Trans-scleral delivery of cytochrome C and lysozyme: iontophoresis as an enhancing strategy

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Purpose

Diseases of the posterior eye segment are a major challenge in ophthalmology and proteins represent powerful tools for their treatment. Despite the potential of these compounds, their non-invasive administration remains a challenge. The high permeability of the sclera could encourage topical application, but the passive flux is often too low to reach therapeutically active concentrations in the target tissue. The purpose of this work was to study the trans-scleral permeation of 2 model proteins with similar MW and charge and to evaluate iontophoresis as an enhancing strategy. Proteins chosen are lysozyme (MW 14.7 kDa, net charge +8 at pH 7.4) and cytochrome C (MW 12.4 kDa, net charge +9 at pH 8.2).

Methods

Quantification was performed by HPLC-UV (cytochrome) and with an enzymatic assay (lysozyme). Impact of magnetic stirring and electric current on proteins stability was assessed. Vertical diffusion cells were employed to evaluate passive and iontophoretic permeation through isolated porcine sclera starting from different buffers (HEPES and citrate). For current assisted experiments, anodal iontophoresis was applied (2 hours, 2.9 mA/cm^2).

Results

Stability tests suggested both proteins were stable for 5 hours under magnetic stirring (loss of protein lower than 10%) and upon current application (more than 90% remaining after 2 hours). Despite similar MW and charge the passive permeation of the 2 proteins differed significantly: at 10 mg/ml permeation occured only for cytochrome (after 5 hours 229.19±54.91 $\mu g/cm^2$); at 40 mg/ml the permeation of lysozyme was 10 times lower than cytochrome. When iontophoresis was applied in HEPES and citrate buffer, a 10 to 20 enhancement factor was found for cytochrome, while a modest (HEPES) or no (citrate) enhancement was found for lysozyme.

Conclusions

Although lysozyme and cytochrome share similar MW and charge, passive and iontophoretic permeation profiles across the sclera were demonstrated to be different: cytochrome permeability resulted higher and highly improved by iontophoresis, while this technique did not represent an efficient method to enhance lysozyme permeation.