Detecting the skin penetration potential of new pharmacological compounds for acne therapy

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Introduction
The skin is the largest organ of the human body, which serves as a barrier against external factors and pathogens. The skin penetration of topically applied compounds is crucial for their efficacy. IP10.C8 is a drug substance developed for the treatment of acne. The aim of this study was to develop methods for evaluating the skin penetration of IP10.C8 as well as for investigating whether IP10.C8 reaches skin layers relevant for acne therapy.

Methods
In vitro skin model
Culture conditions: Human skin explants were obtained from cosmetic surgery and cultured in a transwell system under physiological conditions (100% relative humidity, 37°C, 5% CO2) for 24 h. After the cell cultures were washed with maintenance medium, the skin explants were treated with IP10.C8 in different dilutions. The amount of IP10.C8 in various skin layers was determined by HPLC after 24 h and 48 h of incubation.

Cryosection and homogenization of skin biopsy material
Skin biopsies were taken from different body regions and frozen in liquid nitrogen. The skin pieces were stored at -80°C until further processing. Skin sections were then prepared and homogenized using a microtube homogenizer.

Results of penetration tests using the in-vitro epidermis model EST-1000
DP IV- and APN inhibition assay with maintenance medium
Detection of IP10.C8 in maintenance medium by HPLC

Conclusions
Both methods were found to provide reproducible, time- and concentration-dependent results for the penetration capacity of the test item IP10.C8. The easy handling of the EST-1000 test system suggests the use for screening studies.

The use of ex vivo-treated human skin biopsies with intact complete skin architecture additionally allowed conclusions on the penetration profile for the test item regarding reached skin depth and time course by analyzing the test item content in tangential cuttings. However, a problem represents the high variability between the various skin samples and is currently a limiting factor of the test.

Both methods have the potential for monitoring the penetration of new compounds into the human skin.

Characteristics of newly developed dual inhibitor IP10.C8
Structure
MW=592.68 g/mol

Biological Effects

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<th>Effect on Target Peptidease</th>
<th>IC50 [nM]</th>
<th>IC90 [nM]</th>
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Effect on the proliferative capacity of various cell types:

APN

Innate cell types: PMN-activated and non-activated human monocytes (2000-10,000 cells) 1% IP10.C8 had no effect on cell proliferation. 0.03% IP10.C8 and 0.05% IP10.C8 inhibited cell proliferation of monocytes. 1% IP10.C8 Gel had no effect on cell proliferation of monocytes.

Inhibitory effect of enzyme activity:

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<th>Inhibitor</th>
<th>APN</th>
<th>DP IV</th>
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Penetration of IP10.C8 into human skin-punch biopsies

Concentration-dependent penetration of IP10.C8

Time-dependent penetration of IP10.C8

Detection of IP10.C8 in skin layer by HPLC

[Graphs and tables showing penetration data and analysis]