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Research paper

Characterisation of epidermal lipid composition and skin morphology of animal skin *ex vivo*

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ABSTRACT

Epidermal lipids and skin morphology are assumed to substantially influence skin permeability. Although these parameters have been studied extensively, available data are hard to interpret as data have been gathered at different experimental conditions. Therefore, the aim of this study was to provide detailed information on these parameters for four different mammalian skin types.

Lipids were extracted from heat separated epidermis, the total epidermal lipid content was measured and the epidermal lipid composition was quantitatively determined by high-performance thin-layer chromatography. Furthermore, vertical and horizontal cryostate skin slices were analysed by light microscopy for thickness of the horny layer, epidermal thickness, density, depth of anchorage and diameter of the hair follicles.

The highest total epidermal lipid content was detected in rat epidermis, followed by bovine udder, dog and pig epidermis. Considering the amount of single lipid fractions, cholesterol, cholesteryl ester and free fatty acids were found to be the major constituents of epidermal lipids in all the examined species. However, as confirmed by hierarchical cluster analysis the epidermal lipid profile and morphology showed marked differences between all the examined species.

1. Introduction

In vitro percutaneous permeation studies are performed to obtain detailed information about transdermal drug absorption, an important field of research in toxicology, cosmetics and both human and veterinary medicine. Accepted models of transdermal drug transport are, e.g. Franz type diffusion cells and isolated perfused organs (pig ear and bovine udder) [1–3]. Although these experiments are conducted under reproducible conditions, the results show considerable interspecies and interindividual variances. Therefore, the prediction of drug behaviour in its intended application is complicated and sometimes even impossible based on *in vitro* experiments.

Basically, differences in the barrier strength between different skins may be explained by differences in the skin morphology and in the epidermal lipid composition. Considering the latter, the influence of the total epidermal lipid content on the rate of percutaneous permeation has already been established by a strong correlation between the amount of extracted skin lipids (e.g. by a treatment with acetone or methanol) and the amount of drug permeation [4–9].

The skin permeability barrier is described to be a function of its lipid composition [10]. In many reports [11–14], ceramides (45–50%), cholesterol (25%) and free fatty acids (10–15%) are described to be the main fractions of epidermal lipids. Moreover, other epidermal lipids were found in mammalian skin [15], which potentially influence the percutaneous permeability barrier, although they represent only small lipid classes.

Unfortunately, up to now literature data for the skin lipid composition of commonly used *in vitro* skin types are not consistent, and comprehensive data on skin lipid profiles are only available for neonatal mouse, pig and human epidermis [16–18]. Comparable data for commonly used models are therefore desirable to facilitate the interpretation of results from different permeation studies.

Beyond that, several studies revealed an influence of different morphological skin parameters on percutaneous permeation. Some reports [19–22] described that the rate of transdermal drug transport is related to *stratum corneum* thickness, whereas Elias et al. [23] described no correlation between skin permeability and *stratum corneum* thickness. Several investigations [24–26] showed differences between the percutaneous permeation of physiological skin and scar skin, which seem to be related to the presence and the absence of hair follicles, respectively. Panchagnula et al. [27]

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on the other hand were not able to establish a correlation between hair follicle density in 16 different species and percutaneous permeation of water and cumarin.

Based on these problems, the aim of this study is to provide an extensive description of the epidermal lipid composition and skin morphology of four *in vitro* skin types, which was obtained under standardised conditions [28–34]. It represents the preliminary work for a comparison of physicochemical skin characteristics with results of *in vitro* percutaneous permeation experiments, in order to explain the correlation between skin properties and transdermal drug transport.

2. Materials and methods

2.1. Experimental animals

Skin samples were obtained from rats (abdominal and back skin), cattle (udder skin), pigs, and dogs (both lateral abdominal skin). Cattle skin from adult, lactating holstein frisian cows was obtained from the slaughterhouse, while skin from the other animals (dog: one pug (1 year old), one eurasian (2 years old) and five beagles (5 months old), pig: hybrid (5 months old), rat: wistar (1 year old)) was taken from animals that were sacrificed for reasons not related to this study in the University of veterinary medicine Hannover foundation. All animals were male except the cattle.

2.2. Separation of the epidermis

After clipping the coat, the epidermis was separated by a modified method of Kligman and Christophers [35] (cows: $n = 15$, pigs: $n = 13$, dogs: $n = 8$, and rats: $n = 6$). Therefore, the skin was placed upside down for 120 seconds on a plate heater (Thermoplate S, Desaga, Wiesloch, Germany) at a temperature of 60 °C. Subsequently, the epidermis was isolated from the dermis with a pair of methanol cleaned tweezers. It was dried over several days in an oven (Memmert, Schwabach, Germany) at 30 °C, until a constant weight was reached.

2.3. Lipid extraction

The lipids were extracted following the procedure of Folch et al. [36] that was modified by Melnik [37]. Different lipid extraction methods were compared to get a maximum amount of lipid extracted (e.g. acid and alkaline extraction with chloroform/methanol (2:1) as well as the usage of hexane and ethyl acetate). Since the neutral extraction with chloroform/methanol (2:1) led to the highest lipid amount extracted in all the examined lipid classes, this kind of method was chosen in this study. All solvents were of HPLC grade and were purchased from Merck, Darmstadt, Germany.

According to Melnik [37], 100 mg epidermis (dry substance) was hydrated by incubation with 500 µl NaCl solution (300 mM) for 15 min. Chloroform/methanol (2:1) was added at a volume of 5 ml. After shaking for 2 h at room temperature, 100 µl NaCl-solution (300 mM) was added and was incubated at room temperature for 10 min. Centrifugation at 2500 rpm for 10 min led to the appearance of 2 phases: an upper hydrophilic layer and an underlying layer containing lipid in an organic solution. 3.5 ml of the latter were transferred through cotton wool into a lipid-free glass vial and were evaporated to dryness at room temperature under a stream of nitrogen. The amount of extracted epidermal lipids of each sample was weighed on a precision scale (Kern & Sohn GmbH, Balingen, Germany). Afterwards, 250 µl chloroform/methanol (2:1) was added, followed by air- and lightproof storage at −20 °C until required.

2.4. Analysis of epidermal lipids

The lipid extract of each sample was analysed by planar high-performance thin-layer chromatography (HPTLC).

2.4.1. Lipid standards

Different lipid standards of the highest purity available were purchased from Sigma–Aldrich, Steinheim, Germany. The following lipids were used as a standards on the HPTLC-plates: sphingomyelin and L-α-phosphatidylcholine representative of the phospholipids; oleic acid used as standard for free fatty acids; glyceryl trioleate as representative of triglycerides; cholesterol oleate as cholesteryl ester; ceramide 4 [AS] (hydroxy fatty acid ceramide from bovine brain) and ceramide 3 [NP] (non-hydroxy fatty acid ceramide from bovine brain) [38]; galactocerebrosides, cholesterol sulfate, and cholesterol. To set up a calibration curve four different concentrations of the standard solution (lipids dissolved in chloroform–methanol 2:1, V:V) were used (1.875; 1.250; 0.625; 0.125 µg/µl). Alkanes are represented by homologous hydrocarbon chains (C_{15}–C_{35}) and are of exogenous origin [39,40]. Therefore, they were not analysed in this study.

2.4.2. HPTLC conditions

One microliters of lipid extract was applied on a precleaned and activated HPTLC-plate at a distance of 4 mm from the edge of the plate and of 5 mm to the next sample using a microliter syringe (Hamilton, Bonaduz, Switzerland).

The lipids were separated in a horizontal development chamber (CAMAG, Berlin, Germany) at room temperature using the following solvent systems: I: 1.5 cm chloroform:ethanol:acetic acid (80 %, 18%, 2%), II: 4 cm chloroform:methanol:acetic acid (91.4%, 4.3%, 4.3%), III: 4.5 cm hexane:diethylether:acetic acid (72.7%, 18.2%, 9.1%). All chemicals were of the highest purity available and were received from Labscan, Dublin, Ireland except diethylether (Merck, Darmstadt, Germany) and acetic acid (AppliChem, Darmstadt, Germany). Afterwards, the HPTLC-plate was dried at room temperature for 15 min.

2.4.3. Derivatisation

The plate was dipped into an aqueous solution of copper sulfate pentahydrate (125 g/L) and phosphoric acid (125 g/L) for 30 s (Merck, Darmstadt, Germany). Lipid spots were visualised by heating the HPTLC-plate on a plate heater (Thermoplate S, Desaga, Wiesloch, Germany) at 180 °C for 30 min, resulting in the charring of the lipids.

2.4.4. Densitometrical analysis

Lipid fractions were identified by comparison with the comigrated lipid standards, and were quantified using an ordinary office scanner (Highscreen, Aachen, Germany) along with digital image analysis (ScionImage® 4.0.3.2., Scion Corporation, Maryland, USA). After background subtraction (adjustments: rolling ball 130, horizontal background subtraction), the lipid amount was calculated from the grey value referring to the appropriate calibration curve.

2.5. Histology of the skin

2.5.1. Skin preparation

Fresh excised skin was embedded in tissue freezing medium (Leica Instruments GmbH, Nussloch, Germany), and was frozen in liquid nitrogen. 8 µm thick vertical and horizontal slices were obtained by utilizing a cryostate (HM 560, Microm, Walldorf, Germany). Afterplacing on glass slides (Roth, Karlsruhe, Germany), the skin samples were stained with haematoxylin–eosine in accordance with the standard procedures [41].

2.6.1. Materials

All the chemicals used for histological examination of the skin samples were of analytical grade. The following chemicals were obtained from Merck, Darmstadt, Germany: Eosin G, Mayers haemalaun solution for microscopy, and hydrochloric acid. Furthermore, ethanol (Labscan, Dublin, Ireland) and acetic acid 100% (AppliChem, Darmstadt, Germany) were used for analysis.

2.6.2. Analysis

A light microscope (Axioscop 50, Zeiss, Jena, Germany) was utilized to evaluate the morphological skin parameters. The thickness of both the horny layer and the epidermis as well as the depth of the anchorage of hair follicles was obtained by perpendicular measurement to the skin surface (ScionImage® 4.0.3.2., Scion Corporation, Maryland, USA) (from 10 different areas 100 measurements were recorded for each animal). Furthermore, the density and the diameter of the hair follicles were detected. Different parameters were studied in three animals of each species.

2.7. Hierarchical cluster analysis

Similarities in the complex lipid profiles and in the morphological skin parameters between the examined samples were investigated using hierarchical cluster analysis. Therefore, data were mean centered and data clusters were detected based on the euclidian distance between the data sets using Matlab 5.2 (The MathWorks Inc., Natick, USA). The hierarchical cluster analysis of the epidermal lipid composition was based on all lipids except triglycerides, since they are assumed to represent contaminations from the environment [18,42,43].

2.8. Statistics

All test values are given as mean and standard deviation. Statistical analysis was performed by using GraphPad Prism® 4.01 (GraphPad Software, Inc., San Diego, USA). One-way ANOVA with Tukey's multiple comparison post hoc test was utilized to compare the mean values of the four species with a confidence interval of 95%.

3. Results

3.1. Total lipid content and epidermal lipid composition

The epidermal lipid content of the examined species showed considerable differences (Fig. 1). The lowest amount of lipids was found in pig skin, followed by dog, cattle and rat skin (significantly different results were found between porcine skin and the skin of all other species).

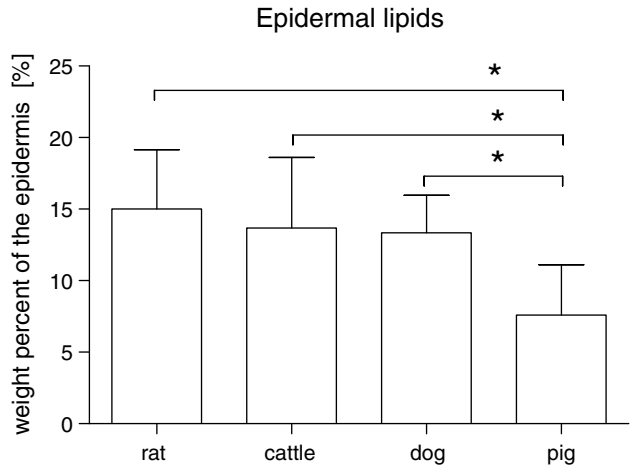

Fig. 1. Total epidermal lipid content of four different species observed by extraction following Folch et al. method [36] that was modified by Melnik [37]; $^{*}p < 0.05$ (one-way ANOVA); (rat: $n = 6$, cattle: $n = 15$, dog: $n = 8$, and pig: $n = 13$).

Furthermore, the lipid profile showed marked differences between the species (Table 1).

3.1.1. Polar lipids

Polar lipids are subdivided into two lipid classes: phospholipids and cholesteryl sulfate. The polar lipids of the four examined skin types represent only a small group of epidermal lipids with amounts smaller than 0.5 wt% of the epidermis. They were found with the highest amounts in pig epidermis (up to 0.46%), followed by dog (0.24%), cattle (0.19 %), and rat skin (0.19 %). Significant differences were found between pig skin and both dog and rat skin for phospholipids, and between rat skin and both cattle and pig skin in the case of cholesteryl sulfate.

3.1.2. Neutral lipids

Neutral lipids with amounts higher than 3 wt% of the epidermis were found in the all examined species in the following order: rat (4.71%) > cattle (3.63%) > dog (3.54%) > pig (3.20%). Significant differences were found between pig skin and both dog skin and rat skin for cholesterol. The content of cholesteryl ester differed significantly between dog and rat, cattle, and pig skin, as well as between rat skin and both cattle and pig skin. No significant differences between the examined species were seen for free fatty acids and triglycerides.

3.1.3. Sphingolipids

The sphingolipids consist of ceramides and galactocerebrosides. Significant differences between cattle skin and rat skin were seen for ceramide 3, while cerebrosides differed significantly between cattle skin and both dog and rat skin. Ceramide 4 was only detectable in porcine skin (Table 1).

3.1.4. Additional lipid fractions

Each individual showed lipid bars next to cholesteryl ester and ceramides on the HPTLC-plate which could not be identified. In Fig. 2, they are marked by the boxes. For both dog and cattle bars next to ceramide 3 and ceramide 4 were detectable. Other unknown lipid bars were found next to cholesteryl ester for rats, cattle and pigs.

3.2. Histological examinations

Both horizontal and vertical skin sections were used to analyse morphological skin parameters (Table 2). Both hair follicle density and thickness could be differentiated for primary and secondary follicles, while these two types showed no difference in the depth of anchorage.

3.3. Hierarchical cluster analysis

Fig. 3 demonstrates the relationship between the examined individuals based on the similarity of the whole epidermal lipid composition. Generally, the similarity in the lipid profile within the four examined species resulted in large clusters of highly similar individuals of the same species (clusters 1, 3, 4, and 6). However, especially in pigs and cattle some individuals exhibit a highly different lipid profile, resulting in the formation of a mixed cluster (cluster 2). As compared to other cattle, these individuals differ considerably in the amounts of cholesteryl ester, galactocerebrosides and ceramide 3, while pigs in this mixed cluster show a different content of the polar phospholipids, cholesteryl sulfate and ceramide 4 than most other pigs contained in cluster 6. In cluster 3 one rat is differentiated from the others

Table 1
Lipid weight percent of the epidermis and the total epidermal lipid content of four different species (rat: $n = 6$, cattle: $n = 15$, dog: $n = 8$, and pig: $n = 13$).

Lipid	Lipid weight percent of epidermis [%]				Lipid weight percent of total epidermal lipid content [%]			
	Rat ($n = 6$)	Cattle ($n = 15$)	Dog ($n = 8$)	Pig ($n = 13$)	Rat ($n = 6$)	Cattle ($n = 15$)	Dog ($n = 8$)	Pig ($n = 13$)
Phospholipids	0.1	0.2	0.1	0.2	1.4	3.1	1.8	6.7
Cholesteryl sulfate	0.1	0.2	0.1	0.2	0.5	2.6	0.9	3.3
Cerebrosides	0.0	0.3	0.1	0.2	0.0	2.6	0.6	2.7
Ceramide 4 [AS]	–	–	–	0.3	–	–	–	4.0
Ceramide 3 [NP]	0.1	0.2	0.2	0.2	0.3	2.0	1.6	2.5
Cholesterol	0.9	1.2	1.0	1.5	5.6	10.3	7.9	20.8
Free fatty acids	1.8	1.5	1.0	1.3	11.1	12.0	7.6	18.3
Triglycerides	1.2	0.6	0.1	0.2	7.6	3.8	1.0	3.1
Cholesteryl ester	0.8	0.4	1.5	0.2	5.2	3.0	11.4	3.2
Total amount of analysed epidermal lipids	5.0	4.8	4.2	4.6	31.7	37.5	32.8	60.8

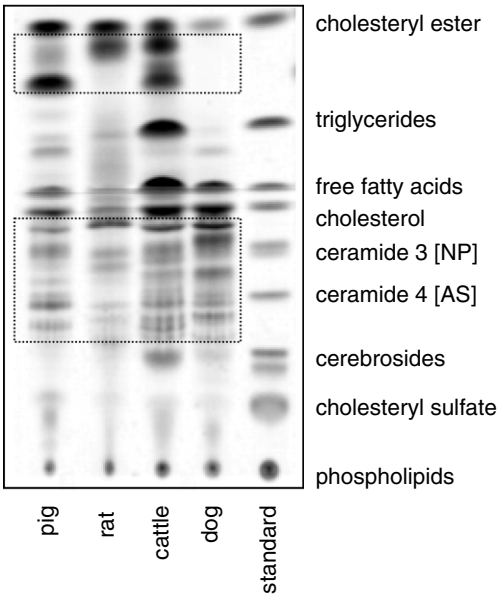

Fig. 2. High-performance thin-layer chromatogram of four different species and the lipid standards; the boxes mark the unknown lipid fractions (except ceramide 3 [NP] and ceramide 4 [AS]).

by the high content of phospholipids. Variabilities in the quantities of cholesterol, free fatty acids and cholesteryl ester in a split of the dog cluster.

Regarding the examined morphological skin properties (Fig. 4), hierarchical cluster analysis demonstrates that rat skin and dog skin as well as bovine udder skin and pig skin, since the thickness of the viable epidermis as well as the hair follicle density differs considerably between cluster 1 and cluster 2.

4. Discussion

This study provides detailed data on physicochemical skin characteristics of four different skin types, which can be used in *in vitro* permeation experiments. In the discussion of similarities and differences to former studies, the high biological variability of these parameters depending on breed, age, season, feeding and sex [43–46] has to be kept in mind. Furthermore, published data have been generated using many different methods, including the use of different skin materials and methods of lipid extraction and quantification. Finally, skin lipid profiles are prone to environmental contamination, e.g. contamination from stable or dermal fat tissue resulting in increased amounts of triglycerides or alkanes [39,42,43].

A detailed summary of the factors influencing the epidermal lipid analysis is therefore provided in the following section to appraise the following discussion on single skin characteristics:

1. *Skin material:* Depending on the kind of material used for extraction, different lipid fractions are found in the lipid samples. In concert with Vicanova et al. [47], Holleran et al. [48], Hedberg et al. [42], and Wertz and Downing [49], the whole epidermis was used for lipid extraction in this study, while other research groups analysed lipids from the *stratum corneum* [15,50] or epidermal cells [16].
2. *Treatment of skin material:* The same skin layer may be obtained by different kinds of treatments. In the present investigation, the epidermis was obtained by heat separation based on Kligman and Christophers [35], while other methods describe the use of cantharidin or salt solutions [35,51]. The influence of these differences remains unclear.
3. *Method of extraction:* Former reports describe different methods of lipid extraction, which may result in different recoveries of single lipid fractions based on individual solubility in the used

Table 2
Results of the histological examination of the skin of four different species ($n = 3$); all data are given as means and standard deviations (STD); PH, primary hair follicle; SH, secondary hair follicle.

Species ($n = 3$)	Mean				STD			
	Rat	Cattle	Dog	Pig	Rat	Cattle	Dog	Pig
Thickness of the viable epidermis [μm]	9.9	57.3	9.8	34.4	0.9	8.5	0.7	3.2
Stratum corneum thickness [μm]	7.2	10.1	12.0	14.6	0.5	0.6	0.0	1.2
Hair follicle								
Diameter PH [μm]	89.3	52.9	54.6	97.9	34.1	4.0	3.4	2.7
Diameter SH [μm]	19.6	16.4	17.5	18.0	0.8	2.2	1.2	3.5
Density PH [amount/cm^2]	336.1	394.2	416.7	47.3	55.5	43.0	192.2	10.9
Density SH [amount/cm^2]	5872.2	365.1	2950.0	4.9	1797.2	80.0	798.6	0.9
Density of all hair follicles [amount/cm^2]	6408.3	759.3	3366.7	52.2	1842.7	83.0	990.8	11.8
Depth of anchorage [μm]	549.6	810.5	938.8	820.0	66.7	139.9	149.0	152.4

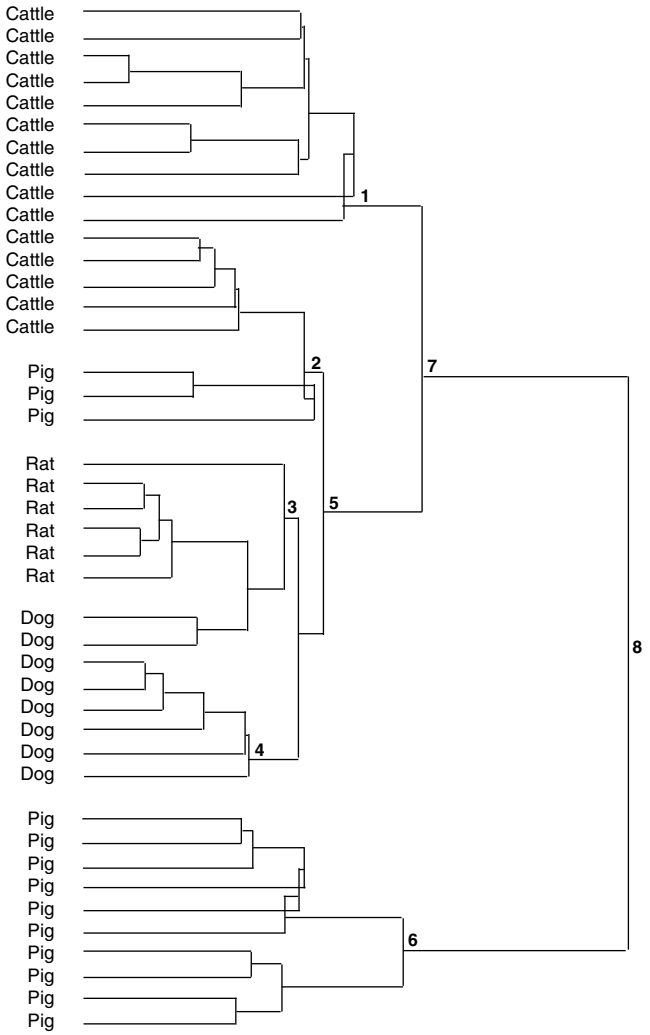

Fig. 3. Hierarchical cluster analysis of the epidermal lipid composition of 42 animals (four different species) after mean centering and centroid linking; the length of the horizontal lines describes the similarity of the epidermal lipid compositions.

solvent systems. For our investigation the method of Folch et al. [36] that was modified by Melnik [37] was used; nonetheless the methods by Bligh and Dyer [52] and Gray [53] are also commonly used. Furthermore, as applied in this study, dried skin material may be used to standardise the weight of raw material [44,54], as well as undried skin to describe the lipid content of skin in its physiological state [36,47,50].

4. *Analytics:* The analytics in this study is based on HPTLC using a sequence of three solvent systems, followed by dipping in a derivatisation solution and ashing. As for this and other methods it may be expected that results are highly influenced by the selectivity and sensitivity of the analytics.
5. *Presentation of results:* Different kinds of result declarations restrict the possibility to compare data in the literature. Wertz and Downing [49], Lampe et al. [15] as well as Gray and Yardley [55] relate the lipid fractions to total epidermal lipid content, while Weerheim and Ponec [50] relate single lipid fractions to main lipid components of the skin. Furthermore, the conversion of single lipid fractions on the dry weight of extraction material is a commonly used method to represent data [56–58]. To provide a good comparability to former reports, we related the lipid weight percent to both total epidermal lipid content and dry weight of the epidermis.

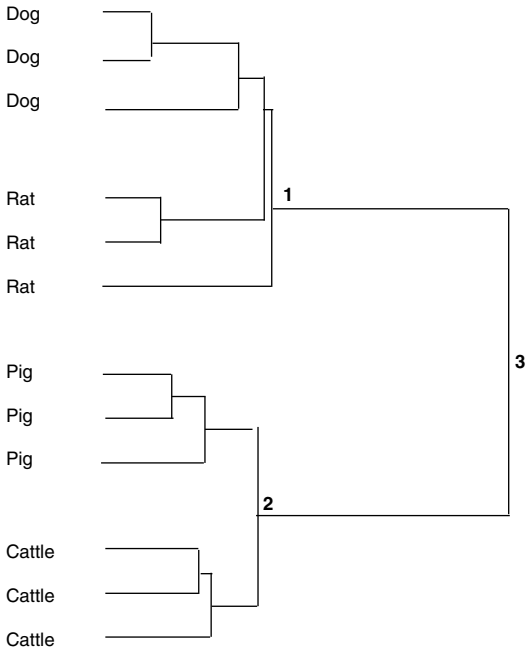

Fig. 4. Hierarchical cluster analysis of all the obtained morphological skin parameters of 12 animals (four different species; $n = 3$) after mean centering and centroid linking; the length of the horizontal line describes the similarity of the morphological skin parameters.

4.1. Total epidermal lipid content

In this study, the highest amounts of epidermal lipids were found in rat back and abdominal epidermis, followed by bovine udder, dog and pig abdominal epidermis. These findings confirm those of Gray and Yardley [16], who described marked interspecies differences in the lipid content of epidermal cells. In these examinations, rat epidermal cells contained 20% lipids, which is more than 2-fold higher than in porcine cells (8% lipids). The striking similarity to the results of this study (16% in rat skin and 7.8% in pig skin) may nevertheless be not overstressed, as Gray and Yardley [16] used isolated epidermal cells only and omitted the horny layer. Nevertheless, a higher lipid content of rodent skin, which seems not to be restricted to the horny layer, is confirmed by the results of both studies. For bovine udder skin, the present study revealed a total epidermal lipid content of 13.7% as compared to 6.5% found in a previous study [29]. Canine epidermis includes 13.4 % lipids, but lipid data about this species are not available in the literature.

4.2. Hierarchical cluster analysis

With regard to the lipid profile, the hierarchical cluster analysis reveals a high number of similar individuals, but also the existence of different groups of individuals within each examined species. Except for the rat, variations in more than one lipid class were responsible for the formation of multiple clusters. Interestingly, cattle from different origins did not vary more than other species, which were held under the same conditions. For example, dogs were divided into two clusters, one with a pug and a beagle, and the other with an eurasian and five beagles, although the six beagles came from the same husbandry. It remains to be clarified whether these differences are correlated to differences in the epidermal barrier strength, and which individual and environmental factors influence the skin lipid profile.

4.3. Lipid composition

Considering the amount of single lipids, cholesterol, cholesteryl ester and free fatty acids were found to be the major constituents of skin lipids in all the examined species. However, it may not be ruled out that other lipids are decisive, as only a small fraction of lipids could be analysed in this study. Additional lipids were detected in the chromatographic analysis, and according to their position on the HPTLC-plate some of these unknown lipids seem to represent several kinds of ceramides [59–64] and highly non-polar wax esters [65]. The source of these lipids remains unclear. Nevertheless, they may influence percutaneous permeation, since they can interact with topically applied test substances. Beyond inherent epidermal lipids or contaminations from dermal lipids, hair lipids may be a part of the unknown lipid content, as hair residuals were still prominent in the heat separated epidermis. Hair cuticula cells offer a lipid envelope, which is comparable to the lipid envelope of epidermal corneocytes [66–69], and the extract of mammalian hair contains ceramides and polar phospholipids [70] and different amounts of glycosphingolipids, cholesteryl sulfate, sterols, and both wax and sterol esters [65,71]. This suggestion is confirmed by the observation of a correlation of hair follicle density with the amount of unknown lipids (pig vs. rat). The identification of these unknown lipids as well as the influence of major and minor lipid skin fractions on barrier properties has to be engaged by further examinations.

4.3.1. Skin morphology

Beyond lipid profiles, this study describes morphological skin characteristics of the four different skin types. The thickest horny layer was found in pig skin, followed by dog, cattle and rat skin. The hierarchical cluster analysis of the morphological skin characteristics showed relations between canine skin and rat skin as well as between dog skin and pig skin caused by the thickness of the viable epidermis as well as the density of hair follicles.

Comparison with the results in the literature [20,29,72–76] show matchable data to some extent, e.g. the epidermis of the pig showed a thickness of 34 μm in concert with Meyer [72], while other data differ from available results. In such a way, the thickness of the horny layer of rat skin can be mentioned, which was measured to be 12–21 μm thick in examinations by Scott et al. [20], whereas a thickness of only 7 μm was found in this study. Meyer [72] described several influences on morphological skin characteristics such as breed, sex or age of the animals. These influences have to be considered to explain the differences in the results of the present investigation with former reports.

In conclusion, this study describes in detail both the epidermal lipid composition and the skin morphology of four different skin types, which have to be compared with percutaneous permeation rates of hydrophilic and lipophilic drugs, to obtain a correlation between skin morphology and transdermal drug transport. A second study will be presented to correlate the obtained physicochemical skin characteristics with *in vitro* percutaneous permeation rates.

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References

[1] T.J. Franz, Percutaneous absorption on the relevance of in vitro data, J. Invest. Dermatol. 64 (3) (1975) 190–195.
[2] M. Kietzmann, W. Loescher, D. Arens, P. Maass, D. Lubach, The isolated perfused bovine udder as an in vitro model of percutaneous drug absorption. Skin viability and percutaneous absorption of dexamethasone, benzoyl peroxide, and etofenamate, J. Pharmacol. Toxicol. Methods 30 (2) (1993) 75–84.
[3] C. Herkenne, A. Naik, Y.N. Kalia, J. Hadgraft, R.H. Guy, Pig ear skin ex vivo as a model for in vivo dermatopharmacokinetic studies in man, Pharm. Res. 23 (2006) 1850–1856.
[4] G.S. Berenson, G.E. Burch, Studies of diffusion of water through dead human skin; the effect of different environmental states and of chemical alterations of the epidermis, Am. J. Trop. Med. Hyg. 31 (6) (1951) 842–853.
[5] H.D. Onken, C.A. Moyer, The water barrier in human epidermis. Physical and chemical nature, Arch. Dermatol. 87 (1963) 584–590.
[6] A.G. Matoltsy, A.M. Downes, T.M. Sweeney, Studies of the epidermal water barrier. II. Investigation of the chemical nature of the water barrier, J. Invest. Dermatol. 50 (1) (1968) 19–26.
[7] T.M. Sweeney, D.T. Downing, The role of lipids in the epidermal barrier to water diffusion, J. Invest. Dermatol. 55 (2) (1970) 135–140.
[8] R. Scheuplein, L. Ross, Effects of surfactants and solvents on the permeability of epidermis, J. Soc. Cosmet. Chem. 21 (1970) 853–873.
[9] R.L. Bronaugh, T.J. Franz, Vehicle effects on percutaneous absorption: in vivo and in vitro comparisons with human skin, Br. J. Dermatol. 115 (1) (1986) 1–11.
[10] P.M. Elias, G.K. Menon, Structural and lipid biochemical correlates of the epidermal permeability barrier, Adv. Lipid Res. 24 (1991) 1–26.
[11] E. Heymann, Haut, Haar und Kosmetik, S. Hirzel Verlag, Stuttgart, Germany, 1994.
[12] T.J. McIntosh, M.E. Stewart, D.T. Downing, X-ray diffraction analysis of isolated skin lipids: reconstitution of intercellular lipid domains, Biochemistry 35 (12) (1996) 3649–3653.
[13] P.M. Elias, Epidermal lipids, membranes, and keratinization, Int. J. Dermatol. 20 (1) (1981) 1–19.
[14] M.L. Williams, P.M. Elias, The extracellular matrix of stratum corneum: role of lipids in normal and pathological function, Crit. Rev. Ther. Drug Carrier Syst. 3 (2) (1987) 95–122.
[15] M.A. Lampe, M.L. Williams, P.M. Elias, Human epidermal lipids: characterization and modulations during differentiation, J. Lipid Res. 24 (2) (1983) 131–140.
[16] G.M. Gray, H.J. Yardley, Lipid compositions of cells isolated from pig, human, and rat epidermis, J. Lipid Res. 16 (6) (1975) 434–440.
[17] P.M. Elias, J. Goerke, D.S. Friend, Mammalian epidermal barrier layer lipids: composition and influence on structure, J. Invest. Dermatol. 69 (6) (1977) 535–546.
[18] M.A. Lampe, A.L. Burlingame, J. Whitney, M.L. Williams, B.E. Brown, E. Roitman, P.M. Elias, Human stratum corneum lipids: characterization and regional variations, J. Lipid Res. 24 (2) (1983) 120–130.
[19] S.T. Horhota, H.L. Fung, Site dependence for topical absorption of nitroglycerin in rats, J. Pharm. Sci. 67 (9) (1978) 1345–1346.
[20] R.C. Scott, M.A. Corrigan, F. Smith, H. Mason, The influence of skin structure on permeability: an intersite and interspecies comparison with hydrophilic penetrants, J. Invest. Dermatol. 96 (6) (1991) 921–925.
[21] C.R. Behl, R.L. Bronaugh, H.I. Maibach, Influence of age on percutaneous absorption of substances, Percutaneous Absorption, Dekker, New York, 1985.
[22] K.A. Holbrook, G.F. Odland, Regional differences in the thickness (cell layers) of the human stratum corneum: an ultrastructural analysis, J. Invest. Dermatol. 62 (4) (1974) 415–422.
[23] P.M. Elias, E.R. Cooper, A. Korc, B.E. Brown, Percutaneous transport in relation to stratum corneum structure and lipid composition, J. Invest. Dermatol. 76 (4) (1981) 297–301.
[24] J. Lademann, N. Otberg, H. Richter, H.J. Weigmann, U. Lindemann, H. Schaefer, W. Sterry, Investigation of follicular penetration of topically applied substances, Skin Pharmacol. Appl. Skin Physiol. 14 (Suppl. 1) (2001) 17–22.
[25] S.N. Tenjarla, R. Kasina, P. Puranajoti, M.S. Omar, W.T. Harris, Synthesis and evaluation of N-acetylprolinate esters – novel skin penetration enhancers, Int. J. Pharm. 192 (2) (1999) 147–158.
[26] F. Hueber, H. Schaefer, J. Wepierre, Role of transepidermal and transfollicular routes in percutaneous absorption of steroids: in vitro studies on human skin, Skin Pharmacol. 7 (5) (1994) 237–244.
[27] R. Panchagnula, K. Stemmer, W.A. Ritschel, Animal models for transdermal drug delivery, Methods Find. Exp. Clin. Pharmacol. 19 (5) (1997) 335–341.
[28] M. Kietzmann, W. Loescher, The use of the organs of slaughtered animals for the testing of drugs: suitability of the bovine udder for the study of substances active through the skin, Dtsch. Tieraerztl. Wochenschr. 100 (2) (1993) 54–57.
[29] F. Netzlaff, U.F. Schaefer, C.M. Lehr, P. Meiers, J. Stahl, M. Kietzmann, F. Niedorf, Comparison of bovine udder skin with human and porcine skin in percutaneous permeation experiments, Altern. Lab. Anim. 34 (5) (2006) 499–513.
[30] A. Nokhodchi, K. Sharabiani, M.R. Rashidi, T. Ghafourian, The effect of terpene concentrations on the skin penetration of diclofenac sodium, Int. J. Pharm. (2006) 97–105.
[31] W. Pittermann, M. Kietzmann, B. Jackwerth, The isolated perfused bovine udder skin, ALTEX 12 (4) (1995) 196–200.
[32] O.I. Sarigullu, K.H. Yesim, G. Kantarci, S. Sozer, T. Guneri, G. Ertan, Transdermal delivery of diclofenac sodium through rat skin from various formulations, AAPS PharmSciTech 7 (4) (2006) 88.
[33] X.M. Wu, H. Todo, K. Sugibayashi, Enhancement of skin permeation of high molecular compounds by a combination of microneedle pretreatment and iontophoresis, J. Control. Release 118 (2006) 189–195.
[34] M. Schaefer-Korting, U. Bock, A. Gamer, A. Haberland, E. Haltner-Ukomadu, M. Kaca, H. Kamp, M. Kietzmann, H.C. Korting, H.U. Krachter, C.M. Lehr, M. Liebsch, A. Mehling, F. Netzlaff, F. Niedorf, M.K. Rubbelke, U. Schafer, E.

Schmidt, S. Schreiber, K.R. Schroder, H. Spielmann, A. Vuia, Reconstructed human epidermis for skin absorption testing: results of the German prevalidation study, Altern. Lab. Anim. 34 (3) (2006) 283–294.
[35] A.M. Kligman, E. Christophers, Preparation of isolated sheets of human stratum corneum, Arch. Dermatol. 88 (1963) 702–705.
[36] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1) (1957) 497–509.
[37] B.C. Melnik, Biochemie und Pathobiochemie des epidermalen Lipidstoffwechsels, Georg Thieme Verlag Stuttgart, New York, 1990.
[38] S. Motta, M. Monti, S. Sesana, R. Caputo, S. Carelli, R. Ghidoni, Ceramide composition of the psoriatic scale 7, Biochim. Biophys. Acta 1182 (2) (1993) 147–151.
[39] J.T. Bortz, P.W. Wertz, D.T. Downing, The origin of alkanes found in human skin surface lipids, J. Invest. Dermatol. 93 (6) (1989) 723–727.
[40] M.L. Williams, P.M. Elias, N-Alkanes in normal and pathological human scale, Biochem. Biophys. Res. Commun. 107 (1) (1982) 322–328.
[41] H.C. Burck, Physikalische und physiko-chemische Faerbemethoden, in: H.C. Burck (Ed.), Histologische Technik, Georg Thieme Verlag, Stuttgart, New York, 1982, pp. 104–141.
[42] C.L. Hedberg, P.W. Wertz, D.T. Downing, The time course of lipid biosynthesis in pig epidermis, J. Invest. Dermatol. 91 (2) (1988) 169–174.
[43] C.L. Hedberg, P.W. Wertz, D.T. Downing, The nonpolar lipids of pig epidermis, J. Invest. Dermatol. 90 (2) (1988) 225–229.
[44] J. Rogers, C. Harding, A. Mayo, J. Banks, A. Rawlings, Stratum corneum lipids: the effect of ageing and the seasons, Arch. Dermatol. Res. 288 (12) (1996) 765–770.
[45] M. Denda, J. Koyama, J. Hori, I. Horii, M. Takahashi, M. Hara, H. Tagami, Age- and sex-dependent change in stratum corneum sphingolipids, Arch. Dermatol. Res. 285 (7) (1993) 415–417.
[46] J.L. Melton, P.W. Wertz, D.C. Swartzendruber, D.T. Downing, Effects of essential fatty acid deficiency on epidermal O-acylsphingolipids and transepidermal water loss in young pigs, Biochim. Biophys. Acta 921 (2) (1987) 191–197.
[47] J. Vicanova, A. Weerheim, V. Swope, M. Westbrook, D. Harriger, S. Boyce, Epidermal lipid metabolism of cultured skin substitutes during healing of full-thickness wounds in athymic mice, Wound Repair Regen. 5 (4) (1997) 329–338.
[48] W.M. Holleran, K.R. Feingold, M.Q. Man, W.N. Gao, J.M. Lee, P.M. Elias, Regulation of epidermal sphingolipid synthesis by permeability barrier function, J. Lipid Res. 32 (7) (1991) 1151–1158.
[49] P.W. Wertz, D.T. Downing, Ceramides of pig epidermis: structure determination, J. Lipid Res. 24 (6) (1983) 759–765.
[50] A. Weerheim, M. Ponec, Determination of stratum corneum lipid profile by tape stripping in combination with high-performance thin-layer chromatography, Arch. Dermatol. Res. 293 (4) (2001) 191–199.
[51] E.R. Cooper, B. Berner, Skin permeability, in: D. Skerrow, C.J. Skerrow (Eds.), Methods in Skin Research, John Wiley and Sons, ohio, 1985, pp. 407–432.
[52] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (8) (1959) 911–917.
[53] G.M. Gray, Phosphatidyl-(N-acyl)-ethanolamine. A lipid component of mammalian epidermis, Biochim. Biophys. Acta 431 (1) (1976) 1–8.
[54] P.W. Wertz, D.T. Downing, Acylglucosylceramides of pig epidermis: structure determination, J. Lipid Res. 24 (6) (1983) 753–758.
[55] G.M. Gray, H.J. Yardley, Lipid compositions of cells isolated from pig, human, and rat epidermis 5, J. Lipid Res. 16 (6) (1975) 434–440.
[56] D.J. Monger, M.L. Williams, K.R. Feingold, B.E. Brown, P.M. Elias, Localization of sites of lipid biosynthesis in mammalian epidermis, J. Lipid Res. 29 (5) (1988) 603–612.
[57] A. Haratake, A. Komiya, T. Horikoshi, H. Uchiwa, S. Watanabe, Acceleration of de novo cholesterol synthesis in the epidermis influences desquamation of the stratum corneum in aged mice, Skin Pharmacol. Physiol. 19 (5) (2006) 275–282.
[58] P. Cox, C.A. Squier, Variations in lipids in different layers of porcine epidermis, J. Invest. Dermatol. 87 (6) (1986) 741–744.
[59] P.W. Wertz, D.T. Downing, Glucosylceramides of pig epidermis: structure determination, J. Lipid Res. 24 (9) (1983) 1135–1139.
[60] P.W. Wertz, D.T. Downing, R.K. Freinkel, T.N. Traczyk, Sphingolipids of the stratum corneum and lamellar granules of fetal rat epidermis, J. Invest. Dermatol. 83 (3) (1984) 193–195.
[61] P.W. Wertz, M.C. Miethke, S.A. Long, J.S. Strauss, D.T. Downing, The composition of the ceramides from human stratum corneum and from comedones, J. Invest. Dermatol. 84 (5) (1985) 410–412.
[62] J.A. Bouwstra, G.S. Gooris, F.E. Dubbelaar, A.M. Weerheim, A.P. Ijzerman, M. Ponec, Role of ceramide 1 in the molecular organization of the stratum corneum lipids, J. Lipid Res. 39 (1) (1998) 186–196.
[63] Y. Uchida, M. Hara, H. Nishio, E. Sidransky, S. Inoue, F. Otsuka, A. Suzuki, P.M. Elias, W.M. Holleran, S. Hamanaka, Epidermal sphingomyelins are precursors for selected stratum corneum ceramides, J. Lipid Res. 41 (12) (2000) 2071–2082.
[64] H. Farwanah, K. Raith, R.H. Neubert, J. Wohlrab, Ceramide profiles of the uninvolved skin in atopic dermatitis and psoriasis are comparable to those of healthy skin, Arch. Dermatol. Res. 296 (11) (2005) 514–521.
[65] D.M. Sharaf, S.J. Clark, D.T. Downing, Skin surface lipids of the dog, Lipids 12 (10) (1977) 786.
[66] P.W. Wertz, D.T. Downing, Integral lipids of human hair, Lipids 23 (9) (1988) 878–881.
[67] P.W. Wertz, D.T. Downing, Integral lipids of mammalian hair, Comp. Biochem. Physiol. B 92 (4) (1989) 759–761.
[68] P.W. Wertz, Integral lipids of hair and stratum corneum 25, EXS 78 (1997) 227–237.
[69] D.T. Downing, Lipid and protein structures in the permeability barrier of mammalian epidermis, J. Lipid Res. 33 (3) (1992) 301–313.
[70] C.S. Birkby, P.W. Wertz, D.T. Downing, The polar lipids from keratinized tissues of some vertebrates, Comp. Biochem. Physiol. B 73 (2) (1982) 239–242.
[71] M.A. Wix, P.W. Wertz, D.T. Downing, Polar lipid composition of mammalian hair, Comp. Biochem. Physiol. B 86 (4) (1987) 671–673.
[72] W. Meyer, Die Haut des Schweines, Archiv fuer tieraerztliche Fortbildung, Schluetersche Verlagsanstalt und Druckerei, Hannover, Germany, 1986.
[73] R. Schwarz, W. Meyer, J. Frewein, B. Vollmerhaus, Haut und Hautorgane, Anatomie von Hund und Katze, Blackwell Wissenschafts-Verlag, Berlin, Oxford, Edinburgh, Boston, London, Melbourne, Paris, Wien, Yokohama, 1994, pp. 316–340.
[74] T. Ludewig, G. Michel, G. Gutte, Histological and histochemical investigations on the structure of udder skin of cattle with special reference to changes during in vivo udder perfusion models 1, Dtsch. Tieraerztl. Wochenschr. 103 (12) (1996) 501–505.
[75] M.A. Hussein, The overall pattern of hair follicle arrangement in the rat and mouse, J. Anat. 109 (Pt. 2) (1971) 307–316.
[76] W. Meyer, G. Huelmann, H. Seger, REM-Atlas zur Haarkutikulastruktur mitteleuropaeischer Saeugetiere, Verlag M&H Schaper, Alfeld, Hannover, Germany, 2002.