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Research paper

The use of small volume ocular sprays to improve the bioavailability of topically applied ophthalmic drugs

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Abstract

It is accepted that the standard eyedrop volume of 25–50 μl is too large to be accommodated within the conjunctival sac, leading to overspill and to rapid drainage into the nasolacrimal duct. This drainage can lead to unwanted systemic absorption of the active agent, which could be avoided if the dropsize were smaller. In this study, we have evaluated the efficiency of small volume ocular sprays in achieving target corneal drug concentrations. A five-way cross-over pharmacodynamic study was initiated using 10 New Zealand white rabbits as the test subjects. The efficacy of a 30 μl instillate of a 1% w/v pilocarpine hydrochloride solution was compared to 5 μl ocular sprays of 1, 2, 3 and 4% w/v solutions. The efficacy of these treatments was determined by measuring the pupillary miotic responses of the rabbits using video imaging. Analysis of the miotic response showed no significant differences between the treatments, e.g. the area under the miosis-time curve for a 30 μl drop of a 1% pilocarpine hydrochloride solution was 3871 c.f 3827 for a 1% spray despite a six-fold reduction in the administered volume and drug loading. This study demonstrates that the spray delivery of 5 μl volumes of pilocarpine hydrochloride, at concentrations of 1, 2, 3 and 4% achieved an equivalent miotic response to that of a 30 μl volume instillate of a 1% solution. © 1997 Elsevier Science B.V.

Keywords: Ocular sprays; Drop volume; Bioavailability; Nasolacrimal drainage-miotic response

1. Introduction

Ocular medication is most frequently administered as eyedrop solutions. The typical volume of an eyedrop has been found to range from between 25 μl and 50 μl, depending on the dispensing nozzle [1]. Under normal conditions, the human tear volume remains relatively constant at around 7 μl with continuous drainage of tear fluid via the nasolacrimal duct balanced by fresh secretions from the tear glands [2]. Without blinking, the tear volume can increase to about 30 μl before overflowing occurs, and the excess fluid is lost, either through nasolacrimal drainage or by spillage onto the cheek. Blinking reduces this maximal volume from 30 μl to < 10 μl, pumping excess volume into the nasolacrimal duct and/or enhancing spillage. The addition of large volumes of liquid, such as those presented in commercial eyedrops, will therefore result in the rapid elimination of the active agents from the eye. Typically 80–90% of an instilled drop is lost in the first minute of dosing [3]. Drug which is drained through the highly vascular and thin-walled nasolacrimal duct can then be rapidly absorbed into the systemic circulation, by-passing hepatic metabolisim

The increasing use of β-blocking agents in ophthalmology has highlighted the disadvantages associated with this rapid absorption process, with serious life threatening side-effects such as bradycardia, bronchospasm and even heart failure being induced in sus-

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ceptible patients [4,5]. Results of studies performed in both rabbits and humans support the hypothesis that administering a given quantity of ophthalmic medication in a reduced drop volume may enhance ocular bioavailability, decrease systemic absorption and improve the therapeutic index of the drug [6–8]. At Scherer DDS, we have evaluated a number of spraying technologies and droplet generation techniques, with the aim of producing a new ophthalmic drug delivery device [9]. A key characteristic of the technology described in this paper is the ability to accurately and reproducibly deliver drug in small volumes over extremely short periods, avoiding delivery to the eyelid during blinking. In this study, a prototype spray device which forces liquid through a small aperture has been used to deliver 5 μl doses of pilocarpine hydrochloride solutions of varying concentration (1, 2, 3 and 4% w/v). The data has been collected using rabbits and the results compared with a standard instillate of 30 μl of a 1% w/v solution. The objective of the study was to determine the minimum amount of drug needed within a 5 μl spray to provide an equivalent miotic response to a standard instillate, reducing the potential risks of systemic absorption and subsequent toxic side-effects.

2. Materials and methods

2.1. Materials

Pilocarpine hydrochloride solutions were obtained from the Bristol Royal Infirmary (UK) in the following concentrations: 1, 2, 3 and 4% w/v. All the solutions were sterile, unpreserved, isotonic, pH 4.5 $\pm$ 0.5, and unbuffered. Minims Fluorescein® (sodium salt BP 1.9% w/v) was manufactured by Chauvin Pharmaceuticals (Romford, U.K). Sterile and non-pyrogenic saline 0.9% w/v (polyfuser grade) was supplied by Fresenius (Basingstoke, UK).

2.2. Test device

The apparatus consisted of a spray nozzle accommodating a molybdenum disc with a 100 μm aperture (Agar Scientific, Essex, UK). The nozzle was connected by P.T.F.E. tubing (0.8 mm internal diameter) to a 2.5 ml Hamilton syringe housed within an aluminium platform and connected to a motorized plunger (see Fig. 1). The motorized plunger was connected to an electronic indexer (Digiplan, model MC 20) which allowed the spray device to propel volumes of liquid ranging from 1 to 100 μl at flow rates from 10 to 200 μl/s.

2.3. Animals and management

Ten young adult male New Zealand white rabbits (Source: Charles River Laboratories) were used in the study. Following arrival the animals were examined to ensure that they were in good general health and free from major physical defects. The animals were allowed to acclimatize for a period of 4–5 days prior to treatment and have free access to food and water. The eyes of each animal were then inspected under a slit lamp using Fluorescein followed by flushing with sterile saline. If no abnormalities were detected, the animals were deemed suitable for inclusion in the study. Prior to dosing, the animals were manually handled over a two day period to condition them to the procedures involved in the study. Each animal was then assigned an identification number and code.

2.4. High speed video imaging

A high speed video camera (Kodak Ekta Pro 1000 HRC operating at 1000 frames/s) was used in a preliminary study to assess the degree of splashback which occurred when ocular sprays impacted upon the eye. The rabbit eyes were manually held open, whilst 5 μl pulses of sterile saline were directed towards the cornea. The degree of splashback was then visually inspected on a monitor in slow motion replay, demonstrating that the degree of splashback was negligible. Additionally, theoretical calculations, based on the formation of uniform spherical droplets, suggested that the number of droplets in a 5 μl volume would be approximately 1300 and thus the loss of a few droplets of liquid could be ignored.

2.5. Study design and dose administration

Animals were randomized to a sequence of 5 treatments. Each animal received each treatment only once. A Williams square design was used so that for all animals, treatment sequences followed a balanced as-

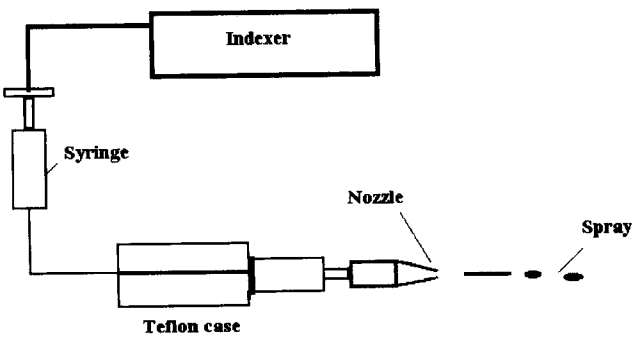

Fig. 1. A schematic diagram of the components of the spray device used in the study.

signment. This design also ensured that each treatment was used in each session an equal number of times. The animals received pilocarpine hydrochloride as a 30 μl instillate of a 1% solution and as a 5 μl spray of 1, 2, 3 and 4% solutions, with the treatments administered into the left eye. A 48 h 'washout period' was introduced between each new dose administration to ensure complete removal of the previous treatment. The right eye was not treated and the total duration of the study was 10 days. The spray device was set to deliver 5 μl doses of drug solution to the centre of the cornea at a flow rate of 100 μl/s over a distance of 2.5 ± 1.0 cm. To facilitate dosing, the animals were manually restrained and the eyelids held open. Massaging of the eyelids was not performed following dose instillation. A BCL 8000 repetitive micropipette (Boehringer Mannheim Diagnostics and Biochemicals) was used to dispense the 30 μl volume into the rabbit eyes. The animals were manually restrained, the head tilted and the eye held open to ensure effective delivery of the eye drop. Following dose instillation, the eyelids were not massaged.

2.6. Measurements of miosis

A metallic rule with a circular aperture of known diameter was orientated perpendicularly to, and at an appropriate fixed distance from, a video camera fitted with a macro lens (Sony V8 Pro-CDD-V100E) throughout the study [10]. During miotic measurements, the animals were positioned such that the left eye was parallel to the ruler and equidistant from the video camera. The video camera was actuated to project and amplify images of both the reference aperture and left eye onto the monitor screen. The diameters of both the reference aperture and pupil were then measured on the screen using a ruler placed on the projected image at an angle of approximately 135°–305°. The value of the pupil diameter was then calculated by multiplying the projected screen pupil diameter by the ratio of the actual reference diameter (8 mm) to the projected screen reference diameter (18 mm).

Pupil measurements were taken at c. 60, 45, 30 and 15 min intervals prior to the administration of pilocarpine hydrochloride solution and at 15 min intervals for the first hour after dosing. Thereafter, the pupil diameter was measured for the left eye at 30 min intervals for a minimum duration of 4 h after dose administration.

2.7. Data-analysis

For the purpose of statistical analysis, the primary endpoint was defined to be the pupillary diameter in the test eye at each time point. The primary endpoint was determined using analysis of variance (ANOVA) including terms for animal, treatment, session, time and the following interactions: session by animal, time by session, time by animal and time by treatment. In addition the following parameters were determined: RR_{max} (the maximum percentage change in pupil diameter), T_{max} (the first time point at which the smallest pupillary diameter was observed) and $AUC_{(0\text{-}4h)}$ (the area under the pupillary diameter vs time curve between 0 and 4 h).

All significance tests were two-tailed and were performed at the 5% significance level. The statistical software SAS V607 and the PROC GLM procedure was used in the analysis.

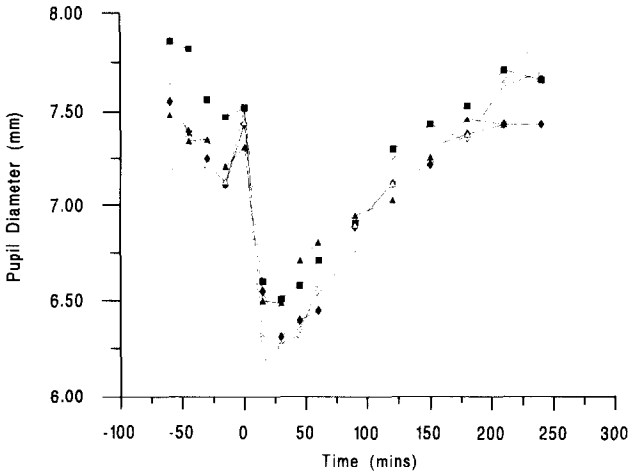

Fig. 2. A plot of the pupil diameter measurements versus time before and after administration of the following pilocarpine hydrochloride formulations: (■) 30 μl of 1% solution, (▲) 5 μl spray of 1% solution, (◆) 5 μl spray of 2% solution, (x) 5 μl spray of 3% solution and (△) 5 μl spray of 4% solution.

3. Results

Fig. 2 summarises the pupil diameter measurements over the time course of the experiments. Some variation in the pupil diameter could be seen in the predose data, with a significant ($P = 0.0001$) decrease in mean diameter being observed for all treatments as a function of time. The Shapiro-Wilk test for normality had a P-value of 0.9723 revealing that the errors associated with the pupil diameter readings were independently and normally distributed. Fig. 3 shows the changes in the pupil diameter. Following pilocarpine administration, where the pupil diameter at time 0 is just prior to dosing, a reduction in pupil diameter was evident for all dose forms at the 15 min time point. A similar reduction to that achieved by the 30 μl 1% instillate was seen for the 1% and 2% 5 μl ocular sprays, whilst a slightly enhanced miotic effect was seen for the 3% and 4% sprays. At 30, 45 and 60 min time points, the 2, 3 and 4% sprays maintained similar miotic responses with the 1% sprays being slightly less effective. At 90 min the

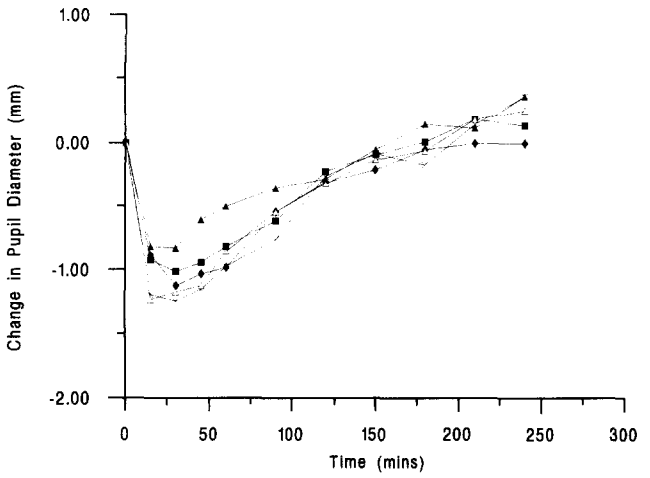

Fig. 3. A plot of the change in pupil diameter (pupil diameter at time t – pupil diameter at time, 0) versus time after the administration of the following pilocarpine hydrochloride formulations: (■) 30 μl of 1% solution, (▲) 5 μl spray of 1% solution, (◆) 5 μl spray of 2% solution, (x) 5 μl spray of 3% solution and (△) 5 μl spray of 4% solution.

level of miosis was similar for all concentrations. Thereafter, pupil diameter increased until predose values were achieved, approximately 120–150 min after dosing for the 5 μl ocular sprays, and approximately 180 min for the 30 μl instillate.

Calculated values of AUC were similar across all dosage administrations. Some variations were apparent for the T_{max} with that of the 2% pilocarpine hydrochloride ocular spray being noticeably longer than that of other formulations. RR_{max} values were similar for the 2, 3 and 4% ocular sprays, achieving 17–18% reduction in pupil diameter. In comparison, a 15% reduction was obtained with the 30 μl instillate of a 1% pilocarpine solution and a RR_{max} value of 12% was determined for the 1% spray (see Table 1).

These results demonstrate that spray delivery of 5 μl volumes of pilocarpine hydrochloride at concentrations of 1, 2, 3 and 4% achieved an equivalent miotic response to that of a 1% pilocarpine hydrochloride instillate of 30 μl volume.

4. Discussion

In spite of the variety of β-blocking agents and carbonic anhydrase inhibitors that exist for the treatment of glaucoma, pilocarpine has remained the first line drug for this condition. Pilocarpine is usually administered as eyedrops and acts by increasing the drainage angle of the trabecular meshwork by contraction of the ciliary muscle. However, problems with compliance may occur because of a short duration of action (2–4 h) and side-effects including brow aches and a loss of accommodative reflexes [11]. Robinson et al. [6,7,12–15] have systematically investigated the precorneal physiological factors responsible for the low ocular bioavailability of topically applied pilocarpine. Factors such as rapid drainage rates, reflex tearing, effects of conjunctival absorption, vasodilation effects of pilocarpine and nonconjunctival routes of elimination (such as the presence of a nictitating membrane in rabbits) were identified as important parameters, with drainage effects accounting for most of the reduced bioavailability. As a consequence of these findings it was predicated that a higher concentration of drug in as small a volume as is practicable would be beneficial. The principle was elegantly demonstrated by Patton and Francoeur [16] who reported equivalent bioavailability of pilocarpine nitrate solutions in the rabbit following ocular instillation of 25 μl of a 0.010M solution (67.8 μg) or 5 μl of a 0.0192M solution (26 μg).

Klein et al. [17] demonstrated a dose-dependent intra-ocular pressure reduction following topical ocular administration of a gel-based formulation containing between 0.7 and 3.4% pilocarpine nitrate. Furthermore, Tanzer et al. [18] showed dose linearity of the pharmacokinetic variables at oral doses between 0.3 and 3.5 MG in man. These data support our contention that the amounts administered are likely to produce a dose-dependent pharmacodynamic response.

In addition to the drop size, the nature of the administered vehicle also affects the bioavailability of ophthalmic formulations. For example; variations from physiological tonicity and pH excipients (e.g., buffers) and the presence of preservatives have all been shown to induce lacrimation due to their irritant effects [19–

Table 1
Summary statistics from pupillary diameter versus time profiles using rabbits ($n = 10$)

Pilocarpine treatment	$AUC_{(0\text{-}240)}$ (mm. min)	T_{max} (min)	RR_{max} (%)
1% 30 μl drop (300 μg dose)	3871 ± 340*	25.50 ± 12.50*	15.22 ± 3.96*
1% 5 μl spray (50 μg dose)	3827 ± 312	24.00 ± 23.66	12.30 ± 5.15
2% 5 μl spray (100 μg dose)	3778 ± 397	34.50 ± 15.89	17.22 ± 5.92
3% 5 μl spray (150 μg dose)	3819 ± 361	21.00 ± 10.49	17.94 ± 6.48
4% 5 μl spray (200 μg dose)	3800 ± 327	19.50 ± 10.12	17.20 ± 6.52

*Values are the standard deviations of the mean.

21]. Induced tearing decreases the concentration of the drug and increases the volume of the lacrimal lake, which in turn increases the drainage rate. In the present study, the pilocarpine solutions were isotonic, unpreserved, unbuffered and of pH 4.5 ± 0.5. The mildly acidic pH is required for stability purposes since pilocarpine is a weak base susceptible to decomposition in alkaline environment [22]. The buffering power of tears is quite low and, therefore, when small (5 μl) volumes of pilocarpine solutions are administered to the eye, adjustment to physiological pH will occur more rapidly than with a 30 μl drop. The drug will therefore be converted more rapidly to the absorbable base form. Additionally, a more rapid adjustment to physiological pH would be expected to decrease induced blinking and lacrimation.

There is a paucity of literature concerning the performance of low volume ocular sprays, although the concept is not entirely new and several ophthalmologists have proposed the spray as a method of increasing the bioavailability of ocular medications [23]. There is little consensus on the volume, although 7–8 μl has been suggested as the ideal volume for an instillate. However, instillates are normally administered directly into the conjunctival sac, with reflex blinking distributing the majority of the solution over the cornea. For small volume instillates, a substantial proportion of the solution may still empty directly into the nasolacrimal drainage system. In contrast, the work investigated in this paper demonstrated that during application of a spray directly onto the cornea, the solution uniformly covered the corneal surface with minimal splash-back upon impact. A gradual pooling of liquid towards the conjunctival sac occurred, but this was distributed over the corneal surface by reflex blinking. Recent studies using high speed video have confirmed that the delivery of the spray is fast enough to beat the blink response in man. Our data indicates that the ocular bioavailability of pilocarpine hydrochloride (assessed by its miotic activity) was maintained when the drop volume, and consequently the drug dosage was reduced by a factor of six. For example, the AUC for a 30 μl drop of a 1% pilocarpine hydrochloride solution was 3871 c.f 3827 for a 1% solution delivered as a 5 μl ocular spray. Therefore, it would appear that drug is utilised for action on the eye more effectively and the ratio of ocular to systemic administration is increased via the use of a low volume ocular spray instead of a conventional eyedrop.

In conclusion, the work reported in this paper has demonstrated that a small volume ocular spray produced an equivalent pharmacodynamic effect to a standard eyedrop, with the same concentration of drug. It is also interesting to note that equivalent miotic responses were seen for a range of sprays with different pilocarpine concentrations. This may have reflected saturation of the pilocarpine receptors in the iris-ciliary body, with all treatments, Thus, in order to more clearly define the relationship between drug concentration and ocular bioavailability for small volume sprays, further studies in which the ocular drug concentration is monitored directly are needed.

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