A 26-week repeated-dose toxicity study of PHMB 0.08% ophthalmic solution in rabbits

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ODAK
Orphan Drug for Acanthamoeba Keratitis

PURPOSE

Acanthamoeba keratitis (AK) is a rare and severe infectious disease caused by *Acanthamoeba* spp. a ubiquitous free living protozoan. In the absence of treatment, the disease progresses to blindness as a result of corneal vascularisation and scarring or corneal perforation. Usually, patients are treated for months with PHMB 0.02% before the resolution of this rare ocular disease (Dart *et al*., 2009). Recently (Asero *et al*., 2015) PHMB 0.08% eye drops was shown to be more effective than PHMB 0.02% in non-clinical efficacy studies against *Acanthamoeba* spp. In addition, PHMB 0.08% eye drops was shown to be safe in a 2-week ocular tolerability study on rabbits (Asero et al., 2016). The objective of this study is to evaluate PHMB 0.08% eye drops following repeated ocular administration in rabbit over a period of 26 weeks.

METHODS

One group of 8 male and 8 female NZW rabbits (Group 2) was treated by ocular application into the right eye of one drop of the test item (PHMB 0.08% eye drops). Animals were treated 16 times/day at approximately 1-hour intervals from Day 1 to Day 5, 8 times/day at approximately 2-hour intervals from Day 6 to Week 3 (Day 21) and 4 times/day at approximately 4-hour intervals from Week 4 to Week 26 (Table 1). The left eye remained untreated. A similarly constituted group was treated in the same manner with the control item and acted as a control (Group 1). All animals were examined with slit lamp and indirect ophthalmoscope prior to allocation, on Day 1 before the first dosing and thereafter at weekly intervals prior to the first daily dosing. Animals with no ocular abnormalities were selected for the study. 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., 1973). All animals of Groups 1 and 2 were sacrificed after 26 weeks of treatment and tissues/organs (Annex 1) were collected to assess local and systemic toxicity. Macroscopic and microscopic examination of treated and untreated eyes were performed in all animals sacrificed at the end of the treatment. All animal procedures were performed according to the guidelines of the ARVO statement for the "Use of Animals in Ophthalmic and Vision Research".

Annex 1

Organs / Tissues	Weight	Fixation Preservation	Microscopic Examination
Abnormalities		✓	✓
Adrenal glands	✓	✓	✓
Aorta		✓	
Bone marrow (from sternum)		✓	
Brain	✓	✓	✓
Caecum		✓	
Colon		✓	
Duodenum		✓	
Epididymides		✓	
Eyes (with attached conjunctivae, e.g. sclera, iris, lens, chorioretina)		✓	✓
Femur with joint		✓	
Gall bladder		✓	
Harderian glands		✓	
Heart	✓	✓	✓
Ileum		✓	
Jejunum (including Peyer's patches)		✓	
Kidneys	✓	✓	✓
Lachrymal glands		✓	
Larynx		✓	
Liver	✓	✓	✓
Lungs (including mainstem bronchi)		✓	
Lymph nodes – cervical		✓	
Lymph nodes – mesenteric		✓	
Mammary area		✓	
Nasal cavity		✓	
Oesophagus		✓	
Optic nerves		✓	
Ovaries	✓	✓	✓
Oviducts[a]		✓	
Pancreas		✓	
Parathyroid glands[b]		✓	
Pituitary gland	✓	✓	✓
Prostate gland		✓	
Rectum		✓	
Salivary glands		✓	
Sciatic nerve		✓	
Seminal vesicles		✓	
Skeletal muscle		✓	
Skin		✓	
Spinal column		✓	
Spinal cord		✓	
Spleen	✓	✓	✓
Stomach		✓	
Testes	✓	✓	✓
Thymus (where present)	✓	✓	✓
Thyroid gland	✓	✓	✓
Tongue		✓	
Trachea		✓	
Ureters		✓	
Urinary bladder		✓	
Uterus – cervix	✓	✓	✓
Vagina		✓	

a: weighed and preserved with ovaries
b: weighed and preserved with thyroid gland

Table 1 - Treatment schedule

Group	Treatment	Administration	Dose level (drop x times daily/Day/Week)	Animal Number	
				Males (even)	Females (odd)
1	Control item	Right eye	1 x 16/Day 1 → 5 1 x 8/Day 6 → Week 3 1 x 4/week 4 → Week 26	2 – 16	1 - 15
2	Test item			18 – 32	17 -31

RESULTS

After 26 weeks of administration no deaths and no significant clinical signs were observed during the study. No treatment-related lesions were detected at the weekly examination in the treated animals. The body weight of animals was not affected by treatment and no relevant changes in food consumption were observed in males and females during the study. Regarding clinical pathology, the haematology and clinical chemistry (at 13 and 26 weeks) did not indicate significant treatment related effects. Similarly, no treatment-related changes were reported at the histopathological examination of the eyes and annexa (Fig. 1) or in the remaining examined organs/tissues.

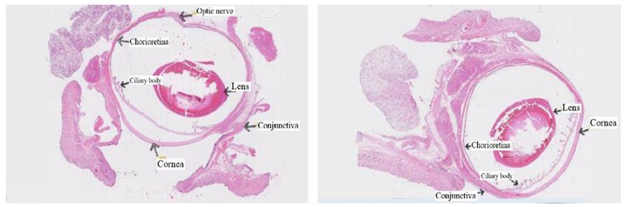

Fig. 1 – Control eye (left) versus 26-week treated eye (right)

The lesions reported in control and/or treated animals, such as congestion and/or oedema in lungs and some other organs/tissues, physiological involution of thymus or pigmentation (hemosiderin like) in the spleen, had comparable incidence in the control and treated groups and/or are known to occur spontaneously in untreated New Zealand White rabbits of the same age, under the experimental conditions.

CONCLUSIONS

Our results indicate that 26-week repeated instillation of PHMB 0.08% ophthalmic solutions in the rabbit eye did not show any relevant treatment-related effect. These findings support the development of PHMB 0.08% eye drops as a safe and effective orphan drug for long-term treatment of *Acanthamoeba* keratitis.

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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. The ODAK Consortium is made of Società Industria Farmaceutica Italiana SpA (SIFI, Italy); University of Rouen Normandy (UoR, France); Research Toxicology Centre SpA (RTC, Italy); PSR Group B.V. (PSR, The Netherlands); Moorfields Eye Hospital NHS Foundation Trust (MEH, UK); Ceratium Limited (CERAT, UK).

Commercial Relationships Disclosure:
Antonino Asero: Società Industria Farmaceutica Italiana (SIFI) SpA: Code E; Michela Salvador: Research Toxicology Centre (RTC) SpA: Code E; Oberto Germano: Research Toxicology Centre (RTC) SpA: Code E; Silvana Venturella: Research Toxicology Centre (RTC) SpA: Code E; Anna Rita Blanco: Società Industria Farmaceutica Italiana (SIFI) SpA: Code E.