The Ocular Surface 18 (2020) 158–169

Contents lists available at ScienceDirect

The Ocular Surface

journal homepage: www.elsevier.com/locate/jtos

Original Research

Dose-dependent benzalkonium chloride toxicity imparts ocular surface epithelial changes with features of dry eye disease

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A B S T R A C T

Purpose: Inclusion of the preservative benzalkonium chloride (BAC) in ophthalmic solutions is prevalent, despite the noted potential for exacerbating dry eye disease (DED). Whilst studies incorporating BAC have assessed its' effects as a mouse model of DED, the impact on limbal epithelia is under-studied. Our investigation aimed to comprehensively assess the impact of different BAC dosing regimens and their suitability as a mouse model of DED.

Methods: C57BL/6J mice ($n = 72$) were administered topical BAC (0.05–0.2%) over 7 days. Fluorescein staining, corneal smoothness index, and immuno-histological analyses were applied to determine architectural and cellular changes on the ocular surface following BAC treatment. The effect of BAC (0.0001–0.01%) on cultivated primary mouse corneo-limbal epithelial cells (CLECs) ($n = 6$) was examined using morphological and functional assays.

Results: Whilst 0.2% BAC induced severe corneal epithelial defects, 0.1% BAC dispensed once daily over 7 days, induced punctate fluorescein staining without detriment to corneal smoothness. Histochemical staining revealed disorganized basal corneal epithelial cells with enlarged cytoplasmic halos. Furthermore, PAS^+ goblet cells were decreased. BAC treatment also modulated K14 expression and distribution within the limbus. In cultured CLEC, BAC triggered cell contraction and vacuolation, increased LDH release and elevated cell necrosis by 4.1-fold. Concentrations of BAC as low as 0.0001% decreased colony formation.

Conclusions: This study describes how exposing C57BL/6 mice to BAC induce some clinicopathological features of DED seen in humans, and therefore provides the foundations to explore the consequences on the ocular surface, particularly on limbal epithelia and its' stem cells.

1. Introduction

Dry eye disease (DED) is a multifactorial condition with a plethora of causal factors, both extrinsic (e.g. lifestyle and environment) and intrinsic (e.g. autoimmunity) [1]. DED is associated with a vicious cycle of activity, including dysfunction of one or more tear components, leading to destabilization of the tear-film, and failure to protect and sustain the ocular surface and chronic inflammation. Ocular irritation, stinging and foreign body sensation are symptoms frequently reported by patients which are exacerbated by using topical eye-drop formulations containing preservatives such as benzalkonium chloride (BAC) [2–6].

BAC is a quaternary ammonium cation widely used to preserve ophthalmic preparations and serves two main purposes. Firstly, it acts as a surfactant to solubilize ionic components into otherwise immiscible solvents. This facilitates effective emulsification and stabilization of medications, and therefore prolonged shelf-life. Secondly, BAC is superior to other preservatives in inhibiting microbial activity [7]. This is critical as patients often lack hygienic administration, and multiple usage of the same eye-drop vial can introduce microbial contamination [4,8].

In recent years, BAC has emerged as an agent for investigating the pathogenesis of DED in animal models. In rabbits, topically applied 0.1% BAC twice daily over 4–14 days results in the development of DED [9,10]. In mice, 0.2% BAC applied twice daily triggers clinical signs of DED including increased fluorescein staining, corneal irregularity and loss of conjunctival goblet cells (GCs) [11–15]. Most mouse studies incorporating BAC have been conducted with the BALB/c strain, however strain-specific responses have been observed [14,16]. Notably, severe ocular surface disease (e.g. limbal stem cell deficiency) develops when > 0.2% BAC is applied at a higher frequency [17]. The mechanisms by which the ocular surface responds to BAC in animal models of DED is not well characterized, and although researchers have examined the corneal epithelium in this model [18] there is minimal information regarding its effect on corneo-limbal epithelial cells (CLECs).

Certainly, the toxic effects of BAC on cultivated corneal and conjunctival cells have been investigated in multiple species [3,6,19–23]. To this end, BAC induces cytoplasmic damage and apoptosis, and

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https://doi.org/10.1016/j.jtos.2019.11.006
Received 1 August 2019; Received in revised form 15 October 2019; Accepted 13 November 2019