Abstract

Penetration enhancers are compounds that facilitate transdermal drug delivery, which is an advantageous pathway of drug administration (compared to conventional methods). However, skin acts as formidable barrier enabling terrestrial life by protecting the inner body from the environmental conditions, microbes and substances. Thus, for drug administration *via* this pathway, some approach to temporary increase the skin permeability (e.g. permeation enhancer) should be employed. Despite the existence of many enhancer classes, there is still a need for new compounds enhancing broader spectrum of drugs, with reversible mode of action and better safety profile.

One of the promising approaches is to explore natural compounds with low toxicity and irritation potential. In this thesis, I first studied various glucose and galactose derivatives bearing structural fragments that were responsible for enhancing activity of previously studied enhancers (e.g. amino acid derivatives, fatty acids and alcohols, modified ceramides). A detailed examination of these sugar-based compounds revealed potent glucoside and galactoside enhancers (**12Glc6N** and **A15**) comparable to or better than Span 20 with reversible mode of action and acceptable toxicity on keratinocyte and fibroblast cell lines.

Another part of my work builds on the previous research of amino acid derivatives, which revealed potent enhancers with reversible mode of action and acceptable toxicity, such as 6-(dimethylamino)hexanoic acid dodecyl ester, DDAK. We prepared new potential enhancers by combining 6-(dimethylamino)hexanoic acid with selected terpenes or cinnamyl alcohol via biodegradable ester bond. This modification resulted in potent (comparable or better than DDAK), reversible and low-toxic enhancers derived from borneol, citronellol and cinnamyl alcohol (**B-DAK**, **C-DAK** and **Ci-DAK**, respectively), which increased the flux of hydrocortisone through human skin up to 82 times (cinnamyl derivative **Ci-DAK**). Noteworthy, **C-DAK**, derived from citronellol, increased the flux values of hydrocortisone and theophylline 56 and 47 times, respectively, which suggest its enhancing activity for both drugs with balanced (theophylline) or more lipophilic (hydrocortisone) properties.

Beside the influence of the prepared enhancers on permeation of theophylline and hydrocortisone, which served simply as model structures with different physical chemical properties, the effect of most promising derivatives on permeation of cidofovir, a potent antiviral with potential clinical use, was investigated. Both sugar and terpene-based enhancers selectively increase cidofovir concentration in the skin (mostly in the superficial layers such as stratum corneum and epidermis) with no effect on the permeation of cidofovir through skin. It means that prepared enhancers could be advantageously used for improvement in local treatment of diseases caused by viruses and carcinomas sensitive to cidofovir, without significant adverse systemic effects of this drug. Similar results were observed in another part of our research for DDAK. Co-application of DDAK with prodrugs of acyclic nucleoside phosphonates highly active against many viruses lead to increased drug retention in the skin with no detectable permeation into the acceptor medium. On the other hand, DDAK was able to increase the delivery of different acyclic nucleoside phosphonates through skin. Therefore, by the application of prodrug or parent drug together with the enhancer, it is possible to target the local or systemic antiviral treatment.

The influence of all prepared enhancers on the skin barrier properties were reversible within 24 h after the enhancer removal as measured by transepidermal water loss and electrical impedance.

Infrared studies using human stratum corneum suggested the interaction of enhancers with barrier lipids with no changes in the stratum corneum proteins.

The last topic of my thesis deals with fluorescent enhancers, which enable direct observation of their behaviour in skin via confocal laser scanning microscopy. These compounds were prepared by a combination of DDAK molecule with fluorescent NBD label (7-nitrobenzo[c][1,2,5]oxadiazol-4-yl). The resulting NBD esters retained permeation-enhancing activity, although lower than that of DDAK. Permeability studies revealed interesting complex interaction between model drug (theophylline, hydrocortisone), enhancer, donor solvent and skin. The enhancer influence on the drug flux was accompanied by various effects of the model drugs on the enhancer penetration. These complex interactions included the skin itself, with rather rapid decomposition of the NBD enhancers by skin esterases. Similarly to saccharide and terpene derivatives, NBD-esters interacted with barrier lipids. Such observation is in good agreement with the fluorescent microscopy results (human epidermis treated by NBD-esters) which confirmed accumulation of NBD enhancers mainly in the intercellular lipid lamellae with negligible entrance into the corneocytes.

Therefore, potent enhancers based on natural compounds were obtained by combination of saccharides or terpenes with structural fragments of previously described enhancers. These low toxic enhancers reversibly interact with lipid part of skin barrier yet maintaining potent enhancing activity toward drugs with different physical chemical properties. The investigation of fluorescently labelled enhancers furthermore revealed more complex interaction between model drugs used, enhancer and the skin. Fluorescent label enabled direct observation of enhancers applied onto skin and description of new interactions occurring during the permeation proces.

The advantageous properties of the enhancers reported in this thesis emphasize the usefulness of natural compounds in further search for new enhancers. We also confirmed the suitability of transepidermal water loss and impedance measurement in distinguishing between reversible and irreversible effect of enhancers and broadened our methods by using the cellular toxicity studies and confocal laser microscopy. Furthermore, the fluorescent enhancers that enable direct observation of enhancer behaviour in skin via confocal laser microscopy certainly deserve further studies.