The pre-corneal tear film and associated lipids, the corneal epithelium, the mucin’s network and the conjunctiva represent the target of Eye Compatibility assessment that concerns very low, low or even non irritant (Non Classified-NC) chemicals or products able to generate early toxicity without clinical signs at eye level as discomfort or itching. Beside the regulatory requirements the assessment of eye compatibility represents a real need for Pharmaceutical and Cosmetic industries: cosmetics that strongly differ for ingredient’s type and concentration, technical form, mode of application and long term ophthalmological treatments involving repeated application, represent the applicability domain of testing methods proposed in this area of toxicology. The investigated relevant endpoints at corneal epithelium level and associated structural components allow to set-up sensitive and predictive testing strategies in the area of NC and mild chemicals and products.

**BACKGROUND**

The Multiple Endpoints Analysis (MEA) Protocol based on the assessment of complementary parameters (cell viability by MTT test, tissue morphology and the release of cytokines) has been modified by including transcriptional regulation of a structural component of the epithelial barrier as an early marker of the effects of sub toxic doses:

**MEA MODIFIED PROTOCOL**

- **EXPOSURE**
  - BAK 0,01% - 0,1% : 30 µL
  - 24h ACUTE
  - ACUTE APPLICATION WITH HIGHER DISCRIMINATING POTENTIAL
  - LONG TERM APPLICATION AND CUMULATIVE EFFECT

- **END POINTS**
  - CELL VIABILITY - MTT TEST
  - ELISA - CYTOKINES RELEASE
  - OCTCLUDIN IMMUNOHISTOCHEMISTRY

**RESULTS**

- **CN**
  - ONLY POLYCARBONATE FILTER
  - BAK 0,01 %
  - BAK 0,1 %

Histological analysis confirms the time and dose dependent effect with the two BAK concentrations: an increasing number of necrotic cells from the apical to the basalar layers is observed with BAK 0,01% at 24h associated to a morphological impairment of the upper layers that became a necrotic zone after the post incubation period without sign of recovery. After 72h the tissue morphology is still observed even in presence of necrosis.

- **OCTCLUDIN IMMUNOHISTOCHEMISTRY**
  - BAK 0,01 %
  - BAK 0,1%

Ocludin membrane staining is detected in the basal and apical layers of the control tissues.

- **OCTCLUDIN GENE EXPRESSION**

**CONCLUSION**

The use of the MEA modified protocol may enlarge the scale for discriminating between NC and mild and very mild chemicals and products. MEA approach has been confirmed as a useful tool for preclinical irritation screening of new ophthalmological or cosmetic products. The study of Ocludin regulation allows a better prediction of the effects of sub toxic doses of chemicals treatment: these are mostly superficial and modify the penetration of the toxicant without inducing a toxic effect at basalar level. Ocludin gene expression seems to be a promising marker to investigate superficial damages at corneal epithelium level and to overcome some limits of MTT test. Thanks to different procedures it is possible to use this new bio-marker to distinguish between a strong irritant that is responsible of the degradation of the tissue (down regulation and early up regulation) and a low irritant able to allows tissue recovery (up regulation followed by any down regulation) or finally to distinguish between different levels of irritation (constant up regulation).

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