Comparative Dermal Penetration Studies in Human Full-thickness and Dermatomed skin

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Aim of the study

Skin Absorption *in vitro* is an alternative method that is accepted by the OECD and for which guidelines (TG 428 and GD 28) give technical guidance how to perform valid experiments. Within these guidelines many experimental parameters can be varied, e.g. the preparation of applied skin barriers. Therefore we investigated the influence of skin preparations on dermal penetration and measured for a given set of compounds dermal penetration with full-thickness skin (FTS) and dermatomed skin (DMS) from the same human donor.

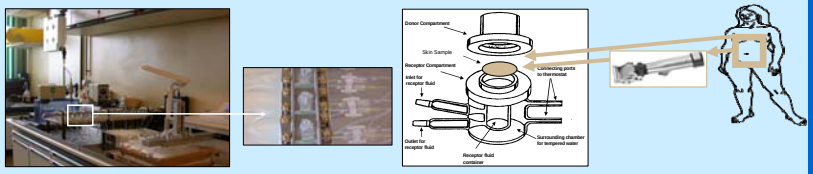

Figure 1: Whole experimental set up and Franz' cell with abdominal human female skin preparation. The skin was used after excision and removing of subcutaneous fatty tissue as "full-thickness skin" or after further preparation with a dermatome as "dermatomed skin".

Materials and Methods

Table 1: Overview of different applications

Test substance (donors)	log Kow	Molecular weight [g/mol]	Dose [µg/cm²]	application volume [µl]	Formulation	Receptor fluid
14C-MCPA-EHE (2)	6,2	312,83	160	10	water	water
14C-Testosterone (1)	3,32	288,43	100	25	ethanol/water 1/1 (v/v)	ethanol:water (1:1, v:v)
3H-Testosterone (4)	3,32	288,43	1,6*10-3	10	water	water
14C-MCPA (2)	2,73	200,62	90	10	as Dimethylamine salt in water	water
14C-Caffeine (2)	-0,07	194,19	100	25	ethanol/water 1/1 (v/v)	ethanol:water (1:1, v:v)
3H-Mannitol (3)	-3,1	182,17	0,01	25	ethanol/water 1/1 (v/v)	ethanol:water (1:1, v:v)

Test-compounds:

Testosterone (Sigma Aldrich, USA), ^{14}C-Testosterone (Perkin-Elmer, USA), ^{3}H-Testosterone (Perkin-Elmer), Caffeine (Sigma Aldrich, USA), ^{14}C-Caffeine (Perkin-Elmer, USA), ^{3}H-Mannitol (Perkin-Elmer), Mannitol (Fluka), ^{14}C-2-Methyl-4-chlorophenoxyacetic acid (MCPA) (BASF), MCPA (BASF), ^{14}C-2-Methyl-4-chlorophenoxyacetyl Ethylhexylester (MCPA-2EHE) (BASF), MCPA-2EHE (BASF).

Skin-Samples:

Human skin was purchased from Biopredic, France. It was received from reductive abdominal surgery from female caucasian patients. The compared full-thickness and dermatomized skin samples were obtained from the same donors. The integrity was determined by measuring the electrical resistance (LCR 400, Thurlby Thandar Instruments, England) and the trans epidermal water loss (TEWL) (Vapometer, Delfin Technologies, Finland). Values > 1 kOhm respectively < 10 g/(cm²*h) were accepted for intact skin preparations.

General Study design:

Franz cells (Laboratory Glass Apparatus Inc, U.S.A.) with automated sampling, a receptor volume of 4 ml and an provided skin surface area of 1 cm² were used. A water jacket brought the skin and receptor medium to the desired temperature of 32°C. The finite dose application was washed of after 6 h. Aliquots of the receptor medium were sampled at defined time points between 0 and 24 hours after application with a fraction collector (Multi-channel peristaltic pump MC 360, Ismatec and fraction collector 222 XL, Abimed). The abstracted receptor was replaced with fresh receptor fluid. Mixing of the receptor fluid was provided by magnetic stirrers (Variomag Telemodul 20C/40C, H + P Labortechnik, Germany) underlying the receptor compartment. At the end of run the Franz' cell was dismantled step by step and the stratum corneum was removed from the skin sample by tape stripping (Scotch Crystal Clear Tape 600). Measurements for amounts of penetrated test substance were carried out by liquid scintillation counting. Radioactivities were used to calculate the maximal penetration rate, the lag time as well as the permeability constant (Kp). The absorbed dose was defined as the penetrated content in receptor fluid added to the residue in skin. An unpaired t-test was used to identify statistical differences (* p-value < 0,05: significant, **p-value < 0,01: highly significant).

Results

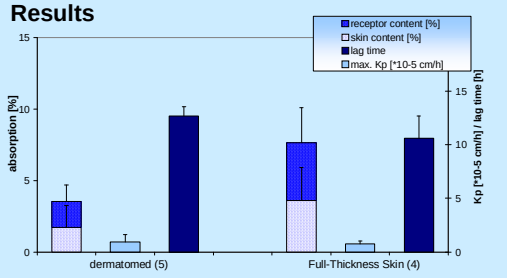

Figure 2: Penetration of ^{14}C-MCPA-2EHE through human FTS and DMS in vitro, 2 different donors were used, number of skin samples in brackets

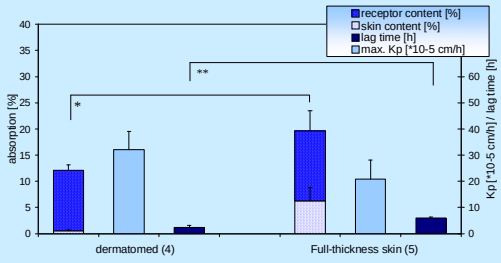

Figure 3: Penetration of ^{14}C-Testosterone through human FTS and DMS in vitro, 1 donor was used, number of skin samples is shown in brackets

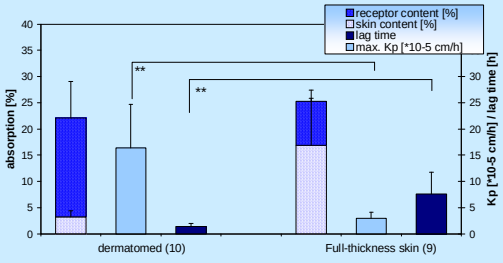

Figure 4: Penetration of ^{3}H-Testosterone through human FTS and DMS in vitro, 4 different donors were used, number of skin samples is shown in brackets

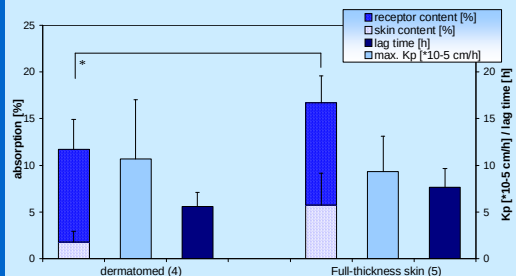

Figure 5: Penetration of ^{14}C-MCPA through human FTS and DMS in vitro, 2 different donors were used, number of skin samples is shown in brackets

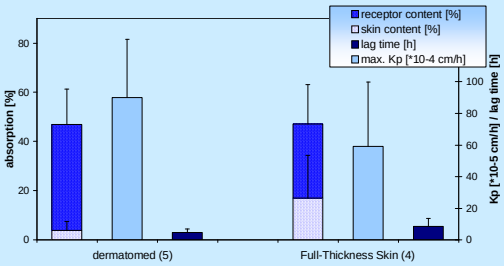

Figure 6: Penetration of ^{14}C-Caffeine through human FTS and DMS in vitro, 2 different donors were used, number of skin samples is shown in brackets

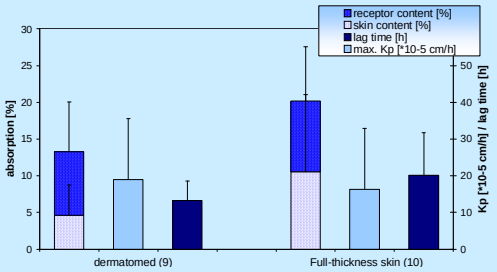

Figure 7: Penetration of ^{3}H-Mannitol through human FTS and DMS in vitro, 3 different donors were used, number of skin samples is shown in brackets

Table 2: Summary of results

	DMS			FTS		
Test substance	**% absorbed**	**Kp [10-5cm/h]**	**lag time [h]**	**% absorbed**	**Kp [10-5cm/h]**	**lag time [h]**
^{14}C-MCPA-EHE	4 ± 2	0.9 ± 0,7	12.7 ± 0.9	8 ± 3	0.8 ± 0.3	10.6 ± 2.1
^{14}C-Testosterone	12 ± 1*	32 ± 7	2.4 ± 0.8	20 ± 5*	21 ± 7	5.9 ± 0.5
^{3}H-Testosterone	22 ± 7	16 ± 8	1.4 ± 0.7	25 ± 9	3 ± 1	7.6 ± 4.1
^{14}C-MCPA	11 ± 2*	11 ± 6	5.6 ± 1.5	17 ± 3*	9 ± 4	7.6 ± 2
^{14}C-Caffeine	47 ± 14	90 ± 37	4.9 ± 2.1	47 ± 12	59 ± 40	8.4 ± 5.2
^{3}H-Mannitol	13 ± 5	19 ± 17	20.2 ± 11.5	20 ± 11	16 ± 17	13.3 ± 5.2

Summary

The dermal penetration behaviour differed for the two preparation types: We observed generally higher amounts of radioactivity remaining in the skin. In the receptor fluid we observed lower amounts of radioactivity with FTS compared to DMS (^{14}C-Caffeine and ^{3}H-Testosterone), equal amounts (^{3}H-Mannitol, ^{14}C-Testosterone, ^{14}C-MCPA) as well as higher amounts (^{14}C-MCPA-2EHE). In sum the absorbed dose was higher with FTS for all compounds except ^{14}C-Caffeine; ^{14}C-Caffeine led to a similar absorption in DMS and FTS. In general the use of FTS led to lower Kp-values and longer lag times. Only ^{14}C-MCPA-2EHE showed a slightly lower lag time with FTS.

Conclusion

Our data demonstrate that FTS and DMS are both applicable for dermal penetration studies in vitro, leading generally to results in the same order of magnitude (e.g. the same categorization scheme as suggested by Marzulli and Brown, 1969). However, a tendency was present that demonstrated slightly higher absorptions of test compounds in FTS than in DMS.

References

OECD Guideline 428 for testing of chemicals – skin absorption in vitro method, OECD, 2004.
OECD GD 28, Guidance document for the conduct of skin absorption studies, OECD series on testing and assessment Number 8, 2004.
Marzulli F.N. & Brown D.W.C.M.H.I. (1969) Techniques for studying skin Penetration. Toxicology and Applied Pharmacology 3, 76-83.