

Poster: free communications

The use of histopathology to improve the predictive capacity of the Bovine Corneal Opacity and Permeability (BCOP) assay

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Introduction

The BCOP assay has gained widespread usage since it was first described in its current form by Gautheron, *et al.* in 1992. The assay combines two mechanistic endpoints – corneal opacity and epithelial permeability – to create an In Vitro Score that is then used to predict ocular irritation potential. However not all eye irritants are detected by these two endpoints, e.g. several strongly irritating materials used in the European Commission/British Home Office study (EC/HO study) did not induce a commensurately high In Vitro Score in the BCOP. Subsequently we were able to show that these underpredicted materials did induce damage that was detectable using histopathology.

Rationale

Although the BCOP assay utilizes “living” (freshly excised; still viable) corneas, we hypothesized that some of the damage seen in *in vivo* treatments could be a consequence of inflammation subsequent to the initial injury. This type of irritation would most likely not be seen in an excised tissue since there would be no opportunity for the recruitment of inflammatory cells from outside the cornea. Jester *et al.* (1998) showed that the depth and area of injury occurring immediately after the initial ocular exposure to irritants was generally predictive of the extent of the final injury and especially of the potential recovery. Therefore we began studies to determine if there was damage to the epithelial, stromal, or endothelial layers of the excised cornea which were not being expressed either in the opacity or permeability scores.

Results

Initially we assessed the results of the EC/HO study on ocular irritation. Although the BCOP assay was arguably the best performing of all the *in vitro* assays, there were still several materials that were under predicted, e.g. parafluoroaniline, quinacrine, and sodium oxalate. When we retested these materials and added a histopathological examination of the corneas, we found that all three induced damage that could be observed microscopically.

Further studies over the next five years revealed additional cases where histopathology of the bovine corneas was important in predicting eye irritation. As a result, the ICCVAM review of “In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants”, suggested creating 1) a reference atlas of histopathology for chemically-induced ocular lesions and 2) a standardized scoring scheme to be used in conjunction with the histopathology atlas to identify decision criteria that could be used in hazard classification.

We have now developed an atlas of ocular lesions observed *in vitro* and have convened a meeting of ocular histopathology experts to standardize the evaluation and lesion nomenclature for bovine corneas. Examples from this atlas and a summary of the histopathology meeting will be presented.

References

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