{sc} was relatively low (<1) for 4-hydroxybenzoic acid and methyl-4-hydroxybenoate where the clog P of these compounds was <2. However, the ratio was much higher (>2) when the length of carbon side chain increased ($n \geq 2$ and clog $P > 2$) and reached the high (>4) at $n = 3$, 4 and 8, respectively. Although there is no linear corre-

Table 4
Mean (±S.D.) partition coefficient values of compounds with similar chemical structures in artificial sebum/water (K_{sebum}) and human stratum corneum (K_{sc})

Compounds	Molecular weight	clog P[a]	K_{sebum}[b]	K_{sc}[c]	K_{sebum}/K_{sc}
4-Hydroxybenzoic acid	138.12	1.42	2.38 ± 0.25	9.28 ± 3.07	0.26
Methyl-4-hydroxybenzoate	152.15	1.87	5.74 ± 0.55	10.46 ± 1.67	0.55
Ethyl-4-hydroxybenzoate	166.17	2.40	13.66 ± 0.98	6.06 ± 1.11	2.25
Propyl-4-hydroxybenzoate	180.20	2.93	51.96 ± 8.40	9.12 ± 2.43	5.70
Butyl-4-hydroxybenzoate	194.23	3.46	151.54 ± 16.72	32.18 ± 1.45	4.71
Amyl-4-hydroxybenzoate	208.25	3.99	471.18 ± 58.16	170.61 ± 11.34	2.76
Hexyl-4-hydroxybenzoate	222.20	4.52	1768.05 ± 266.07	604.50 ± 57.51	2.92
Heptyl-4-hydroxybenzoate	236.31	5.05	3818.10 ± 329.79	1373.11 ± 26.77	2.78
Octyl-4-hydroxybenzoate	250.33	5.58	11078.49 ± 1599.50	2508.53 ± 261.00	4.42

[a] Calculated clog P values.
[b] $n = 6$.
[c] $n = 3$.

lation between the ratio and the length of carbon side chain or clog P, the compounds with the high ratios of K_{sebum}/K_{sc} are likely to selectively partition into sebum, thereby to be desirable for the delivery to hair follicles and sebaceous glands.

In summary, the results of these studies demonstrated that the K_{sebum} of the tested compounds is a different parameter from K_{sc} and $P_{o/w}$. The K_{sebum} and the ratio of K_{sebum}/K_{sc} of the tested compounds provide critical and more relevant information to evaluate the property of drug transport in or through sebum and to select the appropriate candidates for the targeted delivery into the hair follicle and sebaceous glands. When more data are available, further studies are planned so that a mathematical model describing the relationship between K_{sebum} and the calculated $P_{o/w}$ may be developed and the usefulness of K_{sebum} for the follicular drug delivery – both in vitro and in vivo models – may be more accurately estimated.

4. Conclusions

In conclusion, a model artificial sebum sample was formulated and a method was developed to determine the drug partition coefficient between sebum and water (K_{sebum}). A poor correlation ($r \leq 0.59$) was observed in clog P versus log K_{sebum}, clog P versus log K_{sc}, and log K_{sebum} versus log K_{sc} for model compounds with different chemical structures. In contrast, in the 4-hydrxoy benzoate (straight chain) series, a good linear trend ($r = 0.9981$) exists between the clog P and log K_{sebum}. We also theorize that the K_{sebum} and the ratio of K_{sebum}/K_{sc} are the useful parameters to predict the likelihood of targeted delivery to hair/sebaceous follicles.

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