Biomechanical effect of low-intensity and low-frequency ultrasound on cellular transport of retinal pigment epithelium cells

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ABSTRACT SUMMARY

Effects of ultrasound on human retinal pigment epithelium (ARPE-19) cells were examined. Exposure to 40 kHz repeated ultrasound application at Mechanical Index (MI) = 0.20 did not significantly affect cell viability. Enhanced uptake of 120nm folate-decorated nanoparticles was observed up to 4 hours post ultrasound treatment. Insignificant presence of carboxyfluorescein in ARPE-19 cells after ultrasound application indicated that creation of cell membrane pores was not the key mechanism of internalization of substances. These preliminary data suggest that ultrasound may induce mechano-sensitive bioeffects on cell uptake and intracellular trafficking.

INTRODUCTION

Delivery of therapeutics to the intraocular space or to targeted tissues in posterior segment is challenging due to the structural and dynamic barriers surrounding the eye. [1] Previously, we have reported the feasibility of ultrasound (US) irradiation to deliver macromolecules to intraocular space via transscleral route in vivo. [2] The amount of dextran found inside vitreous increased non-linearly with repeating sonication. The result suggested not just dextran could effectively penetrate sclera, but also that Retinal pigment epithelium (RPE) barrier was disrupted to enable the passage. It also suggested an enhancement mechanism other than the physical change of the acelllular structure of sclera may be involved. After two weeks, dextran could not penetrate sclera/RPE and the influx was again undetectable. This suggested the restoration of the barrier function after ultrasound.

In this study, the focus will be the investigation of mechanism of RPE barrier modulation by ultrasound. RPE, being the outer blood-retinal barrier, is formed by closely packed RPE cells with tight junctions formed in intercellular space. This layer presents the rate-limiting step when therapeutics takes transscleral route to reach the back of the eye. There has not been any study about how RPE would respond to low-frequency and low-intensity ultrasound. It is hypothesized that enhanced transport of macromolecules and nanoparticles across RPE is due to ultrasound-triggered biomechanical effects.

EXPERIMENTAL METHODS

Cultured human RPE cells (ARPE-19) cells were maintained at 37°C in humidified atmosphere of 5% CO₂. Cells were grown on 60mm flask and maintained with Dulbecco’s modified Eagle medium (DMEM): nutrient mixture F12 (1:1) supplemented with 10% FBS, 2mM L-glutamine, 100 U/ml penicillin and 100U/ml streptomycin. All the cells within the same passages were grown to 70% confluence prior to application of ultrasound for experiments.

In ultrasound experiment, a 40 kHz non-focusing ultrasound (ISATA = 0.12 W/cm², MI = 0.20) transducer was placed at a pre-designated distance above the cells. Ultrasound was applied for 90s followed by a 5-min break time. This application cycle was repeated as indicated in the result section. During US application, cells were immersed in blank HEPES buffer or HEPES buffer containing 50µM carboxyfluorescein. After US application, cells were immediately lysed with 0.5% Triton X-100 solution prior to fluorescence measurement. For nanoparticles (NPs) uptake study, 0.2 mg/ml Nile red-encapsulated folate-conjugated poly(ethylene glycol)-co-poly(caprolactone) (PEG-PCL) 120nm NPs were added immediately after US application and incubated
for pre-designated duration indicated in Figure 3. Cells were then lysed for fluorescence measurement for NPs uptake quantification. For control experiment, cells were exposed to HEPES buffer for 15 min prior to cell lysis and fluorescence measurement. To assess effect of US on cell viability, MTT assay was conducted immediately on US-treated ARPE-19 cells.

RESULTS AND DISCUSSION

MTT assay on ARPE-19 cells immediately after US application showed that sonication caused a small drop of 13-18% (Figure 1). Thus it implies that US application did not significantly affect cell viability.

![MTT assay of ARPE-19 cells treated with single (1x) or repeated (2x, 3x) ultrasound application. N = 3, error bar = 1 S.D.](image)

Increased uptake of 120nm Nile-red loaded FA-PEG-PCL NPs was observed (Figure 2). The enhancement, lasting for hours, was up to 400-700 % compared to control in which cells without ultrasound treatment were incubated with nanoparticles solution for the same amount of time.

At this frequency and intensity, the calculated MI was far below the critical value of 0.7 for transient cavitation. Considering the size of the particles and the duration of effect, the uptake enhancement is unlikely due to the formation of transient pores or wounding on cell membranes.

![Normalized uptake of carboxyfluorescein of ARPE-19 cells treated with single (1x) or repeated (2x, 3x) ultrasound applications. N = 3, error bar = 1 S.D.](image)

There was 6-28% increase in uptake of cell-impermeable dye carboxyfluorescein compared to control, much lower compared to post-US uptake of NPs. Transient pores can only allow passage of small molecules (~28nm) and last for seconds to minutes after ultrasound application. Hence, transient pore formation is not the dominant effect under the selected US parameters.

CONCLUSION

These preliminary results support the hypothesis that stable cavitation initiates a mechanism other than altering plasma membrane integrity. In future work, the effect of US on ARPE cells tight junction and cellular transport activities will be investigate to confirm if US can modulate RPE barrier property and trigger mechano-sensitive bioeffects.

REFERENCES


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