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Pharmacokinetic Evidence for Improved Ophthalmic Drug Delivery by Reduction of Instilled Volume

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Abstract □ The bioavailability of topically applied pilocarpine nitrate was studied as a function of instilled volume. As the instilled volume decreased, the fraction of dose absorbed increased. The relationship between fraction absorbed and instilled volume was not direct, but appropriate adjustment of instilled volume and concentration should permit substantial dosage reductions without sacrifice of drug concentration in the eye. The implications of these findings from both a therapeutic and toxicity standpoint are discussed.

Keyphrases □ Pilocarpine nitrate—bioavailability, effect of instilled volume □ Bioavailability—pilocarpine nitrate, effect of instilled volume □ Ophthalmic cholinergics—pilocarpine nitrate, bioavailability, effect of instilled volume

The drainage of an instilled drug solution away from the eye is considerable and can affect its biological activity (1). This drainage is dependent upon the volume instilled and increases linearly with instilled volume over a large range (1). This fact (2, 3) has been incorporated into experimental design. However, the concept of reducing drop size to improve ophthalmic drug delivery has not become widespread, perhaps because the pharmacokinetic advantages of such an approach have not been emphasized.

A study of the dynamics of instilled fluid drainage (1) showed that the miotic activity of pilocarpine was enhanced by administering the same amount of pilocarpine in decreasing volumes. No actual measurements of drug penetration to the interior of the eye were made. More recent studies showed that the bioavailability of ophthalmic drugs was improved by slowing the rate of drainage of instilled drug solutions (4–8). The measurement of drug concentration in the eye following the reduction of instilled volume and the resultant pharmacokinetic parameters were not reported previously.

In a recent study (9), the aqueous chamber drug distribution volume for ophthalmic drugs was calculated, thereby making it possible to define more precisely the pharmacokinetics of topically applied ophthalmic drugs. Therefore, the purpose of the present study is to show that, as the instilled volume of pilocarpine nitrate is decreased, the fraction of dose absorbed increases. As a result, the parameters of instilled volume and drug concentration can be quantitated to provide a desired level of drug in the eye.

EXPERIMENTAL

Pilocarpine Solutions—Pilocarpine nitrate was used as received[1]. Tritiated pilocarpine[2], specific activity 4.1 Ci/mmole, was purified prior to use by evaporation as recommended previously (10). The concentration of pilocarpine nitrate used was 1×10^{-2} *M* in all studies. Solutions were prepared in isotonic pH 6.24 Sorensen phosphate buffer and were filtered but not sterile. Procedures for the preparation of the tritiated solutions were described previously (10).

Methods—Male, New Zealand, albino rabbits, 60–67 days old, were used. Prior to experimentation, the animals were maintained in standard laboratory animal cages and allowed food and water *ad libitum*. During the experiments, all test animals were kept in restraining boxes in the normal upright position. The head was unencumbered so that all normal eye movements were maintained. The animals were unanesthetized in all cases.

The volumes of instilled solutions administered were 5, 10, 15, and 25 μl and were delivered accurately with a microliter syringe[3]. At various times postinstillation (5, 10, 15, 20, 30, 45, 60, 90, and 120 min), rabbits were sacrificed with an overdose of pentobarbital sodium injected into a marginal ear vein. Eyes were immediately rinsed and blotted, and aqueous humor (at least 100 μl) was aspirated from the anterior chamber.

One hundred microliters of aqueous humor was quantitatively transferred[4] to scintillation counting vials[5] containing 5 ml of prerefrigerated liquid scintillation cocktail[6]. After storage in the dark at room temperature for at least 24 hr, samples were counted[7]. These counts were converted to micrograms of pilocarpine per milliliter of aqueous humor using suitable standard and blank corrections.

RESULTS

Aqueous humor concentration–time profiles of pilocarpine for the four instilled volumes are shown in Table I. These data were plotted semilogarithmically, and the terminal slopes were calculated *via* linear regression analysis. From these slopes, the elimination rate constants were calculated. By using the trapezoidal rule with extrapolation to infinity, the areas under the aqueous humor concentration–time profiles were calculated (Table II). The apparent maximum in area under the curve with an instilled volume of 15 μl is currently unexplained. This maximum also was observed in other experiments and will be discussed in more detail later.

Using rabbits of similar size and age, Conrad and Robinson (9) calculated the aqueous humor distribution volume of pilocarpine to be 0.575

[1] Sigma Chemical Co., St. Louis, Mo.
[2] New England Nuclear, Boston, Mass.
[3] Hamilton Co., Reno, Nev.
[4] Biopette, Schwarz/Mann, Orangeburg, N.Y.
[5] Mini-vial, ICN Isotope and Nuclear Division, Cleveland, Ohio.
[6] Aquasol, New England Nuclear, Boston, Mass.
[7] Model LS-150 liquid scintillation counter, Beckman Instruments, Fullerton, Calif.

Table I—Aqueous Humor Concentration [a] (Micrograms per Milliliter)–Time Profiles following Instillation of Various Volumes of 1×10^{-2} *M* Pilocarpine Nitrate in Rabbits

Minutes	Volume Instilled, μl			
	5	10	15	25 [b]
5	0.17 (0.03)	0.29 (0.03)	0.29 (0.03)	0.55 (0.06)
10	0.47 (0.05)	0.44 (0.02)	0.79 (0.10)	1.03 (0.14)
15	0.48 (0.07)	0.65 (0.04)	1.03 (0.09)	1.11 (0.11)
20	0.51 (0.05)	0.88 (0.11)	1.16 (0.12)	0.98 (0.12)
30	0.50 (0.03)	0.74 (0.11)	0.92 (0.18)	0.93 (0.12)
45	0.38 (0.05)	0.49 (0.05)	0.67 (0.10)	0.42 (0.04)
60	0.26 (0.03)	0.36 (0.05)	0.33 (0.04)	0.44 (0.04)
90	0.17 (0.02)	0.22 (0.02)	0.21 (0.03)	0.19 (0.02)
120	0.08 (0.01)	0.11 (0.02)	0.15 (0.03)	0.11 (0.01)

[a] Mean with standard error in parentheses. Each point is the mean of at least seven determinations. [b] Data taken from Ref. 11.

ml. When this volume was applied to the data reported here, the fraction of dose absorbed (*F*) for each volume studied, 5, 10, 15, and 25 μl, was 3.01×10^{-2}, 2.23×10^{-2}, 1.92×10^{-2}, and 1.16×10^{-2}, respectively.

DISCUSSION

The smaller the instilled volume, the greater is the fraction of applied dose absorbed. These results were implied previously (1) but were not demonstrated pharmacokinetically. Drainage rate is linear with instilled volume over a fairly wide range (1), but the relationship between fraction absorbed and volume is more complex due to other complicating factors. For example, decreases in the drainage rate (*i.e.*, increasing drug contact time) did not result in proportional increases in aqueous humor levels of drug (7, 8).

This result is true also with the fraction of dose absorbed, as seen from the calculated values. A fivefold decrease in volume results in approximately a threefold increase in fraction absorbed, possibly due to competing routes of drug absorption. For a topically applied drug to reach the aqueous humor, it must penetrate the cornea, but conjunctival or nonproductive absorption also occurs and competes with the cornea for drug uptake (8). Drug absorbed by this nonproductive route is not thought to reach the anterior chamber.

What is apparent from this study is that a considerable decrease in instilled volume coupled with a slight increase in instilled concentration should result in the same amount of drug reaching the interior of the eye as if much larger volumes were administered. A simple calculation illustrates this point. Twenty-five microliters of a 1×10^{-2} *M* pilocarpine nitrate solution resulted in an area under the curve of 60.9 μg min ml^{-1}. The dose administered was 67.82 μg, and the fraction absorbed was 1.16×10^{-2} *M*. If one wishes to match this area with a dose of 5 μl, the following equation allows the calculation to be made:

$$A = \frac{FD}{VK} \qquad \text{(Eq. 1)}$$

or, rearranged:

$$D = \frac{AVK}{F} \qquad \text{(Eq. 2)}$$

The value of *F* for the 5-μl case is 3.01×10^{-2}, and $K = 1.88 \times 10^{-2}$ min^{-1}. The area desired is 60.9 μg min ml^{-1}, and the value for *V* is 0.575 ml (9). The calculated value for *D* is 21.87 μg, which corresponds to a concentration of 1.61×10^{-2} *M*. A 5-μl dose of 1.61×10^{-2} *M* pilocarpine nitrate should result in the same area under the curve as a 25-μl dose of 1.00×10^{-2} *M* pilocarpine nitrate.

Therapeutically, it should be possible, by reducing the instilled volume by a factor of five, to reduce the dose administered by approximately a factor of three without altering drug concentration at the active site. This finding is significant and shows that the doses currently used for ophthalmic drugs are generally much larger than required. Potentially more important, however, are the implications from a toxicity standpoint. In both the 5- and 25-μl cases, only a small fraction of the dose is absorbed into the eye, the remainder being lost by various routes and potentially available to act systemically. By reducing the instilled volume and, hence, substantially decreasing the applied dose, the potential for toxic effects is reduced while drug concentration in the eye is maintained.

By using the data presented here, other combinations of volume and concentration adjustments are possible. Studies are currently in progress to confirm these findings and determine any complicating factors. Any rational approach to the development of ophthalmic dosage regimens should consider the excessive volumes currently used and the advantages from the development of better, small volume delivery systems.

Table II—Elimination Rate Constants and Areas under the Aqueous Humor Concentration–Time Profiles following Instillation of Various Volumes of 1×10^{-2} *M* Pilocarpine Nitrate in Rabbits

Volume Instilled, μl	Dose, μg	*K*, min^{-1}	Area, μg min ml^{-1}
5	13.56	1.88×10^{-2}	37.8
10	27.13	2.05×10^{-2}	51.3
15	40.69	2.13×10^{-2}	63.8
25	67.82	2.25×10^{-2}	60.9

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