Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food and Chemical Toxicology 43 (2005) 155–165
www.elsevier.com/locate/foodchemtox

Eye irritation of low-irritant cosmetic formulations: correlation of in vitro results with clinical data and product composition

Caroline Debbasch [a,*], Catherine Ebenhahn [b], Nadia Dami [c], Marc Pericoi [d], Christine Van den Berghe [b], Martine Cottin [a], Gerhard J. Nohynek [a]

[a] *L'Oréal Research and Development, Worldwide Safety Department, L'Oreal R&D, 25-29 Quai Aulagnier 92600, Asnières-sur-Seine, France*
[b] *L'Oréal Research and Development, Aulnay-sous-Bois, France*
[c] *L'Oréal Research and Development, Chevilly Larue, France*
[d] *Peritesco Recherche Biomédicale, Paris, France*

Received 2 June 2004; accepted 13 September 2004

Abstract

Alternative methods to the Draize eye irritation test, such as the hen's egg test-chorioallantoic membrane (HET-CAM) or the bovine corneal opacity and permeability (BCOP) tests, are currently used to evaluate the irritant potential of cosmetic or consumer products. Although, for strong irritants, the results of these tests correlate well with those of the Draize test, they appear to be less suited to identify mild irritants. In order to improve the sensitivity of alternative eye irritation tests, we developed a novel method that uses a human corneal epithelial cell line (CEPI), and the endpoints of cytotoxicity and IL-8 release. Twelve make-up removers were assessed by the HET-CAM, BCOP and CEPI tests, as well as in a clinical in-use test under ophthalmological control after their application to the external eye lid. In addition, we investigated the impact of osmolality and raw material composition on in vitro and clinical results and compared the in vitro results with those of clinical studies.

Overall, although HET-CAM results were unrelated to eye discomfort and adverse clinical signs, they correlated mainly with the presence and concentration of surfactants in the test articles. BCOP scores were unrelated to clinical signs, but related mainly to glycol and sodium lactate content and concentration in the test articles. Cytotoxicity in CEPI mainly correlated with presence and concentrations of surfactants, and IL-8 release to clinical signs and/or glycol and sodium lactate concentrations. Overall, IL-8 release appeared to be the most sensitive and reliable endpoint to predict human eye tolerance to mildly irritant products. Although our results suggest that the IL-8 assay appears to be a promising screen for borderline-irritant formulations, further experiments are required to confirm and validate these preliminary results.

Keywords: Eye irritation; Inflammation; Hen's egg test-chorioallantoic membrane; Bovine corneal opacity and permeability; Corneal epithelial cell line; Interleukin-8

1. Introduction

For many years the cosmetic and consumer industries have searched for suitable in vitro methods to assess the safety of cosmetic formulations and raw materials. Particular emphasis was given to development of alternative methods to replace the traditional Draize rabbit eye test, the former "gold standard" for assessing eye irritation.

Abbreviations: HET-CAM, hen's egg test-chorioallantoic membrane; BCOP, bovine corneal opacity and permeability; CEPI, corneal epithelial cell line

* Corresponding author. Tel.: +33 1 47 56 82 47; fax: +33 1 47 56 80 50.
E-mail address: cdebbasch@rd.loreal.com (C. Debbasch).

0278-6915/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2004.09.004

In the Draize test, eye irritation is calculated as modified maximum average scores (MMAS), a combination of individual scores of cornea opacity, affected cornea area, iris inflammation, conjunctival erythema, swelling and discharge (Draize et al., 1944; Sina et al., 1995; Moldenhauer, 2003). Given that most current alternative tests are based on cytotoxicity, they have been subject to criticism due to their inability to predict clinical signs associated with human eye irritation (Bruner et al., 1991). A further confounding factor in the validation of in vitro eye irritation tests may be the anatomical dissimilarity of rabbit and human eyes: possibly, some of the problems of comparing in vitro test results with those of the Draize test may be due to the difference of rabbit and human eyes, as well as the poor reproducibility of subjective Draize scores (Cottin, 1995; Sina et al., 1995; Moldenhauer, 2003).

Today the principal challenge for the cosmetic and consumer product industries remains the development of novel alternative methods that permit determination of the mechanism of eye irritation and, at the same time, yield results that are consistent with human data. Given that classical in vitro tests for eye irritation, such as the hen's egg test-chorioallantoic membrane (HET-CAM) (Lüepke, 1985) or the bovine corneal opacity and permeability (BCOP) (Gautheron et al., 1994) tests were shown to identify severe or low-irritant materials, the present challenge is to develop methods suitable to evaluate moderate or mild irritants, or products that produce human eye discomfort in the absence of macroscopic adverse clinical signs.

The HET-CAM test, i.e., the assessment of effects following application of the test material to the chorioallantoic membrane of fertilised chicken eggs, has been shown to be a useful method for assessing the irritant potential of water soluble cosmetic products (Steiling et al., 1999; Doucet et al., 1999). However, given that the scoring of the test relies on visual evaluation, its results may be subject to inter-individual variation.

The BCOP test is frequently performed in parallel to the HET-CAM, and is considered to be a robust predictive tool with a good correlation with Draize test results for finished cosmetic products or water soluble cosmetic ingredients. However, it appears less reliable to identify or rank weak irritants (Swanson and Harbell, 1998; Geerling et al., 2001; Shioda et al., 2002).

In order to avoid the intrinsic variability of visual scoring, we developed a mechanism-based test using a human corneal epithelial cell line. This cell line (CEPI17 clone 5; SV40 transformed), has an extended life span and expresses a series of cytokines, growth factors and enzymes similar to those of the original tissue (Offord et al., 1999). IL-8 was originally discovered as a neutrophil-activating protein, enabling neutrophils to adhere to endothelial cells and to migrate (Baggiolini and Clark-Lewis, 1992). In the inflammatory response of the cornea, Interleukin-8 (IL-8) plays a key role (Baggiolini and Clark-Lewis, 1992; Cooper et al., 2001; Buiatti et al., 1998). Therefore, in the present study, we investigated the irritation potential of a series of cosmetic products: (i) in the standard in vitro tests (HET-CAM, BCOP), (ii) in vitro using a human corneal epithelial cell line including IL-release, and (iii) in clinical tests under ophthalmological control. Our investigation had the aim to investigate the relevance of different in vitro test methods for weak eye irritants and to correlate their results with clinical data and product composition.

2. Materials and methods

Twelve make-up removers were assigned randomised code numbers, 1–12. The products were selected on the basis of their borderline irritant potential. Ten formulations (Nos. 1–8, 11 and 12) were commercially available products, while two formulations were prepared with the aim to produce itching through a high osmolality (addition of sodium lactate) without having irritating potential (product Nos. 9 and 10). Nine test formulations had a known quantitative raw material composition, whereas, for the 3 remaining products, only a qualitative composition was known.

2.1. HET-CAM test

This test was performed according to the official method (JORF, 1996). Nine-day-old fertilised eggs from White Leghorn chicken were incubated on an automatic rotating device (LM12, Mayenne Eclosion, France) for 24 h at 37.5 °C and 55% relative humidity. Eggs without an apparent emerging embryonic vascular system were discarded. The test was performed at 37 °C. The egg shell was opened at the side of the air chamber and the egg white membrane was removed while avoiding any damage to the fine blood vessels. Three hundred microliter undiluted liquid make-up removers was applied to the chorioallantoic membrane. At least 4 eggs were tested by product. After 20 s of contact, the membrane was rinsed with 5 ml of isotonic NaCl solution (Sigma Chemicals, St Louis, USA). The time up to appearance of haemorrhage, coagulation or lysis was noted and the overall irritation scores (0 up to 21) were calculated according to the method recommended by JORF (1996) as the mean sum of individual scores of all endpoints from four replicate assays. The irritant potential of tested product was defined as suggested by the guidelines as follows (Kalweit et al., 1990; JORF, 1996; Steiling et al., 1999):

Score < 1	Non-irritant
1 ⩽ Score < 5	Low irritant
5 ⩽ Score < 9	Moderate irritant
Score ⩾ 9	Irritant