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Comparison of the percutaneous absorption of hydrophilic and lipophilic compounds in shed snake skin and human skin

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The *in vitro* transdermal permeation of eight hydrophilic drugs (antipyrine, L-dopa, dopamine hydrochloride, diclofenac sodium, 5-fluorouracil, isoprenaline hydrochloride, nicorandil and morphine hydrochloride) and eight lipophilic drugs (aminopyrine, cyclobarbital, ibuprofen, indomethacin, isosorbide dinitrate, flurbiprofen, ketoprofen and lignocaine) was determined using shed snake skin of *Elaphae obsoleta* and human skin. The permeation parameters and physiological characteristics of the skin, e.g. the water and lipid content, and the thickness of shed snake skin and human skin were evaluated and compared. In shed snake skin, the permeability coefficients (P) of lipophilic drugs were in the same range as those through the human skin (0.9 to 1.8-times); whereas those of hydrophilic drugs were remarkably lower (3.3 to 6.1-times). The thickness and lipid content of shed snake skin and human stratum corneum were not significantly different ($P > 0.05$), whereas the water content of shed snake skin was significantly lower than that of human stratum corneum ($P < 0.05$). The lower permeability of shed snake skin for hydrophilic compounds might be caused by the lower porosity of skin strata. The results suggested a potential use of shed snake skin as barrier membrane for lipophilic compounds percutaneous absorption studies *in vitro*.

1. Introduction

Theoretically, skin permeability of drugs should be tested in humans. However, ethical considerations are the major problem in using human skin as a model membrane. Therefore, animal skin such as hairless rat, pig, rabbit and shed snake skin is generally used as an alternative (Bisset and Mcbride 1983; Hirvonen et al. 1991; Walker et al. 1983). Shed snake skin of *Elaphae obsoleta* used for skin permeation studies in various transdermal delivery studies because of the similarity to human skin in terms of thickness and composition, the easy storability and the low variation of permeability data. However, shed snake skin differs from human stratum corneum in that it is devoid of appendageal structure such as hair follicles or sweat sebaceous glands (Itoh et al. 1990).

There are at least two permeation pathways existing in the stratum corneum: one is the pathway for hydrophilic permeants or pore pathway; the other is the pathway for lipophilic permeants or lipid pathway (Burnett and Bagniefski 1988; Hatanaka et al. 1990; Inada et al. 1994). There have been many reports on a good correlation between diffusion through shed snake skin and human skin *in vitro* (Higuchi and Konishi 1987; Hirvonen et al. 1991; Harada et al. 1993). However, most publications studied lipophilic permeants. The information about the skin permeability of various hydrophilic permeants is limited. Therefore, species differences in skin permeability of both hydrophilic and lipophilic permeants among these species must be further evaluated.

In this study, we studied *in vitro* permeation of eight hydrophilic drugs and eight lipophilic drugs through shed snake skin. The permeation properties and physiological characteristics of the skin, e.g. the water and lipid content, and the thickness of shed snake skin, were also evaluated and compared with those of human skin. The potential of using shed skin as a model membrane for skin permeability studies was assessed.

2. Investigations, results and discussion

The physicochemical parameters of drugs used in this study are shown in Table 1 (Hatanaka et al. 1990). Eight hydrophilic drugs i.e. antipyrine (ANP), L-dopa (L-DP), diclofenac sodium (DC-Na), dopamine hydrochloride (DPH), 5-flurouracil (5-FU), isoprenaline hydrochloride (IPH), nicorandil (NR) and morphine hydrochloride (MPH) and eight lipophilic drugs i.e. aminopyrine (AMP), cyclobarbital (CB), ibuprofen (IP), indomethacin (IDM), isosorbide dinitrate (ISDN), flurbiprofen (FP), ketoprofen (KP) and lignocaine (LC) were categorized based on distribution coefficient ($\log K_{ow} < 0$ for hydrophilic drugs and $\log K_{ow} \geq 0$ for lipophilic drug). Fig. 1 shows the permeation profiles of drugs, for example, L-DP and ISDN through shed snake skin and human skin. The cumulative amount of drug increased linearly with time after a short lag time (0.11–0.23 h for shed snake skin and 0.89–1.02 h for human skin). This linear accumulation was also

Table 1: Physicochemical parameters of the model drugs[a]

Drugs	Mol. wt	Solubility in water[b] (mg mL^{-1})	Distribution coefficient[c]
Ibuprofen (IP)	206	0.0430	3.94
Flurbiprofen (FP)	244	0.0277	3.86
Indomethacin (IDM)	357	0.0111	3.19
Ketoprofen (KP)	254	0.185	3.11
Lignocaine (LC)	234	3.03	2.37
Isosorbide dinitrate (ISDN)	236	1.34	1.34
Cyclobarbital (CB)	236	3.07	0.873
Aminopyrine (AMP)	231	55.9	0.497
5-Fluorouracil (5-FU)	130	17.1	−0.860
Diclofenac sodium (DC-Na)	296	32.0	−0.962
Nicorandil (NR)	211	39.6	−1.02
Antipyrine (ANP)	188	816	−1.55
Morphine hydrochloride (MPH)	339	82.5	−2.53
Isoprenaline hydrochloride (IPH)	211	345	−2.69
Dopamine hydrochloride (DPH)	153	520	−3.40
Levodopa (L-DP)	197	5.00	−4.70

[a] Hatanaka et al. (1990)
[b] Solubility in water at 37 °C
[c] Logarithmic of octanol/water distribution coefficient at 37 °C

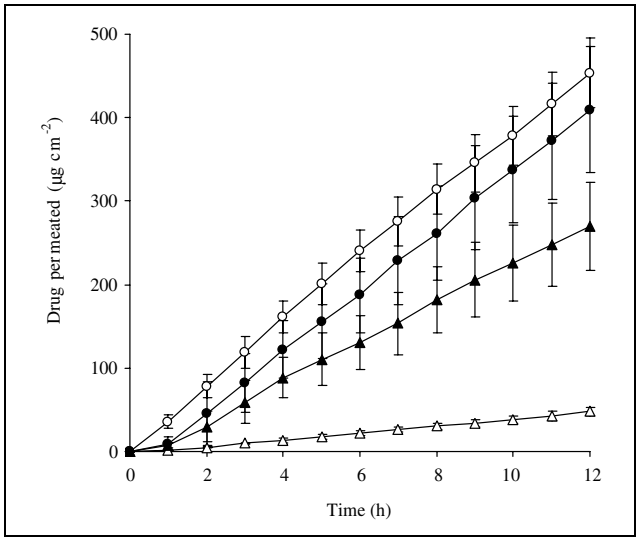

Fig. 1: Permeation profiles of ibuprofen through shed snake skin (△) and human skin (▲) and morphine hydrochloride through shed snake skin (○) and human skin (●). Each point represents the mean ± S.D. of four to six experiments

observed for other permeants (data not shown). Table 2 shows the steady-state permeation rate, lag time and permeability coefficient (P) of permeants through shed snake skin and human skin calculated from permeation profiles. P of hydrophilic drugs (P_H) were lower than those of lipophilic drugs (P_L) in both shed snake skin and human skin. These observations are in agreement with our previous studies using hairless rat skin as model membrane (Hatanaka et al. 1990). To compare P of human skin (P_H) with P of shed snake skin (P_S) of both hydrophilic and lipophilic permeants, the ratio of P_H to P_S was plotted against each permeant (Fig. 2). In shed snake skin, P_L were in the same range as those through the human skin (0.9–1.8-times); whereas P_H were remarkably lower (3.3–6.1-times). A similar result was obtained by Itoh et al. (1990) who compared permeation of the lipophilic compounds phenol, m-cresol, methyl paraben and corticosterone through shed snake skin (1.6-, 1.5-, 1.9- and 0.6-times, respectively, compared to human skin). In contrast, Hirvonen et al. (1990) reported that the permeability of 5-FU through shed snake skin was higher than that through abdominal human skin (2-times). However, one should note that only one hydrophilic compound was used in this investigation; the relationship between skin permeability and lipophilicity was not conclusive.

The coefficient of variation in skin permeability of shed snake skin was small (4.2–13.5%) compared with human skin (14.3–41.0%) (Table 2). These results were in agreement with those reported using methyl paraben (10.2% (snake) and 31.4% (human)), corticosterone (20.2% (snake) and 40.2% (human) (Itoh et al. 1990)). The greater inter-specimen variation might be due to the variation of hair follicles (Bialik et al. 1993). An advantage of using shed snake skin is the lower inter-specimen variation of the permeation results. The mean lag times of drugs across shed snake skin were shorter than for human skin (Table 2). These results agree with those of Pongjanyakul et al. (2000, 2002) for the skin permeation of nicotine.

Table 2: Permeation parameters of drugs across shed snake skin and human skin[a]

	Shed snake skin			Human skin		
	Flux (μg cm^{-2} h^{-1})	P (cm s^{-1})	Mean lag time (h)	Flux (μg cm^{-2} h^{-1})	P (cm s^{-1})	Mean lag time (h)
IP	18.96 ± 1.74 (CV 9.2%)	$1.22 \pm 0.12 \times 10^{-4}$	0.21	26.41 ± 3.77 (CV 14.3%)	$1.72 \pm 0.25 \times 10^{-4}$	0.81
FP	7.16 ± 0.29 (CV 4.2%)	$7.18 \pm 0.31 \times 10^{-5}$	0.12	11.73 ± 4.01 (CV 34.2%)	$1.18 \pm 0.42 \times 10^{-4}$	1.12
IDM	0.44 ± 0.02 (CV 4.6%)	$1.09 \pm 0.05 \times 10^{-5}$	0.13	0.49 ± 0.19 (CV 38.8%)	$1.24 \pm 0.48 \times 10^{-5}$	0.73
KP	4.29 ± 0.46 (CV 10.7%)	$6.44 \pm 0.69 \times 10^{-6}$	0.23	7.25 ± 2.72 (CV 37.5%)	$1.08 \pm 0.38 \times 10^{-5}$	0.83
LC	53.26 ± 6.12 (CV 11.5%)	$4.88 \pm 0.56 \times 10^{-6}$	0.21	76.41 ± 20.24 (CV 26.5%)	$7.01 \pm 1.86 \times 10^{-6}$	1.21
ISDN	37.74 ± 3.28 (CV 8.7%)	$7.82 \pm 0.68 \times 10^{-6}$	0.11	35.75 ± 12.39 (CV 34.7%)	$6.12 \pm 2.13 \times 10^{-6}$	0.89
CB	5.57 ± 0.68 (CV 12.2%)	$5.03 \pm 0.61 \times 10^{-7}$	0.08	4.85 ± 0.88 (CV 18.1%)	$4.42 \pm 0.80 \times 10^{-7}$	0.88
AMP	34.36 ± 4.64 (CV 13.5%)	$1.71 \pm 0.23 \times 10^{-7}$	0.07	62.03 ± 16.68 (CV 26.8%)	$3.09 \pm 0.82 \times 10^{-7}$	1.27
5-FU	0.24 ± 0.02 (CV 8.3%)	$3.84 \pm 0.32 \times 10^{-9}$	0.15	0.79 ± 0.26 (CV 32.9%)	$1.21 \pm 0.39 \times 10^{-8}$	1.15
DC-Na	2.06 ± 0.22 (CV 10.7%)	$1.91 \pm 0.21 \times 10^{-8}$	0.16	8.35 ± 2.71 (CV 32.4%)	$7.46 \pm 2.42 \times 10^{-8}$	1.16
NR	2.17 ± 0.29 (CV 13.4%)	$1.45 \pm 0.19 \times 10^{-8}$	0.27	7.23 ± 2.66 (CV 36.8%)	$5.07 \pm 1.87 \times 10^{-8}$	0.97
ANP	16.45 ± 1.10 (CV 6.7 %)	$5.61 \pm 0.38 \times 10^{-9}$	0.31	54.23 ± 19.25 (CV 35.5%)	$1.85 \pm 0.66 \times 10^{-8}$	0.91
MPH	4.05 ± 0.31 (CV 7.7 %)	$1.26 \pm 0.09 \times 10^{-8}$	0.23	24.95 ± 8.53 (CV 34.2%)	$9.21 \pm 3.15 \times 10^{-8}$	1.02
IPH	2.91 ± 0.28 (CV 9.9%)	$2.34 \pm 0.23 \times 10^{-9}$	0.24	11.74 ± 4.77 (CV 40.6%)	$9.53 \pm 3.87 \times 10^{-9}$	0.84
DPH	7.85 ± 0.95 (CV 12.1%)	$4.22 \pm 0.51 \times 10^{-9}$	0.12	37.73 ± 15.84 (CV 42.0%)	$2.02 \pm 0.89 \times 10^{-8}$	0.92
L-DP	0.073 ± 0.008 (CV 10.9%)	$4.05 \pm 0.44 \times 10^{-9}$	0.27	0.39 ± 0.16 (CV 41.0%)	$2.18 \pm 0.89 \times 10^{-8}$	0.87

[a] Each value represents the mean ± S.D.of four to six experiments
CV = coefficient of variation
P = permeability coefficient

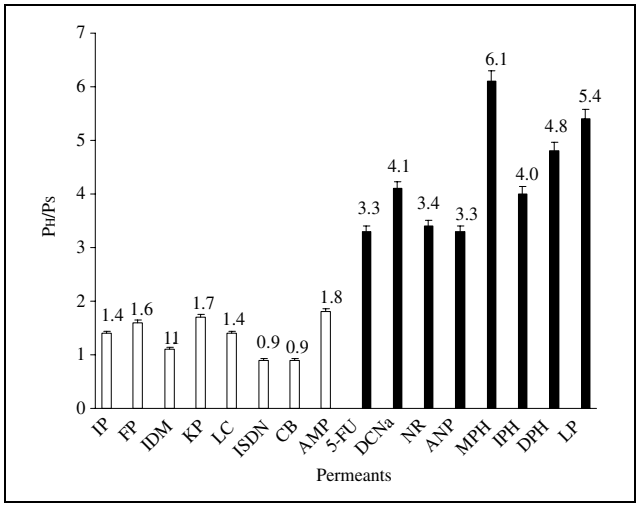

Fig. 2: Ratio of permeability coefficient of human skin (P_H) to permeability coefficient of shed snake skin (P_S) of lipophilic permeants (□) and hydrophilic permeants (■). Each point represents the mean ± S.D. of four to six experiments

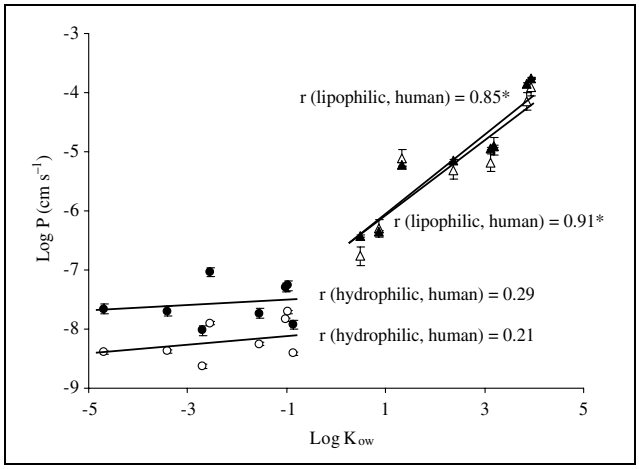

Fig. 3: Relationship between the permeability coefficient and octanol/water distribution coefficient of lipophilic in shed snake skin (△) and human skin (▲) and hydrophilic drugs of shed snake skin (○) and human skin (●). Each point represents the mean ± S.D. of four to six experiments. * $P < 0.05$ compared with zero

Takahashi et al. (1993) also reported that the lag time of neutral, acidic and basic compounds with molecular weights below 200 was not observed when using shed snake skin from *E. obsoleta*. The molecular weight of the compounds used in this study was between 250 and 354. However, the lag time of shed snake skin was found to be small (<0.31 h, Table 2).

In order to validate the skin permeation mechanism in shed snake skin and human skin, the relationship between log P and log K_{ow} of the skin types was plotted (Fig. 3). There was a linear relationship between P and K_{ow} for lipophilic drugs of both shed snake skin ($r = 0.91$, $P < 0.05$) and human skin ($r = 0.85$, $P < 0.05$), whereas for hydrophilic drugs, an almost constant value of P (about 3×10^{-8} cm/s for human and 1×10^{-9} cm/s for shed snake skin), and independence of K_{ow} was found ($r = 0.21$, $P > 0.05$ for shed snake skin; and $r = 0.29$, $P > 0.05$ for human skin). There are two pathways for skin permeation through the stratum corneum (Burnett and Bagniefski 1988; Hatanaka et al. 1990; Inada et al. 1994). One is the pore pathway or pathway for hydrophilic permeants, in which P is independent of lipophilicity and almost constant. The other one is the lipid pathway, the main skin permeation route for lipophilic permeants, in which P is correlated to the partition coefficient (Hatanaka et al. 1990). Therefore, the skin permeation mechanism of shed snake skin and human skin followed the lipid pathway and the pore pathway similar to hairless rat skin (Hatanaka et al. 1990).

Although similar skin permeation pathways were found, a lower skin permeability of hydrophilic permeants through shed snake skin compared to human skin was observed (Fig. 2). To evaluate the reasons for such species differences, we focused on the physiological characteristics of both skin types. Thickness, lipid and water contents were determined (Table 3). The thickness and total lipid content of shed snake skin were not significantly different from those of human stratum corneum ($P > 0.05$), however the composition of ceramides and phospholipids and the water content were significantly different ($P < 0.05$). It was noted that the thickness, the composition of lipids, and the water content of stratum corneum varies among species (Sato et al. 1991; Dick and Scott 1992). Such a difference may be a major factor in the variation in skin permeability between different species. There is no significant difference in the thickness, the total lipid contents, and the content of cholesterol among the two species investigated here. Itoh et al. (1990) also reported that the thickness and lipid content of the human stratum corneum and the shed snake skin of *Elaphae obsoleta* were comparable. The content of ceramide and phospholipid in the shed snake skin and the human stratum corneum are significantly different. The main polar lipids are phospholipids in shed snake skin and ceramides in man (Table 3). These results agree with those reported by Long et al. (1981). Although their lipid composition are remarkably different, Elias et al. (1981) suggested that total lipid content, rather than lipids composition, plays an important role in barrier functions of the stratum corneum. The water content of the shed snake skin was lower than that of the human stratum corneum (Table 2). These results indicate that the volume of pore or porosity of the shed snake skin might be less than that of the human stratum corneum. Cutaneous water promotes the fluidity of lipid bilayer and extends the lipid domain among the lipid polar groups (Barry 1987). As the condition of the stratum corneum is more hydrated, the drug can freely move in the stratum corneum and easily penetrate through the skin. Moreover, lipid and water content can be used as indicators of the ratio of lipid and pore pathways, respectively (Hatanaka et al. 1990). In the shed snake skin and human stratum corneum, differences in permeation pathways may be associated with species differences.

Table 3: Lipid, water content and thickness of the shed snake skin and the human stratum corneum[a]

Subject	Composition	Species	
		Snake	Human
Lipid content (μg/mg)	Ceramides	45 ± 9*	24 ± 4
	Cholesterol	35 ± 4	36 ± 4
	Phospholipids	21 ± 3*	40 ± 5
	Total extract lipid	126 ± 10	119 ± 12
Water content (μg/mg)		61 ± 7*	128 ± 14
Thickness (μm)		17 ± 2	18 ± 3

[a] Each value represents the mean ± S.D of four to six experiments
* $P < 0.05$ compared with human

In this study, a species difference in skin permeation of hydrophilic and lipophilic compounds was verified. In comparison with human skin, P values of lipophilic drugs were similar, however those of hydrophilic drugs were remarkably lower. The discrepancy in skin permeability of drugs in different species may be mainly due to the difference in water content in the skin. To predict the skin permeability of hydrophilic compounds of human skin by extrapolating from the data on shed snake skin, differences in skin permeability should be taken into consideration.

3. Experimental

3.1. Materials

Antipyrine (ANP), aminopyrine (AMP), diclofenac sodium (DC-Na), dopamine hydrochloride (DPH), flurbiprofen (FP), ibuprofen (IP), indomethacin (IDM), isoprenaline hydrochloride (IPH), isosorbide dinitrate (ISDN), ketoprofen (KP), L-dopa (L-DP) and lignocaine (LC) were purchased from Sigma Co. (St. Louis, Mo, USA). Nicorandil (NR) was from Nisshin Flour Milling Co. (Tokyo, Japan). Cyclobarbital (CB) and 5-fluorouracil (5-FU) were obtained from Tokyo Kasei Kagyo Co. (Tokyo, Japan). Morphine hydrochloride (MPH) was obtained from Takeda Yakuhin Industries Co. (Osaka, Japan). The physicochemical properties of drugs used in this study are shown in Table 1 (Hatanaka et al. 1990).

3.2. Skin membrane preparation

The method of the percutaneous absorption study followed Test Guideline 428 of the Organization for Economic Cooperation and Development (OECD, 2000). The shed snake skin of *Elaphae obsoleta* was used as a model membrane. It was a gift from the Pata Department Store Snake Park, Bangkok, Thailand. The shed snake skin was obtained from eight different snakes. Each shed snake skin can be divided into 10–15 pieces. Human skin was obtained following unrelated surgical operations (Department of Surgery, Kanakawa Hospital University, Japan). The source was the chest of female patients (35–75 years old). The number of donors of the skin was twenty. The skin from each donor can be divided in to 3–10 pieces. The skin was stored at −20 °C prior to use in order to maintain the original activity of skin enzymes (Rohatagi et al. 1997). The samples were gradually thawed in 0.9% w/v NaCl solution, and were prepared to be split-thickness skins (0.6–0.7 mm) by a dermatome. At this thickness, the resistance of the dermis to overall skin permeation of drug can be ignored (Hatanaka et al. 1990).

3.3. Skin permeation

The *in vitro* permeation experiments were performed according to Okumura et al. (1989). The skin from different snakes or donors was mounted between two half cells of a side-by-side diffusion cell with a water jacket connected to a water bath at 37 °C, each having 4.0 ml volume and 0.78 cm^2 effective diffusion area. The receiver and donor compartments were filled with distilled water and stirred with a Teflon magnetic stirrer at 600 rpm. After 1 h of equilibration, the receiver compartment was filled with freshly distilled water in the case of hydrophilic drugs or 20% w/w polyethylene glycol 400 in the case of lipophilic drugs to maintain a sink condition in the receiver solution. The skin permeability was not changed using these receptor solutions (Hatanaka et al. 1993). The donor compartment was replaced with a drug suspension in distilled water (at 2–10 times higher concentration than the solubility of each drug) to assure constant thermodynamic activity throughout the course of the experiment. Skin permeation was run at 37 °C for at least 12 h. A part (1.0 ml) of receiver solution was withdrawn and replaced with the same volume of distilled water to keep the volume constant. The concentration of drugs in the samples was assayed by HPLC (Perkin Elmer) using a 4.6 mm × 250 mm stainless steel column packed with Spherisorb ODS-2 (C18) (Scitronic Co., Thailand) under the conditions previously reported by Hatanaka et al. (1990). The concentration of drugs and their cumulative amount was plotted against time. The permeability coefficient (P) of permeants was determined by dividing the slope of the steady-state portion (6–12 h) of these plots by the solubility of permeants at 37 °C. Metabolites were not detected in either the donor or the receiver solution throughout the experiment.

3.4. Isolation of human stratum corneum

The stratum corneum of human skin was isolated by trypsin treatment (Knutson et al. 1985). The dermis side of whole skin was placed on a filter paper saturated with 1% (w/v) trysin solution (1000 ATEE units ml^{-1} in phosphate buffered saline (PBS), at pH 7.4) at 37 °C in a sealed petridish. After 8 h, the stratum corneum was carefully separated from the viable epidermis and rinsed throughly with distilled water. The stratum corneum samples were dried for 24 h and stored in a desiccator at room temperature (about 25 °C) until used.

3.5. Skin thickness

The thickness of shed snake skin and human stratum corneum was determined according to Evans and Rutter (1986) with a minor modification. The skin specimens were fixed in 3% formaldehyde in 0.1 M phosphate buffer pH 7.2 and then dipped in paraffin. The sections were cut and stained with hematoxylin-eosin, then observed under a light-microscope (BHS-324, Olympus, Tokyo).

3.6. Lipid contents of skins

The content of total lipid and lipid composition of ceramides, cholesterol and phospholipids in the shed snake skin and the human stratum corneum was determined. After weighing, samples of the skin (about 10 mg) were placed in a 10 ml glass tube with a screw cap containing 7 ml of a 2 : 1 chloroform/methanol mixture and shaken for 20 h at room temperature. The delipidized skin samples were transferred to another tube, rinsed with fresh chloroform/methanol mixture and dried to constant weight. The total lipid content of the skin was determined from the difference in its weight before and after solvent extraction. The lipid composition in the extracted solvent was also analyzed. Ceramides content was determined according to Lauter and Trams (1982). Cholesterol and phospholipids were measured by cholesterol CII-testWako® and phospholipid B-testWako® (Wako Pure Chemical Industries, Osaka, Japan). The lipid content was calculated in μg of the lipid per mg of the dry skin.

3.7. Water content of skins

The water content in the shed snake skin and the human stratum corneum was determined according to Potts et al. (1985). Briefly, the water content in the skin was quantified by measuring the intensity of the weak O−H stretch near 2100 cm^{-1} in infrared spectroscopic spectra. The shed snake skin and human stratum corneum were placed and analyzed by Fourier Transform Infrared Spectroscopy with an ATR sampling device (FT/IR-230, JASCO corporation, Tokyo). All *in vitro* spectra were obtained under ambient laboratory conditions of approximately 50% relative humidity and at 25 °C. The water content in the skin was calculated as the ratio of mass of water to dry mass of the skin (μg/mg).

3.8. Statistics

Statistical significance was evaluated by a paired t-test.

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