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Ocular tolerability assessment of PHMB (Polyhexanide) 0.8%, 0.25% and 0.08% ophthalmic solutions in rabbits

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PURPOSE

Acanthamoeba keratitis (AK) is a rare but severe infectious disease caused by *Acanthamoeba* spp. a ubiquitous free living protozoan. The incidence is uncertain but probably <500 cases/year based on the EU population of about 500 million, and as low as 0.15-0.18 per million in the USA (Acharya *et al*., 2007). In the absence of treatment, the disease progresses to blindness as a result of corneal vascularisation and scarring or corneal perforation. PHMB 0.02% eye drops is a unlicensed product which is empirically used to treat AK. Recently (Asero *et al*., IOVS 2015) it has been identified in PHMB 0.08% eye drops a potential effective drug product for treating AK. The objective of this study is to establish if PHMB 0.08% eye drops is sufficiently safe as a selected concentration to be tested in healthy human volunteers.

METHODS

In accordance with the European Medicine Agency Guideline on repeated dose toxicity CPMP/SWP/1042/99 a low dose (0.08%) of PHMB eye drops with established therapeutic effect, together with a high dose (0.8%) of PHMB, eye drops selected to enable identification of toxicity and an intermediate dose (0.25%) of PHMB eye drops, such as the geometric mean between the high and the low dose, have been selected for conducting a two-week tolerance/toxicity study in rabbit. All animal procedures were performed according to the guidelines of the ARVO statement for the "Use of Animals in Ophthalmic and Vision Research".

A total of 21 male and 21 female New Zealand White rabbits, approximately 8 weeks old, were distributed in four Groups with 8 animals each (4 male and 4 female rabbits) in Groups 2 and 3; 12 animals each in Groups 1 and 4 (6 male and 6 female rabbits). Rabbits were instilled into the right eye with 50 µL of PHMB vehicle (Group 1), PHMB 0.08%, 0.25% and 0.8% eye drops (Groups 2, 3 and 4), 13 times a day at approximately 1 hour intervals from Day 1 to 7 (first week) and 7 times a day at approximately 2 hours intervals from Day 8 to 14 (second week). The left eye remained untreated. Two animals per sex of Groups 1 and 4, were sacrificed after 1 week of recovery. Ocular irritation assessment was performed daily, before first dosing, in all animals during the treatment and once daily during the recovery period. In addition, fluorescein staining of cornea, slit-lamp examination and ophthalmoscopy were performed at weekly intervals in all animals during the study (Baldwin *et. al*.). Macroscopic and microscopic examination of treated and untreated eyes were performed in all animals sacrificed at the end of the treatment.

REFERENCES
1. Acharya NR, Lietman TM, Margolis TP. Parasites on the rise: a new epidemic of Acanthamoeba keratitis. Am J Ophthalmol 2007;144:292-3.
2. Asero A; Sudano Roccaro A; Favennec L; Gueudry J; Le Goff L; Blanco AR. The efficacy of Polihexanide (PHMB) eye drops against Acanthamoeba polyphaga investigated by an ATP-bioluminescence assay and a rat model of keratitis. Invest Ophthalmol Vis Sci. June 2015, Vol.56, 1890.
3. Baldwin, H. A. McDonald, T. O. and Beasley, C. H., J,. Slit-Lamp Examination of Experimental Animal Eyes - Grading Scales and Photographic Evaluation of Induced Pathological Conditions. Soc. Cosmet. Chem., 24, 181-195, 1973).

RESULTS

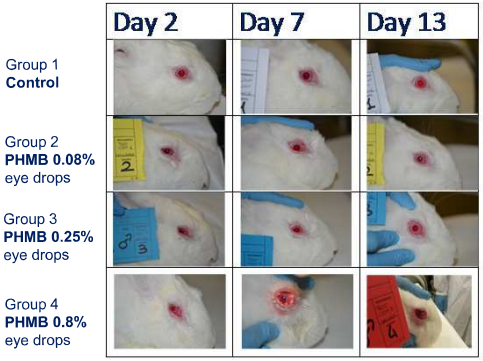

Figure 1- Photos of rabbits eyes at 3 different days of the two-week treatment period.

A photographic overview of the ocular observations during the two-week study is represented in Figure 1.

Slight conjunctival redness with slight/moderate discharge was noted in few animals treated with PHMB 0.08% and 0.25% eye drops (Groups 2-3). Recovery occurred in all animals after the treatment period. No treatment-related changes were observed in the eyes of animals of these two Groups after microscopic examination.

Assessment of ocular irritation, including fluorescein staining of the cornea, slit lamp examination and ophthalmoscopy, together with microscopic examination of the treated eyes and associated tissues, did not suggest any relevant treatment-related effect of PHMB ophthalmic solutions at 0.08% and 0.25%. PHMB 0.8% eye drops (Group 4) induced moderate/severe treatment-related effect, increasing with the treatment period, causing an irreversible damage to the eye. However, even in the Group 4 (10-fold the anticipated dose in Phase I clinical trials), histopathological evaluations of the eye lens showed no degenerative changes. Moreover, for all tested PHMB eye drops no indication of systemic effects were observed during the period of the study.

CONCLUSIONS

Our results indicate that 2-week repeated instillation of PHMB 0.08% and 0.25% ophthalmic solutions in the rabbit eye did not show any relevant treatment-related effect. Only animals treated with PHMB 0.8% eye drops showed moderate/severe treatment-related effect with irreversible ocular toxicity during the follow-up period. The observed microscopic changes in the Group 4 were from minimal to mild severity (i.e., grade 1 or 2 out of 4) and consisted of combination of conjunctival/corneal oedema, necrosis, iris congestion and acute inflammation. However, even in Group 4 (PHMB 0.8% eye drops), histopathological evaluations of the eye lens showed no degenerative changes. Moreover, for all tested PHMB eye drops no indication of systemic effects were observed during the period of the study.

These findings support our plan to investigate with sufficient safety margin PHMB 0.08% eye drops in Phase I clinical trials as a selected orphan drug for the treatment of *Acanthamoeba* keratitis.

The research leading to these results has received funding from the European Union Seventh Framework Programme [FP7/2007-2013] for developing the ODAK project under the grant agreement n° 305661.