ABSTRACT

Background: Limbal stem cells (LSCs) play a crucial role in maintaining corneal epithelial homeostasis and regeneration. Their damage leads to disruption of ocular surface integrity and deterioration of visual acuity. Preservatives used in eye drops may exert cytotoxic effects, with benzalkonium chloride (BAC) being one of the most commonly used. Long-term exposure to BAC may result in LSC dysfunction. In recent years, increasing interest has been focused on the use of stem cell secretome as a potential therapeutic strategy due to its cytoprotective and regenerative properties.

Hypothesis and main goal: The hypothesis assumes that BAC exerts toxic effects on human LSCs, leading to impaired viability and disruption of their basic biological functions. In contrast, conditioned medium (CM) derived from epithelial (hAEC), mesenchymal (ADSC), and mixed epithelial–mesenchymal phenotype (hAC) cells may mitigate the adverse effects of BAC and support cellular regeneration. The effectiveness of this action may depend both on the phenotype of the source cells and on the timing of CM application relative to exposure to the toxic agent.

The aim of the study was to evaluate the effect of ADSC-CM, hAEC-CM, and hAC-CM on the viability, migration, proliferation, apoptosis, and inflammation of human LSCs exposed to BAC in vitro.

Material and Methods: The study was conducted on human LSCs cultured in vitro and exposed to BAC. Subsequently, the effects of ADSC-CM, hAEC-CM, and hAC-CM were analyzed when applied simultaneously with BAC (model 1) and after prior cellular damage (model 2). Cell viability was assessed using the MTT assay, while proliferation and migration were evaluated using the colony formation assay (CFA) and scratch assay. Apoptosis was analyzed by RT-qPCR (*BAX, BCL2, TP63, CASP3, CASP7*) and flow cytometry. Expression of cell cycle-related genes (*E2F2, CCND1, CCND2, CDKN2A, CDKN2C, CCNE1, CCNE2, RB1*) and inflammatory and NLRP3 pathway markers (*IL-1β, IL-6, IL-4, IL-10, NLRP3, CASP1, IL-18, MMP3, MMP9, TIMP1*) was assessed by RT-qPCR, while cell cycle distribution was analyzed using flow cytometry.

Results: BAC was shown to exert a significant, dose-dependent cytotoxic effect on LSCs. The 0.0002% BAC concentration significantly reduced cell viability, inhibited proliferation, and

increased apoptosis. Treatment with stem cell secretome partially reversed the adverse effects of BAC. The strongest protective effect was observed for ADSC-CM, which significantly improved cell survival, reduced apoptosis, and supported regeneration. The therapeutic effect of all CMs was more pronounced in the co-treatment model (simultaneous exposure to BAC and CM) compared to the post-injury regenerative model.

Conclusions: BAC exerts a strong, dose-dependent toxic effect on LSCs. Stem cell secretome, particularly ADSC-CM, represents a promising therapeutic strategy for corneal limbus protection. Simultaneous application of CM with BAC may be more effective than its administration after cellular injury has occurred.

Key words: limbal stem cells; benzalkonium chloride; stem cell secretome; adipose-derived stem cells; amniotic epithelial cells; conditioned medium