Biophasic Availability of Ophthalmic Carbachol I: Mechanisms of Cationic Polymer- and Surfactant-Promoted Miotic Activity

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Abstract ☐ The influence of the topical application of cationic benzalkonium chloride and diethylaminoethyl dextran (mol. wt. $\simeq 2 \times 10^6$) adjuvants on the time variation of the miotic response intensity elicited by intraconjunctivally and intraaqueously administered carbachol was studied. The inclusion of either adjuvant in a 0.1% carbachol ophthalmic solution topically administered in the eye was observed to augment the miotic response activity attributable to direct transcorneal absorption into the biophase of the treated eye; the effect was nearly comparable to that achieved with similar dosing of a 2.0% solution containing carbachol alone. Simultaneous miotic activity in untreated control eyes of the rabbits, as well as other cholinergic side effects observed with the 2.0% carbachol solutions, did not occur with the 0.1% adjuvant-containing solutions, indicating that the enhanced local effects were not accompanied by a concomitantly promoted undesirable systemic absorption. The topical application of the adjuvants to the cornea following injection of the carbachol into the anterior chamber of the eye also appreciably promoted the miotic activity of the drug when it was administered by this mode. The principal mechanism of the observed adjuvant effects on carbachol biophasic availability is postulated as attributable to a promoted tissue permeability and release of the drug from corneal tissue binding sites; these occurrences result from an inductively implemented diminution of carbachol tissue binding affinities. The inductive effects originate and are propagated from fixed anionic corneal sites which interact with the cationic adjuvants. The impermeability of the cornea prevents the penetration of the adjuvant molecules to the deeper tissue layers where they could more directly influence carbachol tissue interactions.

Keyphrases ☐ Carbachol ophthalmic solutions—effect of cationic adjuvants on miotic activity ☐ Adjuvants, cationic—effect on miotic activity of carbachol solutions ☐ Miosis—effect of cationic adjuvants on carbachol activity ☐ Cationic adjuvant effect—miosis of carbachol ophthalmic solutions ☐ Ophthalmic solutions, carbachol—effect of cationic adjuvants on miotic activity ☐ Benzalkonium chloride—effect on miotic activity of carbachol solutions ☐ Diethylaminoethyl dextran—effect on miotic activity of carbachol solutions

Studies in this laboratory have been directed toward the elucidation of factors that influence the dynamic pharmacological response behavior of ophthalmic drugs (1–9) and the biophysical mechanisms by which they are operative (3, 6, 9). Since they are salutary to the development of an ultimate predictive capability with respect to the optimal control (7, 8) of drug response dynamics, quantitative treatments of observed phenomena in terms of pharmacokinetic and biophysical models have been developed and implemented whenever practicable. The requisite quantitative data have been derived from the implementation of two unique experimental methodologies, which allow results generally considered to be attainable only by destructive and *in vitro* techniques to be derived *in vivo* under normal physiological drug product use conditions without causing any injury. These methods thereby allow repeated measurements to be performed on the same subjects which can also serve as their own control. These techniques include a pharmacological method of drug absorption analysis (1–8, 10, 11) and a bioelectrometric method (9, 12–18) for the study of drug and other solute interactions with tissues or any other electrolytically conducting materials.

The bioelectrometric technique permits the determination of adsorption isotherms (12, 18) to describe drug–tissue interactions and the performance of kinetic studies of sorption and desorption (12, 18); the results can be quantitatively characterized in terms of thermodynamic and kinetic parameters (9, 12, 14, 18). The interpretation of results provided by the bioelectrometric method, concerning the dependency of the fixed-charge density of tissue surfaces on the composition of applied drug formulations, provides valuable insights into the submolecular mechanisms operative in drug–tissue interactions and transport across biological barriers; such information is generally unattainable by other techniques.

The use of transformed temporal pharmacological data (10) coupled with conventional pharmacokinetic approaches and/or techniques of engineering systems analysis and optimization (7, 8) provides a powerful approach to performing drug absorption analysis (1–5). The use of pharmacological data, as contrasted to the use of direct assay data derived by chemical or radiological assay, in addition to allowing the time course and rates of cumulative amounts of drug absorbed systemically to be determined, also permits the biophasic availability of the drug, *i.e.*, the amounts of drug that gain access to their sites of action, to be studied for any route of administration. The capability to compute the time course of drug absorption to a vicinal biophase adjacent to a site of topical administration, as well as the drug absorption profile for the quantities of drugs that simultaneously become systemically absorbed, renders this approach particularly valuable for ophthalmic drug bioavailability studies (1–6). Similar to the bioelectrometric method for the study of drug–tissue interactions, the use of pharmacological data for the study of drug transport is nondestructive and noninvasive.

The present report is the first of a series concerned with the kinetics and mechanisms of the biophasic availability of ophthalmically administered carbachol. Carbachol is an effective drug for the treatment of glaucoma. The seemingly erratic and sensitive dependency of its pharmacological response behavior on the formulation of the ophthalmic vehicle has contributed to its not being more commonly employed. However, it was hypothesized that the same and similar formula-
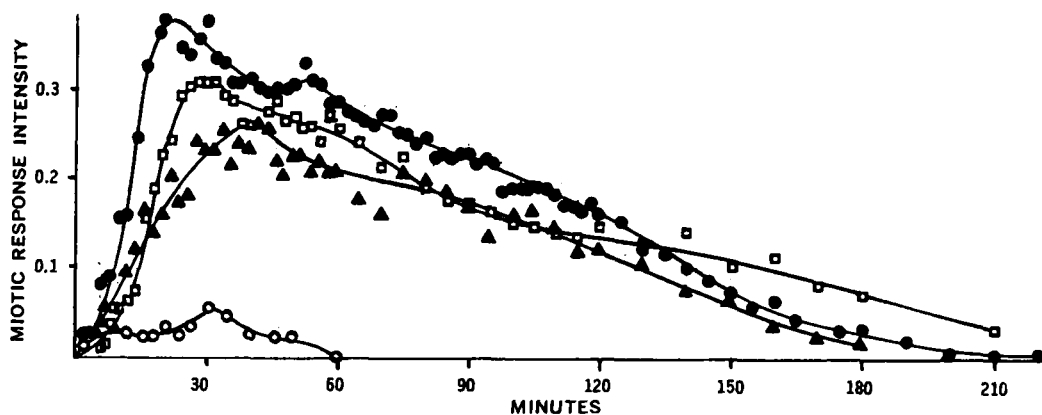
Figure 1—*Time variation of miotic response intensity following topical administration of 0.1 ml. of 0.1% carbachol* (O), *2.0% carbachol* (●), *and 0.1% carbachol solutions containing 0.02% benzalkonium chloride* (▲) *and 0.06% diethylaminoethyl dextran* (□). *Each point represents the average of four replications performed on separate rabbits.*

tion factors to those responsible for the apparent erratic response behavior of ophthalmic carbachol, when properly understood, could conceivably provide the means by which to implement an optimal predictive control of the pharmacological response dynamics; this capability would permit a rational approach to the design of carbachol ophthalmic vehicle formulations.

The present report describes the results of relatively simple preliminary experiments concerned with the gross mechanistic nature of the very appreciable enhancement of the miotic activity observed to be induced by the inclusion of benzalkonium chloride (I) and diethylaminoethyl dextran (II) in carbachol ophthalmic vehicles. Subsequent reports will describe the quantitation of such results through the implementation of the bioelectrometric technique and the pharmacological method of drug bioavailability analysis.

EXPERIMENTAL

Materials—The ophthalmic solutions containing carbachol[1], I[2], and II[3], which were employed for topical and/or intraaqueous administration to the eye, were each prepared in a pH 7.4, isotonic phosphate buffer containing 0.1884 g./l. $NaH_2PO_4 \cdot H_2O$, 1.8293 g./l. $Na_2HPO_4 \cdot 7H_2O$, and 7.8910 g./l. NaCl. Distilled water was used and all the inorganic components were of reagent grade. The cationic dextran polymer has a supplier-reported molecular weight of approximately 2×10^6. The molecular weight of I was determined in this laboratory as 370 and 370.2 by UV spectrophotometry and chloride electroanalysis, respectively. It possessed a *critical micelle concentration*, determined by a liquid junction potential technique (19), of 8×10^{-4} M. The carbachol was at least USP grade. The solutions were prepared fresh prior to experimentation.

The apparent equivalent weight of the cationic polymer, II, was determined from Donnan potential measurements to have a value of 704 g. per equivalent of charge. The method involved separating a 5% (w/v) solution of II in pH 7.4 buffer from the buffered vehicle itself across a dialyzing membrane[4]. Dialysis equilibrium was achieved by storing the dialysis cell arrangement in a 37° water bath for 30 hr. with constant agitation until the Donnan potential, measured with a digital pH/voltmeter[5] using saturated calomel electrodes[6], became constant. The concentration of cationic charge in the solution resulting from the polymer and its equivalent weight was computed using previously reported (9, 12) theoretical expressions. By using these results, the 0.06% (w/v) II solution used in the present studies may be computed to be 8.5×10^{-4} N

in polymer-contributed cation c charge. The normalities of the 0.02 and 0.03% I solutions are 5.4×10^{-4} and 8.1×10^{-4} equivalents of cationic charge per liter, respectively.

Approximately 4-month-old, male, New Zealand white rabbits, weighing 3.3–3.6 kg., were used as experimental animals. Twenty rabbits were screened on the basis of their similitude in pupillary accommodation to varying light intensity, magnitude of peak miotic response to test doses of carbachol administered intravenously, and clarity of pupil definition. Four of the rabbits were selected for use in the study.

Topical Administration to the Eye—The solutions administered intraconjunctivally to the rabbits contained 0.1 or 2.0% carbachol or 0.1% carbachol with 0.03% I or 0.06% (w/v) II. Solutions of 0.03% I and 0.06% II alone were also administered topically following intraaqueous dosing with carbachol.

Topical doses of 0.1 ml. of solution were instilled into the rabbit's eye using a 1.0-ml. syringe; the lower eyelid was retracted from the cornea and the solution was dropped into the conjunctival sac. The eyelids were held closed for 30–45 sec. following instillation to keep the rabbit from blinking, thereby preventing a large initial loss of the solution from the eye.

Intraaqueous Administration of Carbachol—Approximately 1 ml. of a 1%, isotonic, xylocaine solution[7] was dropped into the rabbit's eye to anesthetize the cornea. A 4-mcg./kg. dose of carbachol, contained in volumes of 0.05% (w/v) solutions ranging between 0.020 and 0.029 ml., was carefully injected through the cornea into the anterior chamber of the right eye. A similar injection of the same volume of solution vehicle alone provided a control. The influence of the adjuvants was studied by the topical instillation of 0.1 ml. of the 0.03% I or 0.06% II solution immediately following the intraaqueous injection.

Miotic Response Monitoring—The details of monitoring the time variation of pupillary diameters following dosing of rabbits were reported previously (1, 2). Briefly, however, the rabbits were restrained in a box during the procedure in which the diameter of the pupil was measured with a vernier caliper supported at a constant distance from the eye. A standard initial value of 4.5 mm. was obtained by adjusting the light intensity produced by two microscope lamps[8] using a rheostat. Subsequently, the eye was illuminated only during the time of the measurements (30–45 sec.) to avoid accommodation of the pupil to the light. The minimum pupil diameter during the period of illumination was recorded along with the time at which it was observed. Experience has demonstrated that this procedure, coupled with screening the rabbits prior to their selection for the study, considerably decreases both intrasubject and intersubject variations in the results. The miotic response intensity, I, was computed as: $I = 1 - (d_t/4.5)$, where d_t is the pupillary response at time t following dosing, and the value of 4.5 refers to the millimeter value of the pupillary diameter at time zero. The overall average relative error in the values of I obtained in this study is less than 6%. Since the variation in replicate experiments performed on the same animals was of the same magnitude as for replicates performed on different rabbits, results from different animals could be combined as averages.

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[1] Alcon Labs, Inc., Fort Worth, Tex.
[2] K & K Labs., Plainsview, N. Y.
[3] Pharmacia, Uppsala, Sweden.
[4] Brasite Machine Co., New York, N. Y.
[5] Orion model 601.
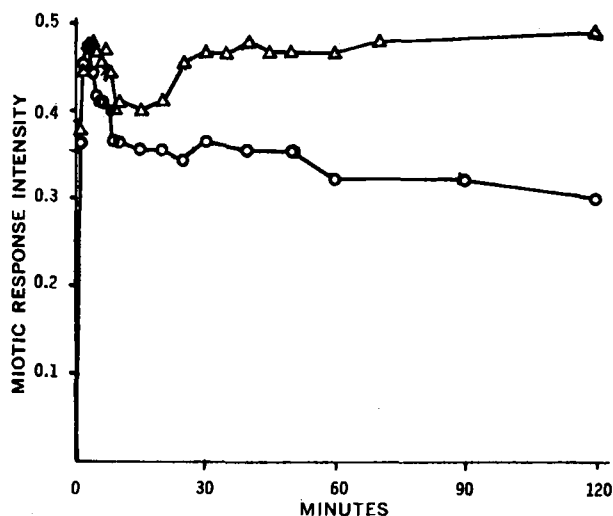[6] Beckman.

[7] Astra Pharmaceutical.
[8] Bausch & Lomb.

Figure 2—*Time variation of miotic response intensity following intra-queous administration of 4 mcg./kg. of carbachol to two rabbits with (Δ) and without (O) the topical administration of 0.1 ml. of 0.03% benzalkonium chloride immediately afterward.*

Figure 3—*Time variation of miotic response intensity following intra-aqueous administration of 4 mcg./kg. of carbachol to two rabbits with (Δ) and without (O) the topical administration of 0.1 ml. of 0.06% diethylaminoethyl dextran immediately afterward.*

RESULTS AND DISCUSSION

Adjuvant-Promoted Miotic Activity of Topically Administered Carbachol—Figure 1 presents the results of monitoring the time variation of miotic response following intraconjunctival administration. The results represent the averages of four replications performed on separate animals. The lowest curve depicts the miotic activity observed following the administration of 0.1% carbachol, which may be compared to the approximately eightfold increase in peak response intensity observed with an equal volume dose of a 2.0% carbachol solution. The middle curves demonstrate the influence of the inclusion of 0.02% I or 0.06% II in the ophthalmic solutions. The response *versus* time profiles clearly demonstrate the appreciable enhancement of miotic activity induced by the relatively small concentrations of these cationic adjuvants. Concentrations of II other than 0.06% were not studied. However, increasing the concentration of I beyond 0.02% up to 0.04%, which was the maximum level studied, causes further, although less pronounced, enhancement to the point where the response to the 0.1% carbachol solution is approximately tantamount to the results of administering the 2.0% carbachol solution alone. The details of all results with I will be presented in a subsequent report in the present series. It may be expected that an increase in the concentration of II would behave similarly to I in augmenting the response.

Except for topical dosing with the 2.0% carbachol solution without adjuvants, which produced a peak response in the left eye of 0.055-0.06 unit and a duration of approximately 100 min., the 0.1% carbachol solutions with and without adjuvants did not elicit any miotic activity in the left eyes of the rabbits used as car-bachol-untreated controls. This observation and the observed relative lack of other systemic effects clearly indicate that the adjuvant-induced promotion of the pharmacological activity is attributable to an enhanced transcorneal biophasic availability directly to the sites of action as compared to an increase in the systemic bio-availability as can occur through schleral absorption, aqueous flow, and volume loss through the lacrimal-nasal drainage duct (3, 5, 6). By the latter routes, the drug enters the systemic circulation prior to gaining access to its biophase. Such systemic absorption is obviously undesirable because of the cholinergic side effects that can result from the systemic presence of carbachol. Inclusion of the adjuvants in the topically administered 0.1% carbachol solutions induced a local pharmacological activity nearly equivalent to the 2.0% carbachol, without apparent systemic activity. The significance of this observation with respect to the possibilities for the formulation of both safer and more effective carbachol ophthalmic preparations is apparent.

Adjuvant-Promoted Miotic Activity of Intraaqueous Injected Carbachol—Figure 2 presents the average results of the intraaqueous administration of 4-mcg./kg. doses of carbachol alone (lower curve) and the same dose followed by the topical administration of 0.1
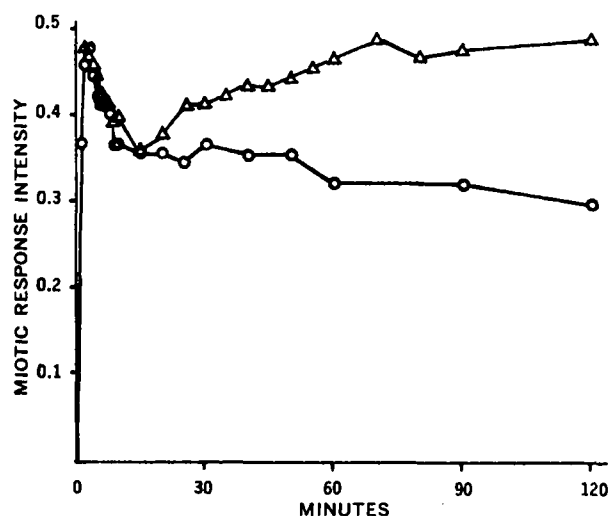
ml. of 0.03% I (upper plot). Intraaqueous dosing results similarly obtained with the topical administration of II are shown in Fig. 3. In each case, a peak miotic response intensity was observed within 2-4 min. following dosing, after which an initially rapid decline in effect was apparent. The effects of the treatment with intraaqueous carbachol alone continued to diminish in a second phase, appearing between approximately 5 and 10 min., during which the decay was considerably slower. However, the miotic response observed in ad-juvant-treated eyes again rose to a secondary maximum and plateau for a prolonged period in excess of the 180-min. interval over which the miotic activity was monitored. The pattern and magnitude of the influence of I and II were similar. Although the molar concentrations of I and II solutions are quite different, their normality with respect to cationic charge is nearly the same.

Mechanisms of Adjuvant-Promoted Miotic Activity—The topical application of the cationic adjuvants to the cornea has been demonstrated by the discussed results to augment the miotic activity of both topically and intraaqueously administered carbachol. Non-specific physical mechanisms operative at the corneal surface—such as an increased viscosity of the vehicle or a Donnan exclusion effect which may be expected to be imparted by the positively charged polymeric II, or a lowering of surface tension attributable to the inclusion of the surface-active I—which could conceivably contribute to promoting the transcorneal absorption of carbachol following topical dosing, cannot reasonably be considered responsible for the enhanced activity observed following intraaqueous administration. Any explication in terms of a principal mechanism common to both routes of administration must obviously account for apparent transmission of the influence of the adjuvants through the thickness of the cornea to effect the disposition of the bolus of carbachol injected directly into the anterior chamber in such a manner as to promote the availability of pharmacologically active drug to its sites of action. The adjuvants themselves are miotically inactive; any appreciable penetration of the adjuvants across the minute lesional area in the periphery of the cornea resulting from the needle puncture may be considered as unlikely. The permeation of the highly charged II, possessing a molecular weight of approximately 2 million, across the corneal barrier to influence directly the interaction of carbachol with deeper tissue structures and thereby effect its disposition is untenable.

As a remaining alternative, it may be hypothesized that it is not necessary for the adjuvant molecules themselves to be physically transported into and through the cornea to effect changes in the nature of the interaction of carbachol with affected tissue structures; it is only required for the cationic adjuvants to interact with anionic corneal tissue surface sites. Such interactions can allosterically operate to induce changes which are inductively propagated (20, 21) by delocalization of mobile electrons to neighboring sites and deeper tissue levels where they alter tissue binding affinities for carbachol. These changes, in turn, result in the release of free, pharmacologi-

cally active drug. Although this postulation concerning the propagation of seemingly ethereal influences over relatively long ranges through tissues may initially appear highly speculative, it is consistent with theoretical considerations appearing in reports by other investigators (20–25) as well as from this laboratory (12, 13, 15, 16, 18). A further discussion and the details of experimental results which lend considerable credence to this hypothesis will be presented in subsequent reports. Briefly, however, it was found through the application of the pharmacological method of drug absorption analysis that larger quantities of carbachol were computed to be ultimately absorbed following topical administration with adjuvant-containing vehicles than were actually contained in the volume of solution with which the rabbits were dosed. This obviously violates the requirement for material balance. This anomaly does not occur when carbachol is administered alone, suggesting that the adjuvants act to displace and/or cause a release of tissue-bound, miotically inactive, carbachol from tissue binding sites. This action of the adjuvants is manifest in a lesser "apparent volume" of distribution; therefore, a higher concentration of free carbachol is available to its sites of action relative to that occurring in the absence of the influence of the adjuvants.

The above phenomenon may be further understood by considering an analogy to drug binding to plasma proteins. The volume of the plasma compartment computed as the ratio of an amount of drug known to be present in the plasma to the concentration of free drug for an extensively protein-bound drug will be determined as quite large. An adjuvant-induced release of the drug from its binding sites will obviously cause an increased free concentration of drug. When this concentration is multiplied by the value for an apparent volume of distribution determined in the absence of the adjuvant effect, values of apparent quantities of drug in excess of those which may be known to be actually present will be obtained. This is, in effect, analogous to what was observed with carbachol. The results of applying the bioelectrometric method to the study of adjuvant and carbachol interactions (to be reported later) also clearly imply further evidence that binding of cationic adjuvants to anionic corneal surface sites can profoundly influence the extent to which gangs of other anionic sites are occupied by cationic sorptates such as carbachol. Briefly, the results reveal a complex behavior in which I was observed to: (a) create new anionic tissue binding sites for carbachol through causing a cooperative, all-or-none type, deprotonation and/or release of other cations, e.g., Na^+ and K^+, which can normally occupy these sites, (b) compete with carbachol for tissue binding sites, and (c) cause a sudden release of carbachol, as well as possibly other cations. This release occurs over nearly undiscernably small concentration ranges; such behavior is characteristic of cooperative mechanisms (21). The contribution of each of these phenomena to the tissue interaction behavior at any time was found to be dependent upon the relative magnitudes of carbachol and I concentrations simultaneously present.

SUMMARY AND CONCLUSIONS

In summary, the mechanism of the adjuvant effects is tentatively postulated on the basis of the present evidence as follows. Carbachol enters the corneal tissue through its surface following topical administration or back-diffuses into the cornea after intraaqueous dosing; the carbachol strongly binds to the tissues to a considerable extent. The bound, miotically inactive, drug could serve as a reservoir to prolong the activity, except that, under normal circumstances in the absence of adjuvants, the strongly bound drug is released too slowly to induce or contribute appreciably to observable levels of pharmacological activity. The interaction of the cationic adjuvants with anionic corneal surface sites results in the competitive displacement of bound carbachol from the surface and a diminution in the affinities of surface and deeper tissue binding sites. There is also a probable enhancement of the permeability of carbachol through the cornea (21). These effects permit the existence of relatively higher concentrations of the free drug to be available to its site(s) of action.

It is particularly notable that the observed adjuvant-induced augmentation of the miotic response activity apparently occurred by a promoted transcorneal absorption route rather than an enhanced systemic availability, which appeared to remain unaffected. The significance of this observation resides in an ability to formulate safer carbachol ophthalmic preparations using smaller concentrations of the drug, with a resulting diminution in systemic side effects; this can be accomplished without impairing the ophthalmic potency of the formulations.

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