CHOOSING THE APPROPRIATE SOLVENT FOR SOLID MATERIALS TESTED IN THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) IN VITRO ASSAY

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ABSTRACT

In compliance with OECD Test Guideline 437 for eye irritation (BCOP assay), non-surfactant solid materials are typically tested as 20% dilutions prepared in 0.9% sodium chloride solution, distilled water, or other solvent that has been demonstrated to have no adverse effects on the test system. However, the limited solubility of some chemicals adds technical challenges in finding a vehicle that would ensure the material’s availability to the excised corneas and that itself would not affect the test system. In this study, we evaluated five solvents frequently used in the BCOP assay: distilled water, mineral oil, corn oil, polyethylene glycol (PEG)-400, and methanol solution (0.5%). Based on the available classification systems, our preliminary data showed that water, methanol, mineral oil and corn oil were perceived as non-irritants, while PEG-400 was predicted as a mild irritant. To demonstrate the influence of the type of solvent on the outcome/prediction of the BCOP assay for solid materials, we tested a 20% suspension of benzoic acid (BA) prepared in these solvents. BA has a non-polar benzene ring that would presumably dissolve in non-polar solvents and a polar acidic group with affinity for polar solvents, thus making it a good model for testing its effect on corneas when dissolved in various solvents. Previous animal tests reported moderate to severe eye irritation induced by BA. Our results demonstrated that when mixed in water, mineral oil, corn oil, or methanol, BA was predicted to be a corrosive/severe irritant, while it was predicted to be a moderate irritant when mixed in PEG-400. These results support the need for further investigation of the solvent’s influence in the BCOP assay to allow the correct prediction of the irritation potential of solid materials.

INTRODUCTION

To investigate the influence of vehicle in the in vitro eye irritation Bovine Corneal Opacity and Permeability (BCOP) assay, we assessed and compared the eye irritation of five solid materials when dissolved in water (also the assay’s negative control), mineral oil, corn oil, PEG-400 and 0.5% methanol (Methyldihydroxilol) solution. These solvents are routinely utilized for the Draize rabbit eye test when the test substance is not directly applicable. These solvents were subsequently used to prepare the 20% dilution of benzoic acid (BA) tested per OECD TG 437 (1). Benzoic acid is used in topical drugs such as anti-fungal creams or in cosmetics, thus having the potential to induce irritation through ocular exposure to the eye. Previous animal tests reported moderate to severe eye irritation induced by BA (2-6). BA has a non-polar benzene ring that would presumably dissolve in non-polar solvents and a polar acidic group with affinity for polar solvents, thus making it a good model for testing its effects when dissolved in various solvents.

MATERIALS & METHODS

- In Vitro Score = Mean Opacity Value + (15 x Mean OD 90 Value) Score
- Histology analysis: Hematoxylin & Eosin (HE) staining
- Prediction models (7-8):
  - in Vitro Score: 0 to 3 = non-irritant
  - 3.1 to 25 = mild irritant
  - 25.1 to 55 = moderate irritant
  - above 55 = corrosive/severe irritant

CONCLUSIONS

1. The results showed that solvents used in the study, deionized water, mineral oil, corn oil and 0.5% methanol were predicted to be non-irritant. PEG-400 was predicted to be a mild irritant. The histology analysis confirmed the BCOP data by showing sloughing of the squamous layer of corneas treated with PEG-400.

2. When used to prepare the BA dilutions, all solvents induced high mean opacity scores and very low permeability scores, with the exception of PEG-400 which in reversed induced the lowest opacity score and the highest permeability score of all solvents used in the study.

3. BA was predicted by the BCOP assay as severe irritant when dissolved in deionized water, mineral oil, corn oil or 0.5% methanol and induced damage up to the mid-depth stroma. When dissolved in PEG-400, BA was predicted to be a moderate irritant and limited its damaging effects to the epithelium. Furthermore, the stroma appeared better organized in corneas treated with BA dissolved in PEG-400 than in the negative control-treated corneas.

FUTURE CONSIDERATIONS

4. Our study emphasizes the importance of choosing the appropriate vehicle for solid materials when tested in the BCOP assay. The solvent may need to be selected based not only on previous animal data or on chemical properties but also on the formulation/dilution of the test material that the end-user comes in contact with in order to better reflect the effects upon human exposure.

5. Our results support the need for thorough investigation on the solvent’s choice and influence in the BCOP assay to allow the correct prediction of the irritation potential of solid materials, particularly when validating the in vitro results against animal data.

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