In vivo ocular tolerance of a new preservative-free eye drop formulation in rabbits

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Introduction

Due to low water solubility of drugs, solubilizers have to be added to the ocular formulation. Macrogol 15 hydroxystearate (MGH 40) is frequently used in pharmaceutical formulation as solubilizer and it is recently used for new formulation in eye drops. This new formulation seems to be well tolerated. Pauly et al. recently reported that preservative free (PF) latanoprost (Monoprost®) containing a concentration of 5% MGH 40 had a good tolerability, when applied topically in a rabbit model or in 3D HCE cell construct model. A clinical phase III trial with two parallel groups Monoprost® versus Xalatan® showed that Monoprost® was non inferior for intraocular pressure lowering efficacy with less frequent and severe conjunctival hyperhemia. However, recent *in vitro* studies investigating PF formulations in glaucoma drugs have indicated that Monoprost® had induced signs of cellular damage. The concentration of the solubilizer MGH 40 in Monoprost® was presumed to cause many of the detrimental cellular effects in HCE-2 cells, although mildly increased apoptotic cells were detected and on rabbit cornea cultured on artificial anterior chamber (EVEIT system).

In order to assess the accuracy of these models, we performed a long term study in rabbits with high level of MGH40.

Material and method

Twenty-eight (28) male and female New Zealand White albino rabbits were involved in this study and assigned to two groups of 14 animals (7 male and 7 female) corresponding to the 3-month and 6-month time-points. A formulation containing 10% MGH 40 was instilled in the right eye, three times daily. The left eyes served as control.
Ocular signs were evaluated daily over a 4 –week period and once a week for the following time using the slit-lamp and indirect ophthalmoscopy.
Intraocular pressure data were collected up to 6 months.
The corneal sensitivity of both eyes was tested using an esthesiometer of Cochet-Bonnet (nylon thread; 0.12 mm diameter; 7.5 mm longer). Corneal sensitivity was evaluated by the number of stimuli necessary to induce a blinking reflex.
Eye globes were collected and examined by histology at 3 months and 6 months.

Results

	3-month groups (=Day-90 +/- 3 days)	6-month group (=Day-180 +/- 5 days)
Body weights	Pre-test, weekly and one day before sacrifice	Pre-test, weekly and one day before sacrifice
Food and water consumption	Weekly	Weekly
Clinical signs	Daily	Daily
Ophthalmoscopy (Draize's scale)	Pre-test, twice daily[a] over 4-week period, then once daily[b].	Pre-test, twice daily[a] over 4-week period, then once daily[b].
Slit-lamp examination (McDonald-Shadduck's scale)	Pre-test, once a week over 4-week[b] period, then once a month[b].	Pre-test, once a week over 4-week[b] period, then once a month[b].
Corneal Sensitivity	Pre-test, on Day 2 and the last week (week 13).	Pre-test, on Day 2 and the last week (week 26).
Intraocular Pressure	Pre-test, on Day 1 and the last week (week 13).	Pre-test, on Day 1 and the last week (week 26).
Hematology and Biochemistry	Pre-test, at 1 month and the day of sacrifice.	Pre-test, at 1 month and the day of sacrifice.
Euthanasia and necropsy	End of 3 months (=Day 90 +/- 3 days).	End of 6 months (=Day 180 +/- 5 days).
Histopathology	Treated and untreated eyes + Lacrimal and Harderian glands.	Treated and untreated eyes + Lacrimal and Harderian glands.

Body weight, Hematological and Biochemical Parameters

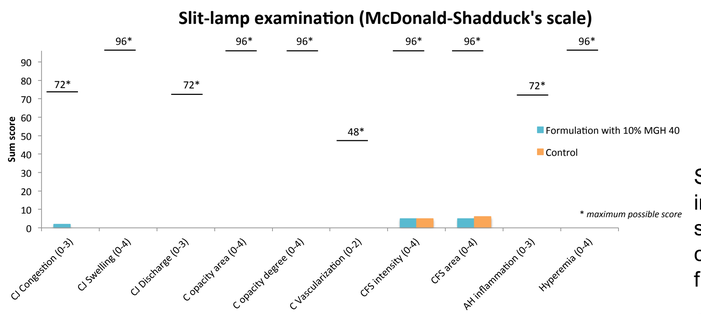
The body weight was measured once a week throughout the study period.
Blood was collected at 3-month and 6-month time-points and hematological and biochemical parameters were analyzed.
There were slight alterations in the biochemical parameter (slight increase of urea at 3 months, which was not observed at 6 months), however they were within the normal range.
All animals had a normal body weight evolution.

Corneal sensitivity

The corneal sensitivity was measured using an esthesiometer of Cochet-Bonnet.
No difference was observed between treated and control group. All animals had need less than 4 mechanical stimuli to have a blinking reflex which is commonly observed.

Ocular examination

Each eye was examined weekly with a slit-lamp using the McDonald-Shadduck's scale. The following ocular structures were evaluated:
-conjunctiva: congestion, swelling and discharge;
-cornea: opacity (degree and area), vascularization and fluorescein staining (intensity and area);
- anterior chamber inflammation;
- Iris hyperemia.

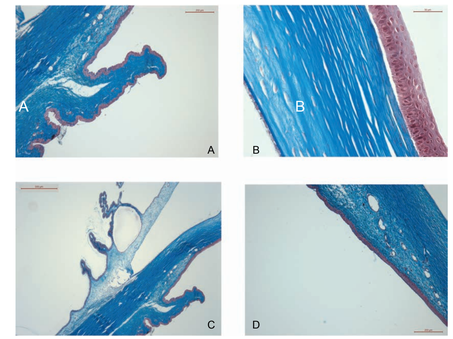


No ocular finding was observed for all animals excepted very slight and transient conjunctival congestion in one out of 14 treated eyes over the two first week. Slight corneal fluorescein staining in treated as well in control eyes was observed. This ocular finding is commonly observed for this specie.

Intraocular pressure

The intraocular pressure was measured on the treated and control eyes using a pneumatonometer during the first week then on 3-month and 6-month time-points.
No difference was observed between treated and control group. The values were similar between treated and control eyes.

Histological analysis



Paraffin sections from MGH 40-treated eyes stained with Trichrome Masson,(A) Bulbar conjunctiva, (B) cornea, (C) Iris-Ciliary bodies, (D) corneal limbus

Some minor focal changes attributed to repeated instillations were observed. Theses changes consisted of slight signs of irritation (extravasated lymphocytes) in conjunctival or limbus stroma. No other microscopic findings were observed in the ocular structures.

Conclusions

A formulation with MGH 40 10% 3 times a day was well tolerated and the ocular surface remained normal after 6-month exposure. Thus, results obtained *in vitro/ex vivo* with the new prostaglandin preservative free formulation containing 5% MGH 40 seem to be not predictive of *in vivo* tolerance. MGH 40 is a good solubilizer for topical use. This may be useful for the formulation of a variety of lipophilic and hydrophobic drugs that hitherto have not been available as eye drops.

References

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