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# Methylprednisolone esters of hyaluronic acid in ophthalmic drug delivery: in vitro and in vivo release studies

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## Summary

Films and microspheres were prepared from various esters of hyaluronic acid. A model drug, methylprednisolone, was either physically incorporated into the polymer matrix or chemically bound to the polymer backbone through an ester linkage. In vitro release from films with covalently bound drug was much slower ($t_{50\%}$ = 71 h) than that for physically dispersed drug ($t_{50\%}$ = 2.5–17 h). Methylprednisolone concentrations in the tear fluid of New Zealand rabbits were measured after ocular application of drug (approx. 420 $\mu$g) in different dosage forms. When methylprednisolone was physically dispersed in the polymer matrix, in vivo drug release from matrices was slower than that observed in vitro. Compared with a suspension control, peak methylprednisolone concentrations in tear fluid were 9–14 times lower after administration of drug in polymer films and $AUC_{0-8\ h}$ values were 4–7 times higher. These results imply that hyaluronic acid ester preparations can increase the residence time of methylprednisolone in the tear fluid of rabbits.

## Introduction

The ocular bioavailability of several drugs can be improved by prolonging the ocular contact time (Lee and Robinson, 1986). Several investigators (Chrai and Robinson, 1974; Patton and Robinson, 1975) have reported that low-viscosity ophthalmic solutions, while able to improve the amount of drug that penetrates the cornea, generally do not provide a sustaining effect. Conversely, the administration of ophthalmic drugs in gels (Patton and Robinson, 1975; Schoenwald et al., 1978) and in polymer matrices (Urtti et al., 1984; Finne et al., 1990) has been shown to sustain drug delivery to the cornea.

Hyaluronic acid is a naturally occurring mucopolysaccharide consisting of residues of D-glucuronic acid and *N*-acetyl-D-glucosamine. The polymer is particularly attractive for ophthalmic

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use because it is a major component of both the vitreous and aqueous humors. Currently, hyaluronic acid is used for intra-ocular injection during eye surgery. Various dosage forms based on hyaluronic acid have also been reported to increase the bioavailability of ophthalmic drugs (Saettone et al. 1989a,b, 1991).

Ester derivatives of hyaluronic acid have been prepared by several groups (Sparer et al., 1983; Della Valle and Romeo, 1987). Esterification provides a means of altering the polymer's physical properties, such as aqueous solubility. More interestingly, by linking drugs to the polymer either directly or through spacer groups, a polymeric drug carrier or 'macromolecular prodrug' can be formed. The pharmacologic and therapeutic properties of esterified hyaluronic acid are thought to be similar to those of the parent polymer (Della Vale and Romeo, 1987). The specific objective of this study is to evaluate various devices formed from hyaluronate esters for drug delivery to tear fluid.

Earlier studies have shown that in vitro hydrocortisone release from various hyaluronic acid ester films (Joshi, 1991) and microspheres (Benedetti et al., 1990) is highly dependent on the method of drug incorporation. For example, when hydrocortisone was physically incorporated into microspheres of the benzyl ester of hyaluronic acid, in vitro release was complete in approx. 10 min. In contrast, when hydrocortisone was covalently bound to the polymer, release was sustained for more than 100 h (Benedetti et al., 1990). In the current study, the release of methylprednisolone from hyaluronic acid ester films and microspheres in vitro and in the tear fluid of New Zealand non-pigmented rabbits was investigated. Methylprednisolone was either physically dispersed in the polymer matrix or covalently linked to hyaluronic acid.

## Materials and Methods

### *Materials*

All hyaluronic acid esters were supplied by Fidia S.p.A. (Abano Terme, Italy), and were used as received. The ester derivatives studied included ethyl (HYAFF7), benzyl (HYAFF11) and partial methylprednisolone (HYC41) esters. The manufacturer reported that the ethyl and benzyl esters have 100% of their carboxyl groups esterified as determined by either the saponification or Zeisel methods. The partial methylprednisolone ester (HYC41), in which a nominal 50% of the carboxyl groups were esterified to the drug and the remaining groups were present as the sodium salt, was supplied in dry powder form for film preparation (30% methylprednisolone, w/w) or formulated into microspheres. The latter were produced using a spray-drying process; the diameter of the resulting microspheres was between 1 and 10 $\mu$m. All microspheres used in this study were from the same lot. HPLC assay of the solubilized microspheres indicated a methylprednisolone content of $22.2 \pm 0.3\%$. It should be noted that the ethyl and benzyl esters are virtually insoluble in water, while the partial steroid ester is water soluble. 6$\alpha$-Methylprednisolone and 1,1,1,3,3,3-hexafluoro-2-propanol (hexafluoroisopropanol, HFIP) were used as received from Sigma Chemical Co. (St. Louis, MO). Acetonitrile and glacial acetic acid were purchased from Fisher Scientific (Fair Lawn, NJ) and physiological saline from LyphoMed (Rosemont, IL). All reagents were analytical grade and were used without further purification.

### *Production of dosage forms*

Methylprednisolone suspensions (16.8 $\mu$g/$\mu$l) were prepared in physiological saline; the pH of the resulting suspensions was approx. 5.0 (range: 4.5–5.4). The microspheres were administered as a dry powder (2.0 mg; dose of methylprednisolone, approx. 420 $\mu$g). Drug-loaded films were prepared by casting on glass plates, using a modification of an existing procedure (Joshi, 1991). Briefly, 500 mg of either the ethyl or benzyl ester were admixed with 215 mg of methylprednisolone, then dissolved in 15 ml of HFIP. This solution was poured onto glass plates in a fume hood and the HFIP was allowed to evaporate at room temperature for approx. 48 h. Films of the methylprednisolone ester were prepared by dissolving 500 mg of polymer in 9 ml of water. The film was dried in a microwave oven according to

an established procedure (Joshi et al., 1989). The approximate thicknesses of the resulting films as measured with a micrometer (Ames, Waltham, MA) were: HYAFF11, 250 $\mu$m; HYAFF7, 140 $\mu$m; and HYC41, 80 $\mu$m. Circular matrices with a 4 mm diameter were then cut from the dry films with a cork borer. Each matrix contained $420 \pm 38$ $\mu$g of methylprednisolone, as determined using the HPLC procedure described below. The approximate total masses of the final films were: ethyl ester, 2.0 mg; benzyl ester, 2.3 mg; methylprednisolone ester, 1.2 mg.

*Drug release in vitro*

The release of methylprednisolone from films and microspheres was studied using a USP rotating bottle apparatus (Vanderkamp sustained release apparatus, Van-Kel Industries, Inc., Edison, NJ) at 25 rpm. The dissolution medium was 50 ml of 2 mM phosphate buffer at pH 7.4; the ionic strength was adjusted to 0.5 M with sodium chloride. Release experiments were performed at 32°C to mimic conditions in the tear fluid. At preset time intervals, 1.0 ml samples were removed from the bottles, diluted with acetate buffer (0.5 ml, 1 M, pH 4.3) and stored at 4°C until analysis. The volume of liquid removed was replaced with fresh buffer. When release from microspheres was studied, samples were withdrawn using a syringe fitted with a 0.45 $\mu$m filter (Gelman Sciences, Ann Arbor, MI) and fresh buffer was added by flushing back through the filter. This procedure prevented removal of microspheres from bottles. The concentration of methylprednisolone in the samples was determined using the HPLC assay described below.

*Methylprednisolone in tear fluid*

Male New Zealand non-pigmented rabbits weighing 2.8–4.1 kg were used to measure the in vivo release of methylprednisolone in tear fluid. The rabbits were placed in restraining boxes during tear fluid sampling. The animals were allowed to move their heads freely and their eye movements were not restricted. Between experiments, the rabbits were housed singly and allowed food and water ad libitum.

Solution and suspension dosage forms were instilled in 25 $\mu$l volumes on the upper corneoscleral limbus. Matrix dosage forms and dry microspheres were applied in the lower conjunctival sac. Dosage forms were applied to the left eye of each rabbit. Tear fluid samples were collected from the lower marginal tear strip using 1 $\mu$l disposable glass capillaries (Drummond 'Microcaps', Fisher Scientific, St. Louis, MO) as described previously (Urtti et al., 1990). The capillaries were emptied into microcentrifuge tubes containing 200 $\mu$l of water and flushed several times. The methylprednisolone concentration in the resulting tear fluid samples was determined using the HPLC assay described below.

*HPLC analysis of methylprednisolone*

The high-performance liquid chromatography (HPLC) system, UV detector (model SPD-6A), pump (model LC-6A), controller (model SIL-6A) and the integrator (Chromatopac C-R4A) were all from Shimadzu (Tokyo, Japan). The column was a C18 reversed phase (0.46 cm i.d. $\times$ 25 cm, ODS, Hypersil®, 5 $\mu$m silica) from Keystone (State College, PA). The methylprednisolone concentration in the samples was measured according to a modification of the HPLC assay of Smith (1979). Chromatographic separations were achieved using a mobile phase of glacial acetic acid : distilled water : acetonitrile (2 : 65 : 35). The mobile phase was filtered under reduced pressure through a 0.45 $\mu$m filter (Gelman Scientific, Ann Arbor, MI) before use. The flow rate was held constant at 1.6 ml/min. The column effluent was monitored at 254 nm at an attenuation of 0.02 absorbance units full-scale. Under these conditions, the retention time for methylprednisolone was 5.8 min. Methylprednisolone concentrations were determined by measuring peak areas and comparing with a calibration curve prepared using known standards. The lower limit of detection for the assay was approx. 50 ng/ml.

*Data analysis*

For the in vitro drug release studies, the slopes ($k$) of the log (percent drug released) vs log (time) plots were calculated from the fitted linear regression lines. A slope of 1.0 indicates zero-

order release kinetics, while a slope of 0.5 indicates diffusional square-root-of-time release (Schwartz et al., 1968). The time required to release 50% of drug ($t_{50\%}$) was estimated from percent released vs time data for individual experiments.

For the in vivo studies, the tear fluid concentration vs time profiles were characterized with several different parameters for each animal and dosage form studied. The largest sampling time that drug was detected in tear fluid was recorded. In many cases, drug was detectable at the end of the 8 h experiment, and the 'largest time' value was recorded as 8 h. The maximum drug concentration in tear fluid ($C_{max}$) and the time at which this sample was drawn ($t_{max}$) were also noted. Due to the scatter in the data and the variability in profile shapes, the ($t_{max}$, $C_{max}$) value does not necessarily correspond to a well-defined 'peak' in the tear fluid concentration vs time profile. Finally, the area under the tear fluid concentration vs time profile from time 0 to 8 h ($AUC_{0-8}$) was calculated using the trapezoidal rule. Since the clearance of drug from tear fluid may depend on the type of dosage form administered, this $AUC_{0-8}$ value should not be considered a measure of 'bioavailability' in the classical pharmacokinetic sense (Gibaldi and Perrier, 1982), but rather a measure of the cumulative exposure of the cornea to the drug.

The mean values of various parameters in the in vitro and in vivo studies were compared using the software package StatView$^{tm}$ SE + graphics (Abacus Concepts, Inc., Berkeley, CA) and a Macintosh SE computer. A one-factor analysis of variance (ANOVA) was performed for each parameter; significance in the differences in the means was tested using Fisher's Protected Least Significant Difference (PLSD) at 95% confidence.

## Results and Discussion

### *In vitro release*

Methylprednisolone release from films and microspheres in vitro was highly dependent on the ester derivative of hyaluronic acid included in the preparation (Table 1, Fig. 1). When the drug was physically incorporated into films (HYAFF7, HYAFF11), the release into buffer solution was much faster than the release of covalently bound

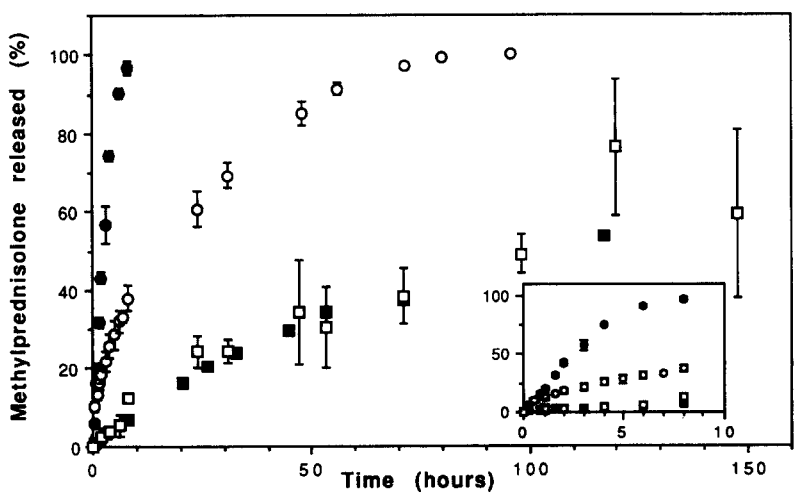


Fig. 1. In vitro methylprednisolone release from films and microspheres of hyaluronic acid esters. Data for films of the ethyl (HYAFF7) (●), benzyl (HYAFF11) (○) and methylprednisolone (HYC41) (■) esters and for microspheres of the methylprednisolone ester (HYC41) (□). Release experiments were conducted in 2 mM phosphate buffer, pH 7.4 and 32°C. Means ± S.E. are presented ($n$ = 3–4).

TABLE 1

*Release of methylprednisolone from films and microspheres of hyaluronic acid esters in phosphate buffer (2 mM, pH 7.4, 32°C)*

| Dosage form | $k$ [a] ± S.D. | $t_{50\%}$ (h) [b] ± S.D. | Number of trials ($n$) |
|---|---|---|---|
| Films | | | |
| Ethyl ester (HYAFF7) | 0.92 ± 0.08 | 2.6 ± 0.4 | 4 |
| Benzyl ester (HYAFF11) | 0.47 ± 0.04 | 17 ± 5 | 4 |
| Steroid ester (HYC41) | 0.62 ± 0.14 | 71 ± 27 [cd] | 3 |
| Microspheres | | | |
| Steroid ester (HYC41) | 0.70 ± 0.09 | 96 ± 12 [cd] | 3 |

[a] Slopes of log (percent drug released) vs log (time) plots.
[b] Time to release 50% of drug.
[c] Significantly different from the value for the ethyl ester film at 95% confidence.
[d] Significantly different from the value for the benzyl ester film at 95% confidence.

drug (HYC41). A similar sustaining of release was observed when the polymer-drug conjugate (HYC41) was formulated into microspheres.

The type of hyaluronate ester used for physical incorporation of the drug also affected the rate of release. After 8 h, 100% of the incorporated methylprednisolone was released from ethyl ester films, while only about 40% was released from benzyl ester films (Fig. 1). Complete release of the drug from benzyl ester films required about 80 h. In general, over a wide range of permeant molecular weight, the permeability values in the ethyl ester films are greater than in the benzyl ester films (Hunt et al., 1990; Papini et al., 1991). This may be due to the significantly greater hydration of ethyl ester films (259% vs 48%) (Hunt et al., 1990). The more rapid release of methylprednisolone from the ethyl ester films in this study is consistent with their greater permeability.

Methylprednisolone was completely released from benzyl ester films according to square-root of time kinetics ($k = 0.47$, Table 1). In contrast, about 80% of the drug was released from ethyl ester films according to near zero-order kinetics ($k = 0.92$, Table 1). This difference in the order of release suggests a difference in mechanism. The amount released is proportional to the square-root of time in both the Higuchi moving boundary model (Higuchi, 1961) and in models based on a semi-infinite domain (Crank, 1975). In either case, the diffusion of the drug through the hydrated polymer is rate-limiting. In contrast, a number of mechanisms may result in zero-order release. These include certain systems with rate-limiting membrane or aqueous boundary layer control of drug release, and systems in which the dissolution of dispersed solid drug is rate-limiting. The zero-order release observed here for the ethyl ester films may suggest that the dissolution of suspended solid drug is rate-limiting. However, these films are highly hydrated and appear translucent after drug incorporation. The aqueous boundary layer resistance has been found to be a significant fraction of the total resistance to drug release for ethyl ester films of similar thickness in Franz diffusion cells (Joshi, 1991). Since the magnitude of the boundary layer resistance cannot be determined in the rotating bottle apparatus used here, the possibility that the aqueous boundary layer resistance is responsible for the observed zero-order release cannot be ruled out.

When the drug was covalently bound to the polymer and formed into films or microspheres, drug release followed neither zero-order nor square-root of time kinetics ($k = 0.6$–$0.7$, Table 1). Various rate processes, including hydration and ester hydrolysis, may interact to give this 'mixed' release. These processes may exhibit non-linear dependences on pH, temperature and ionic strength (Goei et al., 1989). The cause for the large run-to-run variation in release observed for the microspheres (Fig. 1) is not known at this time.

*Release in tear fluid*

Data on the concentrations of drug in tear fluid are presented in Table 2 and in Figs 2 and 3. Figs 2 and 3 show the mean tear fluid concentration vs time profiles for all the dosage forms studied. The hyaluronate ester formulations appear to offer a significant sustaining of drug release relative to the suspension control. High initial tear fluid concentrations of approx. 1000

TABLE 2

*Release of methylprednisolone into rabbit tear fluid following ocular administration of 420 μg of drug in various dosage forms: summary of tear fluid concentration vs time profiles*

| Dosage form | Suspension | Microsphere HYC41 | Film HYC41 | Film HYAFF11 | Film HYAFF7 |
|---|---|---|---|---|---|
| Largest time drug detected (min) ± S.D. | 190 ± 45 | 270 ± 103 | 345 ± 158 [a] | 480 ± 0 [a] | 480 ± 0 [a] |
| $t_{max}$ (min) ± S.D. [b] | 1.5 ± 0.5 | 42 ± 52 | 89 ± 90 [a] | 49 ± 60 | 33 ± 58 |
| $C_{max}$ (μg ml$^{-1}$) ± S.D. [c] | 1600 ± 1800 | 102 ± 132 [a] | 117 ± 48 [a] | 146 ± 65 [a] | 168 ± 53 [a] |
| AUC (μg ml$^{-1}$ min) ± S.D. [d] | 4300 ± 3600 | 11600 ± 17600 | 17600 ± 14100 [a] | 29700 ± 13700 [a] | 23500 ± 6500 [a] |
| Number of trials ($n$) | 6 | 4 | 10 | 9 | 9 |

[a] Significantly different from the suspension control at 95% confidence.
[b] Time of largest measured tear fluid concentration.
[c] Largest tear fluid concentration measured; does not necessarily correspond to a well-defined peak in the profile.
[d] Area under the tear fluid concentration vs time curve from $t = 0$ to $t = 8$ h as determined by the trapezoidal rule.

μg/ml were observed for the methylprednisolone suspension; these decreased to less than 10 μg/ml within 30–60 min, and to undetectable levels in less than 200 min. In contrast, the hyaluronate ester formulations showed concentrations of 10–80 μg/ml for up to 8 h, with no initial peaks as observed for the control. In addition, it appears that the hyaluronate dosage forms also provided near-constant tear fluid concentrations. While this is true in the mean, constant tear fluid concentrations were only observed in about half the individual animals studied; others displayed concentrations that increased or decreased with time. Thus, the hyaluronate ester dosage forms do provide a significant sustaining of release relative to the suspension control, but cannot claim to provide constant tear fluid concentrations in a given animal.

Key parameters describing the tear fluid concentration vs time profiles are presented in Table

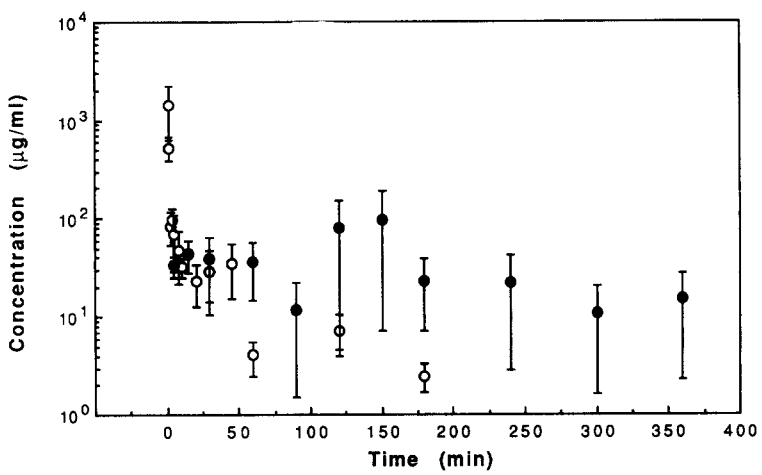


Fig. 2. Concentration of methylprednisolone in rabbit tear fluid vs time following ocular administration of 420 μg of the drug in microspheres. Data for microspheres of the methylprednisolone ester (HYC41) (●) and for a control methylprednisolone suspension (○). Note that the scale for tear fluid concentration is logarithmic. Means ± S.E. are presented ($n = 6$, suspension; $n = 4$, microspheres).

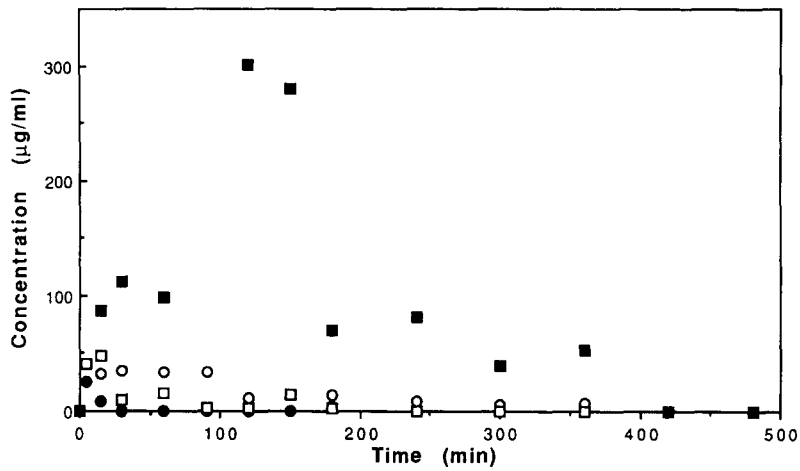


Fig. 3. Concentration of methylprednisolone in rabbit tear fluid vs time following ocular administration of 420 μg of the drug in microspheres. Data of Fig. 2, showing variability in the four individual experiments. Each symbol represents a different trial.

2. All of the hyaluronate ester formulations showed significantly lower peak tear fluid concentrations than the control. In addition, the latest time drug could be detected and the AUC values were significantly greater for the hyaluronate films than for the control; the microsphere formulation did not provide a significant increase in these parameters. Taken together, this quantitative information provides further evidence that drug release from hyaluronate ester films is sustained relative to the conventional methylprednisolone suspension. Furthermore, the differences in the AUC values for the films suggest that the cumulative exposure of the cornea to the drug is increased with film formulations.

When microspheres were administered as a dry powder, methylprednisolone was detected in the tear fluid for an average of 270 min (Table 2, Fig. 2). The tear fluid concentrations were characterized by a very high variability (Fig. 3). In two

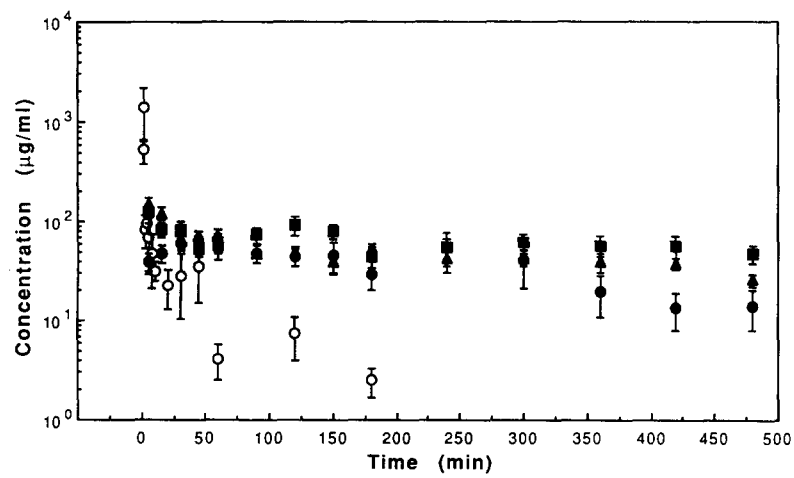


Fig. 4. Concentration of methylprednisolone in rabbit tear fluid vs time following ocular administration of 420 μg of the drug in films. Data for films of the ethyl (HYAFF7) (▲), benzyl (HYAFF11) (■) and methylprednisolone (HYC41) (●) esters of hyaluronic acid, and for a control methylprednisolone suspension (○). Note that the scale for tear fluid concentration is logarithmic. Means ± S.E. are presented ($n$ = 6, suspension; $n$ = 9 or 10, films).

of the four rabbits studied, the microspheres appeared to hydrate forming a gelatinous matrix approx. 5 min after administration, and were retained in the eye for up to 360 min. In the remaining rabbits, no polymer gel was visible and essentially no drug was detected 30 min after administration. It should be noted that administration of microspheres as a dry powder was only a model system to study the behavior of the microspheres in the eye and is not intended for human treatment.

The release of methylprednisolone into tear fluid from films of the various esters is presented in Table 2 and in Fig. 4. In addition to the numerical data, several visual observations are noteworthy. Films of the methylprednisolone ester (HYC41) appeared to swell after about 5 min in the eye. HYC41 films were visible in the eyes of six of the ten rabbits studied for the full 8 h of the study; in three additional rabbits, no films were observed after 90 min. Since the rabbits were not restrained throughout the entire experimental period, the absence of the methylprednisolone ester films may suggest that the animals removed them. In general, the detection of methylprednisolone in tear fluid corresponded with the observation of HYC41 films in the conjunctival sac. In contrast, films formed from the ethyl (HYAFF7) and benzyl (HYAFF11) esters with drug physically incorporated did not appear to swell. Ethyl and benzyl ester films were visible in the eyes of the 18 rabbits studied for the full 8 h of the experiment.

The largest time point at which drug was detected in tear fluid is reported in Table 2 for the methylprednisolone, benzyl and ethyl ester films. In general, drug was detectable for longer periods for films in which drug was physically incorporated (HYAFF11 and HYAFF7) than for the film in which the drug was covalently bound (HYC41). In addition, the mean AUC values were greater for films with physically incorporated drug. These differences may be due to the longer retention of physically incorporated films in the eye. Alternatively, they may reflect very slow release of drug from the methylprednisolone ester films, as observed in vitro.

From the in vitro $t_{50\%}$ values (Table 1), one might expect that drug release in tear fluid would be most rapid from ethyl ester films, somewhat slower from benzyl ester films, and slowest from methylprednisolone ester films and microspheres. The $t_{max}$ values listed in Table 2 suggest that this order is preserved in general, but that the differences in release rate are not as dramatic as in vitro. There are several possible reasons for the differences between the in vitro and in vivo release profiles. First, the drug release rate and tear fluid clearance combine to give the in vivo concentration profile; clearance factors are absent in vitro. In addition, hydrodynamic conditions in the thin film of tear fluid and in the rotating bottle apparatus are quite different. Also, the tear fluid volume may vary from day-to-day and from animal-to-animal, adding variability to the in vivo studies. Finally, it is possible that the films do not achieve their full equilibrium hydration values in tear fluid due to the limited volume of tears present. The equilibrium weight increases of the benzyl and ethyl ester films on hydration are 48 and 259%, respectively (Hunt et al., 1990).

Taken as a whole, the results suggest that administration of methylprednisolone in films or microspheres of hyaluronic acid esters can increase the residence time of the drug in rabbit tear fluid relative to a suspension control. Drug delivery devices based on hyaluronic acid derivatives may prolong the absorption time and increase the ophthalmic delivery of other steroids.

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## References

Benedetti, L.M., Topp, E.M. and Stella, V.J., Microspheres of hyaluronic acid esters-fabrication methods and in vitro hydrocortisone release. *J. Control. Rel.*, 13 (1990) 33–41.

Chrai, S.S. and Robinson, J.R., Ocular evaluation of methylcellulose vehicle in albino rabbits. *J. Pharm. Sci.*, 63 (1974) 1218–1227.

Crank, J., *The Mathematics of Diffusion*, Clarendon, Oxford, 1975, pp.32–38.

Della Valle, F. and Romeo, A., Hyaluronic acid esters and their medical and cosmetic uses and formulations. *Eur. Patent. Appl. EP216453*, April 1, 1987, 129 pp.

Finne, U., Väisänen, V. and Urtti, A., Modification of ocular and systemic absorption of timolol from ocular inserts by a buffering agent and a vasoconstrictor. *Int. J. Pharm.*, 65 (1990) 19–27.

Gibaldi, M. and Perrier, D., *Pharmacokinetics*, 2nd Edn, Dekker, New York, 1982, pp. 169–180, 445–449.

Goei, L., Benedetti, L., Topp, E.M. and Stella, V.J., Drug release from hydrocortisone esters of hyaluronic acid. *Pharm. Res.*, 6 (1989) S–94.

Higuchi, T., Rate of release of medicaments from ointment bases containing drugs in suspension. *J. Pharm. Sci.*, 50/10 (1961) 874–875.

Hunt, J.A., Joshi, H.N., Stella, V.J. and Topp, E.M., Diffusion and drug release in polymer films prepared from ester derivatives of hyaluronic acid. *J. Control. Rel.*, 12 (1990) 156–169.

Joshi, H.N., In vitro characterization of membranes of hyaluronic acid and its esters. Ph.D. dissertation, The University of Kansas, June 24, 1991.

Joshi, H.N., Kral, M.A. and Topp, E.M., Microwave drying of aqueous tablet film coatings: a study on free films. *Int. J. Pharm.*, 51 (1989) 19–25.

Lee, V.H.L. and Robinson, J.R., Topical ocular drug delivery: recent developments and future challenges. *J. Ocul. Pharmacol.*, 2 (1986) 67–108.

Papini, D., Stella, V.J. and Topp, E.M., Diffusion of peptides and proteins in polymer films prepared from ester derivatives of hyaluronic acid, *Proceedings of the 18th International Symposium on the Controlled Release of Bioactive Materials*, Amsterdam, The Netherlands, July 8–11, 1991.

Patton, T.F. and Robinson, J.R., Ocular evaluation of polyvinyl alcohol vehicle in rabbits. *J. Pharm. Sci.*, 64 (1975) 1312–1316.

Saettone, M.F., Chetoni, P., Torracca, M.T., Burgalassi, S. and Giannaccini, B., Evaluation of muco-adhesive properties and in vivo activity of ophthalmic vehicles based on hyaluronic acid. *Int. J. Pharm.*, 51 (1989a) 203–212.

Saettone, M.F., Giannaccini, B., Chetoni, P., Torracca, M.T. and Monti, D., Evaluation of high- and low-molecular-weight fractions of sodium hyaluronate and an ionic complex as adjuvants for topical ophthalmic vehicles containing pilocarpine. *Int. J. Pharm.*, 72 (1991) 131–139.

Saettone, M.F., Monti, D., Torracca, M.T., Chetoni, P. and Giannaccini B., Muco-adhesive liquid ophthalmic vehicles-evaluation of macromolecular ionic complexes of pilocarpine. *Drug. Dev. Ind. Pharm.*, 15 (14–16) (1989b) 2475–2489.

Schoenwald, R.D., Ward, R.L., DeSantis, L.M. and Roehrs, R.E., Influence of high-viscosity vehicles on miotic effect of pilocarpine. *J. Pharm. Sci.*, 67 (1978) 1280–1283.

Schwartz, J.B., Simonelli, A.P. and Higuchi, W.I., Drug release from wax matrices. I. Analysis of data with first-order kinetics and with the diffusion-controlled model. *J. Pharm. Sci.*, 57 (1968) 274–277.

Smith, M.D., High-performance liquid chromatographic determination of hydrocortisone and methylprednisolone and their hemisuccinate esters in human serum. *J. Chromatogr.*, 164 (1979) 129–137.

Sparer, R.V., Ekwuribe, N. and Walton, A.G., Controlled release from glycosaminoglycan drug complexes. In Roseman, T.J. and Mansdorf, S.Z. (Eds), *Controlled Release Delivery Systems*, Dekker, New York, 1983, pp. 107–119.

Urtti, A., Pipkin, J.D., Rork, G., Sendo, T., Finne, U. and Repta, A.J., Controlled drug delivery devices for experimental ocular studies with timolol.2. Ocular and systemic absorption in rabbits. *Int. J. Pharm.*, 61 (1990) 241–249.

Urtti, A., Salminen, L., Kujari, H. and Jäntti, V., Effect of ocular pigmentation on pharmacology in the rabbit eye.II. Drug response. *Int. J. Pharm.*, 19 (1984) 53–61.