Dermal Application of Chloramphenicol - The Effect of Liposomes and Chitosan Hydrogel Formulations on *ex vivo* Permeation and Antimicrobial Activity

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Purpose

Liposomes solubilize lipophilic drugs in the phospholipid membrane, and entrap hydrophilic drugs in its aqueous core. These vesicles provide a sustained- and targeted- drug release, and protect from degradation. Liposomes retain the drug onto/in the skin and improve skin drug deposition. Due to their liquid nature, a secondary vehicle, such as a hydrogel, is needed to obtain a necessary retention and bioadhesion onto the skin surface. Chloramphenicol (CAM) is an antimicrobial drug that, due to its bone marrow toxicity, is mainly applied in the treatment of eye and ear infections. When applied dermally to treat skin infections, systemic absorption should be avoided. The chitosan (CS) hydrogel with inherent antimicrobial activity might improve the antimicrobial activity. In this study, we investigated the effect of both the liposomal carrier and the CS-hydrogel on the retention and permeation of CAM through pig skin *ex vivo*. Four different formulations were compared; CAM aqueous solution (CAM-Sol), CAM in a liposome dispersion (CAM-Lip), CAM dissolved in chitosan hydrogel (CAM-CS) and CAM in a liposome-in-hydrogel formulation (CS-CAM-Lip). Finally, the antimicrobial activity of CS-CAM-Lip and CAM-Sol was compared.

Methods

Liposomes were prepared from CAM, Lipoid E-80 phospholipids, propylene glycol and dH_2O in a dual centrifugation (ZentriMix 380R, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). Liposome size, polydisperity index (PI) and zeta potential were measured in a Zetasizer nano ZS (Malvern, UK). The free drug was removed from the liposomes by dialysis, and the CAM-entrapment quantified by HPLC. The CS-hydrogel, contained 2.5% (w/w) high molecular weight chitosan (310-375 kDa) and 10% (w/w) glycerol, was mixed with dialyzed liposome dispersion or a CAM stock solution in propylene glycol to obtain a final CAM concentration of 0.5 mg/g. The *ex vivo* permeation was conducted for 8 hours at 32 °C in Franz

diffusion cells (PermeGear, Bethlehem, USA) with pig ear skin as a permeation barrier. Drug formulation remaining on the skin surface was collected using the HPLC mobile phase, and the amount of drug in the skin was extracted in mobile phase. The collected solutions were analyzed by HPLC. Concentration of CAM in the Franz diffusion acceptor chamber was measured every hour for 8 hours. The antimicrobial activities (zone of inhibition) of CS-CAM-Lip and CAM-Sol was determined by the agar diffusion testing after 24 hours incubation at 37 °C in four different bacteria strains; two strains of both *Staphylococcus epidermidis* and *Staphylococcus aureus* were applied.

Results

Liposomes with a CAM-entrapment of 55.2 ± 5.9%, mean diameter of 120.9 ± 3.2 nm, PI of 0.126 ± 0.02 nm, and zeta potential of -27.8 ± 1.9, were processed. The CS hydrogel and liposomes assured a reduced permeation of CAM through the skin, when applied in combination (CS-CAM-Lip), but also when applied as the sole delivery system (CAM-Lip and CS-CAM). After 8 hours, 22.9 ± 3.3% CAM had permeated the skin from the CAM-Sol formulation, whereas 9.2 ± 2.0%, 10.3 ± 1.1% and 11.9 ± 3.0% CAM permeated the skin from the CAM-Lip, CS-CAM-Lip and the CS-CAM formulation, respectively. CAM-Sol left 69.8 ± 4.8% of the applied drug on the skin surface after 8 hours, whereas less drug was recovered on the skin surface from the test formulations; 44.2 ± 5.5% (CS-CAM), 48.9 ± 2.4% (CS-CAM-Lip) and 55.3 ± 4.0% (CAM-Lip), respectively. The liposomes retained more drug on the skin surface and assured a sustained release of the drug, whereas the hydrogel acts as penetration enhancer. Thus, the tested formulations can be ranked in the following order regarding the skin penetration: CAM-Lip < CS-CAM-lip < CS-CAM, and in the opposite order regarding the drug depot on the skin surface. The tested formulations also increased 6-7 fold the amount of drug recovered in the skin as compared to from CAM-Sol. The antimicrobial evaluation showed that all the CAM-containing formulations induced a zone of inhibition of 21-29 mm, whereas the vehicles (controls) had a zone of inhibition between 6.7- 8.0 mm. Three out of eight liposome-in-hydrogel samples had a significantly improved antimicrobial activity as compared to the corresponding CAM-Sol, whereas none of liposome-in-hydrogel formulations had a smaller inhibition zone as compared to the corresponding CAM-Sol.

Conclusion

This study demonstrates that a liposome-in-hydrogel formulation preserve the antimicrobial activity of CAM and assures an improved sustained drug release and reduces skin drug

permeation. Thus, the formulation is promising regarding both avoiding systemic drug exposure and obtaining efficient local treatment of infectious skin disorders/wounds.