Evaluation of the Effects of Dexamethasone-PAMAM Dendrimer Complexation on Ocular Permeability: In Vitro and Ex Vivo Transport Studies
B. Yavuz, S. Bozdag Pehlivan, I. Vural, N. Unlu
Hacettepe University

Purpose
Ocular barriers such as cornea and sclera-choroid-retinal pigment epithelium (SCRPE) are one of the biggest challenges in retinal drug delivery. Dexamethasone (DEX) is a corticosteroid that often employed for the treatment of posterior segment diseases of eye, although it’s poor ocular permeation limits ocular bioavailability and leads to invasive applications such as intravitreal injection or implantation.

The purpose of this study is to investigate the effects of poly(amidoamine) (PAMAM) dendrimer complexation on DEX ocular permeation. Thus, in vitro and ex vivo transport studies were performed across Human retinal pigment epithelial cell line (ARPE-19), rabbit cornea and SCRPE tissues in order to evaluate the effects of different type of dendrimers on DEX ocular permeation. Cytotoxicity of the formulations on ARPE-19 cells was also investigated.

Methods
All PAMAM dendrimers, acetonitrile and methanol were purchased from Sigma-Aldrich (USA). ARPE-19 was purchased from ATTC (USA). Albino rabbit eyes were supplied from Pel-Freez (USA). Fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM:F12), trypsin–EDTA, and penicillin–streptomycin were purchased from Biochrom (Germany).

Six different types of PAMAM dendrimers [G3, G4 (cationic); G3-OH, G4-OH, G3.5, and G4.5 (anionic)], were used in order to compare the effect of the charge and the generation of dendrimers. Preparation and in vitro characterization of the formulations were previously reported.

MTT cytotoxicity test and in vitro permeability studies were performed using ARPE 19 cell lines. Statistical analysis was performed using One Way ANOVA using SPSS (p < 0.05). Medium was prepared by adding 10% (v/v) FBS and 50 Unit/mL penicillin–streptomycin to DMEM:F12. After DEX-PAMAM exposure for 24 hours, MTT assay was performed and absorbance values were measured at 570 nm and percentage of viability was calculated (n=3).

150000 cells per well were seeded apically and incubated at 37 °C in 5% CO₂ for permeability studies. 15 days after seeding, experiments were performed when the cell monolayer had reached confluence. Trans-epithelial resistance was measured using Millicel® ERS to confirm cell monolayer integrity. Cell monolayers were used for transport studies (n=3), when the resistance reached about 200–250 Ω cm². Apparent permeability co-efficients (Papp, cm/s) were calculated.

In order to evaluate DEX transport across ocular barriers, transport studies across cornea and SCRPE were performed for PAMAM-DEX complexes using modified Ussing chambers. Cornea and SCRPE were isolated from fresh New Zealand albino rabbit eyes. Assay buffer was used as the medium, the thermostat was set to 37 °C and air with 5% CO₂ was used for bubbling the bathing fluids. Samples were collected from acceptor side every 30 minutes for 3 hours and DEX amount in the samples were quantified using LC-MS/MS analysis. Ex vivo experiments were carried out in quadruplicate. Apparent permeability co-efficients (Papp, cm/s) were calculated.

Results
MTT assay indicated that all groups resulted in cell viability comparable to DEX solution (87.5%), with the cell viability being the lowest for cationic G3 complex at 73.5%, which was the only statistically significant difference. Compared to DEX solution, all formulations resulted in enhanced delivery, with the permeability being the highest for G4.5 and G4-OH complexes. The differences between all groups were found statistically significant (p < 0.05). Also it was observed that higher generation dendrimers (PAMAM G4.5, G4-OH and G4) showed higher DEX permeation rates than lower generations (PAMAM G3.5, G3-OH and G3).

Ex vivo transport studies across cornea and SCRPE tissues were carried out for DEX-PAMAM complex formulations in comparison with DEX solution. The approximate rank order for cumulative DEX transport across cornea and SCRPE at 3 hours was all anionic dendrimer complexes (G3.5, G4.5, G3-OH and G4-OH) > DEX solution and the cationic dendrimers, whereas highest transport rate was obtained with G4.5 dendrimers. These results indicated that surface charge of the dendrimer has significant importance on ocular transport of the drug. Furthermore, higher DEX transport rates were achieved with generation 3 than generation 4 dendrimers across both cornea and SCRPE. Also as it was expected based on the structures of cornea and SCRPE, transport from SCRPE has been found higher than corneal.

Conclusion
The results indicated that DEX-PAMAM complex formulations were able to enhance in vitro permeability and ex vivo transport of DEX. Especially DEX-PAMAM complex formulations prepared with PAMAM G3.5 and G4.5 (anionic dendrimers with -COOH functional groups) were found to be promising drug delivery systems. In vivo ocular tissue distribution studies following topical or subconjunctival applications might be helpful for further evaluation of the effects of dendrimer complexation on ocular DEX delivery.