Transcutaneous Immunization using Iontophoresis - *In vivo* Preliminary Studies

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**ABSTRACT SUMMARY**

The potential of iontophoresis in promoting transcutaneous immunization using ovalbumin (OVA) as a model antigen has been investigated in mice. The animals were immunized twice with topical iontophoretic administration of OVA-loaded liposomes. The formulation was put in contact with the negative electrode due to the anionic characteristic of OVA. An electric current of 1.5 mA was applied in 3 cm² for 15 min. The specific anti-OVA immunoglobulin (Ig) G1 antibody levels were determined in the serum after 8 and 36 days. Iontophoresis of OVA-loaded liposomes lead to IgG1 titers similar to that produced with the traditional subcutaneous OVA immunization, indicating its potential to induce an immune response.

**INTRODUCTION**

Transcutaneous immunization is a promising vaccination technique in which the formulation containing the antigen is applied on the skin to induce immune response. This immunization via can be very interesting once the antigen presenting cells located in the viable epidermis are stimulators of the lymphocyte T cells¹. However, passive penetration of antigens through the stratum corneum, the primary skin barrier to drug penetration, is unlike due to their high molecular weight and hydrophilic characteristics. Therefore, it is a challenge to make antigens to reach the viable epidermis in an adequate concentration to induce the immune response². Iontophoresis, a physical method used to increase the transdermal penetration of different drugs³, may be an alternative to overcome this problem. We assume that iontophoresis, i.e., the application of an electrical current of low intensity, can increase the antigen skin penetration and also may act as an immune adjuvant. In this preliminary study, the potential of iontophoresis in facilitate the OVA penetration and induce an immune response was evaluated. Because OVA can degrade in contact with the skin and with the electric current, in this first experiments it was protected by encapsulation in liposomes. Liposomes can also help the drug to be retained in the skin after iontophoresis. The appropriated control of formulation and the iontophoretic parameters could increase the penetration of OVA-loaded liposomes to the specific place of the skin where the antigen presenting cells are located, without causing irreversible damage to the cells while protecting the antigen against degradation in this tissue.

**EXPERIMENTAL METHODS**

Liposome was prepared by the lipid film hydration method using soy phosphatidylcholine and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) in the proportion of 3:1. The lipid film was hydrated with OVA in PBS at 5 mg/mL. Liposomes dispersion was characterized using the Zetasizer Nano ZS 90, for particle size distribution, polydispersity index and zeta potential. Atomic force microscopy (AFM) in intermittent contact mode (tapping mode) was used to analyze the morphology and the size of the vesicles using 3 v5.12b43 NanoScope software for processing the images. The OVA encapsulation efficiency (EE) was determined using a 50 k Amicon® filter to separate the free drug by centrifugation at 4000 g for 40 minutes.

The *in vivo* immunization studies were done using female BALB/c mice of 8–12 weeks old. The animals were subdivided in 2 groups: Group 1 - immunized only at the first day of the study and euthanized at the eighth and Group 2 – immunized at the first day, challenged in the twenty-second day and euthanized in the thirty-sixth days. Groups 1 and 2 were subdivided in 5 groups each: Positive Control Group: animals were treated with subcutaneous injection of 100 µg/mL of OVA in saline (n=2), Negative Control Group: the animals were not submitted to any treatment (n=2), Group A: treated with iontophoresis (n=2), Group B: treated with liposome and iontophoresis (n=2), Group C: treated with liposome containing OVA and iontophoresis (n=2). The liposome formulation (1 mL) was applied at the 24 h-before trichotomyzed abdominal area of the anesthetized animal, with the aid of Iomed® iontophoretic adhesives (area of 3 cm²), and connected to the negative pole of a power supply.
(Kepco Power Supply®, model 500 APH MD). The tail of the animal was also surrounded by another patch containing the positive electrode to close the electric circuit. An electric current of 0.5 mA/cm$^2$ was applied during 15 minutes. For the group 2, this procedure was repeated after 22 days (second immunization). After 8 (group 1) or 36 (group 2) days the animals were euthanized and their blood used to determine anti-OVA IgG1 levels in the serum by Enzyme Linked Immuno Sorbent Assay (ELISA). The animal protocol was approved by the University of São Paulo Animal Care and Use Committee (Authorization number: 10.1.1052.53.9).

RESULTS AND DISCUSSION

The mean particle size of liposome containing OVA was 58.87 ± 0.42 nm, the polydispersity index was 0.2 and the zeta potential was -9 mV. OVA encapsulation efficiency was around 61%. The AFM was able to generate high resolution morphological images of the liposome adhesion on a mica surface, without causing particles collapse. The range of particles size determined corroborate with light scattering results.

Figure 1 shows the anti-OVA IgG1 titers found in the animals that were immunized only in the first day. It is possible to notice that none of the iontophoretic treatments led to IgG1 antibody titers similar to the subcutaneous antigen treatment.

However, animals that were challenged in the 22 day with iontophoresis of OVA-loaded liposomes presented IgG1 antibody titers very similar to the positive control (Figure 2).

CONCLUSION

Iontophoresis of OVA-loaded liposomes was able to generate specific antibodies against the OVA, inducing a humoral immune-response. However, as the study was performed with only two animals per group, additional studies with at least 5 animals per group will be done to obtain more representative results.

REFERENCES


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