

Human Breast Milk Enhances Cellular Proliferation in Corneal Wound Healing

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Introduction

- Corneal wounds are often treated with epithelium debridement, allowing regrowth of new epithelial cells.¹
- Cornea wound healing requires a cascade of signaling molecules, including epithelial growth factor (EGF) and growth modulating cytokines; however, a topical post-operative treatment with these components is not available.^{2,3}
- Human breast milk (HBM) offers a potential, novel treatment as it contains growth factors and cytokines that may play a role in epithelial cell migration and proliferation.^{4,5,6}

Purpose

- This study seeks to investigate the role of human breast milk in enhancing re-epithelialization of the cornea after mechanical wounding.
- Human breast milk may be a promising treatment for a variety of eye diseases and thus can be a cheaper alternative to pharmaceutical therapy.

Methods

Wound Model:

- Male and female Balb/C mice, 8 to 12 weeks old, were anesthetized prior to creating a 2mm central cornea defect with a 0.5mm Algerbrush (Figure 1).

Treatment:

- After wounding, mice were randomly assigned to one of three treatment groups: HBM, triple antibiotic ophthalmic ointment containing neomycin, polymyxin B, dexamethasone (RxTx), or saline and treated 4x/day for 2 days.
- Epithelial defect area was quantified by fluorescein and ImageJ at 0, 8, 24, and 48 hrs post wounding. Eyes were used for histology, RT-qPCR, and ELISA.

FIGURE 1. Algerbrush for corneal epithelial cell removal.

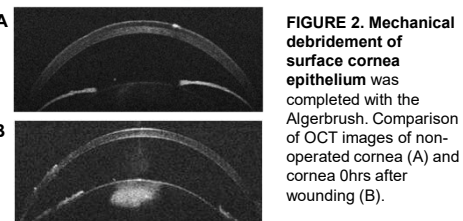


FIGURE 2. Mechanical debridement of surface cornea epithelium was completed with the Algerbrush. Comparison of OCT images of non-operated cornea (A) and cornea 0hrs after wounding (B).

Results

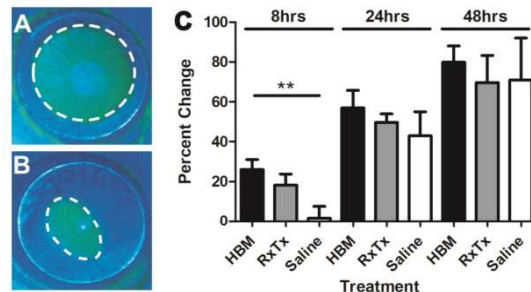


FIGURE 3. HBM treatment enhances cornea wound healing at 8 hours. Fluorescein staining of representative mouse cornea at (A) 0h and (B) 24h post wounding with (C) percent reduction in fluorescein-staining corneal epithelium defect area at 8, 24, and 48h post wounding for three treatment groups: HBM, RxTx (neomycin, polymyxin B, dexamethasone), and saline (n=6 to 10, **p=0.0253).

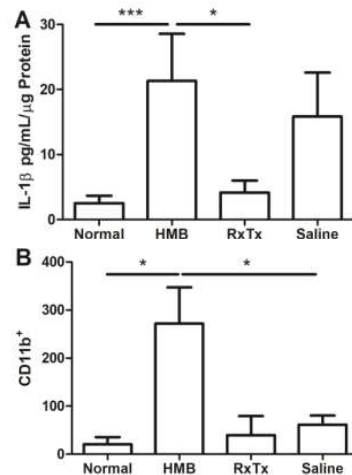


FIGURE 5. (A) ELISA of IL-1 β in corneas treated with HBM vs. RxTx vs. saline drops at 48h post wounding (n=3 per group). (B) Cell counts of macrophage marker, CD11b, on treated cornea tissue sections at 48h post mechanical wounding (minimum of 3 consecutive sections, n=3 per group). Normal represents unwounded, age-matched eyes.

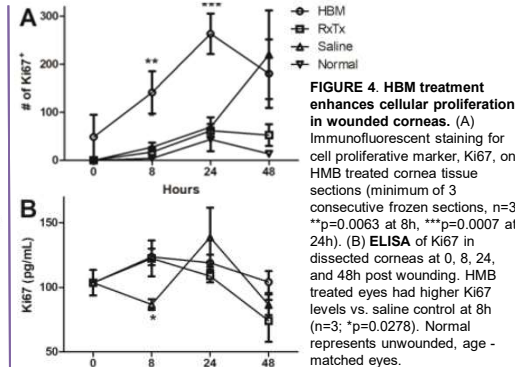


FIGURE 4. HBM treatment enhances cellular proliferation in wounded corneas. (A) Immunofluorescent staining for cell proliferative marker, Ki67, on HBM treated cornea tissue sections (minimum of 3 consecutive frozen sections, n=3; **p=0.0063 at 8h, ***p=0.0007 at 24h). (B) ELISA of Ki67 in dissected corneas at 0, 8, 24, and 48h post wounding. HBM treated eyes had higher Ki67 levels vs. saline control at 8h (n=3; *p=0.0278). Normal represents unwounded, age-matched eyes.

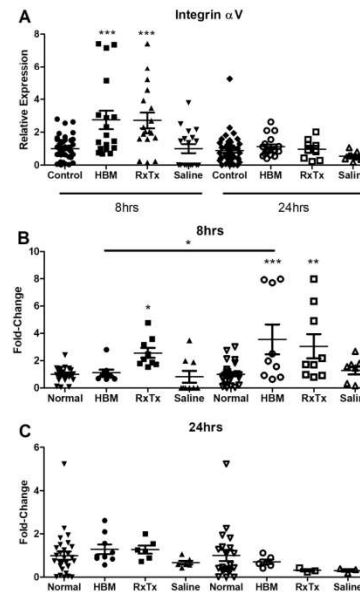


FIGURE 6: Abundance of Integrin αV gene transcripts measured by RT-qPCR following injury of corneal tissue. (A) Transcript levels measured at 8h and 24h following injury. Groups comprised approximately equal representation from males and females. Transcript levels from male (filled symbols) and female (unfilled symbols) mice are shown separately at (B) 8h and (C) 24h following injury. Normal are untreated and unwounded. Significance determined from ANOVA with Tukey's post hoc analysis. (n=6 per group; 3 males and 3 females per treatment group).

Conclusions

- In mouse models, topical HBM leads to a significantly smaller residual wounded surface area at 8h post wounding, similar to treatment with RxTx.
- HBM treatment enhanced proliferation of epithelial cells at 8h in the ocular surface.
- HBM may lead to enhanced numbers of macrophages (CD11b+ cells) that migrate into the cornea after mechanical wounding.
- LESC marker, Integrin αV , was upregulated in HBM - and RxTx -treated mice at 8h post wound. This was specifically noted in female mouse populations which may indicate that females are more responsive to HBM treatment via stimulation of LESC populations.

Future Directions

- Future studies could investigate the LESC response to different signaling molecules in human breast milk to understand the best therapeutic strategies and timing of those treatments in relation to injury.

Disclosures

The authors have no financial interests to disclose.

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