*International Journal of Pharmaceutics*, 12 (1982) 147–152
Elsevier Biomedical Press



# Effect of poloxamer 407 gel on the miotic activity of pilocarpine nitrate in rabbits

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(Modified version received March 9th, 1982)
(Accepted March 11th, 1982)

## Summary

This study was designed to evaluate poloxamer 407 gel, relative to its suitability for use as a vehicle for ophthalmic drug delivery. Pilocarpine nitrate was incorporated into 25% poloxamer gel and the formulation was administered topically to rabbit eyes. The ocular activity of pilocarpine was assessed using the pharmacological response of miosis. The change in pupillary diameter versus time curve was compared to that obtained with an aqueous pilocarpine solution, dosed under similar conditions. As indicated by the miotic response, the gel formulation appeared to enhance the activity of pilocarpine when compared to the aqueous solution.

## Introduction

Results of recent studies, in rabbits and humans, indicate that aqueous-based gels appear to offer several advantages over traditional ophthalmic dosage forms, either in terms of improved ocular drug bioavailability (Schoenwald and Boltralik, 1979) or enhanced therapeutic response (Saettone et al., 1980). Many of the ophthalmic gels investigated to date have been formulated with either carbomers or cellulose derivatives (Bottari et al., 1978). Poloxamers, a class of gel-forming polymers, do not appear to have been evaluated as semi-solid vehicles for ophthalmic use. These polymers, however, have been used in contact lens products, artificial tears, and ophthalmic drug solutions (BASF Wyandotte, OS-3012(765); Waring and Harris, 1979; Facts and Comparisons, 1982).

Poloxamers possess several properties which appear to make them particularly suitable for use in the formulation of ophthalmic dosage forms. These include their

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low toxicity, mucomimetic properties, and optical clarity (BASF Wyandotte, OS-796; Waring and Harris, 1979). Because of these favorable characteristics, a poloxamer was chosen for this study. In particular, poloxamer 407 was chosen because it is reported to be the least toxic of commercially available poloxamers (BASF Wyandotte, OS-3012(765)).

## Materials and methods

### *Materials*

Male albino rabbits were used in the study and prior to experimentation were housed in standard laboratory cages and allowed food and water ad libitum. Four rabbits were used and during the time course of the study (10 weeks) their weights varied between 2.2 and 3.6 kg. Pilocarpine nitrate was obtained from Sigma Chemicals, St. Louis, MO. Poloxamer 407 (Pluronic F-127) was a gift from BASF Wyandotte, Wyandotte, MI. All other chemicals were of reagent grade and distilled water was used in all formulations.

### *Preparation of drug formulations*

Pilocarpine nitrate solutions, 0.25 M, were prepared on the day of use in Sorensen's phosphate buffer (pH 7.4). The gel formulations were 0.25 M pilocarpine nitrate and 25% w/v poloxamer. To prepare the gels, an appropriate amount of pilocarpine nitrate was placed in a volumetric flask and dissolved in buffer. The required amount of poloxamer was then added to the drug solution and the flask was stored at 5°C until poloxamer dissolution was complete (approximately 24–36 h). The formulation was then brought to volume with buffer and thoroughly mixed while cold. Upon warming the mixture to room temperature (RT), a clear viscous gel formed. Gel formulations were prepared 1–2 days prior to use and a separate formulation was prepared for each experiment.

### *Test procedure*

During the experiments, test animals were kept in restraining boxes in the normal upright position. The box was placed on a swivel-stool which allowed the rabbit to be easily positioned for measurement of pupillary diameter. Experiments were conducted in a dimly lit room, isolated from outside disturbances. The room was illuminated by a 15 W cool white fluorescent lamp which was placed horizontally above the rabbit, with the light source directed behind the animal. This allowed for adequate and constant illumination of both corneas; the light source rotated with changes in animal position. Pupillary diameters were measured using a cathetometer, positioned 1.1 m from the rabbit.

Rabbits were acclimatized to the test room for approximately 30 min before beginning the experiments. After this time period, to establish a baseline, pupil diameters were measured continuously for approximately 20 min, alternating between the right and left eye. For each pair of readings, the difference in pupil diameter (control minus test eye) was determined. These predosing differences were

averaged and the mean was used to convert post-administration data to "baseline corrected" values as described below. This minimized both "animal" and "day" variation.

Drug formulations were administered topically, using a gas/liquid tight, microliter syringe. The gels were chilled prior to filling the syringes to facilitate this procedure. Formulations were administered at RT and, in all cases, 10 $\mu$l were instilled quantitatively. The dose was placed in the lower conjunctival sac, approximately midway between the inner and outer canthus. The aqueous solution was administered to the test eye through a 27-gauge needle, 10 $\mu$l of buffer being administered to the opposite eye which served as a control. The gel formulation was administered through an 18-gauge needle, the end of which had been cut at 90°, producing a blunt, flat-ended bore. Non-medicated 25% poloxamer gel served as the control vehicle.

*Study design and data treatment*

Both the aqueous and gel formulations were tested in each of the 4 rabbits. A minimum of 1 week elapsed between tests in the same rabbit. The formulation testing order and the control eye for each experiment were randomly assigned. In all cases, to avoid experimental bias, the assigned formulation or control vehicle was first administered to the left eye, followed by the appropriate application to the right eye. After administration of both the control vehicle and test formulation, pupil diameters of both eyes were measured, starting with the right eye, every 5 min for 90 min and every 10 min thereafter. Due to blinking, head movements, etc., the exact time required for measurement of pupil diameters varied. Thus, it was not possible to obtain measurements precisely at predetermined times. The exact times at which measurements were made were recorded, however, and an average time point was calculated based on the test and control eye. For each time point, the difference in pupil diameter (control minus test eye) was calculated. The data were converted to baseline corrected values by subtracting the average baseline difference in pupil diameter, which was calculated, for each experiment, on the basis of readings obtained prior to dosing.

To obtain an estimate of the variance of the response to the aqueous and gel formulations, the study duration was divided into a series of 5 and 10 min intervals. For each formulation, pupillary diameter difference data (obtained from all 4 rabbits) were assigned to an appropriate time interval and the values within each interval were averaged.

## Results and discussion

The results of the study are shown in Fig. 1, where the average differences in pupil diameter are plotted against the midpoints of the time intervals. From this diagram, it can be seen that at all times post-administration, the change in pupil diameter was greater for the poloxamer gel formulation than for the aqueous solution. The general shape of the two profiles, however, was similar. These results indicate that the

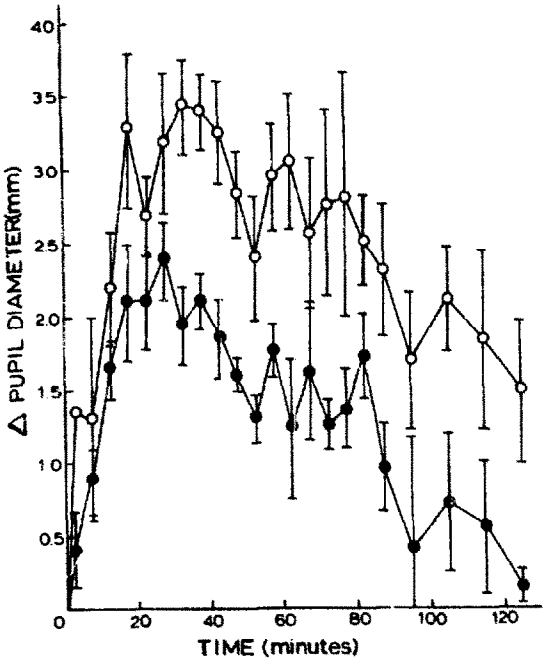


Fig. 1. Change in pupil diameter versus time following the topical administration of 10 μl of $2.5 \times 10^{-1}$ M pilocarpine nitrate formulations; ○, 25% poloxamer 407 gel; ●, aqueous solution. Bars indicate standard error of the mean.

overall miotic response was greater for the poloxamer gel formulation than for the aqueous solution of pilocarpine nitrate. In all cases, rabbits received the same dose of pilocarpine nitrate (678 μg) in a 10 μl dosing volume.

The individual areas under the curve (AUC), for baseline corrected differences in pupilliary diameter as a function of time, were also calculated for each experiment. The results of these calculations are summarized in Table 1. Based on truncated areas (0 → 125 min), results indicated that a 1.9-fold increase in miotic response was

TABLE 1

AREA [a] UNDER THE PUPILLARY DIAMETER [b] VERSUS TIME CURVES (0→125 MIN) FOLLOWING THE TOPICAL DOSING OF 10 μl OF $2.5 \times 10^{-1}$ M PILOCARPINE NITRATE FORMULATIONS

| | Aqueous solution | Poloxamer 407 gel |
|---|---|---|
| Rabbit A | 13.9 | 32.5 |
| Rabbit B | 14.2 | 26.9 |
| Rabbit C | 15.0 | 26.7 |
| Rabbit D | 19.4 | 33.5 |
| Average | 15.6 | 29.9 |

[a] Area was calculated by trapezoidal integration and units are cm·min. [b] Pupillary diameter refers to the difference in diameter between eyes, control minus test eye, corrected for pre-dosing baseline differences in pupil diameter.

obtained with the gel formulation when compared to the aqueous solution. Results of a $t$-test ($\alpha = 0.05$) indicated that the observed difference in area between the two formulations was statistically significant ($P < 0.001$). No attempt was made to estimate the AUCs ($0 \rightarrow \infty$).

Other investigators have shown that the vehicle in which a drug is formulated can effect its ocular bioavailability. Studies by Chrai and Robinson (1974) and Patton and Robinson (1975) indicated that the ocular bioavailability of an aqueous solution of pilocarpine could be increased approximately 2-fold by adding polymers to the solution to increase its viscosity. A later study, by Sieg and Robinson (1977), showed that relative to an aqueous solution of pilocarpine, a 3–4-fold increase in bioavailability could be obtained when the drug was administered in a petrolatum-based ointment. It must be pointed out, however, that in all 3 of those studies, aqueous humor levels of pilocarpine were determined directly and drug concentrations were used to assess ocular bioavailability. Thus, although the enhancement of miotic response observed in this study with poloxamer gel was similar in magnitude to the increase in bioavailability observed with viscous solutions, the results of the studies are not directly comparable. In fact, if the ability of pilocarpine to induce miosis follows a typical log dose–response relationship, the increase in drug bioavailability may actually be greater than the 2-fold difference based on miotic response.

Relative to their physical properties, poloxamer gels possess some characteristics that are ointment-like and others that are similar to those of a viscous, aqueous solution. Poloxamer gels exhibit reverse thermal gelation; their macroscopic viscosity increases with an increase in temperature. Preliminary studies in this laboratory indicate that the rheological properties of poloxamer formulations are markedly dependent on composition and temperature. Shear forces operative in the eye are likely to alter gel viscosity, a property that can affect its disposition.

At room temperature, the poloxamer gels used in this study were soft, semi-solids. It was observed, however, that after dosing, a semi-solid was present in the rabbit's eye for less than 5 min. Thus, it appeared that the aqueous-based gel mixed with and was readily dissolved by the tears. This is in contrast to what has been observed with petrolatum-based ointments. Studies by Sieg and Robinson (1977), using a pilocarpine-containing ointment, indicated that there was limited mixing of the ointment base with the tears and only drug present at the ointment surface was available for absorption.

In conclusion, it is likely that prolongation of contact time is the mechanism responsible for the results observed with the 25% poloxamer gel used in this study. While poloxamer gels appear to be a promising new vehicle for ophthalmic drug delivery, additional studies are required before optimal gel formulations can be developed for specific therapeutic agents. A better understanding is needed of how dynamic processes operative in the eye, such as blinking and tear turnover, influence gel disposition, which, in turn, will govern the rate at which a drug is made available for absorption.

## Acknowledgments

This work was supported in part by a Young Investigator Research Award from the 3M Foundation, St. Paul, MN and a Biomedical Research Support Grant from the National Institutes of Health.

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