Observations on the Use of the Bovine Corneal Opacity and Permeability (BCOP) Assay to Evaluate the Eye Irritation Potential of Prototype Cosmetic Formulations Containing Salicylic Acid, Glycolic Acid and Ethanol

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ABSTRACT

Prototype cosmetic formulations containing 2% salicylic acid (SA) or ethanol (10% or 50%) were classified as having minimal eye irritation potential in the BCOP assay. In contrast, three formulations containing 2% SA and ethanol (10%, 15% or 30%) resulted in a classification of moderate eye irritation potential (opacity scores were higher and histopathological injury more pronounced than 100% ethanol control). The eye irritation potential of formulations containing glycolic acid (GA) is very low (EpiOcular™ ET_{50} = 35 to 55 minutes). Inclusion of ethanol (5%, 10% or 15%) in formulations containing 3.2 to 3.9% GA had no impact on BCOP opacity or permeability scores (each classified as having minimal eye irritation potential); however, formulations containing either 20% or 30% ethanol, 3.9% GA and 2% SA resulted in a classification of severe eye irritation potential. In both cases opacity and permeability scores were significantly higher than for the formulations containing SA and ethanol (opacity scores were also higher than for 100% ethanol and histopathological damage extended beyond the lower stroma into the endothelium). Further work is required to understand the significance of these observations but SA may potentiate the eye irritation potential of some cosmetic formulations containing ethanol (and in particular ethanol in combination with GA).

INTRODUCTION

The Bovine Corneal Opacity and Permeability (BCOP) assay has found widespread utility as an alternative method for the evaluation of the eye irritation potential of various product types including personal care and cosmetic formulations (Curren & Harbell, 2002). Moreover, the BCOP assay has also achieved acceptance by OECD (OECD, 2013) and a number of regulatory agencies for various applications (see eg., European Chemicals Bureau, 2004; NIH, 2008).

We routinely use the BCOP assay in conjunction with histological examination of treated corneas to evaluate the eye irritation potential of prototype cosmetic formulations (e.g., those with high surfactant load; >10% alcohol; low or high pH) we suspect may produce moderate to severe eye irritation. Depending upon product type we use the data from the BCOP to provide direction to Product Development on reformulation or, if the histological evidence is indicative of damage which is reversible, on the need for the provision of eye warning statements on product labels to alert consumers to avoid contact with eyes and rinse eye thoroughly with water if contact occurs (EPA, 2013).

We report here our observations on the use of the BCOP assay to evaluate the eye irritation potential of prototype cosmetic formulations containing salicylic acid (SA), glycolic acid (GA) and ethanol.

MATERIALS & METHODS

Fifteen prototype cosmetic formulations representing various product types (hydroalcoholics, astringents, and lotions) and containing various concentrations of ethanol, salicylic acid and glycolic acid were evaluated. The pH range for all the formulas was between 3-4.5 except for Test Formulation 5 which had a pH value of 8.5. In each case, 100% ethanol was used as a positive control.

BCOP methodology (including determination of opacity and permeability) was based on the protocol developed by Gautheron *et al* (1992). BCOP *in vitro* irritation score (IVIS) was calculated according to Sina *et al* (1995):

BCOP IVIS = mean corrected opacity + 15 (corrected permeability value)

Using the IVIS, each material was then assigned an irritation classification using the classification scheme (Table 1) adopted by ICCVAM (ICCVAM, 2006).

Table 1: Irritation Classification Based on BCOP IVIS

IVIS	Classification
0 - 3.0	Minimally Irritating
3.1 – 25	Mildly Irritating
25.1 – 55	Moderately Irritating
≥ 55.1	Severely Irritating

Depth of injury analysis was conducted on some but not all formulations using histopathological evaluation. Corneas were fixed for at least 24 h in 10% buffered formalin. Each cornea was bisected, paraffin-embedded and the two halves mounted in the paraffin block so that sections cut from each half could be placed on a single slide. Each slide was then stained with hematoxylin and eosin. Histopathological evaluation of the epithelium, stroma and endothelium (relative to the negative control) was performed on the central region of each cornea whenever possible. Evaluation of the stroma included identification of any swelling of the extracellular collagen matrix and keratocyte damage; estimation of stromal thickness was based on measurement of the distance from Descemet's Membrane to Bowman's Membrane. Photomicrographs were prepared and thickness measurements determined using a Spot Insight digital camera and associated software (Spot Diagnostic Instruments, Sterling Heights, MI).

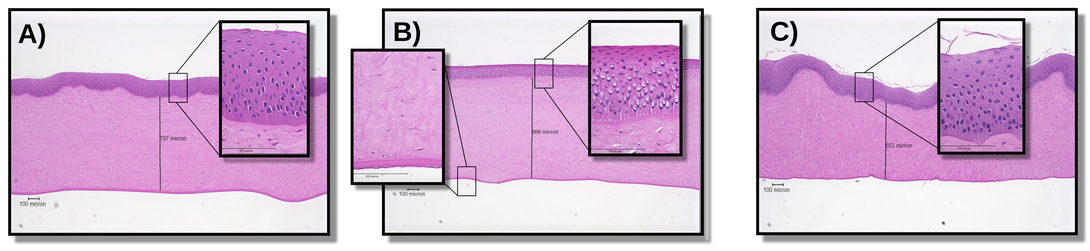
RESULTS

Mean opacity, mean permeability and BCOP *in vitro* irritancy score (IVIS) results are summarized in Table 2. Examples of histological sections from corneas treated with the negative control (0.9% NaCl), positive control (100% ethanol) and various test formulations are illustrated in Fig 1A, Fig 1B and Fig 1C-E, respectively.

Table 2: Summary of the Mean Opacity, Permeability and In Vitro Irritation Scores for Prototype Cosmetic Formulations Containing Ethanol, SA and GA

Test Formulation	pH	Ethanol Concentration (%)	Glycolic Acid Concentration (%)	Salicylic Acid Concentration (%)	Mean Opacity (O)	Mean Permeability (P)	In Vitro Irritation Score (IVIS)
1	4.0	----	----	2	3.2	0.001	3.2
2	4.0	----	----	2	1.7	-0.004	1.6
3	3.5	----	----	2	3.7	0.000	3.7
4	4.5	10	----	----	0.3	-0.009	0.17
5	8.5	50	----	----	18.5	0.189	21.3
6	4.5	10	----	2	41.9	0	41.9
7	4.0	15	----	2	44.4	0.01	44.6
8	4.0	30	----	2	78.1	0.064	79
9	4.0	----	3.9	----	4.6	-0.001	4.6
10	3.0	5	3.9	----	2.9	0.012	3.1
11	4.5	10	3.2	----	3.2	0	3.2
12	4.5	15	3.2	----	3.4	0.01	3.6
13	4.5	20	1.4	1.5	66.1	1.059	85
14	4.0	30	3.9	2	117.2	0.147	119.4
Positive Control		100	----	----	32.4 ± 5.1	1.2 ± 0.2	50.3 ± 3.2

Figure 1. Corneal Histopathology †



Negative Control (DI H_2O) | **Positive Control (Ethanol, 100%)** | **Formulation containing only SA (2.0%)**

Formulation containing SA and ethanol | **Formulation containing only GA** | **Formulation containing SA, GA and ethanol**

†Full thickness sections (x48); Insert magnification (x475)

Formulations containing 2% SA (Test Formulations 1-3) or either 10% or 50% ethanol (Test Formulations 4, 5) were classified as producing minimal to mild eye irritation in the BCOP model. For formulations containing 2% SA, IVIS was driven by opacity. For formulas with 10% or 50% ethanol, there was a dose response effect in both opacity, permeability and IVIS values relative to the concentration of ethanol. These results are consistent with the reported results of testing in vivo (SCCP, 2001; Gettings *et al*., 1991). The depth of injury in corneas treated with the 100% ethanol positive control extended to the upper stroma (Figure 1B). In contrast, the depth of injury from formulation containing only SA was confined to the epithelium (Figure 1C).

Three formulations (Test Formulations 6-8) containing both 2% SA and ethanol (10%, 15% or 30%) resulted in a classification of moderate eye irritation potential using BCOP. The primary driver of IVIS was a high opacity score. In each case the opacity scores were significantly higher than for formulations containing only SA (Test Formulations 1-3) or only ethanol (Test Formulations 4 and 5) and higher even than for 100% ethanol. In contrast, the permeability scores were surprisingly low compared with what we might anticipate based on eg., the permeability score for a formulation containing 50% ethanol (Test Formulation 5) and inconsistent with the depth of injury (including to the stroma and endothelium) we observed for Test Formulation 8 (Figure 1D).

In contrast to the apparent enhanced injury from SA due to inclusion of ethanol in test formulations, the addition of ethanol to formulations containing GA (Test Formulations 10-12) had no impact on eye irritation and opacity and permeability scores when compared to the formulation containing only GA (Test Formulation 9). Both resulted in minimal eye irritation and injury was confined to the epithelial layer (Figure 1E).

Formulations containing 20 or 30% ethanol, 1.4 or 3.9% GA and either 1.5 or 2% SA (Test Formulations 13 and 14) resulted in a classification of severe eye irritation. Opacity scores were significantly higher than for the formulations containing only 2% SA or 3.9% GA and at the high end of the range for the formulations containing both SA and ethanol. Similarly, permeability scores were also higher than for the formulations containing only SA or GA or both SA and ethanol and depth of injury was much greater extending into the lower stroma and endothelium (Figure 1F).

DISCUSSION

Reasonable to assume that the inclusion of *either* SA or ethanol would drive increased irritation potential (due primarily to increased opacity). The higher irritation potential than anticipated with inclusion of *both* SA and ethanol is suggestive of a synergistic response.

Although with such limited data we can't rule out the possibility that SA may potentiate the eye irritation potential of some cosmetic formulations containing ethanol (and in particular ethanol in combination with GA), our findings may be related to the propensity for ethanol to act as a penetration enhancer (William and Barry, 2004). The mechanism of enhanced penetration by ethanol is not thoroughly understood (Trommer and Neubert, 2006). Proposed mechanisms include:

- Insertion of ethanol between the hydrophobic lipid tails of the cell membrane of keratocytes in the stratum corneum (Panchagnula *et al*., 2001, Manabe *et al*., 1996).
- Extraction of lipids from the cell membrane of keratinocytes in the epidermis formation of "pores" in the stratum corneum (Kurihara-Bergstrom *et al*., 1990, Levang *et al.,* 1999).

Enhanced penetration of SA through the cornea may explain greater corneal opacity and depth of injury than was observed from formulations containing only SA or from 100% ethanol.

- It does not explain why we did not see a corresponding increase in permeability score commensurate with the greater depth of injury.

The addition of ethanol to formulations containing GA had no impact on eye irritation and opacity and permeability scores when compared to a formulation containing only GA.

- Unlike SA, which is lipophilic and has been shown to have a high rate of dermal penetration (Harada *et al*., 1993), GA has a lower partition coefficient (Barratt, 1996) and a low rate of dermal penetration (Jiang and Qureshi, 1998). It may be reasonable to assume ethanol penetration enhancement of GA will be lower than for SA.

For formulations containing ethanol, SA and GA, it seems unlikely that our findings are attributable solely to enhanced penetration of SA.

- Further work is required to understand the significance of these observations but the eye irritation potential of formulations containing both SA and ethanol may be much greater than anticipated.

Significantly, in our hands the IVIS of one formulation containing SA and ethanol (Test Formulation 6) was lower than might be expected given the extent of damage observed histologically and underscores the value of conducting histopathological evaluation of treated corneas following completion of permeability and opacity measurements.

REFERENCES

A reprint of this poster and the complete list of references can be obtained at www.iivs.org or by scanning the QR code: