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COMMUNICATIONS

Lack of Influence of Rabbit Nictitating Membrane on Miosis Effect of Pilocarpine

Keyphrases □ Pilocarpine hydrochloride—miosis induction, effect of rabbit nictitating membrane □ Nictitating membrane, rabbit—effect on miosis induction by pilocarpine hydrochloride □ Ophthalmic cholinergics—pilocarpine hydrochloride, effect of rabbit nictitating membrane on miosis induction

To the Editor:

The rabbit frequently is used in ophthalmological research even though it possesses some ocular anatomical dissimilarities to the human. There are differences in the normal tear volume and the tear turnover rate between humans and rabbits (1, 2). A prominently different anatomical feature of the rabbit is the nictitating membrane, a so-called third eyelid. This epithelially covered sheet of cartilaginous tissue retracts into the nasal canthus secondarily to retractions of the globe with contraction of the retractor bulbi muscles (3). Because this membrane is well vascularized and occupies part of the precorneal space, it conceivably could influence the dynamics of instilled material in the eye (3, 4).

Ten New Zealand albino rabbits of either sex, 1.5–2.5 kg, were used in the present study. A 50-μl dose of 2% pilocarpine hydrochloride[1] was administered to the right eye by slightly pulling away the lower eyelid from the globe and allowing the measured drop to fall onto the cornea and collect into the lower conjunctival sac. After a few seconds, the eyelid was carefully returned to its normal position. Pupil diameters were measured under controlled lighting conditions using a micrometer caliper held at a constant distance between the animal's and the observer's eyes.

At the completion of the first experiment, in which the control response to the drug was recorded, the nictitating membranes were surgically removed at the base from the test eyes of all rabbits. After 1 week, the operated animals were handled in the same manner as before and were treated with the same lot of drug as in the first experiment. Pupil size measurements were made in the usual manner for comparison with the previous week's results. The nictitating membrane had no apparent effect on the response parameters of pilocarpine-induced miosis following topical dosing of an aqueous solution (Table I).

These results are surprising for a number of reasons. First of all, the nictitating membrane can be moved approximately halfway across the cornea. In so doing, the membrane occupies a significant portion of the precorneal space. Therefore, one would expect a reduction in the fluid volume in the normal rabbit eye and a concomitant loss of drug available for absorption. However, the movement of the membrane across the eye is accompanied by a retraction of the eye into the orbit. Thus, with its movement, there conceivably could be no net change in the volume of fluid that the rabbit eye can hold.

Second, the membrane is well vascularized and there are many lymphatic nodules in the connective tissue (3). Therefore, the presence of the membrane might provide a competitive depot to channel drug away from the corneal absorption process. Third, tear secretions from the nictitans and Harderian glands containing oil and mucus empty into the nictitating membrane (3). The removal of the membrane carries with it the contribution of the nictitans glandular secretions to the composition of the tears and its possible effect on steady-state tear volume and/or turnover rate. It may be that some of these processes are operative but in effect cancel one another.

An alternative explanation could be that a relative lack of movement of the nictitating membrane occurs throughout drug absorption. We observed that, after dosing, the rabbits may close their eyelids for 15–30 sec but that the membranes remain retracted. Quantitatively, Chrai *et al.* (2) showed that 1 min after dosing a 50-μl aqueous drop, only 28.2% of the original instilled volume remained. Consequently, pilocarpine hydrochloride absorption may be rapid relative to the nictitating membrane activity. Thus, our experiments indicate that the membrane in its fully retracted state has no effect on the response to pilocarpine solution as observed under these

Table I—Miosis Induced by 50 μl of 2% Pilocarpine Hydrochloride Solution in Albino Rabbit Eyes before and after Removal of the Nictitating Membrane

Area under Curve, mm/mm × hr	Peak Intensity[a]	Total Duration, hr	Baseline Diameter, mm
Before Removal of Nictitating Membrane (n = 10)			
0.9194[b] ±0.2058	0.4275 ±0.0445	4.38 ±1.02	4.975 ±0.611
After Removal of Nictitating Membrane (n = 8[c])			
0.9388 ±0.3057	0.3911 ±0.0641	4.32 ±0.96	5.181 ±0.575

[a] Peak intensity = $[(\text{pupil diameter})_{t_0} - (\text{pupil diameter})_{t_{max}}]/(\text{pupil diameter})_{t_0}$. [b] Mean ± *SD*. [c] One rabbit suffered from debilitating diarrhea and another died; therefore, only eight rabbits were used after removal of the nictitating membrane.

[1] Isopto Carpine 2%, Alcon Laboratories, Fort Worth, TX 76101.

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experimental conditions. However, under conditions where the membrane may cover the cornea or by its movement may mechanically remove instilled materials, *e.g.*, suspensions and inserts, absorption kinetics may possibly be altered.

Miller and O'Conner (4) found that dexamethasone sodium phosphate inhibited limbal wound healing in normal rabbit eyes but did not do so when the nictitating membrane had been surgically removed. Our results, as well as the results obtained by Miller and O'Conner, indicate that the influence of the membrane may vary for each preparation and/or experimental condition.

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Louis M. DeSantis
Ronald D. Schoenwald ˣ
New Product Research
Department of Ophthalmology
Alcon Laboratories, Inc.
Fort Worth, TX 76101

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Determinations of Amygdalinamide, Amygdalin Acid, and 2-Propanol in Amygdalin Dosage Forms from Mexico

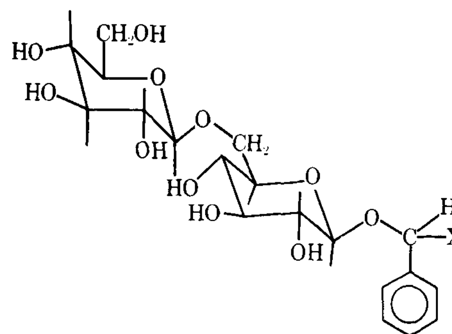
Keyphrases □ Amygdalinamide—synthesized, high-pressure liquid chromatographic analysis in amygdalin dosage forms □ Amygdalin acid—synthesized, high-pressure liquid chromatographic analysis in amygdalin dosage forms □ 2-Propanol—GLC analysis in amygdalin dosage forms □ Impurities—amygdalinamide, amygdalin acid, and 2-propanol, analyses in amygdalin dosage forms □ High-pressure liquid chromatography—analyses, amygdalinamide and amygdalin acid in amygdalin dosage forms

To the Editor:

Assay results for amygdalin dosage forms from Mexico were reported earlier (1). In addition to amygdalin[1] (I), the injectable dosage forms contain significant amounts of amygdalinamide[1] (II) and amygdalin acid[1] (III), hydrolysis products of amygdalin, plus 2-propanol. This communication reports the determination of these three impurities in the amygdalin dosage forms.

Amygdalinamide and amygdalin acid in the amygdalin dosage forms were quantified by an assay that required reference standards of the amide and acid of known purity. Since these standards were unavailable commercially, they were prepared.

Amygdalinamide was prepared from (*R*)-amygdalin by treatment with ammonium hydroxide. The crude product



I: X = CN

II: X = $\overset{O}{\overset{\|}{C}}NH_2$

III: X = $\overset{O}{\overset{\|}{C}}OH$

was purified by a preparative high-performance liquid chromatographic (HPLC) procedure that used a C_{18}-bonded silica[2] column and an aqueous methanol mobile phase. When this mobile phase was changed to 0.1 *M* KH_2PO_4, the purified amygdalinamide was separated into two major components of nearly equal intensity plus two very minor impurities and a trace of another. On the basis of peak enhancements, these impurities were identified as (*R*)- and (*S*)-amygdalins and amygdalin acid, respectively. Identity of the principal components in purified amygdalinamide was based on spectral evidence. IR data show an intense band near 5.95 μm assigned to the primary amide, broad absorptions between 9 and 10 μm assigned to carbohydrate, and bands near 13.2 and 14.3 μm assigned to phenyl. The ^{1}H-NMR data were consistent with the proposed structure. Only one peak was found in the region where the benzylic protons were expected; its area was one-fifth that of the phenyl protons. The ^{13}C-NMR data were consistent for a mixture of (*R*)- and (*S*)-amygdalinamides. The amide carbon appeared as two bands centering near 136.6 ppm[3], the phenyl carbons appeared as a group of at least six bands centering near 129.4 ppm, and the two anomeric carbons in the glucose moieties appeared as three bands centering near 101.9 ppm. The benzylic carbon was among the nonanomeric glucosido carbons, which appeared as a complex of many bands. These observations were consistent with those reported (2) for a mixture of (*R*)- and (*S*)-amygdalins. GLC–mass spectral analysis of the trimethylsilyl derivative on a phenyl methyl silicone column (OV-17) showed only one major peak; the mass spectral data for this peak were consistent for the proposed structure as the octatrimethylsilyl derivative.

The purity of the amygdalinamide sample was estimated at 90 ± 1.5% on the basis of chromatographic and elemental data and the results of an NMR assay[4] with pyrocatechol[5] as the internal standard.

[1] Amygdalin is mandelonitrile-β-D-glucosido-6-β-D-glucoside (I). Amygdalinamide is mandeloamide-β-D-glucosido-6-β-D-glucoside (II). Amygdalin acid is mandelic acid-β-D-glucosido-6-β-D-glucoside (III). In the amygdalin dosage forms, all three materials exist as nearly equal mixtures of the respective (*R*)- and (*S*)-epimers.

[2] Porasil C_{18}.

[3] Relative to tetramethylsilane as 0.00 ppm.

[4] This assay measured the total amount of phenyls in the sample. Since the HPLC data showed approximately 1% phenyl-containing impurities, this amount was subtracted from the total phenyls to give a sample purity of 90 ± 1.5%, which is consistent with the elemental data. The remainder of the sample was presumed to be 8.5% water, an amount derived by difference.

[5] Pyrocatechol, Aldrich Chemical Co., analyzed 99+% pure based on chromatographic, titrimetric, and elemental data.

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