A Novel Approach for Investigations into Skin Barrier Function and Drug Penetration

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Background

The penetration of agents into human skin is difficult to investigate in vivo. Two methods are commonly used to obtain samples from the dermis of the skin:

1) Multiple skin biopsies, an invasive procedure being not well tolerated by patients or
2) dermal microdialysis, which is less invasive, continuously delivers a dialysate of interstitial fluid (ISF) but is limited to a rather small spectrum of well accessible low molecular weight and hydrophilic substances.

We are developing an alternative approach based on membrane-free sampling probes to overcome those limitations. Lately, we investigated whether the novel approach also enables the assessment of the pharmacokinetics and pharmacodynamics of a topically applied lipophilic drug.

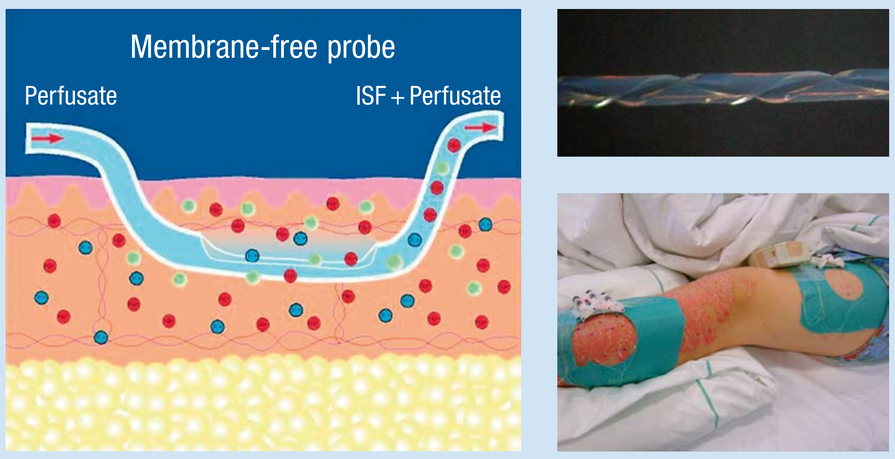

Figure 1: Concept of continuous sampling of the entire dermal interstitial fluid matrix using minimally-invasive membrane-free probes (left) – picture of the probe (top right) – portable sampling setup (bottom right)

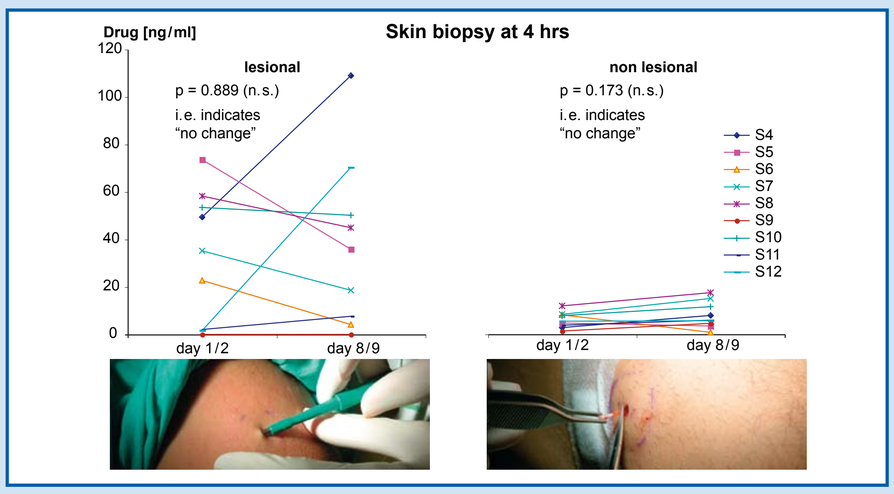

Figure 2: Total dermal drug levels at 4 hrs post-dose on day 1 and 8 as assessed by invasive 3 mm skin punch biopsies (state of the art) in lesional and non-lesional sites following ~ 6-fold tape stripping

Methods and Materials

The principle of Open-Flow Microperfusion (OFM) was utilized and adapted for skin. OFM is a continuous sampling approach similar to microdialysis. OFM, however, uses probes without semi-permeable membranes and thus enables a direct access to ISF with all its constituents for subsequent assay (Fig. 1). Its membrane-free probes had previously shown superior properties in investigations of adipose and skeletal muscle tissue biochemistry, in particular in investigations for peptides and proteins. In the transfer of the method to dermatological applications a series of basic animal experiments and in vitro experiments (adsorption properties) were performed.

The recent trial for lipophilic sampling was performed with a topical lipophilic drug (LogP = 3.1, MW 310 Da) selectively inhibiting p38. The p38 pathway is suggested to be critically involved in both the innate as well adaptive immune system, in the production and signalling of the main pro-inflammatory cytokines and thus be crucially involved in the psoriasis pathology.

12 patients diagnosed with untreated psoriatic lesions have been enrolled in a single center, open label exploratory trial. Four defined skin sites of 2.54 cm² (2 lesional + 2 non-lesional) were treated once daily with the lipophilic drug in a 0.5 % cream formulation from day 1 to day 8. On day 1 and day 8 six dermal OFM-probes were inserted into the dermis (3 lesional + 3 non-lesional) to continuously obtain dermal interstitial fluid (ISF) in fractions (15 µl / hr / probe) from pre-dose up to 24 hrs post-dose (Fig. 1). In addition state of the art skin punch biopsies (diam. 3 mm) were taken 4 hrs post-dose on day 1 and 8 following tape stripping (6 stripes) (Fig. 2). ISF samples were analysed for drug (Fig. 3) and the cytokine TNFα (Fig. 4). Skin biopsies were analysed for total drug concentrations (Fig. 2). High-sensitive, validated, bioanalytical methods prior tailored in house for lowest volume anwd concentration were used (drug: capLC / MS / MS, LLOQ: 0.033 ng / ml; TNFα: ELISA, LLOQ: 10 pg / ml, 10 µl of sample volume).

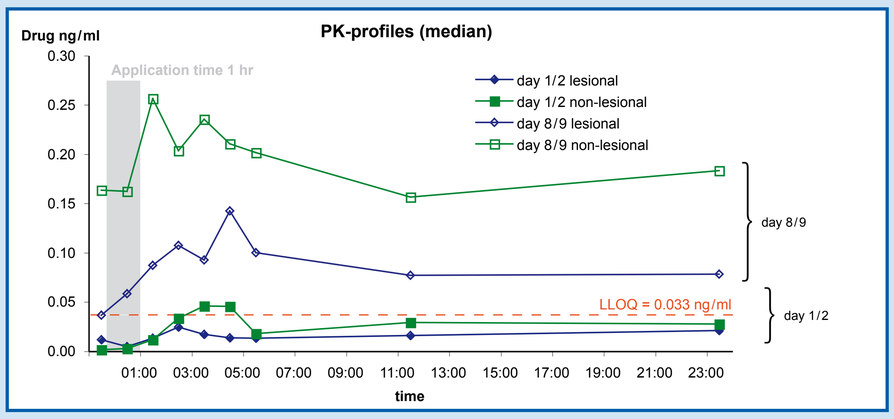

Figure 3: 24 hrs dermal interstitial drug kinetics as assessed from OFM samples

Results

In vitro data showed a low level of drug adsorption to the probes. In the recent trial on intradermal lipophilic sampling more than 500 dermal interstitial fluid samples (by OFM) and biopsies from lesional and non-lesional skin were obtained and analysed during the trial. On day 1 the drug was already detected (> LLOQ) in ISF samples around ~3 hours post-dose in many patients (Fig. 3). On day 8 pre-dose drug levels were above LLOQ and further increased up to a maximum of ~0.25 ng / ml after last drug application. TNFα levels were low on day 1, peaking at ~12 hrs post-dose at approx. 20 pg / ml and remaining below LLOQ on day 8, with the decrease being significant in lesional skin (Fig. 4). Biopsy drug levels in lesional skin (~40 ng / ml) were significantly higher than in non-lesional skin (~8 ng / ml) on day 1 and 8 (Fig. 2). In general biopsy drug levels showed high variability and thus gave no trend information.

Conclusion

The trial demonstrated that dermal OFM provides access to lipophilic drugs. Thus OFM has again proven to provide continuous access to virtually all molecules of interest in the ISF at target tissue level, regardless of molecular size or electrochemical properties (Fig. 5).
We conclude that OFM can overcome limitations of state of the art methods and thus represents an alternative for the assessment of skin barrier function and the drug penetration from topically applied formulations.

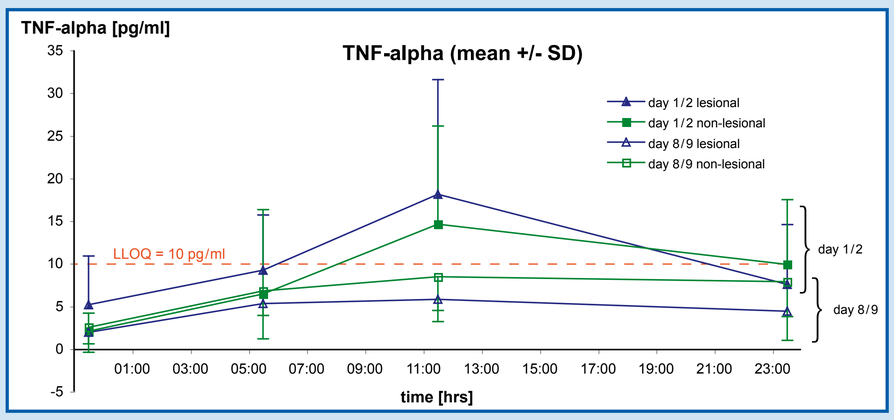

Figure 4: 24 hrs dermal interstitial TNFα profiles (4-points: pre-dose, 6 hrs, 12 hrs, 24 hrs post dose) as assessed from OFM samples

Classes of substances / drugs …	µ-Dialysis	OFM
… small	Yes	Yes
… hydrophilic (small)	Yes	Yes
… middle + large	Yes / No	Yes
… lipophilic	No	Yes ✔
… protein-bound	No – C_{free}	Yes – C_{total}
… (nano)carrier / cells	No	Yes

Figure 5: Range of applications for µ-Dialysis and the membrane-free method of Open-Flow Microperfusion (OFM)

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