In vitro evaluation of sodium hyaluronate protective effect against benzalkonium chloride toxicity

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BACKGROUND

- 70% of eye drops contain Benzalkonium chloride (BAK) as preservative.[1]

  50% of glaucoma patients treated with BAK-preserved antiglaucomatous eyedrops develop ocular surface disease (OSD) after 2 years of treatment.[2]

- OSD is triggered by BAK toxicity and decreases both quality of life and treatment compliance of glaucoma patients.[3,4]
- Sodium hyaluronate (SH) has been postulated as a potential neutralizing agent of BAK-induced toxicity.[5]

PURPOSE

The goal of this work was to evaluate the protective effect of different concentrations of SH on BAK-induced toxicity using an *in vitro* model.

METHODS

The NAV14 cell line (SV40-Immortalized murine conjunctival epithelium) was used. Cell monolayers were exposed to different combinations of BAK (0.001%; 0.005%; 0.01%) and SH (0.2%; 0.3%; 0.4%) for 15 minutes; then, cells were washed, and fresh culture media was added.

Cell viability was evaluated after 2 h by resazurin reduction and lactate dehydrogenase (LDH) enzyme release. Also, **cell migration and proliferation** over 24 hours were determined by the scratch wound-healing assay.

Data were analyzed by two-way ANOVA and are shown as mean±SD of two independent experiments with 4-6 replicates each.

METHODS

Cell viability

BAK induced a concentration-dependent decrease on cell viability ($p<0.001$) and an increase in LDH release ($p<0.001$). Conversely, SH neutralized these effects also in a concentration-dependent manner ($p<0.001$).

In the presence of SH 0.4% (highest effect), cell viability was for BAK 0.001%: 104±22%, for BAK 0.005%: 109±9% and for BAK 0.01%:75±13% of control cells ($p<0.001$ for BAK 0.005-0.01%) while LDH release was for no BAK: 0.24±0.03, for BAK 0.001%: 0.26±0.01, for BAK 0.005%: 0.37±0.02 and for BAK 0.01%: 0.49±0.22, (vs no SH: $p<0.001$ for BAK 0.005-0.01%).

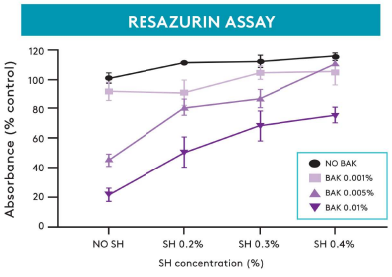


Figure 1. Metabolic activity by sodium hyaluronate concentration.

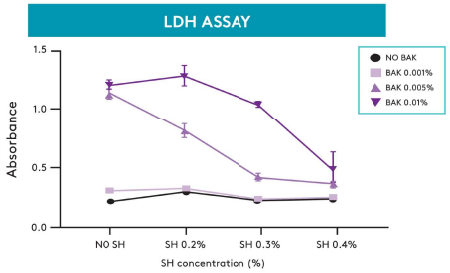


Figure 2. Cell death by sodium hyaluronate concentration.

Cell migration and proliferation

BAK also reduced in vitro wound closure ($p<0.001$). Conversely, SH neutralized this effect in a concentration-dependent fashion ($p<0.001$).

In the presence of SH 0.4% (highest effect), wound closure at 24 h was: for no BAK: 81±15%, for BAK 0.001%: 58±6%, for BAK 0.005%: 63±10%, for BAK 0.01%: 60±8% (vs no SH: $p<0.001$ for BAK 0.005-0.01%).

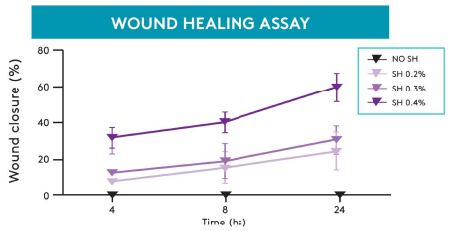
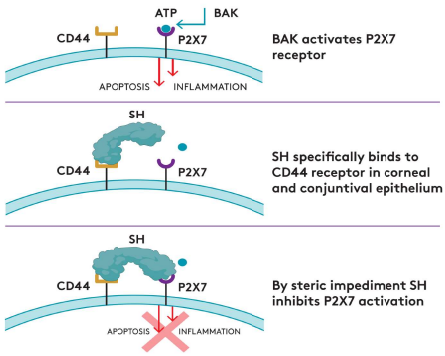


Figure 3. Wound healing of cell monolayers exposed to BAK 0.01% by time.

Figure 4. Representative photographs of wound healing assay at 8 hs. obtained by electron microscopy

CONCLUSIONS

- **SH neutralized BAK toxicity on conjunctival epithelial cells in a concentration-dependent manner.**
- **SH 0.4% was even protective at the highest preservative concentration.**
- **These findings support the use of SH to mitigate BAK toxicity in long-term anti-glaucomatous medication treatment, although more studies are needed.**



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Access this poster online here:

Bibliography:

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