Summary

The purpose of this work is to contribute to the understanding of the structure-activity relationships and behavior of the skin ceramides and transdermal penetration enhancers based on dimethylamino acid esters.

First two chapters provide a theoretical background for understanding the main principles of transdermal drug delivery as well as composition and function of stratum corneum barrier. The experimental work is presented in the form of individual publications in chapters three to five.

The first chapter describes transport routes through the human skin and gives an overview of enhancers of transdermal permeation with main accent on their chemical structure, mechanism of action, interaction with stratum corneum components and advantages and drawbacks of their use.

The second chapter brings more detailed information on the specific content and composition of stratum corneum. The main attention is paid to the ceramides - a complex group of lipids that play a crucial role as cell signaling molecules and skin barrier constituents. In the skin, these sphingolipids form a major part of the stratum corneum intercellular lipid matrix, which is the barrier for penetration of most compounds. The development of such a protective layer was a critical step in the evolution of life on a dry land. Moreover, prominent skin diseases such as psoriasis and atopic dermatitis are associated with diminished ceramide levels and may be effectively improved by exogenous ceramides or their analogues. Since ceramides are not obtained from natural sources in pure form, they are of synthetic interest since 1950's. In this chapter, we describe sphingosine syntheses from 1998 until 2008, and the synthetic approaches to the unique epidermal ceramides, including the 6-hydroxysphingosine-based ones, the alpha- and omega-hydroxy forms and the omega-acyloxy species. Moreover, the structural requirements of ceramides for a competent skin barrier are discussed, including acyl chain length, *trans* double bond, acyl alpha-hydroxyl, stereochemistry, omega-linoleyloxy species and ceramide conformation.

Chapter 3 focuses on the synthesis, evaluation of transdermal permeation-enhancing potency, biodegradability and reversibility of action of series of *N*,*N*-dimethylamino acid esters. Effects of chirality, linking chain length and polyfluorination together with the enhancer-drug interaction are discussed. No differences in activity were found between (*R*), (*S*) and racemic dodecyl 2-(dimethylamino)propanoate (DDAIP). Substitution of hydrocarbon tail by fluorocarbon one resulted in loss of activity. Replacement of branched linking chain between nitrogen and ester of DDAIP by linear one markedly improved penetration-enhancing activity with optimum in 4-6C acid derivatives. Dodecyl 6-(dimethylamino)hexanoate (DDAK) was more potent than clinically used skin absorption enhancer DDAIP for theophylline (enhancement ratio of DDAK and DDAIP was 17.3 and 5.9, respectively), hydrocortisone (43.2 and 11.5) and adefovir (13.6 and 2.8), while DDAIP was better enhancer for indomethacin (8.7 and 22.8). DDAK was rapidly metabolized by porcine esterase, and displayed low acute toxicity. Electrical resistance of DDAK-treated skin barrier promptly recovered to control values, showing the reversibility of action of the enhancer. These results suggest that DDAK, a highly effective biodegradable transdermal permeation enhancer for a broad spectrum of drugs, is a promising candidate for future research. 7

As mentioned above, stratum corneum ceramides are major determinants of skin barrier function. Although their physiological and pathological role has been widely investigated, to date no structure-activity relationships have been established. Study described in chapter 4 concentrates on the synthesis of a series of short-chain ceramide analogues with polar head structure identical to ceramide NS, sphingosine length of 12 carbons and acyl chain length from 2 to 12 carbons. Their effect on skin permeability is evaluated using porcine skin and two model drugs, theophylline and indomethacin, and compared to that of a physiological ceramide NS. The results showed that ceramide chain length was crucial for their barrier properties. Ceramides with 4-8C acyl chain were able to increase skin permeability for both drugs up to 10.8 times with maximum effect at 6C acyl. No increase in permeability was found for ceramide analogues with 2C and 12C acyl and ceramide NS. The same relationships were obtained for skin concentrations of the model drugs. The relationship between ceramide acyl chain length and its ability to perturb skin barrier showed striking similarity to the behavior of short-chain ceramides in sphingomyelin/phospholipid membranes and confirmed that short-chain ceramides do not act as natural ceramides and their use as experimental tools should be cautious.

Topical skin lipid supplementation may provide opportunities for controlling ceramide deficiency in diseases such as atopic dermatitis or psoriasis. However, the exact mechanisms by which exogenous ceramides correct the barrier abnormalities are not known. Although exogenous short-chain NBD-labeled C6-ceramide was shown to rapidly traverse stratum corneum (SC) and to be uptaken and metabolized by viable epidermal layers, no such evidence is available for long-chain ceramides. Thus, the study described in chapter 5 aims at comparing skin penetration of fluorescent NBD-labeled C6, C12, and C24- ceramide and pseudo ceramide 14S24. Fluorescent lipids were synthesized from sphingosine or L-serine ester and omega-NBD-labeled hydroxysuccinimide-activated acids. 24-NBD-lignoceric acid was prepared from dodecan-1,12-diol using lithium tetrachlorocuprate coupling. NBD- ceramide at two concentrations, either alone or in equimolar mixture with cholesterol and lignoceric acid, were applied on viable human skin for 2 and 12 h, respectively. Only short-chain NBD-C6- ceramide reached viable epidermis; NBD-C12- ceramide and both NBD-C24 lipids penetrated solely into several upper SC layers of both intact and acetone-treated skin. These results show that the skin penetration of exogenous ceramide is chain length-dependent and that exogenous long-chain NBD-labeled ceramide and their analogues do not enter viable epidermis. This supports our hypothesis that short-chain ceramide cannot be used as general long-chain ceramide mimics.