LIPOSOMES LOADED WITH THE PNEUMOCOCCAL ENDOLYSIN MSLYS: FROM *IN VITRO* CHARACTERIZATION TO *EX VIVO* PERMEATION ACROSS THE TYMPANIC MEMBRANE

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Introduction: The increasing antibiotic resistance triggered interest in novel antimicrobials as well as delivery systems that allow a topical and targeted delivery. However, biological barriers, such as the skin or the tympanic membrane (TM), may hinder the success of the therapy. Drug permeation has been extensively studied in the context of transdermal delivery, but only recently started to be explored for transtympanic applications. *Ex vivo* Franz diffusion cell permeation tests have been used and validated for the permeation of compounds through the skin prior to *in vivo* studies, but their exploitation for transtympanic delivery is limited. Endolysins, peptidoglycan hydrolases derived from bacterial viruses, are attractive antimicrobials, with promising action against the otitis media pathogen *Streptococcus pneumoniae.* Liposomal systems, such as transfersomes or PEGylated liposomes, have been shown to enhance drug permeation across the TM. Here, we describe the *in vitro* characterization of the endolysin-loaded liposomal carriers as well as their *ex vivo* permeation through TMs.
Objectives: The main objective was to develop a delivery system containing an endolysin for a targeted transtympanic treatment of otitis media. To achieve this, it was necessary: i) to encapsulate the endolysin into liposomes for a controlled delivery; and ii) to evaluate the transtympanic permeation ability of the formulations.
Materials and Methods: Liposomes composed of 4 mM of L-alpha-lecithin and sodium cholate (5:1) (L:SC) or L-alpha-lecithin and PEG2000 PE (10:1) (L:PEG) loaded with the MSlys endolysin were prepared. The size, polydispersity index (PDI), zeta potential, stability, deformability, encapsulation efficiency, and *in vitro* MSlys release were determined. The cytotoxicity against fibroblasts and keratinocytes and the efficacy against pneumococcal planktonic and biofilm cells were also evaluated *in vitro*. Permeation studies were performed in Franz diffusion cells using porcine skin, sheep TMs, and cadaveric human TMs. The amount of MSlys permeated and its antipneumococcal activity were evaluated, and the protein integrity was analyzed by SDS-PAGE.
Results and Discussion: The MSlys endolysin was encapsulated into liposomes, with an average efficiency of about 35%. Liposomes with ca. 100 nm and relatively low PDI were produced, with L:PEG formulations being smaller and less polydisperse than L:SC. Both characteristics remained stable for one year at 4 ˚C. Liposomes were shown to be deformable and to provide a controlled release of MSlys over time following a first-order kinetics. No cytotoxicity was observed. Endolysin-loaded liposomes interacted with *S. pneumoniae* cells, reducing both planktonic and biofilm cultures. The potential of L:PEG over L:SC formulations to transport MSlys was demonstrated in preliminary transdermal assays. The permeation of MSlys across the TMs was enhanced when loaded in PEGylated liposomes. Samples were shown to significantly reduce pneumococcal cells after 2 h of permeation through the human TM. Nonetheless, loss of antipneumococcal activity after 4 h of permeation and protein hydrolysis at 48 and 72 h were observed.
Conclusions: This work reports the delivery of an endolysin through an intact TM using liposomes. However, further optimization is needed to expand the overall therapeutic efficacy of this strategy for use in otitis media.

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