In vivo enhancement of transscleral penetration of macromolecules by low-intensity and low-frequency ultrasound

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ABSTRACT

This study aims to determine in vivo effectiveness of low-frequency ultrasound in mediating the transport of macromolecules to posterior segment of the eye via transscleral route. Investigations were carried out to examine, if any, damage is caused by ultrasound at the tested operation parameters on the posterior ocular tissues and visual function.

INTRODUCTION

Posterior eye diseases can lead to impaired vision and blindness. Current clinical practice by repeated intravitreal injection, though effective, increases the risk of possible complications. Sclera, the outermost barrier along the transscleral path, the sclera, is a hydrated fibrous network of collagen, elastin and proteoglycan. It is permeable to hydrophilic molecules. Transscleral delivery is gaining popularity due to its proximity the retina and circumvents the corneal epithelium and the lens. However, the low scleral permeability to macromolecules, together with the dynamic clearance limits the utility of transscleral route for delivering therapeutics. In previous study, we have shown that short duration of low-frequency, low-intensity ultrasound (US) enhanced the transport of dextran and albumin protein significantly across rabbit sclera in ex vivo experiments. Here, we aim to provide evidence in live rabbits to support our hypothesis that ultrasound is an effective and non-invasive approach for transscleral delivery.

The amount of macromolecular probe (70 kDa dextran) transported from the scleral surface to vitreous, with and without US treatment, was compared to assess the effect and duration of ultrasound. Full-field electroretinography (ffERG), binocular indirect microscopy (BIO), and histology were employed to evaluate whether there is any negative impact of US on the visual function and integrity of posterior ocular tissues.

EXPERIMENTAL METHODS

New Zealand white rabbit was anesthetized prior to US applications. A cycle of sonication process consists of 2 steps: 1) continuous sonication with dextran solution (40 kHz for 90s) inside the adaptor at acoustic power (ISATA, spatial average – temporal average intensity) 0.12 W/cm² were delivered via transscleral route; 2) after sonication, dextran solution remained in contact with the sclera for 5 min. Step 1 and 2 were repeated up to a total of 3 cycles in experiments. US parameters here corresponded to mechanical index (MI) of 0.20.

Vitreous was extracted for fluorescence detection within 10 min. The control group was done without placing US transducer above the eye. For the sclera recovery experiment, PBS solution was used to fill the adaptor during ultrasound application. Continuous ultrasound wave at 40 kHz and 0.12 W/cm² was applied to the eye for 90s 3 times with a break of 5 minutes between each time. After a lag time of 1d, 7d and 14d, dextran solution was placed in contact with the sclera around the sonication zone for 15 minutes. The vitreous was extracted for fluorescence detection. At the same time points, visual function of ultrasound-treated rabbit was examined by ffERG and BIO. Histology of ocular tissues were performed to examine presence, if any, of US-induced cellular level changes.

RESULTS AND DISCUSSION

Single sonication cycle was capable of overcoming the transscleral barrier to deliver a
detectable amount of dextran into the vitreous. Notably, there is a dramatic, nonlinear enhancement in the effectiveness of delivery after repeated US application. (Figure 1) The concentration of macromolecular probe found in vitreous was at a range that, when applied to protein therapeutic, is sufficient to produce biological effects.

Figure 1 Effect of ultrasound applications on transscleral delivery of macromolecules in vivo 70kDa dextran (n = 3). The difference between any two groups shown is statistically significant (p< 0.05, unpaired two-tailed student’s t-test).

Transscleral barrier against dextran transport was restored to normal about 2 weeks after multiple sonication. When the lag time was increased to 14 days, dextran could not be detected inside vitreous, comparable to the observation in control without ultrasound treatment.

ffERG provides insight on the global electrical response of retina to photic stimulation. Results indicated that ultrasound did not affect either the cone or the rod system. ffERG results at all the time points sampled did not show any abnormality that further supporting that ultrasound at the chosen parameters did not influence the electrical response of retinal cells upon photic stimulation. Ocular tissue was examined by BIO for macroscopic image, and then by histology for microscopic morphology. No damage was found. BIO images indicated that the overall structure of the posterior segment of the treated rabbit eyes remained intact up to 14 days after US application (Figure 2). Microscopically, the overall morphology and thickness of the retina and sclera of ultrasound-treated eyes was not found to be modified when compared with unoperated eyes under 100x and 400x magnification (Figure 3).

Figure 2 Representative BIO images of rabbit retina-choroid before (A) and 1 day (B), 7 days (C) and 14 days (D) after ultrasound applications

Figure 3 H&E staining of H&E-stained cross sections of the area behind the limbus in superior-temporal quadrant of rabbit left eyes; A: retina, normal un-operated; B: retina, US- treated, scale represents 210 μm; C: sclera, normal un-operated; D: sclera, US- treated. Scale bar = 10 μm (A and B), scale bar = 100 μm (C and D).

CONCLUSION
This study showed for the first time that it is feasible and non-invasive to use low-frequency, low intensity US to deliver macromolecules into posterior segment of the eye via the transscleral route without inducing severe damages to ocular tissues. Transscleral barriers were found to be restored in 2 weeks after US application.

REFERENCES

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