Long-term tolerance of preservative-free eye drops containing macrogol hydroxystearate as an excipient

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Summary
Purpose. — This in vivo animal study was conducted to assess the tolerability of macrogol hydroxystearate 40 (MGH 40), commonly used as a solubilizing excipient in prostaglandin F₂α analogue eye drops without benzalkonium chloride.
Methods. — Twenty-eight (14 males and 14 females) New Zealand white albino rabbits in good health and with no signs of ocular irritation were randomly assigned to receive 25 μL instillations of a solution containing 10% MGH 40 in the right eye 3 times daily for either 3 or 6 months. Ocular examinations of the conjunctiva, cornea and iris (using an ophthalmoscope and slit-lamp), corneal sensitivity, and intraocular pressure were assessed in both the right (treated) and left (untreated) eyes throughout the study. General characteristics, hematology and serum biochemistry parameters were also assessed throughout the study and necropsy examinations were performed at study completion.

The data included in this manuscript have been accepted for poster presentation at the annual meeting of The Association for Research in Vision and Ophthalmology (ARVO), Denver, Colorado, USA, 3–7 May 2015.

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Introduction

Excipients are inactive ingredients that can be intentionally added to pharmaceutical products and intended to be inert, i.e. to not exert a therapeutic effect. These are routinely used in a wide range of pharmaceutical products, including fillers, diluents, preservatives, flavoring or coloring agents. There have been occasional historical cases of excipients that have proven toxic, e.g. a sulfanilamide preparation using toxic diethylene glycol as a solvent (the so-called ‘elixir sulfanilamide’) that caused numerous mortalities in 1937. Cases such as this have led to the implementation of guidelines for the rigorous pre-clinical testing of excipients for safety in vitro and in animal models before any product is tested clinically [1].

New prostaglandin F₂α analogue, preservative-free eye drops containing macroglycolglycerol hydroxystearate 40 (MGH 40) are increasingly popular [2,3] as medication for the treatment of glaucoma since preservatives — such as benzalkonium chloride (BAK) — contained in earlier formulations have been shown to be inflammatory and poorly tolerated [4–6]. MGH 40 is used as a solubilizer in such formulations due to the low water solubility of prostaglandin analogues.

Results. — There were no treatment-related effects on the cornea, conjunctiva, iris, or intraocular pressure. Transient findings were generally seen in the untreated as well as the treated eye. Similarly, there were no treatment-related findings in either the hematolary or serum biochemistry data or at necropsy. There were no differences based on gender.

Conclusions. — Long-term administration of a 10% MGH 40-containing formulation three times per day in a standard in vivo animal model was well tolerated and had no ocular or other effect. © 2016 Elsevier Masson SAS. All rights reserved.
2.382 kg (females) (supplied by Hypharm, Roussay, France), in good health, and with no signs of ocular irritation were used in this study. Animals were ear-tagged and also identified in their ears using indelible ink. They were individually housed in standard cages, with a mean ambient temperature of 19.3 ± 0.9 °C and relative humidity of 57.9 ± 5.1%. Rooms were continuously ventilated (≥15 air volumes per hour) and had a light and darkness controlled cycle (light from 7 am to 7 pm). All animals had free access to food and water, and were fed a standard dry pellet diet (LASQCdiet® Rab14-H [batches 12-2012, 02-2013, 04-2013, 05-2013]) that was distributed every day. All animals were treated according to the French decree, the European Directive, and the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

The animals were randomly assigned to receive the test product for either 3 or 6 months (n = 14 animals [7 males and 7 females] per group). In each group the test product was administered 3-times daily as 25 µL in the right eye (treated eye), with each administration separated by 3 to 4 hours and approximately 8 hours between the first and last administration each day (6 hours at weekends and public holidays).

Animals were assessed throughout the study for general characteristics, ocular examinations, corneal sensitivity, intraocular pressure, and hematology/biochemistry. At study completion animals were fasted for 1 day prior to sacrifice for necropsy examination. In each group, 4 animals (2 males and 2 females) completed a further 1-month observation period without any treatment prior to sacrifice. Animals were anesthetized with a mix of xylazine and ketamine for blood sampling and then euthanized by cardiac injection of pentobarbital (as recommended by the European authorities [11,12]). The study design is presented in Fig. 1.

**Test product**

The test product was a solution (batch Y455) containing (per 100 mL) MGH 40 (10.00 g), carboxymethylcellulose (2.00 g), polyvinyl alcohols (0.50 g), NaH₂PO₄, 1H₂O (0.520 g), Na₂HPO₄, 2H₂O (0.652 g), and water for injection (qs 100 mL). Except the concentration of MGH 40, all other excipients are common in this range of concentration in eye drops and are frequently used and well-tolerated.

**Assessments**

Observations were performed by technicians, which were trained by and for ophthalmology for which they performed daily assessments.
General

General clinical examination (general clinical signs and appearance) was conducted daily; food and water consumption were recorded weekly; and body weight was recorded pre-test, weekly, and the day before sacrifice.

Ocular examinations

The conjunctiva, cornea and iris of each eye were examined using a slit-lamp at pre-test, then twice daily for 4 weeks (before first and after the last daily administration), then once a month (before the first daily administration). Daily ocular clinical data were reported according to the Draize scale [13,14] and monthly slit-lamp data were reported using the McDonald-Shadduck scale [15,16].

Corneal sensitivity

Corneal sensitivity of each eye was tested using a Cochet-Bonnet esthesiometer (nylon thread: 0.12 mm diameter and 7.5 mm length) at pre-test, 10 minutes and 1 hour after the first administration on Day 2 and after the last administration either at the end of the study or at the end of the recovery period. The maximum number of stimuli to be used was 10 (to limit corneal damage) and the number of stimuli needed to induce a blinking reflex was recorded.

Intraocular pressure

The IOP of each eye was assessed at pre-test, Day 1 (20 and 40 minutes after the first administration), and during the last week (1–2 hours after the first installation of the day). This was performed under topical anesthesia (30 μL/eye of Cebesine® [0.4% oxybuprocaine] diluted to 1/10) using a tonometer (Model 30 Classic–Riechert).

Hematology and biochemistry

Whole blood was collected for hematological analysis (hemoglobin, hematocrit, mean hemoglobin concentration, total erythrocyte count, mean cell volume, mean cell hemoglobin concentration, total leucocyte count, platelets, and differential leucocyte count [neutrophils, lymphocytes, monocytes, eosinophils, basophils]) and biochemical analysis (sodium, potassium, chloride, calcium, glucose, cholesterol, triglycerides, urea, creatinine, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bilirubin) at pre-test, 1 month, and on the day of sacrifice. At pre-test and 1 month the sample was taken from the jugular vein and on the day of sacrifice the sample was taken by intracardiac puncture after anesthesia and before euthanasia.

Hematology samples were collected into K3-EDTA tubes and stored at room temperature; biochemistry samples were collected into lithium heparin tubes and plasma was extracted and stored at +5 ± 3 °C.

Necropsy, histological and microscopic analysis

Immediately after sacrifice both eyeballs were sampled and processed for histological and microscopic analysis. The eyeballs (including optic nerves, extraocular muscles, lachrymal glands and Harderian glands, and conjunctivae) were fixed in Bouin-Hollande solution, dehydrated, embedded in paraffin wax, cut into four 5–7 μm sections, and stained using the Masson trichrome procedure. The lenses were removed 24–48 h after fixation, frozen in Optimum Cutting Temperature compound, cut into four 7–10 μm sections, and stained with hematoxylin-eosin. The following organs were assessed macroscopically: adrenal glands, brain, heart, kidney, liver, lungs (with mainstem bronchi), spleen, and ovaries or testes (with epididymis). If any abnormal findings were observed the organ was to be analyzed histologically.

Data analysis

There were no statistical comparisons: all analyses were descriptive.

Results

General

All animals remained in good health throughout the treatment phase of the study. Minor clinical observations were recorded in 6 animals at isolated time points, but none of these findings was considered to be treatment-related. Overall food and water consumption was normal and regular.

Ocular examinations

From the macroscopic examinations, slight and transient conjunctival redness (grade 1 on a scale of 1–3) was found in the right (treated) eye of one male on Day 1 before the first instillation, Day 7 before the first and after the last instillation, Day 11 before the first and after the last instillation, Day 12 before the first and after the last instillation, Day 13 before the first and after the last instillation, Day 14 before the first instillation, and Day 73; in one male on Day 11 before the first instillation; in one male on Day 23 and Day 24 after the last instillation; in one male on Day 51 (also found in the left [untreated] eye at the same time point); and in one female after the last instillation on Day 18.

From the slit-lamp examinations (Table 1 and Fig. 2), slight and transient fluorescein corneal staining was found at isolated time points in the right (treated) eye of four males (1, 2, and 17 weeks) and one female (26 weeks) and was also found in the left (untreated) eye of two males (2 weeks) and two females (2 and 13 weeks). Slight and transient conjunctival congestion was observed in the right (treated) eye of one male (1 and 2 weeks). No animal had any other corneal or iris findings, nor any aqueous flare.

Corneal sensitivity

No difference was observed between treated and untreated eyes. All animals required fewer than four mechanical stimuli to have a blinking reflex except for one male (in the 6-month group, that required four stimuli on Day 2 at
Table 1  Slit-lamp examinations.

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<th>Week</th>
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<th>Fluorescein corneal staining</th>
<th>Conunctival congestion</th>
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<td>Intensity of staining</td>
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<td>Treated eye</td>
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<td>Treated eye</td>
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<tr>
<th>Week</th>
<th>n</th>
<th>Baseline (w0)</th>
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<th>w2</th>
<th>w3</th>
<th>w4</th>
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<th>w13</th>
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n: number of animals; w: week; M: male; F: female; --: no animal with observation.
Ocular tolerance of eye-drops containing macrogol hydroxystearate

10 minutes after the first administration) which is commonly observed.

**Intraocular pressure**

No difference was observed between treated and untreated eyes throughout the study, with IOP measurements being similar in treated and untreated eyes. At baseline, the mean IOP in the right (treated) eye was 23.3 ± 3.8 mmHg and 21.9 ± 3.9 mmHg in the left (untreated) eye. At 3 and 6 months (right eye vs. left eye), mean IOP was 18.5 ± 2.9 mmHg vs. 18.7 ± 2.6 mmHg and 20.4 ± 3.4 mmHg vs. 19.5 ± 4.1 mmHg, respectively. In the recovery animals (right eye vs. left eye), mean IOP was 18.8 ± 2.5 mmHg vs. 18.2 ± 1.8 mmHg (3-month group) and 20.3 ± 3.3 mmHg vs. 20.9 ± 3.2 mmHg (6-month group).

**Hematology and biochemistry**

There were no clinically important findings in any hematological or biochemical parameter for any animal.

**Necropsy, histological and microscopic analysis**

There were no remarkable findings in either group at necropsy, and no important findings in the histological analyses.

**Discussion**

Previous in vitro data have been inconclusive and contradictory regarding the potential tolerability of MGH 40-containing products such as Monoprost® at a concentration of 5%. For example, recently it was suggested that Latanoprost® seemed to increase LDH leakage from HCE cell lines but no effect on mitochondrial metabolic activity (MTT assay) was reported [7]. These results are contradictory since LDH leakage would be expected to mirror mitochondrial metabolic activity. Inconclusive data such as these led to the design of the present study, which was conducted to assess the long-term tolerability of MGH 40 using an in vivo animal model.

As in different models, different results were observed, it was decided to adopt GLP assay performed usually for marketing authorization and validated by Health Authorities. Furthermore tests that were not qualified as GLP were excluded (in vivo confocal microscopy which would avoid animals sacrifice). This would allow the study to be not questionable.

The albino rabbit is recommended and commonly used for ocular tolerance studies as the ocular surface is more sensitive that in the pigmented rabbit [13,17–20] and so was chosen for this study, which evaluated the long-term effects (3 and 6 months) of multiple instillations (3 times per day) into the right eye of a formulation containing 10% MGH 40. The left (untreated) eye was assessed in the same way as the right eye to provide controlled comparative data.

The findings of the present study showed no treatment-related effects of the MGH 40-containing solution, with few findings that were often seen in the untreated as well as the treated eye. Overall, in terms of the ophthalmological results there was no damage to the cornea, conjunctiva, or iris, no reduction in corneal sensitivity, and no effect on IOP due to the instilled eye drops. There were some minor and transient responses to the corneal fluorescein test and conjunctival congestion, but such ocular effects are usual for multiple instillations tolerance studies — e.g. a minimal response to the corneal fluorescein test is often seen in rabbits — and since they occurred with a very low incidence and usually in both the treated and untreated eyes these findings were not considered to be treatment-related [21–23]. Furthermore, there were only minor changes in serum biochemistry and hematology parameters; these were within the ranges of normal physiologic variation during the period of time of the study [24] and so not considered to be of clinical importance or treatment-related. There were no findings at necropsy, nor histology findings that could not be accounted for by normal occurrences in housed rabbits, and there were no differences in any endpoint based on gender.
These findings underline the inherent problems in extrapolating from a non-validated ex vivo (EVEIT) or simple in vitro toxicological model, such as the HCE model with only one layer of corneal epithelial cells with no stroma and endothelium to in vivo, to clinical situations [7]. The results of this study confirm the data obtained using a more sophisticated three-dimensional HCE cell line and rabbit model [25] and are consistent with pivotal clinical trials data [26].

Conclusion

Overall, the administration of this 10% MGH 40-containing formulation 3 times per day for 3 or 6 months in a standard in vivo animal model had no ocular or other effect. These results indicate that MGH 40, which is included as a solubilizing excipient at a lower concentration (5%) in marketed preservative-free eye drop formulations containing F2 analogues, is well tolerated, supporting extensive and reproducible clinical trials data.

Author’s contribution

K.V.-Q., S.L.-J., L.F. and P.-P.E. are employees of Iris Pharma, France, who conducted this study.

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Disclosure of interest

The authors declare that they have no competing interest.

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