Human Breast Milk Enhances Cellular Proliferation in Corneal Wound Healing

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Introduction

- Corneal wounds are often treated with debridement of the cornea epithelium, allowing new epithelial cells to grow in their place.[1]
- Corneal 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 sedated with intraperitoneal ketamine, xylazine and given 0.5% ophthalmic proparacaine prior to creating a 2mm **central cornea defect** with a 0.5mm Algerbrush (Figure 1).

Treatment:

- Immediately post epithelial cell layer removal, mice were randomly assigned to one of three **treatment groups**: HBM, triple antibiotic ophthalmic ointment containing neomycin, polymyxin B, dexamethasone (TAB), or saline.
- Operated eyes were treated 4 times per day for up to 48hrs. **Epithelial defect** size was ascertained with fluorescein staining and **ImageJ** area analyses at 0, 8, 24, and 48 hrs post wounding. Subsets of mice from each treatment group were used for **histology** and **ELISA** cytokine analyses.

FIGURE 1. Algerbrush for corneal epithelial cell removal.

Results

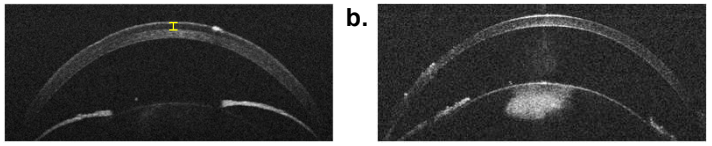

FIGURE 2. Mechanical debridement of surface cornea epithelium (yellow bar) was completed with the Algerbrush to make a central 2mm defect. **a**. and **b.** Comparison of OCT images of non-operated cornea (a) and cornea 0hrs after Algerbrush epithelium removal (b).

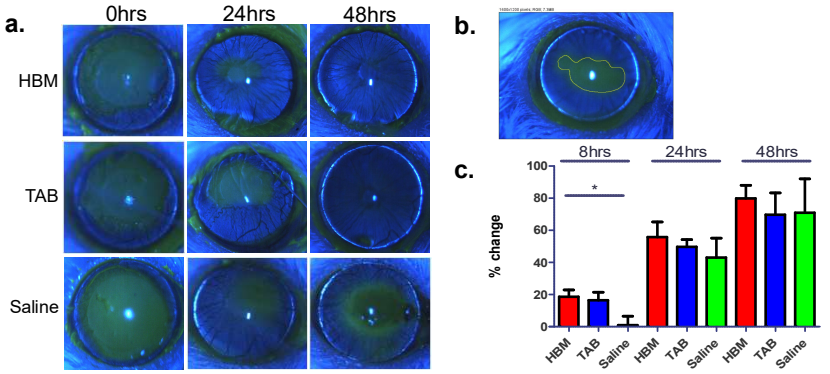

FIGURE 3. Topical HBM may lead to accelerated wound healing. a. Representative images of fluorescein stained mouse corneas at 0, 24, and 48hrs post mechanical epithelial removal for three treatment groups. **b.** Fluorescein stained mouse cornea outlined with ImageJ software for area quantification (yellow outline). **c.** Percent change in fluorescein area at 0, 24, and 48 hours post wounding for 3 treatment groups: HBM, TAB, and saline. (N=6 to 10 per group) (p= 0.02)

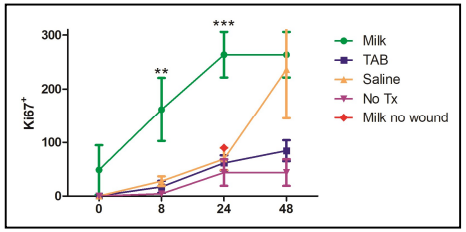

FIGURE 4. HBM treatment enhances cellular proliferation in wounded corneas. Immunofluorescent staining for cell proliferative marker, Ki-67, on HMB treated eye tissue sections showed significantly higher positive cell numbers over TAB treated eyes (N=3 per group; p=0.0063 at 8hrs, p=0.0007 at 24hrs, and p=0.0014 at 48hrs).

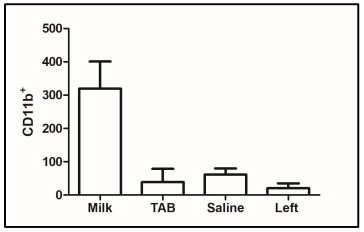

FIGURE 5. Human breast milk treatment enhances CD11b+ cell number in the cornea following mechanical wounding. IHC staining for CD11b in operated eye tissue sections for HBM, TAB, and saline treated eyes. Left eye is untreated contralateral eye (N=3 per group).

Conclusions

- In mouse models, we observed that human breast milk treated eyes showed improved rate of re-epithelialization at 8 hrs post wounding over saline treatment.
- Application of human breast milk leads to significantly increased numbers of Ki-67+ cells, suggesting that components in HBM enhance cellular proliferation.
- HBM may lead to enhanced numbers of macrophages (CD11b+ cells) that migrate into the cornea after mechanical wounding.

Future Directions

- Future studies will investigate the potential effect of HBM on endogenous limbal epithelial stem cells in the cornea.
- Additionally, further investigation into the role of growth modulating cytokines is needed to see if HBM enhances phagocytosis of damaged epithelium and thus recycling of their products.

Disclosures

The authors have no financial interests to disclose.

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