Ophthalmic Bioavailability I: Corneal Penetration of Aceclidine (3-Acetoxyquinuclidine) into the Rabbit Eye Using a Perfusion Technique

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Abstract □ A rapid, efficient, and sensitive method was developed for extraction and subsequent quantitation of aceclidine (3-acetoxyquinuclidine) hydrochloride and 3-quinuclidinol hydrochloride from biological fluid. After adjustment of pH and salt concentration, chloroform extracts of serum, urine, or aqueous fluid could be quantitated by GLC without derivative formation. The analytical procedure was used to determine the corneal absorption of aceclidine in the rabbit eye. Aceclidine, a drug used topically in glaucoma treatment, was found to enter the anterior chamber of the eye by penetration exclusively through the cornea. In experiments on conjunctival absorption, the amount found in the anterior chamber was less than 50 ng. The corneal absorption experiments gave cumulated absorption of from 1 to 8.5 μg in 30 min.

Keyphrases □ Aceclidine—ophthalmic bioavailability, corneal penetration into rabbit eye, perfusion technique, analytical determination □ Bioavailability, ophthalmic—aceclidine analysis, rabbit eye, perfusion technique, corneal penetration □ Ophthalmic drugs, aceclidine—bioavailability, corneal penetration, rabbit eye, perfusion technique, analysis □ 3-Quinuclidinol hydrochloride—analysis, biological fluids, ophthalmic bioavailability of aceclidine, corneal penetration

For a drug to be effective in topical glaucoma therapy, it should have good penetrability into the anterior chamber so that direct action on the parasympathetic end-organs in the iris, the trabecular meshwork, and ciliary body is accomplished to influence intraocular pressure. Since aceclidine hydrochloride (I) is a cholinomimetic drug and has properties in common with acetylcholine, it was of interest to determine the amount of absorption of the drug through the cornea and/or conjunctival membrane into the anterior chamber of the eye.

These absorption studies necessitated the development of a rapid, efficient, and sensitive method for the extraction and quantitative analysis of drug from aqueous fluid or alternative biological fluid such as serum and urine. Since deacetylation of I by the biological system to yield 3-quinuclidinol (II) seemed probable, the analytical method required a selective procedure with equal efficiency for both I and II. Such a method is reported in this paper[1].

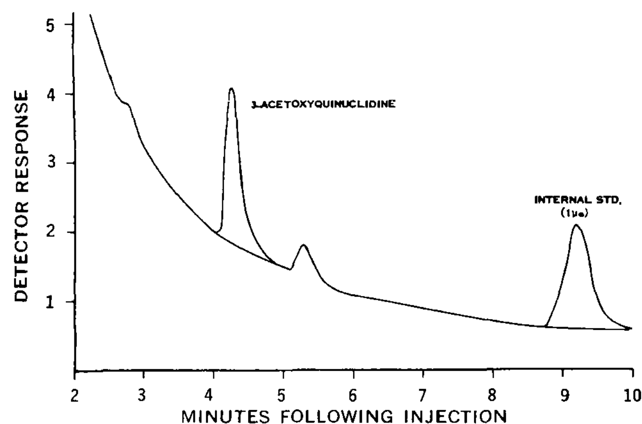

Figure 1—*GLC scan of a 4-ml serum sample containing 1 μg aceclidine (3-acetoxyquinuclidine).*

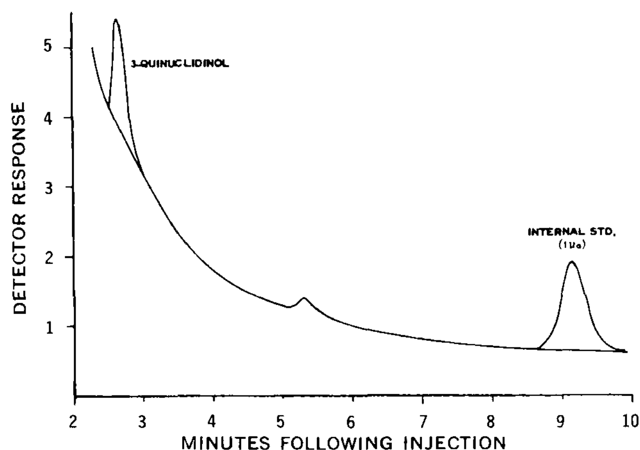

Figure 2—*GLC scan of a 4-ml serum sample containing 1 μg 3-quinuclidinol.*

Consideration was given to which of the two topical routes tested, conjunctival or corneal, would produce a measurable amount of drug in the anterior chamber of the rabbit eye and to the correlation, if possible, of the presence of drug with cholinomimetic symptoms.

EXPERIMENTAL[2]

Quantitation of I in Aqueous Fluid, Serum, or Urine—Aceclidine hydrochloride[3] (I) and 3-quinuclidinol[4] (II) were employed. The GLC internal standard was 2,4-dichloro-3-methyl-5-ethylphenol[4]. All solvents were spectrophotometric grade.

Serum or aqueous fluid containing I was adjusted to pH 8–9 and extracted three times with 1.5 volumes of chloroform in a separator. The chloroform extracts were combined and evaporated to dryness under reduced pressure. The internal standard was added prior to dissolving the residue in 30 μl chloroform.

Urine samples containing I were first acidified to pH 2–3 with hydrochloric acid and then were washed three times with 1.5 vol-

[1] A preliminary report appeared in Abstracts of the 9th National Meeting, APhA Academy of Pharmaceutical Sciences, Nov. 15–18, 1970, p. 61.

[2] GLC was performed with 0.82-m × 0.31-cm (6-ft × 0.12-in.) stainless steel columns packed with 10% UCC-W-982 (methyl vinyl silicone gum rubber) on Diataport S (80–100 mesh) operated at 170°. The injector ports were at 230°, and hydrogen flame detectors were at 250°. Helium carrier flow was 30 ml/min, hydrogen was at 40 ml/min, and air was at 500 ml/min. Peak areas were quantitated by multiplication of peak height and width at one-half peak height.

[3] Laboratories Chibret, France.

[4] Aldrich Chemical Co.

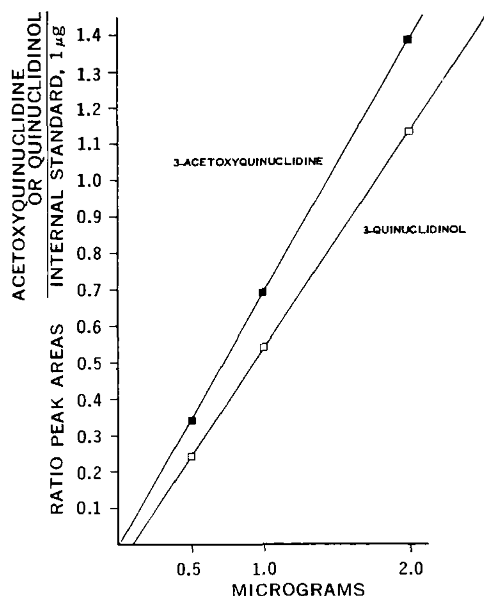

Figure 3—*Response curves of aceclidine (3-acetoxyquinuclidine) hydrochloride and 3-quinuclidinol (0.5–2 µg) relative to 1 µg internal standard using GLC flame detector.*

umes of chloroform. The chloroform extracts were discarded, and the urine was adjusted to pH 8–9 with ammonium hydroxide. The alkaline urine was then treated identically to the serum.

To extract II, solid sodium chloride was added to make a 12.5% solution in the chloroform-extracted serum and the solution was shaken vigorously for 1 min. One volume of sodium chloride-saturated concentrated ammonium hydroxide was added, and the mixture was extracted three times with 1.5 volumes of chloroform. The chloroform was removed, the internal standard was added, and the residue was taken up in 30 µl chloroform for injection into the gas chromatograph.

The extraction of II from human urine requires that 4 ml chloroform-extracted urine, 1 g sodium chloride, and 1 g potassium carbonate be added prior to addition of the sodium chloride-saturated ammonium hydroxide. From this point, the procedure was the same for the urine as for the serum or aqueous fluids.

A typical chromatographic scan for a spiked serum sample is depicted in Figs. 1 and 2. Recovery of I and II from biological samples averaged 91 and 83%, respectively.

Prior to analysis of extracted compounds, response curves for I and II (0.5, 1, and 2 µg each) were determined relative to 1 µg internal standard (Fig. 3).

Perfusion Method—Rabbits weighing 2 kg were anesthetized with 50% urethan solution intravenously, and then two cannulas (No. 20 needles) were inserted at the limbus carefully into the anterior chamber of the eye. The input was under a hydrostatic head of pressure of 20 mm Hg, and the outflow was at atmospheric pressure. The anterior chamber perfusate was collected in a graduated tube to determine volume per unit time. Heparin (1 unit/ml) was added to 0.15 *M* NaCl input solution to prevent coagulation in the outflow tube if bleeding occurred.

Corneal Absorption—Eye retractors were placed under the eyelid to expose the eyeball fully. A low viscosity petrolatum vehicle was placed into the eye socket using a syringe until the petrolatum was a few millimeters over the limbus. The "eye well," a modified plastic contact lens having a 4.5-mm hole and to which was cemented a plastic cylinder having a volume of approximately 0.1 mm^3, was set on top of the cornea. A high viscosity petrolatum was used to seal the well to the cornea. The well prepared in this manner was "leakproof" for 30–50 min. One hundred microliters of 2% I in 0.15 *M* NaCl was added to the well, and the collection of perfusate was started. The perfusion flow rate under these conditions varied from 0.1 to 0.3 ml/min.

Conjunctival Absorption—The rabbit was anesthetized and the eyeball was exposed with retractors. The head of the rabbit was positioned so that the lachrimal duct was away from the area where drug was to be placed. The eyeball could be shifted slightly using sutures under the inferior and superior latis rectus muscles to prevent the cornea from being immersed in the drug. The scleral conjunctiva was bathed in 100 µl drug solution.

RESULTS AND DISCUSSION

GLC of 3-quinuclidinol and aceclidine was performed without derivative formation of either compound. Some peak tailing occurred with both compounds but was more pronounced with 3-quinuclidinol. Columns containing a higher percentage of stationary phase (10%) prevented the formation of active sites caused by injection of entrained acid or base in the chloroform extracts, and both periodic silylation[5] and conditioning of the column overnight at 250° were required to obtain linear response curves with high sensitivity.

Compound I was extracted efficiently at low concentrations with chloroform from aqueous solution at pH 8 but remained in the aqueous phase under acidic conditions (pH < 6) for both serum and urine. It was necessary to extract chloroform-soluble materials from urine under acidic conditions before extraction of I due to the many peaks observed on the chromatogram as chloroform-soluble compounds. Acid extraction of serum was not necessary because the major serum peak occurred at 25 min.

Sternbach and Kaiser (1) required the use of a very alkaline pH and high salt concentration to extract 3-quinuclidinol from aqueous solutions. For this study, a procedure for extraction required compatibility of the final sample with GLC analysis as well as ability to extract very small amounts of compound (1 µg or less) efficiently.

Sodium chloride was an effective salting-out agent in the extraction of 3-quinuclidinol. Additionally, ammonium hydroxide was an ideal base for controlling the required pH during the extraction. Neither sodium chloride nor ammonium hydroxide affected the GLC analysis. Both I and II may be extracted together by using the procedure for extraction of II. This alternative is feasible when large numbers of samples are to be analyzed. This method was used to analyze samples obtained in the topical absorption studies.

Figure 4 describes the cumulative absorption of I into the anterior chamber. During the first 15–20 min, there is a rapid penetration of the drug which then decreases proportionally with time. Cumulative absorption of I can range from 1 to 8.5 µg in the first 30 min. Conjunctival absorption experiments did not give a measurable amount of I (<50 ng) in the anterior chamber. In preliminary experiments, some drug was detected in the anterior chamber due to exposure of drug to the corneal surface.

The rate of penetration of I into the anterior chamber has been assessed as a function of miosis (2). Whether cholinomimetic symptoms such as miosis, peristalsis, and sialismus are directly related or can be used as an index of corneal penetration is arguable.

Table I shows one cholinomimetic reaction, salivation, as a

Table I—Correlation of Aceclidine in the Anterior Chamber with Salivation Resulting from Topical Administration

Type of Absorption Experiment[a]	Minutes	
	First Trace of Drug	Salivation
Corneal	2	4
Corneal	10	45
Corneal	2	16
Corneal	2	14
Corneal	2	30
Corneal	7	—
Conjunctival	16	6
Conjunctival	25	5
Conjunctival	10	4
Conjunctival	None	31
Conjunctival	None	14
Conjunctival	None	26

[a] No miosis was noted in the opposite eye.

[5] Silyl 8, Pierce Chemical Co.

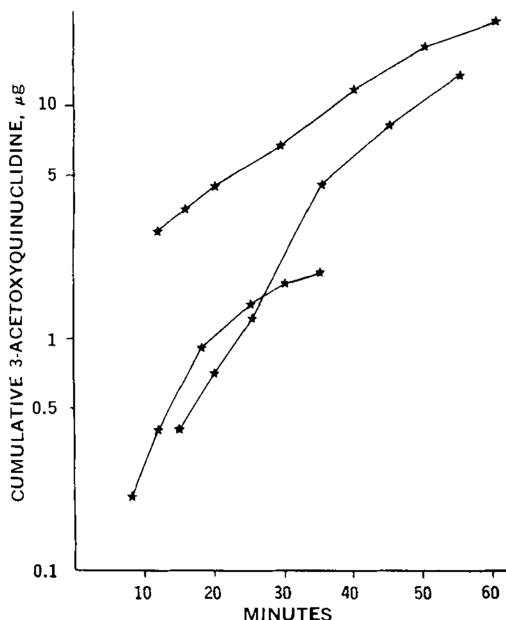

Figure 4—*Cumulative absorption of aceclidine (3-acetoxyquinuclidine) into the anterior chamber of the rabbit eye.*

function of the time of drug detection in the anterior chamber. Statistical analysis of the significance of the means between the times for onset of salivation resulting from corneal or conjunctival absorption was found to be insignificant at the 95% level. It has been assumed (2) that salivation is an index of corneal penetration of I. This assumption is not warranted from the observations reported here. Support for the lack of correlation is found in Table I (corneal absorption) where it can be seen that drug in the anterior chamber can precede salivation from 2 to 35 min. It is conceivable that pharmacological assay with sensitivities considerably greater than reported here would show the different symptomatic relationships to be significant. The drawback in either chemical or pharmacological assay was the subjectivity involved in defining the onset of symptoms as a function of drug intake. For example, observation of salivation was partially subjective since the end result and not the initial action was observed.

In these experiments, there was no evidence for deacetylation of I to II. Therefore, as would be predicted, the intact drug (I) penetrates primarily through the cornea into the anterior chamber where it can act to influence intraocular pressure. The experiments indicated that the scleral conjunctival membrane was not a major absorbing surface for I into the anterior chamber.

SUMMARY

Aceclidine (I), after topical administration, was detected in the anterior chamber. The penetration was primarily, if not exclusively, through the cornea and not the conjunctiva. In a sensitive GLC assay, only I was detected. There was no evidence of hydrolysis to the corresponding 3-quinuclidinol (II).

REFERENCES

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Effect of Washing on Physicochemical Properties of Aluminum Hydroxide Gel

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Abstract □ The negative electrophoretic mobility of aluminum hydroxide gel approaches zero logarithmically as the gel is washed and is related to the elution of chloride ion. Chloride ion is eluted at a constant rate and appears to be associated with the gel by a single mechanism. The viscosity of the gel increases with increased washing.

Keyphrases □ Aluminum hydroxide gel—effect of washing on physicochemical properties, loss of electrophoretic mobility, elution of chloride ion □ Gels, aluminum hydroxide—effect of washing on physicochemical properties, loss of electrophoretic mobility, elution of chloride ion □ Electrophoresis—loss of mobility of aluminum hydroxide gel, effect of washing, elution of chloride ion

An important step in the preparation of aluminum hydroxide gel is washing the gel free of reactants and soluble components following precipitation. The USP (1) places a limit on the chloride and sulfate content of aluminum hydroxide gel. The effect of washing on the physicochemical properties of aluminum hydroxide gel has not been extensively reported, although precipitation factors such as pH (2), temperature (3, 4), and order of addition and concentration of reactants (4–6) have been studied. The effect of washing on such dosage form-related properties as electrophoretic mobility and viscosity is examined in this report.

EXPERIMENTAL

Materials—All chemicals used were either official or reagent grade.

Preparation of Aluminum Hydroxide Gel—Aluminum hydroxide was prepared by the reaction of aluminum chloride, sodium carbonate USP, and sodium bicarbonate USP at pH 6.5 (2). Doubly distilled water was used for both precipitation and washing. Fractions of the wash water were collected for conductivity and chloride determinations. The extent of washing is expressed as the volumes of wash water eluted per volume of finished gel.