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Ocular delivery of progesterone using a bioadhesive polymer

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Summary

Polymers of acrylic acid crosslinked (0.3% w/w) with divinyl glycol and 2,5-dimethyl-1,5-hexadiene were synthesized and examined as to their utility in ocular drug delivery. Both showed excellent bioadhesion to the conjunctival mucin/epithelial layer as determined by visual inspection and a sensitive in vitro method. The densities and extent of hydration of the polymers were also determined. Progesterone was used as a model drug which was entrapped in the polymer of acrylic acid crosslinked with 2,5-dimethyl-1,5-hexadiene. A suspension without polymer was prepared at the same progesterone concentration and used as a control for ocular bioavailability comparison. From the 1 h determination through the end of the study, the aqueous humor drug levels showed a statistically significant difference between the test and control formulations. The bioadhesive dosage form showed an area under the curve 4.2 times greater than the results obtained for the suspension preparation, over the time course of the study.

Introduction

A significant problem accompanying instillation of a drug solution or suspension into the eye is the extensive drug loss that occurs due to highly efficient precorneal loss processes. The most important of these is drainage loss of the instilled solution which reduces the total amount of drug present in the cul-de-sac and magnifies the importance of the dilution effect of incoming tears (Lee and Robinson, 1979).

In drug therapy, it is sometimes desirable to maintain drug levels in tissues, within

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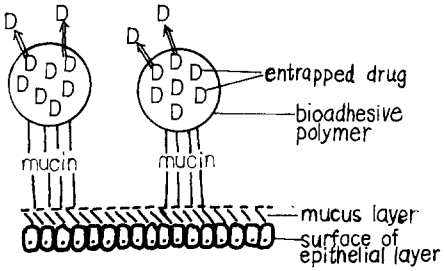

Fig. 1. The bioadhesive ocular drug-delivery system adheres to the mucus lining of the conjunctiva for an extended period of time.

a certain range, over a specified period of time. In terms of ocular drug delivery by topical administration, an obvious approach is to eliminate or reduce the precorneal loss factors with a concomitant adjustment in dose. Decreasing the precorneal loss rate constant will result in an increase in contact time between the drug and absorbing tissue, thereby improving ocular drug bioavailability. Thus, the typical small corneal absorption rate constant permits sustaining of levels to occur provided relatively constant levels of drug are maintained in the precorneal area. One possible approach to accomplish this goal is to employ polymeric substances that adhere to precorneal surfaces through non-covalent bonds, i.e. bioadhesive polymers. Such polymers have been exploited for similar purposes elsewhere in the body (Longer et al., 1984).

Earlier work (Park and Robinson, 1984) had examined a broad range of both water-soluble and insoluble polymers as to their binding affinity to mucin/epithelial surfaces. From this initial work, it was decided that water-insoluble polymers offered the greatest range of strategies to develop controlled drug delivery systems. Typical polymers are lightly crosslinked, acrylic acid polymers with extensive capacity to attract and hold water. A pictorial representation of an ocular, bioadhesive, drug delivery system is shown in Fig. 1.

In the present study, polymers of acrylic acid lightly crosslinked with two different agents, divinyl glycol and 2,5-dimethyl-1,5-hexadiene, were examined as to their utility in ocular drug delivery. Both showed excellent bioadhesion to the conjunctival mucin/epithelial layer. Progesterone was used in the present study because of its commercial availability in a ^{14}C-labeled form and its limited water solubility.

Experimental

Materials

Acrylic acid, benzoyl peroxide, progesterone and magnesium sulfate heptahydrate were obtained from Aldrich Chemicals. Divinyl glycol and 2,5-dimethyl-1,5-hexadiene were from Polyscience. All of the above chemicals were either reagent or analytical grade and were used as received.

[4-^{14}C]Progesterone was obtained as a toluene solution from Amersham (Arlington Heights, IL). Its specific activity was 56 mCi/mmol while its radiochemical purity was 99%. It was subject to vacuum distillation immediately prior to use to remove the solvent.

Male, albino rabbits (Klubertanz, Edgerton, WI) weighing between 2.5 and 3.0 kg were used throughout the studies. Lighting and auditory backgrounds were maintained constant within the caging facilities, and the animals were fed a regular diet with no restriction on the amount of food or water consumed.

Determination of aqueous solubility of progesterone

Two different values have been reported for the solubility of progesterone in distilled water at 37°C, which may be accounted for on the basis of different polymorphic forms of the drug. They are 8.59×10^{-5} M (Sundaram and Kincl, 1968) and 3.63×10^{-5} M (Roseman, 1972). In order to determine the correct value, the following experiment was conducted. Aqueous solutions of progesterone were prepared at concentrations of: 0.05, 0.1, 0.25, 0.5, 1.0 and 2.0 ($\times 10^{-5}$ M). UV absorbances of these solutions were measured at a wavelength of 241 nm, and a Beer's law plot was constructed.

Fifty ml of saturated progesterone solution was prepared and kept at a constant temperature of 37 ± 0.5°C while stirring. After 48 h, 5 ml of the supernatant was withdrawn using a 5.0 ml volumetric pipette fitted with a glass wool filter and then transferred to a 10-ml volumetric flask. Five ml of doubly-distilled water was immediately added to the flask, and the UV absorbance of the diluted supernatant was then measured.

In order to ensure that maximum solubility was reached, another 5 ml of the supernatant was transferred to a 10-ml volumetric flask 24 h later and treated as before. By extrapolation using the Beer's Law plot, the solubility of progesterone in double-distilled water at 37°C was then determined.

Preparation of polymer

The procedures for polymer synthesis were modified from that of Markus (Markus et al., 1965). A mixture of 2 g acrylic acid, 20 mg benzoyl peroxide, and 6 mg crosslinking agent was added with stirring to a solution containing 16 g of magnesium sulfate ($MgSO_4 \cdot 7H_2O$) in 2 ml of distilled water refluxed at 95°C. Polymerization occurred within a short period of time, but the mixture was maintained at the same temperature with stirring for 4 h. At the end of this time, the mixture was placed onto a 40-mesh stainless steel mesh screen and repeatedly rinsed with distilled water. The washed crosslinked polymer was completely dried in a hot air oven at 90°C before being ground to the required size. Polycarbophil met USP standards for purity and identification.

Determination of polymer density and hydration

The density of each polymer was determined in a 2 ml specific gravity bottle at 25°C. Benzene of known density (0.874 g/ml) was used as the medium. No swelling of the polymer in benzene was evident.

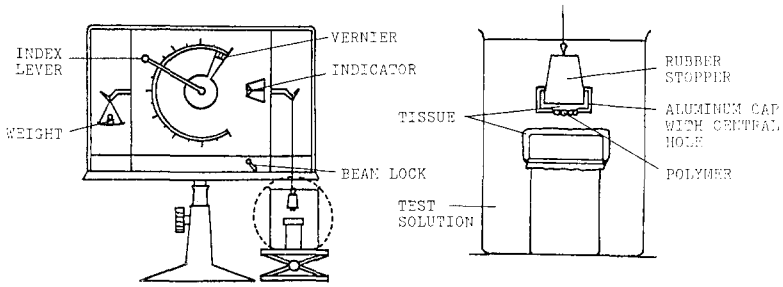

Fig. 2. A diagram of the modified precision balance for in vitro evaluation of polymer bioadhesion.

100 mg of each polymer was allowed to hydrate in 10 ml of pH 7.4 isotonic Sorensen buffer in a 10 ml graduated cylinder, which was placed in a jacketed beaker, thermostated at 37°C. The volume of the polymer was measured at 5-min intervals until equilibrium swelling was achieved.

In vitro evaluation of polymer bioadhesion

The force required to separate a polymer specimen from freshly excised rabbit conjunctival membrane was measured using a modified precision balance (Roller Smith, Biolar, North Grafton, MA) as described earlier (Ch'ng et al., 1984). A diagram of the apparatus is shown in Fig. 2.

Prior to surgery, the hair on the eyelids was clipped. The rabbit was then sacrificed by rapid injection of sodium pentobarbital into a marginal ear vein. The lower eyelids of both eyes were surgically removed and placed in an aerated saline solution until used. One of the eyelids was secured over a weighted glass vial using a rubber band so that the conjunctiva faced outwards. The vial was then placed in a jacketed beaker thermostated at 37°C containing 500 ml pH 7.4 isotonic Sorensen buffer, and positioned under the scale.

Another eyelid was placed over a rubber stopper so that the conjunctiva faced outwards. The tissue was secured with an aluminum vial cap which had a uniform size opening. Four mg of polymer, previously fully hydrated with pH 7.4 Sorensen buffer, was carefully spread over the tissue on the rubber stopper.

The stopper was then suspended from the scale into the beaker containing the buffer solution and weighted vial. The scale was zeroed with the tissue on the vial at a depth equal to the layer of polymer. The scale was locked and the beaker repositioned so that the vial was directly below the rubber stopper. The beaker was then slowly raised until the tissue came into contact with the polymer, then elevated slightly so that polymer mucus contact would be made with the force of the rubber stopper (1.8 g in this system). After 1 min, the lock was released and a force was applied to remove contact between polymer and tissue. The force was increased at a constant rate of 10 mg/s until the polymer became detached from the mucus.

Incorporation of [^{14}C]progesterone powder into the polymer

An alcoholic solution containing 133 mg nonradioactive progesterone was added to a flask containing purified [^{14}C]progesterone. This solution was evaporated to

dryness under vacuum distillation. A mixture of 2 g acrylic acid, 20 mg benzoyl peroxide, and 6 mg 2,5-dimethyl-1,5-hexadiene was added to the flask containing the dried ^{14}C-labeled progesterone powder, and then sonicated so that the progesterone powder dispersed evenly in the acrylic acid. The whole mixture was then added, with stirring, to a flask containing magnesium sulfate in distilled water refluxing at 95°C. The preparation procedure was carried out as described earlier for preparation of the polymer.

Determination of the amount of drug trapped in the polymer

A weighed amount of polymer–drug mixture was placed in a measured volume of methanol and shaken for 24 h on a mechanical shaker. An aliquot of the supernatant was then obtained and transferred to a scintillation vial. Another aliquot was obtained 24 h later to make sure all of the drug had been extracted. Scintillation cocktail (Aquasol, New England Nuclear, Boston, MA) was added, and the vial was stored in the dark for 24 h before counting in order to minimize chemiluminescence. Counting was conducted in a liquid scintillation counter (Packard Model 2002, Packard Instruments, Downers Grove, IL), and after appropriate corrections, the final count was converted to milligrams of drug per milliliter of aliquot through the use of a standard.

In vitro release studies

The in vitro release rate of progesterone from the polymer was determined by a modification of the United States Pharmacopeia rotating-basket method (U.S. Pharmacopeia, 1980). A known amount of the polymer containing progesterone was placed in a basket assembly (Van-Kel Industries, Chatham, NJ) that was connected to a stirrer motor. The assembly was lowered into a jacketed beaker, containing 500 ml pH 7.4 isotonic Sorensen buffer, to a depth approximately 2.5 cm from the bottom. The solution was equilibrated at 37 ± 0.5°C, and sink conditions were maintained throughout the study. The basket assembly was rotated at 120 rpm, and samples were withdrawn at regular intervals with a 5.0 ml volumetric pipette equipped with a glass wool filter. The sample was replaced with an equal volume of buffer pre-equilibrated to temperature. Collected samples were assayed by liquid scintillation counting using Aquasol as a liquid scintillation cocktail. Counts were converted to concentration of drug, using an appropriate standard.

Bioavailability studies

A calculated amount of polymer containing progesterone was dispersed in a volume of pH 7.4 isotonic Sorensen buffer so that a 0.3% w/v suspension of progesterone was prepared, which would give a dose of 0.15 mg progesterone/3 mg loaded polymer/50 μl dose. The above drug–polymer suspension was stirred for 48 h to make the dispersing buffer solution saturated with progesterone. Thus a suspension of drug-entrapped polymer dispersed in a saturated drug solution was prepared.

During the experiments, all rabbits were placed in restraining boxes to minimize movement. During dosing, the lower eyelid was pulled away from the globe while

the upper eyelid was elevated slightly, and a 50 μl dose of the above suspension was placed into the lower cul-de-sac. Immediately after dosing, both eyelids were released and no further mechanical manipulation was performed.

Animals were sacrificed at various times postdosing by rapid injection of sodium pentobarbital solution through a marginal ear vein. The corneal surface was rinsed with buffer solution and carefully blotted dry with tissue. Aqueous humor samples were then obtained with a 1-ml syringe fitted with a 27-gauge needle. Aqueous humor samples were then transferred to glass vials, and 15 ml scintillation cocktail added. Vials were dark-adapted for 24 h before counting to minimize chemiluminescence. After correcting for background and quenching, the data in disintegrations per minute were converted to micrograms of drug. The above studies were repeated using a 0.3% w/v suspension of progesterone powder without polymer.

Metabolism studies

Aqueous humor sample was extracted with 2 ml of chloroform and then centrifuged. The recovered chloroform was evaporated under nitrogen and 10 μl of methanol added. The methanol solution was spotted on silica gel plates (polygram Sil G/UV 254, Brinkmann Instruments, Westbury, NY) and developed in a solvent system of ethyl acetate/chloroform (1 : 1). Dried plates were cut into 1-cm sections and the sections placed in scintillation vials containing 1 ml of methanol and mixed for 5 min. Aquasol was added and the samples counted after 24 h storage in the dark. Results were compared to TLC plates which had been spotted with nonradioactive solutions and examined under UV light in order to determine the RF value for the drug in the solvent system.

Results and Discussion

Aqueous solubility of progesterone

The solubility of progesterone in distilled water at 37°C was found to be 3.8×10^{-5} M, i.e. 11.95 μg/ml which is in good agreement with the published value by Roseman (1972). Progesterone is known to exist in at least two different forms. The alpha form consists of orthorhombic prisms (m.p. 127–131°C), while the beta form consists of orthorhombic needles (m.p. 121°C). In the present study the alpha form was used.

Densities and hydration of the polymers

Two crosslinked polyacrylic acid polymers were synthesized. Reasonably high yields were obtained for both polymers, i.e. 85% for acrylic acid–divinyl glycol and 98% for acrylic acid–2,5-dimethyl-1,5-hexadiene. Both polymers were examined as to densities and extent of hydration. No additional characterization of the polymer was made. For the acrylic acid–divinyl glycol polymer, its density is 1.56 g/ml at 25°C and its maximum extent of hydration in pH 7.4 isotonic Sorensen buffer is 3.8 ml for 100 mg of polymer at 37°C. For comparison, the density of the acrylic acid–2,5-dimethyl-1,5-hexadiene polymer is 1.63 g/ml at 25°C, and 100 mg of this

TABLE 1

IN VITRO EVALUATION OF POLYMER BIOADHESION TO RABBIT CONJUNCTIVA USING A MODIFIED SURFACE TENSIOMETER

All studies used 4 mg of approximately 75 μm mesh material. Six pairs of conjunctiva were used for each polymer.

Test material	Weight required for detachment (mg)	Force/area (dyne/cm)
I	167.2 ± 40.5	207.5 ± 50.3
II	186.3 ± 35.9	231.2 ± 44.5
III	39.8 ± 4.0	49.4 ± 5.0

I = Polymer of acrylic acid crosslinked with divinyl glycol.
II = Polymer of acrylic acid crosslinked with 2,5-dimethyl-1,5-hexadiene.
III = Gelatin microcapsules crosslinked with formaldehyde.

polymer is able to swell to a volume of 1.6 ml at 37°C. For 335 μm size polymer particles, the equilibrium state of swelling was reached in about 25 min, while it took about 20 min for 75 μm size polymer particles. An earlier publication from this laboratory (Ch'ng et al., 1984) reported a more comprehensive picture, in which the effect of percent of crosslinking on the density, the effect of structural modification on extent of hydration, and the equilibrium swelling of polymer at different pH's were discussed.

In vitro bioadhesion studies

In the present study, the modified surface tensiometer method gives a reasonably sensitive and reproducible method to measure adhesion of polymer to animal tissue, provided precautions are taken to ensure that the conditions of each test are the same. 4 mg of fully swollen polymer were sufficient to provide a good range of forces for detachment. In Table 1, the detachment forces of the two polyacrylic polymers, as well as crosslinked gelatin microcapsules, are shown.

As has been found previously for gastric mucosa, crosslinked gelatin beads showed little bioadhesion to conjunctival tissue. The two polymers used in this study showed approximately one-fourth the bioadhesion to conjunctival tissue as compared to gastric tissue. Several explanations can be offered for this finding. (a) The cohesive property of conjunctival mucin is less than gastric mucin. (b) A primary mechanism of bioadhesion involves intercalation of the polymer with the mucin coating the tissue. Conjunctival mucin thickness is considerably less than that of gastric mucin, and thus the polymer has less opportunity for intimate contact and hence less bioadhesion. (c) During the in vitro test, a proportionally greater amount of conjunctival mucin dissolves in the bathing fluid of the in vitro apparatus as compared to gastric mucin. The authors favor explanation (b) as the most reasonable based on other unpublished findings in this laboratory. As will be shown subsequently, the adhesive force of the polymers for the conjunctival surface is still sufficient to maintain long contact time.

Amount of drug entrapped in the polymer

From replicate assays, it was determined that 0.15 mg progesterone was entrapped in approximately 3 mg of polymer. The reproducibility of the data suggested that progesterone was distributed uniformly in the drug–polymer system. Thus 3 mg of drug–polymer per 50 μl would be equivalent to a 0.3% progesterone suspension dose.

In vitro release studies

The acrylic acid–2,5-dimethyl-1,5-hexadiene polymer is a hydrophilic glassy hydrogel matrix. In the dry state, diffusion of the drug will be essentially zero. Upon contact with water, the matrix swells and drug diffusion increases by several orders of magnitude, eventually reaching a limiting value determined by the structure of the equilibrium-swollen gel.

Fig. 3 shows the result of the in vitro release studies. Reproducibility in duplicate runs was good. For example, the amount of drug released with the largest deviation was $x = 0.176$ and RSD = 2.8%. In these studies, different particle sizes of polymer–drug complex were used. They are 75 μm and a 50 : 50 mixture of 335 μm and 75 μm particles. In the latter case, 335 μm particles were added to see if the presence of larger particles would reduce the release rate of progesterone. The data showed that in the first 20 min, the amount of progesterone released was higher from the 75 μm particles than that from the mixed-size particles. At later times, the amount of progesterone released was similar in both cases.

According to the hydration studies described earlier, the polymer being used in this study can absorb a significant amount of water to form an elastic gel and, at the same time, release the dissolved entrapped drug by diffusion through swollen regions of the gel. For the smaller particle-size polymer, the total surface area in contact with water is larger. Thus in the first 20 min, the area of the swollen region is larger than that of the larger particle-size polymer and the amount of progesterone released

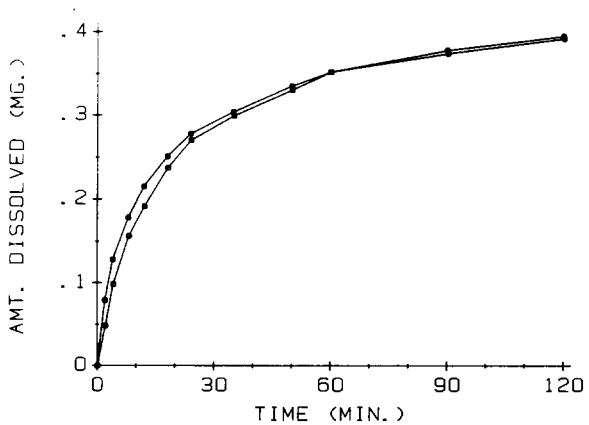

Fig. 3. In vitro release profiles for progesterone from the acrylic acid–2,5-dimethyl-1,5-hexadiene polymer. ●, represents approximately 75 μm particle size; and ○, represents 50 : 50 mixture of 75 μm and 335 μm particle size.

from the 75 μm particles is higher than that from the mixed-size particles.

For 75 μm particles of polymer, the fully swollen state would be reached in about 20 min, whereas it took about 25 min for the 335 μm particles. Thus, from 25 min onwards, polymers of both sizes would become fully swollen and entrapped drug would be dissolved by water in the swollen region. In the present case, the progesterone release rate was similar from the 'fully swollen' polymer of both particle sizes, suggesting that the release was dissolution-controlled rather than diffusion-controlled since the particle size of the 'fully swollen' polymer had no effect on the release rate, and the drug was uniformly dispersed. Indeed, Korsmeyer and Peppas (1983) had published that in a highly swollen hydrogel, drug diffusivity may approach a value observed in pure water.

It should be noted that progesterone was specifically chosen because of its low water solubility and slow dissolution rate. Recognizing that these hydrogels offer little resistance to diffusion of small molecules, it is necessary to either use a sparingly soluble drug, i.e. dissolution-controlled, or incorporate the drug into a separate rate-controlling release system which is interfaced with the polymer.

The in vitro release studies implied that the swollen polymer does not present a diffusion barrier to the entrapped drug. Nevertheless, this polymer possesses good bioadhesive properties, and solid drug particles entrapped in the polymer remain in the cul-de-sac for an extended period of time postinstillation. In comparison, when a suspension of drug particles, in the absence of a bioadhesive, is instilled in the cul-de-sac, most of the drug particles are rapidly lost through drainage.

Bioavailability studies

Ocular metabolism studies indicated that there is little metabolism of progesterone in albino rabbits since less than 10% metabolites were detected. One published study (Southren et al., 1976) utilizing steroid alcohols and ketones reported metabolites of 15% or less from various ocular tissue incubates. Our findings are in agreement with this work.

Fig. 4 shows aqueous humor levels of progesterone obtained after dosing with 50 μl of 0.3% progesterone suspension and 50 μl of the 0.3% progesterone suspension in a bioadhesive–polymer system. The open circles represent the suspension, whereas the closed circles represent the bioadhesive system. From the 1-h determination through to the end of the study, a statistically significant difference between each formulation was obtained for each aqueous level of drug. Table 2 lists the probability values calculated from the data. The bioadhesive dosage form showed an area under the curve 4.2 times greater than the results obtained for the suspension preparation, over the time course of the study. According to Fig. 4, the time to peak for the bioadhesive dosage was found to be about 2 h. Meanwhile, the peak time for the suspension formulation was maximally 1 h, since earlier sampling periods might have indicated an earlier peak time. The difference in peak time suggests that the bioadhesive dosage form is dissolution limited, whereas the suspension is not.

From the semilogarithmic representation of the data in Fig. 4, it is visually apparent that the aqueous humor steroid levels obtained from the bioadhesive dosage form declined slower than the simple suspension formulation. This is also

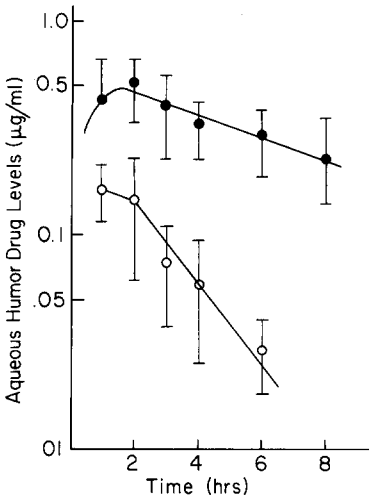

Fig. 4. Progesterone levels in aqueous humor following topical administration of 0.3% suspension of progesterone-entrapped polymer (●) and 0.3% suspension of progesterone without polymer (○). The error bars represent standard deviation.

evident by subjecting the data to statistical treatment. Thus, linear regression analysis of the data for the declining portion of the bioadhesive system gives a correlation coefficient of 0.965 and, from the slope of the line, a rate constant of 0.002 min^{-1} was obtained. Similar analysis for the suspension case gives a correlation coefficient of 0.983 and a slope of 0.006 min^{-1}.

Under normal circumstances, particles less than 20 μm are instilled into the eye for both patient comfort and to allow faster dissolution of suspended drug. In the present case, larger particles were employed as an experimental convenience to the authors. Nevertheless these larger particles gave no noticeable discomfort to the test animals, presumably attributable to the swollen nature of the particles as well as their ease of compression upon blinking.

TABLE 2

MEANS, STANDARD DEVIATIONS, AND *t*-TESTS ON AQUEOUS HUMOR LEVEL OF PROGESTERONE

Time (h)	Polymer system		Simple suspension system		*P*
	Mean (μg/ml)	S.D.	Mean (μg/ml)	S.D.	
1	0.42	0.26	0.16	0.05	< 0.05
2	0.51	0.15	0.14	0.08	< 0.01
3	0.39	0.16	0.07	0.04	< 0.01
4	0.32	0.09	0.06	0.03	< 0.01
6	0.29	0.10	0.03	0.01	< 0.01
8	0.22	0.16			

Progesterone was selected as the drug for this system because it provides rate-limited dissolution and thus overcomes the rapid diffusion attendant with small water-soluble molecules in a swollen hydrogel. Placement of this progesterone containing polymer in a saturated solution of progesterone produces a sustained release suspension, provided the polymer is retentive in the eye. A potential problem associated with such a system is precipitation of the drug during temperature fluctuations. Thus, higher temperatures can cause the drug to leave the solid state and go into solution, and when the temperature drops, the now insoluble drug can precipitate out rather than re-enter the polymer. Such an effect was not observed in the present case, but represents a potential problem for commercial application of these systems.

In conclusion, the present study has shown that the ocular bioavailability of progesterone was significantly enhanced by delivery using a bioadhesive polymer. That the polymer was present in the eye for extended periods is evident by the drug study data and also by visual observation of the animal eye using simple magnification. It is suggested that this relatively primitive dosage form is indicative of the powerful potential of ocular bioadhesives in drug delivery.

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