Effect of pH and BAK on Pupil Dilation Following Low-dose Atropine Administration in Dutch Belted Rabbits

Tim Murphy[1], Linda Gieschen[1], David Baker[1,2], Janet K Cheetham[1,3], Patrick Johnson[1]

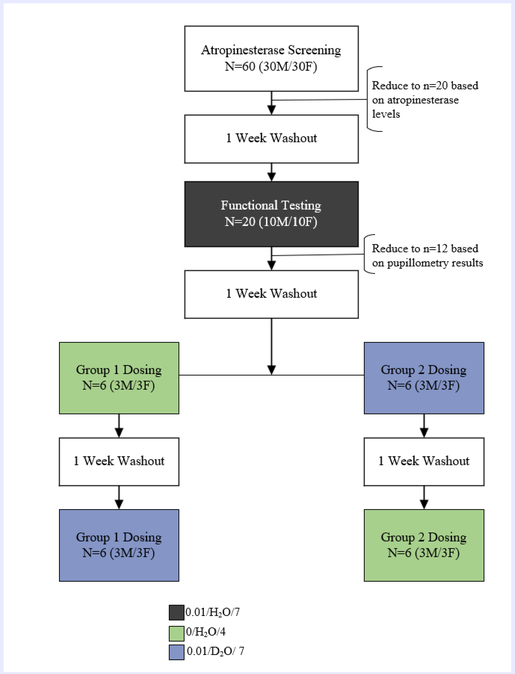
[1] Sydnexis, Inc. Del Mar, CA, United States, [2] Writeresource Inc., Carlsbad, CA, United States, [3]Celeris Consulting, Laguna Niguel, CA, United States

ABSTRACT/SCIENTIFIC PROBLEM

- Over the past two decades, numerous clinical studies have shown that low-dose atropine can effectively slow myopia progression in children[1,2,3,4,5,6,7,8] but three recent studies utilizing the same formulation found the efficacy to be less than previously reported.[9,10,11] The inconsistency in clinical outcomes for low-dose atropine highlights the need for further investigation into formulation differences.
- The formulation of atropine eye drops is critical, as factors such as pH and the presence of preservatives like BAK can significantly influence drug stability and corneal penetration.[12,13,14]
- Pupil dilation is a well-known indicator of drug uptake for mydriatics such as atropine. Atropine blocks the contraction of the circular sphincter muscle of the iris when administered topically.[15]
- This study aims to compare pupil dilation responses following the administration of two low-dose atropine formulations in Dutch Belted rabbits.

STUDY DESIGN

Figure 1 Study Design



METHODS

- This non-GLP crossover study was conducted at Pharmaron Lab Services (PLSS), an AAALAC International-accredited facility. The Protocol and Amendments were reviewed and approved by PLSS's Institutional Animal Use and Care Committee (IACUC).
- See Figure 1 for study design.

Atropinesterase Screening

- Sixty Dutch Belted (DB) rabbits were screened for atropinesterase activity by measuring tropic acid levels in their plasma.

Phase I Functional Testing

- Eighteen rabbits with the lowest plasma tropic concentration and two rabbits with the highest were selected for functional testing.
- Test article formulation: 0.02% atropine with 0.01% BAK, buffered NaCl, pH 7.
- Atropine Sulfate Ophthalmic Solution was obtained from Amneal Pharmaceuticals and diluted with Bausch & Lomb Advanced Eyewash (0.01% BAK, buffered NaCl, pH 7).
- All 20 rabbits received one 35 µL drop of the test article in each eye.
- Ophthalmic exam and Draize scoring were performed at 1-minute post-dosing.
- Pupillometry using the Neuroptics VIP400 pupillometer was conducted at baseline, 30, 60, 90, 120, 150, 180, 240, 300, and 360 minutes post-dose.
- Twelve rabbits were selected and underwent a 7-day washout period before Phase II testing.

Phase II Cross-Over Testing

- On Day 1, rabbits were randomized in a 1:1 ratio to either Group 1 or Group 2, with each group consisting of 3 males and 3 females.
- **Group 1 received the following test article:**
  0.02% atropine containing 0.9% buffered NaCl, **0% BAK, H_2O, pH 4** (hereafter called 0/H_2O/4)
- **Group 2 received the following test article:**
  0.02% atropine containing 0.9% buffered NaCl, **0.01% BAK, D_2O, pH 7** (hereafter called 0.01/D_2O/7)
- Each group received 1 drop (35uL) of the respective test article to each eye at baseline.
- Schirmer's testing without anesthesia was performed 2 minutes after baseline, followed by clinical ophthalmic examination and Draize scoring at 15 minutes.
- Pupillometry measurements were taken at baseline, 30, 60, 120, 240, 360, 480, 600 and 720 minutes after instillation of the test article.
- Cross-over Phase: After a 7-day washout, each rabbit received the alternate test article on Day 8.

Analysis

- Pupil diameters for both eyes were averaged, and the mean pupil diameter was calculated at each timepoint for the six rabbits in each group.
- Data Analysis: Change from baseline in pupil diameter was calculated at all time points, and mean changes for Days 1 and 8 were pooled by treatment.
- Differences in pupil diameter changes from baseline between formulations were compared using a one-tailed, paired t-test at all time points.
- Changes in pupil dilation between Day 1 and Day 8 were analyzed using an analysis of variance (ANOVA) test for each formulation.
- The slope of the pupil diameter–time curve between 360 and 720 minutes (elimination phase) was used to estimate the time to return to baseline for both formulations. Area under the curve (AUC) was calculated by summing the areas using the trapezoidal method to estimate the total area.

RESULTS

Pupillometry

- Changes from baseline in pupil diameter showed that eyes treated with 0/H20/4 had statistically significantly less pupil dilation ($p<0.01$) at all post-baseline time points compared to those treated with 0.01/D2O/7. See Figure 2.
- Pupil diameters did not return to baseline for either formulation group at the final 720-minute test point. At 720 minutes, eyes treated with 0.01/D2O/7 remained 1.40 mm larger than baseline, while those treated with 0/H20/4 remained 0.38 mm larger than baseline.
- When extrapolated to the time required to return to baseline, the AUC shows a stronger and more sustained mydriatic effect for the 0.01/D2O/7 formulation. See Table 1 and Figure 3.

Figure 2 Change from Baseline in Pupil Diameter

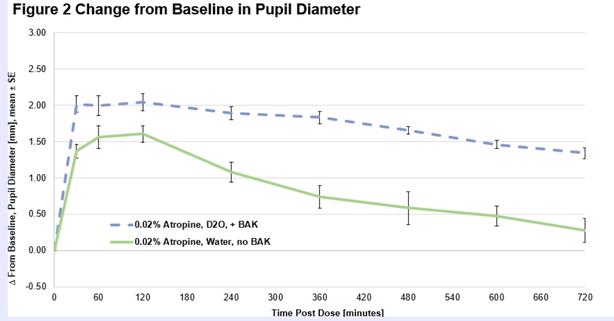


Figure 3 Change from Baseline in Pupil Diameter - Extrapolated

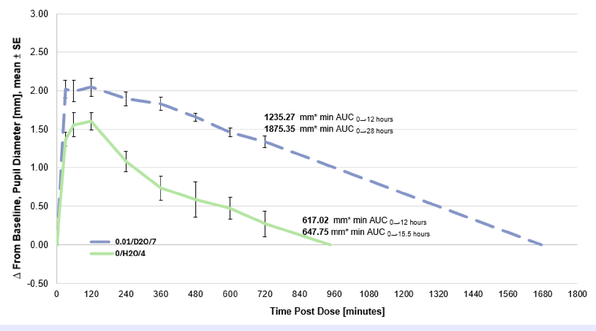


Table 1 AUC for 0/H_2O/4 and 0.01/D2O/7

	0/H_2O/4	0.01/D2O/7
$AUC_{0\text{-}720}$ (mm*min)	617.02	1235.27
$AUC_{0\text{-}Baseline}$ (mm*min)	647.75	1875.35

Ocular Exam, Schirmer's and Draize Results

- Overall, there were no significant differences in ocular findings, irritation or tearing between the two formulations.

CONCLUSION

- The results of this study indicate that an equivalent atropine concentration at neutral (near physiologic) pH, including BAK, increases mydriasis relative to a lower pH and BAK-free formulation, which may be due to increased bioavailability.
- Consistent with the findings of previous clinical trials that evaluated low-dose atropine for myopia control and utilized similar components, pH and BAK likely play a role in the overall efficacy of the drug product.
- These results may help explain why recent clinical studies that used an acidified atropine formulation without BAK reported fewer cases of photophobia and mydriasis and were less effective in slowing myopia progression.[9,10,11,14]
- These results indicate that ocular drug product formulation can play a critical role in efficacy, leading to enhanced bioavailability of atropine.

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- Funded by Sydnexis, Inc.
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For additional questions please contact: sydcorrespauth@sydnexis.com