ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Training Workplace	Department of Pharmaceutical Technology
Doctoral Degree Program	Pharmaceutical Technology
Candidate	Mgr. Martin Juhaščik
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Title of Doctoral Thesis	Study of conjugates of sphingolipids with hyaluronic acid and their application on the skin

The main goal of this study was to systematically design and subsequently prepare conjugates of hyaluronic acid (HA) with selected sphingolipids, which could find application in their topical administration during physiological decline of skin functions. In this study, phytosphingosine and ceramide were chemically linked to HA via biocompatible linkers, enabling successful modification of HA into a hydrophobized conjugate of sphingolipid-linker-hyaluronate.

The preparation of succinylceramide-hyaluronate conjugate was found to be the most effective. The developed method of preparing the conjugate using mixed anhydrides brings several advantages, including the possibility of scaling up the preparation and conducting reactions in an aqueous environment. The method was effective over a wide range of molecular weights, from 6 to 442 $kgmol^{-1}$, achieving a degree of substitution of up to 14.2 %, surpassing the originally defined target of 2.5 to 3.5 %. Successful modification of HA into the conjugate of sphingolipid-linker-hyaluronate was confirmed by NMR, infrared spectroscopy, and mass spectrometry.

Succinylceramide-hyaluronate conjugates showed no cytotoxic effects. However, cell growth was observed, which could be beneficial for potential compensation of dermal atrophic changes. Furthermore, the conjugate demonstrated a significant reduction in the level of the pro-inflammatory cytokine interleukin 6, up to 95 %, at relatively low concentrations. Aggregation properties were studied using critical aggregation concentration, pyrene binding constant, dynamic light scattering, and binding of the model lipophilic substance curcumin, which confirmed the self-assembling of conjugates into polymeric nanoparticles with sizes up to 40 nm.

After application of polymeric nanoparticles of succinylceramide-hyaluronate conjugates with encapsulated fluorescent marker Nile red on porcine skin *ex vivo*, fluorescence was observed in the *dermis*. Prepared conjugates applied to *ex vivo* human skin had no negative impact on skin barrier permeability, as evaluated by transepidermal water loss, electrical impedance, and skin permeability to the model substance theophylline. Infrared spectroscopy suggests that the applied succinylceramide-hyaluronate conjugates did not significantly affect the conformation of skin lipids but influenced the secondary structure of keratin in favor of the β-sheet conformation.

Self-assembled polymeric nanoparticles of prepared conjugates are soluble in water and therefore could be incorporated into the aqueous phase of liquid or semi-solid topical therapeutic/cosmetic preparations for the treatment of inflammatory skin diseases or could be used as carriers for hydrophobic active substances with application in (trans)dermal drug delivery.