Development of PAMPA model for skin penetration of drugs

Synthesis and application of ceramide analogues

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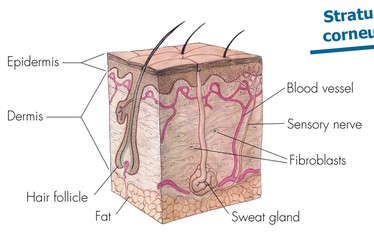
Introduction: Transdermal drug delivery has extensively grown in the past decades. Advantages of transdermal application dosage forms like plasters in certain drug families (steroid hormons, NSAIDs, major analgetics, antianginal agents etc.) have been proved. Efforts in drug research have been devoted to find a useful model for predicting the skin penetration of new molecules. The parallel artificial membrane permeability assay (PAMPA)[1] as a passive permeation screen method can be used for the ADME screening of molecules. So far this method has been applied for prediction of human intestinal absorption and the penetration through the blood-brain barrier. In the literature there is only one application of PAMPA for skin absorption, but that model uses a membrane made of silicone oil and IPM (isopropyl-myristate), materials that are not ingredients of the real human skin[2].

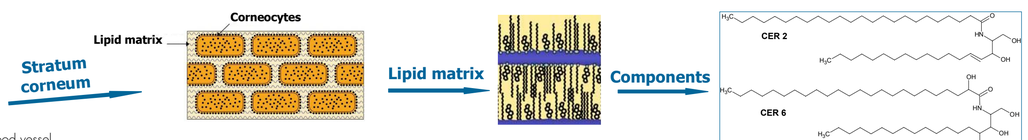
Our purpose is to develop an effective skin PAMPA model using similar ingredients are present in the real human skin. We plan to study the effect of ceramide analogues on this model.

The outer-most layer of the skin, the stratum corneum plays the most important rule in the barrier function of skin. It contains a relatively high level of ceramides, which are present mainly in the extracellular domain and are accompanied by nearly equimolar amounts of cholesterol and free fatty acids. Besides the penetration the ceramides also mediates diverse cellular effects like differentiation or proliferation. The synthetized ceramide analogues are able to mimic the barrier function of real ceramides without having proapoptopic effect[3].

1: Avdeef et al. Expert Opin. Drug Metab. Toxicol. (2005) 1 (2): 325-342; 2: Ottaviani et al. Journal of Medicinal Chemistry 49 (13) (2006) 3948-3954; 3: Benjamin et al. Biochemica et Biophysica Acta 1585 (2002) 114-125

Skin

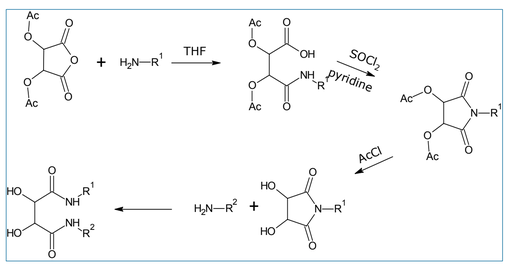




Stratum corneum lipid matrix components %		
Cholesterol	Free fatty acids	Ceramides
35	15	50

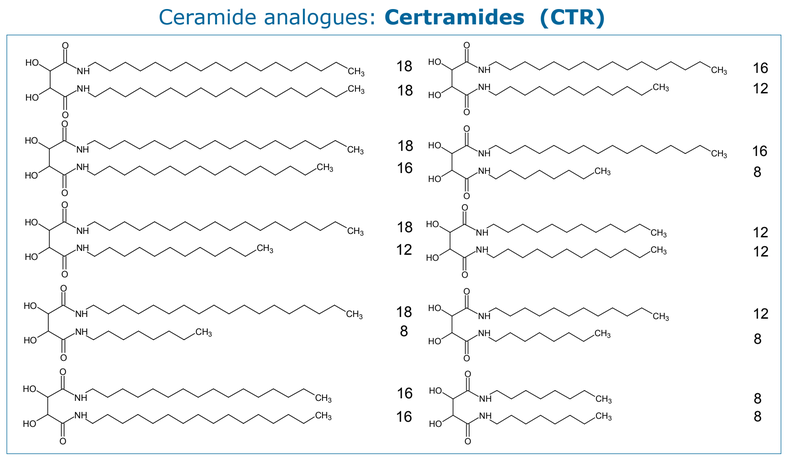
The main barrier of the skin is the stratum corneum lipid matrix.

Synthesis:



All the structures were proved by NMR spectroscopy and Mass spectrometry.

Ceramide analogues: **Certramides (CTR)**



Method, Chemicals:

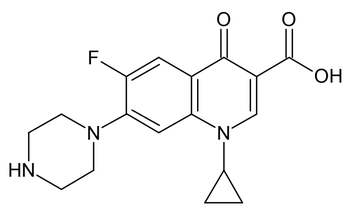
The PAMPA method was used, having designed the experiments according to "pION's guide to improve Double-Sink PAMPA" with the PAMPA Explorer™ software for all the calculations. TECAN Infinite M200 was the applied UV plate-reader. All the chemicals were purchased from Sigma-Aldrich.

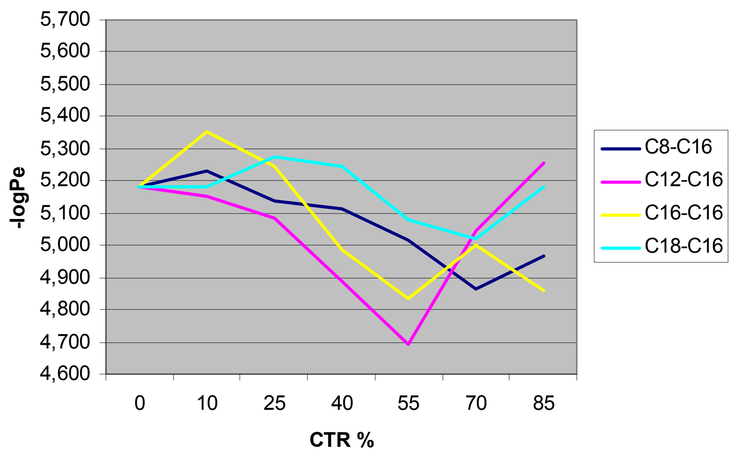
Experiments:

- substances: ciprofloxacin, nifedipine, verapamil HCl
- membrane:
  - no phospholipids were used
  - CTR chain lenght: (8;16), (12;16), (16;16), (18;16)
  - CTR concentration.: 0-100 %
  - rest of the membrane: Chol : FFA = 1:1
  - Solvent: chloroform (evaporated after lipid painting)
- PAMPA incubation: T= 25 °C, t= 6 hours
- pH: donor phase: 5.5; acceptor phase: 7,4 (B-R buffer)
- parallel measurements: n= 6 – 12 SD= 0,03 – 0,09
- results are expressed in – logPe (the higher value indicates lower permeability)

Results:

Ciprofloxacin

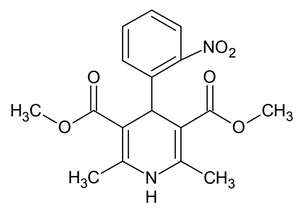


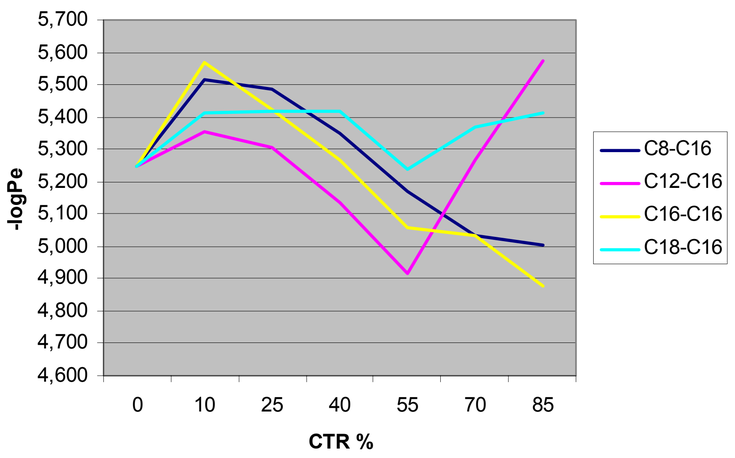


	Alkil chain lenght			
	8-16	12-16	16-16	18-16
0	5,180	5,180	5,180	5,180
10	5,230	5,150	5,351	5,181
25	5,137	5,083	5,243	5,276
40	5,114	4,888	4,985	5,247
55	5,016	4,691	4,835	5,081
70	4,866	5,047	5,001	5,021
85	4,965	5,253	4,857	5,180
100	10,000	10,000	10,000	10,000

In case of ciprofloxacin the effect of the CTR (12;16) is the strongest on the permeability, and at the rate of 55% the permeability reach the maximum value. All the analogues are able to increase the permeability at 55 or 70 %, but over that a strong decreasing effect appear.

Nifedipine

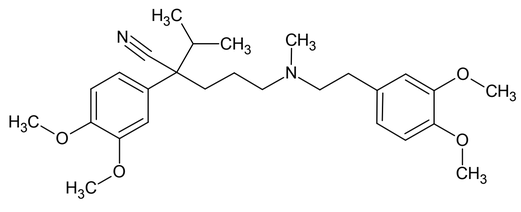


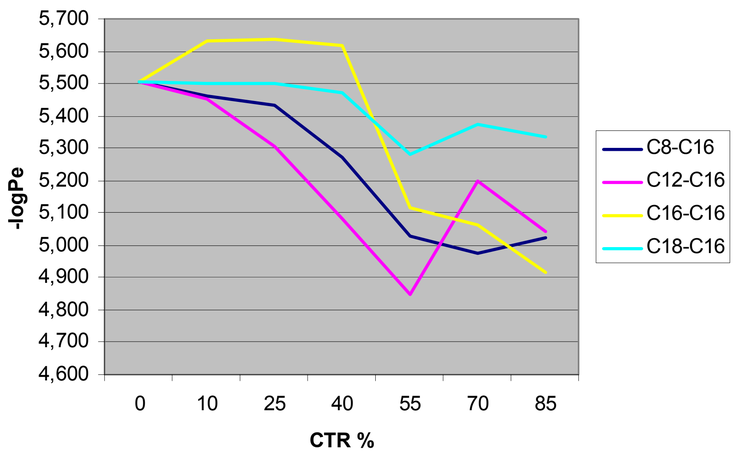


	Alkil chain lenght			
	8-16	12-16	16-16	18-16
0	5,245	5,245	5,245	5,245
10	5,517	5,352	5,567	5,412
25	5,485	5,305	5,425	5,418
40	5,349	5,137	5,267	5,420
55	5,169	4,914	5,056	5,239
70	5,032	5,267	5,031	5,370
85	5,004	5,572	4,879	5,412
100	10,000	10,000	10,000	10,000

All of the investigated ceramide analogues have a very strong decreasing effect in a low rate. The highest penetration value were measured if the CTR (12;16) was used. At higher ratio a decreasing effect was experienced.

Verapamil





	Alkil chain lenght			
	8-16	12-16	16-16	18-16
0	5,505	5,505	5,505	5,505
10	5,460	5,451	5,630	5,502
25	5,434	5,308	5,639	5,502
40	5,272	5,081	5,619	5,473
55	5,026	4,849	5,115	5,280
70	4,975	5,201	5,064	5,375
85	5,024	5,044	4,917	5,336
100	10,000	10,000	10,000	10,000

In case of verapamil the CTR (12;16) has the strongest increasing effect. Comparing to nifedipin, none of the analogues has a significant decreasing effect. The CTR (18;16) do not have an effect on permeability until the rate of 40 %, over 55 % it increase the permeability as well.

Summary:

- The synthetized molecules can be able to replace the real ceramides in the PAMPA model.
- These precise, reproducible experimental PAMPA permeability data provide a good basis for the correlation study with human skin epidermal permeability data.
- With skin-PAMPA model without phospholipids, containing certramides, cholesterol and FFAs we obtained well reproducible data with small SD.
- The effect of investigated certramides on the permeability of model substances is different, the CTR (12;16) has the strongest increasing effect in every case. At the rate of 100 % the permeability was undetectable in every case.

Future plans:

- In vitro human skin measurements are in progress (in collaboration with University of Valencia).