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

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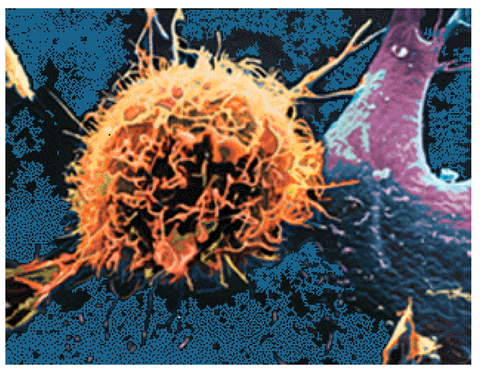
Immunopharm

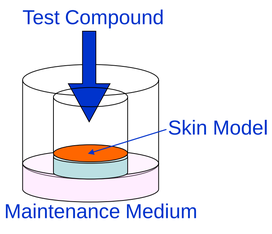


Introduction

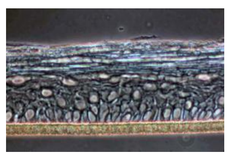
The dual inhibitor of dipeptidyl peptidase IV (DP IV) and alanyl aminopeptidase (APN) activity IP10.C8 with anti-proliferative and anti-inflammatory effects on cell types crucial in acne pathogenesis represents a novel promising drug substance for topical acne treatment. The aim of this study was to 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
The EST-1000 (CellSystems, Germany) consists of normal epidermal keratinocytes in an air-lift culture and shows significant similarity to the skin found on thorax, abdomen and extremities of adult humans. Penetration of gel formulated IP10.C8 (concentrations 0,1 % and 1 %) were analyzed by exposure on the top of the epidermal layer in model. The amounts of test item passing the epidermal layer of the EST-1000 model (after 4 h, 24 h and 48 h) were quantified in the maintenance medium by HPLC and in a target enzyme inhibition assay. Viability of the skin model cell layers was proved with standard MTT-assay (OECD TG431). 1% IP10.C8 had no effect on the viability of the EST-1000 three-dimensional cell layers after 3 min or one hour. After 24h and 48h the cytosolic enzyme lactate dehydrogenase was measured and there was no difference between the controls and the IP10.C8 treated skin models.



(Picture and Info from CellSystems website)



Skin biopsies
Human skin material from different body regions and various skin types was kindly provided by the Clinic of Dermatology and Venereology of the Otto-von-Guericke University Magdeburg. Small pieces of healthy skin obtained as dispensable material during dermatosurgical excisions were used after patients' written informed consent. The skin pieces were shortly stored for transport in steril petri dishes under humid conditons. Punch biopsies (6 mm) were prepared ex-vivo and placed in microtiter plate wells with maintenance medium.

Ex-vivo-treatment of skin biopsies with the test item
The skin biopsies were incubated with 0,1 %, 0,3 %, 0,6 % and 1 % IP10.C8 in gel formulation for 16 h.

With an IP10.C8 concentration of 1 %, the penetration assay was replicated to various time points. The applicated batch of IP10.C8 to each skin-punch biopsy amounted to approximately 30 µg and the incubation took place at room temperature.

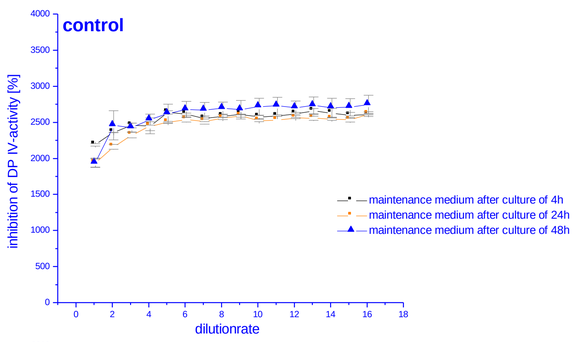
Cryosection and homogenization of skin biopsy material
After incubation 50 µm horizontal cryosections were prepared, homogenized and the test item amount in each cryosection homogenate was determined as follows:

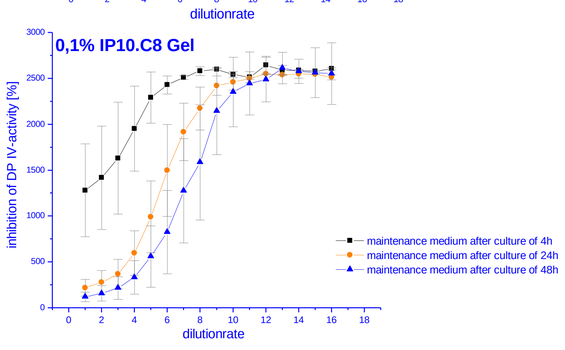
Target enzyme assay
For IP10.C8 penetration analysis we used DP IV- and APN inhibition assays. In these assays DP IV and APN were added at a concentration range of 10^{-9} M. The DP IV-activity was measured by using 2 µM Ala-Pro-Rhodamine 110 , APN-activity was detected with 2 µM Ala-Rhodamine 110.

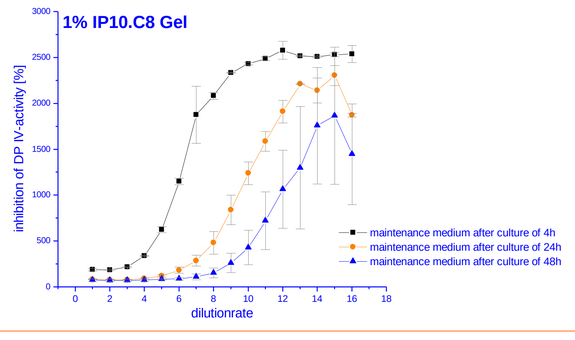
HPLC analysis
The amount of IP10.C8 in various skin layers was determined by HPLC.
HPLC-conditions: Column: LiChroCART Supersper RP60 selectB 75-4 with precolumn (MERCK)
Eluents: acetonitril in water, Phosphoric acid, sodium 1 –heptane-sulphonate,
Gradient: 1,2 ml/min
Path length: 320 nm
Injection: 10 µl

Results of penetration tests using the in-vitro epidermis model EST-1000

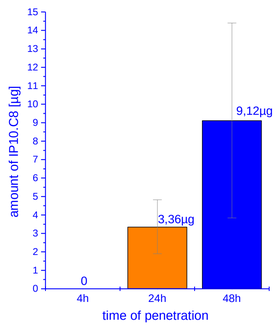
DP IV- and APN inhibition assay with maintenance medium

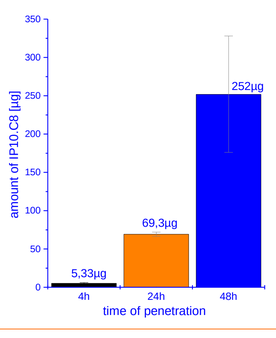


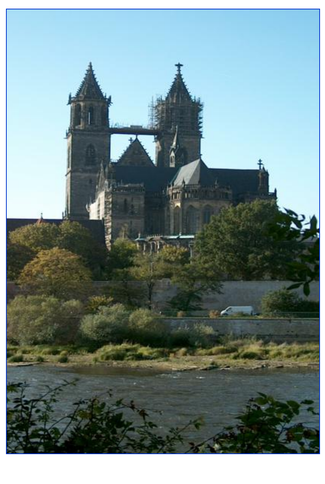




Detection of IP10.C8 in maintenance medium by HPLC







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 human skin biopsies from different body regions and skin types.

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: synthetic low molecular weight molecule containing a DPIV inhibitory and an APN inhibitor motif
MW=592.68 g/mol
water soluble

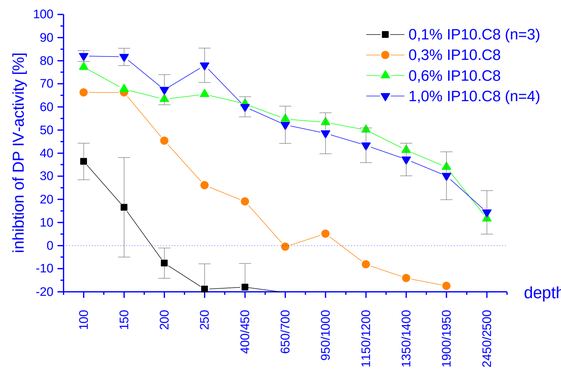
Biological Effects:

Effect on Target Peptidases: $IC50_{DPIV}$ = 8 nM ; $IC50_{APN}$ = 1,9 nM

Effect on the proliferative capacity of various cell types:

		IC50 [µM] after 48h
Immune cell types	PHA-stimulated peripheral blood mononuclear cells (PBMC)	5,95
	PHA-activated peripheral blood T-lymphocytes	12,5
Skin cell types	Primary keratinocytes- NHEK (Normal Human Epidermal Keratinocytes)	17,3
	SZ95 (Immortalized sebocytes)	19,3

Penetration of IP10.C8 into human skin-punch biopsies



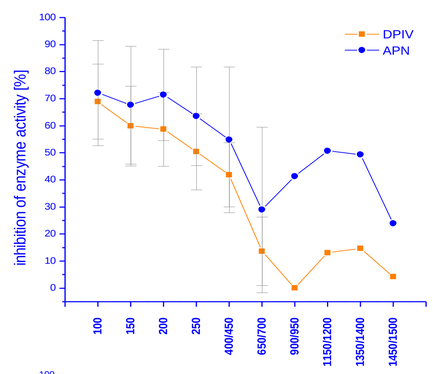
Concentration-dependent penetration of IP10.C8
(HPLC values not detected)

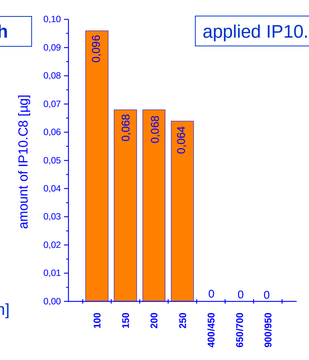
Time-dependent penetration of IP10.C8

DP IV- and APN inhibition assay with skin-layers

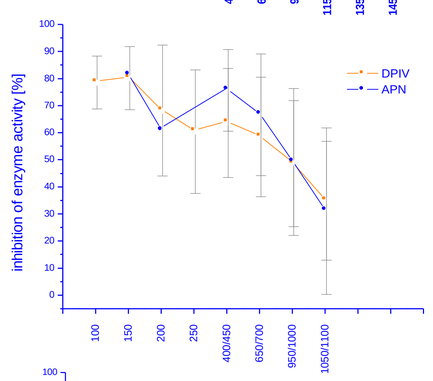
Detection of IP10.C8 in skin-layer by HPLC

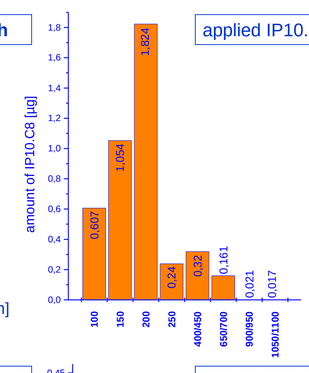
Time of incubation: 2h — applied IP10.C8 concentration: 17 µg



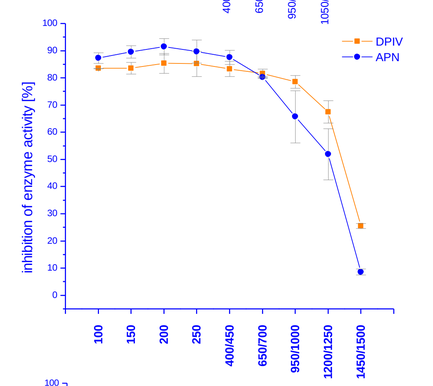


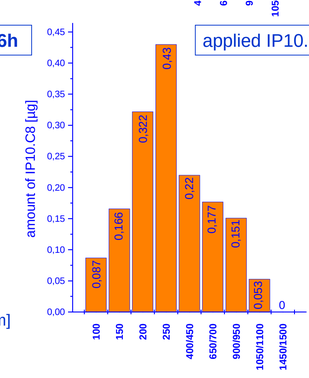
Time of incubation: 6h — applied IP10.C8 concentration: 51 µg



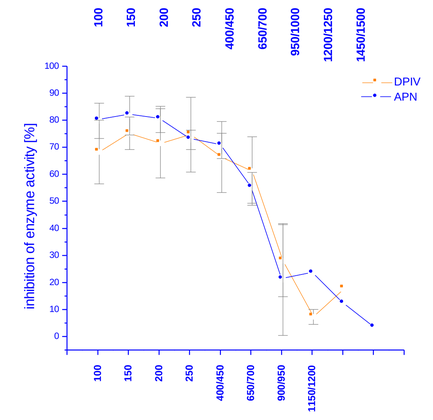


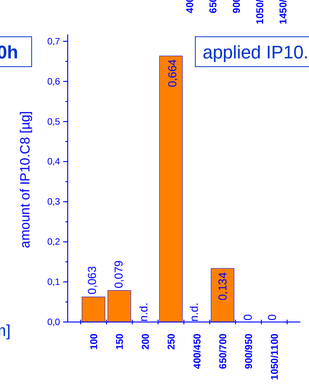
Time of incubation: 16h — applied IP10.C8 concentration: 35 µg



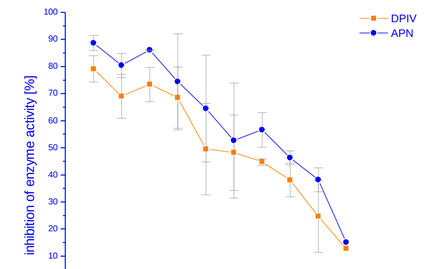


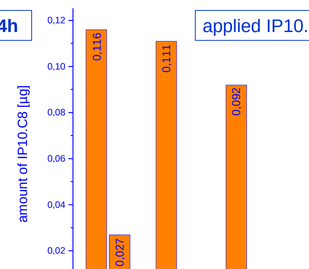
Time of incubation: 20h — applied IP10.C8 concentration: 26 µg





Time of incubation: 24h — applied IP10.C8 concentration: 22 µg





Time of incubation: 48h — applied IP10.C8 concentration: 26 µg