Introduction

Surfactants, or surface-active agents, are compounds used to decrease surface tension of a liquid, with wide-ranging applications from household and personal care products to industrial and agricultural use. Surfactants exhibit a wide range of responses based on concentration, consumer or industrial use, chemical structure, charge, etc. Surfactants can be classified based on the charge found on the head group of the molecule, and are generally divided into positively charged cationic, negatively charged anionic, and zwitterionic non-ionic surfactants.

Here we present the observed responses of surfactants for each of these charge groups in the Bovine Corneal Opacity and Permeability (BCOP) assay with respective histopathological evaluations to provide context and insight into addressing ocular irritation in vitro and in vivo methodologies. Using methods established in OECD TG 437, as well as in the Guidelines for Histopathological Evaluation of the BCOP Assay (2016), we present a quantitative and mechanistic understanding of the ocular irritation caused by each surfactant group. The BCOP assay utilizes a full thickness corneal model to evaluate ocular irritation which allows for preservation of treated corneas for further histopathological evaluation of the epithelial, stromal, and endothelial layers of the cornea.

Materials & Methods

The BCOP assay was performed as outlined in TG 437, as shown in Figure 1 below. Three corneas were used per treatment group, including the negative control (distilled water) and positive control (ethanol). The change in opacity was determined by subtracting the final opacity value (i.e., opacity value after treatment followed by 2-hour post-exposure incubation) from the initial opacity value (i.e., baseline opacity prior to treatment). The permeability changes were quantified as an optical density (OD) of 490 nm. Both the opacity and permeability scores were adjusted by the changes in the opacity and permeability for the negative control (NC) treated corneas. The in vitro Irritation Score (IVIS) for each treatment group was calculated by adding the mean opacity score to the mean permeability score (Figure 2) [15]. The histopathology was performed and evaluated under the guidance of the Histopathology Guidelines [2016] (Figure 14-17).

Results

The General behaviors of surfactants in the Bovine Corneal Opacity and Permeability (BCOP) assay were summarized in Table 1. A general observation of surfactant type was conducted in the BCOP assay.

Conclusions & Future Directions

This data supports the central tenet that surfactants exhibit a range of responses, and illustrates how different surfactant types have different mechanistic irritating events resulting in different modes of irritation in the eye. As expected, the IVIS was driven by opacity in cationic surfactants and permeability in anionic surfactants. Non-ionic surfactants generally were least irritating, with some exceptions. In some cases, such as Triton X-100, a more conservative prediction was obtained when tested at 10%, rather than 5%, as shown in Figure 15 [19]. Histopathology analysis provides additional data and may elucidate damage not picked up in the assay alone. Similarly, opacity and permeability changes should be independently reviewed and considered in addition to IVIS scores. Future investigations on the structural differences between mild non-ionic surfactants like PEG 400 and non-ionic surfactants like Triton X-100 may provide additional insights. Our findings provide a reference for industry or research in formulation development, highlight structural or chemical-based mechanisms for ocular irritation, and demonstrate surfactant behavior.

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