Epidermis-target by iontophoresis of ovalbumin-loaded liposomes for transcutaneous immunization

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ABSTRACT SUMMARY
Iontophoresis potential to improve the delivery of ovalbumin (OVA) loaded in liposomes into the skin have been investigated in vitro. The iontophoretic permeation of OVA dispersion in sodium phosphate buffer (PBS) and OVA-loaded liposome were studied. The pig ear skin was mounted in “Franz” diffusion cells where the formulations were placed in contact with the negative electrode (cathodal iontophoresis) due to the anionic characteristic of OVA. An electric current of 0.5 mA/cm² was applied for 15 minutes. After iontophoresis, 79.9 ± 5.6 µg/cm² and 126.5 ± 17.7 µg/cm² of OVA from OVA dispersion and OVA-liposome, respectively, were recovered from the viable epidermis. The association of liposomes and iontophoresis seems to have target OVA delivery to the viable skin.

INTRODUCTION
Transcutaneous immunization is a promising vaccination technique in which the immune response is acquired by topical administration of the antigen in the skin. OVA is a protein commonly used as a model antigen in immunization studies. To induce an immune response when topically applied, OVA has to penetrate the stratum corneum (SC) and reach the viable epidermis and dermis to stimulate lymphocyte T cells. However, very few drugs are suitable for topical delivery, limited to small molecules (<500 Da) which are neutral and relatively lipophilic¹. Therefore, innovative methods are necessary to enhance drugs absorption across the SC. Iontophoresis is a physical method that provides electrical driving forces for transport of compounds across the skin² and should improve OVA permeation. To retain the antigen into the epidermis, its encapsulation in liposomes can be useful due to liposomes depot effect. Moreover, proteins like OVA can degrade in contact with the skin so its protection by the liposomes can overcome this phenomenon. The aim of this work was to design an appropriate formulation together with well-defined iontophoretic parameters to increase OVA penetration to the epidermis.

EXPERIMENTAL METHODS
Two formulation were prepared; a simple dispersion of OVA in PBS pH 7.4 10 mM and a OVA liposome prepared by the lipid film hydration method using phosphatidylcholine (PC) and 1,2-dioleoylglycerolphosphoethanolamine (DOPE) in the proportion of 3:1 and a PBS solution containing silver nanoparticles (for a prospective function as immunologic adjuvant). Liposomes were characterized using the Zetasizer Nano ZS 90, for particle size distribution, polydispersity index and zeta potential.

The pig ear skin was placed in a modified “Franz” diffusion cell between the donor and receptor compartment (n = 6). The receptor compartment was filled with PBS pH 7.4 10 mM at 37°C. In the donor compartment, 2 mL of the formulation was added. Cathodal iontophoresis was performed using Ag/AgCl electrodes connected to a power supply and submitted to a constant electric current of 0.5 mA/cm² during 15 minutes.

At the end of the experiment the skin was removed from the diffusion cells and placed into a smooth surface with the SC facing up. The region of skin in contact with the formulation was removed through the tape stripping technique. The epidermis without the SC and dermis (viable skin) were perforated and OVA
was extracted using PBS. The amount of OVA recovered from the SC and from the viable skin was determined by ELISA using a commercial kit specific for OVA from white egg (Alpha Diagnostic - Texas, USA, catalogue number 6050). For that, a calibration curve, in the range of 0.25 to 4 ng/mL, was done using skin samples contaminated with OVA in PBS as a matrix.

RESULTS AND DISCUSSION

The mean particle size of the liposomes containing OVA was 58.87 ± 0.42 nm, the polydispersity index was 0.2 and the zeta potential was -9 mV. OVA dispersion in PBS showed a particle size of 25.55 ± 3.22 nm and a zeta potential of -21 mV.

Figure 1 shows the OVA amount recovered from the viable skin and the SC after 15 min of iontophoresis from the two formulations studied; OVA dispersion in PBS and OVA-loaded liposome.

Figure 1. OVA recovered from the SC and viable skin after 15 min of cathodal iontophoresis.

The OVA recovered from the skin after iontophoresis of OVA dispersion was 1.3-fold higher in the SC than in the viable skin. On the other hand, 2-fold more OVA were found in the viable skin than in the SC when liposomes were used. Iontophoresis of OVA-loaded liposomes improves 1.6-fold OVA penetration in the viable skin when compared with OVA dispersion in PBS (P<0.05, t-test). However, iontophoresis of OVA dispersion was able to deliver the protein in the receptor medium, i.e., across the skin (554.86 ± 201.59 ng/cm²) while no OVA was detected after iontophoresis of the liposomes (values were below the quantification limit of the method).

So far, it has not been possible to compare the passive and iontophoretic permeation of the studied formulations. However, the potential of physical methods to increase the permeation of drugs and macromolecules is well-established.

CONCLUSION

The results of the iontophoretic permeation of OVA dispersion and liposome indicated high concentrations of the protein in the SC and in the viable skin. Iontophoresis of OVA-loaded liposomes seems to have target OVA to the viable skin, suggesting a potential applicability of the association of iontophoresis and liposomes in the transcutaneous immunization.

REFERENCES


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