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ORIGINAL ARTICLE

Characterization of new eye drops with choline salicylate and assessment of their irritancy by *in vitro* short time exposure tests

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Abstract The aim of our study was to examine the irritation potential of new eye drops containing 2% choline salicylate (CS) as an active pharmaceutical ingredient (API) and various polymers increasing eye drop viscosity (hydroxyethylcellulose, hydroxypropyl methylcellulose, methylcellulose, polyvinyl alcohol, polyvinylpyrrolidone). The standard method for assessing the potential of irritating substances has been the Draize rabbit eye test. However the European Centre for Validation of Alternative Methods and the Coordinating Committee for Validation of Alternative Methods recommend, short time exposure (STE) *in vitro* tests as an alternative method for assessing eye irritation. The eye irritation potential was determined using cytotoxicity test methods for rabbit corneal cell line (SIRC) after 5 min exposure. The viability of cells was determined using two cytotoxicity assays: MTT and Neutral Red Uptake. According to the irritation rankings for the short time exposure test, all tested eye drops are classified as non-irritating (cell viability >70%).

1. Introduction

From a number of pharmaceutical dosage forms applied topically to the eye, the most common and widely used remain continuously eye drops (Lu, 2010). Their preparation requires special consideration with regard to sterility, preservation, isotonicity, buffering, viscosity and ocular bioavailability. In addition to physiological factors affecting ocular bioavailability, other factors such as physicochemical characteristics of the active pharmaceutical ingredient (API) and excipients are important. The evaluation of irritation potential of API as well as of the excipients is essential to secure the patient safety. For several years the method of choice to determine eye irritation potential was the Draize rabbit eye test (Wilhelmus, 2001). However, ethical considerations and the limited value of animal models including lack of reproducibility and overestimation of

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human responses became the impetus for the development of alternative *in vitro* tests (Vinardell and Mitjans, 2008). As the ocular surface is a very complex system (corneal and conjunctival epithelial cells, the underlying stroma, and associated cells), it is difficult to develop the tests with a physiological and mechanistic base that are capable of eliminating the need for animals. Generally the *in vitro* model has been designed for only one tissue but in the eye there are more tissues. This is useful in obtaining more detailed data on the mechanical irritation of the eye. However, experiments on animals have to be replaced with several *in vitro* studies, such as: red blood cell test, isolated cornea and eye tests, as well as culture cell tests, which have different targets (da Nobrega et al., 2012; Donahue et al., 2011; Gerner et al., 2005; McNamee et al., 2009).

The European Centre for Validation of Alternative Methods (ECVAM) and the Interagency Coordinating Committee for Validation of Alternative Methods (ICCVAM) supervise the development of alternative methods. Both organizations recommend short time exposure (STE) *in vitro* test using a rabbit corneal cell line (SIRC) as an alternative method for assessing eye irritation (EVCAM, 2007; Li et al., 2009; Prinsen, 2006; Sakaguchi et al., 2011; Takahashi et al., 2008).

The aim of our study was to apply two *in vitro* tests (MTT and NRU) for assessing the irritation potential of new eye drops containing choline salicylate as API and various polymers improving eye drops viscosity (Repetto et al., 2008).

2. Materials and methods

2.1. Chemicals

Choline salicylate (CS) was kindly obtained from ICN Polfa Rzeszów S.A. The following polymers: hydroxyethylcellulose (HEC), hydroxypropyl methylcellulose (HPMC), methylcellulose (MC) and polyvinylpyrrolidone (PVP) were supplied from Sigma–Aldrich. Polyvinyl alcohol (PVA) and disodium ethylenediaminetetraacetate (Na_2EDTA) were purchased from POCH SA. Sodium chloride from Pharma Cosmetics, sodium bicarbonate from PPH Galfarm, and sodium metabisulfite from Fluka were used. Ultrapure water was produced by Millipore Simplicity UV system.

The Dulbecco's Modified Eagle's Medium (DMEM) without phenol red, fetal bovine serum (FBS), 0.25% trypsin EDTA solution, phosphate buffered saline (PBS), methylthiazolyldiphenyl-tetrazolium bromide (MTT), neutral red were obtained from Sigma Aldrich (St. Louis, Mi, USA). Penicillin–streptomycin and L-glutamine solution were obtained from Gibco Invitrogen Corp. (Grand Island, NY, USA). Dimethyl sulfoxide was obtained from POCh, S.A. (Gliwice, Poland).

2.2. Eye drops

Three series of eye drops without polymers, containing increasing quantities of choline salicylate (1.0% CS, 2.0% CS, and 3.0% CS) were prepared. Appropriate amounts of choline salicylate were dissolved in water containing suitable quantities of tonicity adjusting agent (sodium chloride). After mixing, the drops were filtered through filter Schott G3, poured into infusion bottles of 100 ml and sterilized in the Exacta M.O.COM autoclave at the temperature of 122 ± 2 °C for 20 min. under steam pressure 101.4 kPa. After 12 h of sterilization physicochemical parameters of drops were tested.

In the next stage six types of eye drops with increased viscosity (A-F) containing 2.0% choline salicylate were prepared. Received eye drops differed in type and concentration of the polymer used, and comprised respectively: 0.25% hydroxyethylcellulose (A), 0.5% hydroxyethylcellulose (B), 0.5% hydroxypropyl methylcellulose (C), 0.25% methylcellulose (D), 2.0% polyvinyl alcohol (E) or 5% polyvinylpyrrolidone (F). Their compositions are presented in Table 1. As in the case of eye drops mentioned above, eye drops of increased viscosity (A-F) were poured into infusion bottles and sterilized in the Exacta M.O.COM autoclave at the temperature of 122 ± 2 °C for 20 min. under steam pressure 101.4 kPa. After 12 h of sterilization physicochemical parameters of eye drops were tested.

2.3. Cell culture

The cytotoxicity study was determined for rabbit corneal cell line (SIRC) obtained from European Collection of Cell Cultures (Salisbury, UK). The cell line was cultured in DMEM medium, without phenol red supplemented with 10% (v/v) FBS, 1% penicillin–streptomycin and 1% L-glutamine. The cells were cultured at temperature 37 °C, in a humidified atmosphere containing 5% CO_2. The cytotoxicity assays for three eye drops formulations (1% CS, 2% CS and 3% CS) and new eye drops (A-F), with 2% choline salicylate and different

Table 1 Composition of examined eye drops with choline salicylate.

Active ingredient/excipient	Content in eye drops (g)					
	A	B	C	D	E	F
Choline salicylate (CS)			2.00			
Hydroxyethylcellulose (HEC)	0.25	0.50				
Hydroxypropyl methylcellulose (HPMC)			0.50			
Methylcellulose (MC)				0.25		
Polyvinyl alcohol (PVA)					2.00	
Polyvinylpyrrolidone (PVP)						5.00
Sodium chloride	+	+	+	+	+	+
Sodium bicarbonate	+	+	+	+	+	+
Disodium ethylenediaminetetraacetate (Na_2EDTA)						+
Sodium metabisulfite						+
Water ultrapure			ad 100			