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Ocular disposition of poly-hexyl-2-cyano[3-^{14}C]acrylate nanoparticles in the albino rabbit

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Summary

The disposition of poly-hexyl-2-cyanoacrylate nanoparticles in tears, aqueous humor, cornea and conjunctiva of albino rabbits was studied using radiotracer techniques. Results indicate that the majority of nanoparticles were drained away rapidly with a small percent adhering to corneal and conjunctival surfaces. The nanoparticles underwent degradation in collected tear samples. Within the time course of the study, the conjunctival nanoparticle level was fairly constant, while the corneal nanoparticle level decreased slowly. The radioactivity observed in the aqueous humor was presumably due to degradation of the nanoparticles rather than endocytosis of the intact nanoparticle. The mucolytic agent, N-acetyl-L-cysteine, had no effect on either the corneal nanoparticle level nor the radioactivity level observed in the aqueous humor, but it increased the conjunctival nanoparticle level.

Introduction

Topical application of a drug to the eye in a conventional solution results in extensive drug loss. Indeed, usually only a small amount (1–3%) actually penetrates

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the cornea and reaches intraocular tissues (Patton and Robinson, 1976). The reasons for this are primarily due to complex tear fluid dynamics, such as tear turnover, lacrimal drainage, and drug dilution by tears (Lee and Robinson, 1979). One approach that has been taken to improve ocular drug absorption is to decrease the rate constant governing drainage. This can be accomplished by the use of viscous solutions (Li, 1984; Patton and Robinson, 1975; Chrai and Robinson, 1974), suspensions (Sieg and Robinson, 1975; Schoenwald and Stewart, 1980), polymeric inserts (Katz and Blackman, 1977; Bensinger et al., 1976; Waltman et al., 1970; Shell and Baker, 1974), ointments (Li, 1984; Sieg and Robinson, 1979), gels (Schoenwald and Boltriak, 1979; Miller and Donovan, 1982), and, more recently, microencapsulated drug particles such as liposomes and biocompatible polymeric materials (Stratford et al., 1983; Singh and Mezei, 1983; Schaeffer and Krohn, 1982; Gurny, 1981). 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. The present work investigates the ocular disposition of poly-hexyl-2-cyanoacrylate nanoparticles in albino rabbits.

Nanoparticles are colloidal particles, ranging in size from 10 to 1000 nm, in which drug may be entrapped, encapsulated, and/or adsorbed. To date, it has been demonstrated that nanoparticles are useful as adjuvants for vaccines (Kreuter and Speiser, 1976; Kreuter et al., 1976; Kreuter and Liehl, 1978, 1981) and are able to enhance the antitumor activity of some cytostatic agents (Brasseur et al., 1980).

Materials and Methods

Preparation of the nanoparticles

Hexyl-2-cyano-[3-^{14}C]acrylate with specific activity of 2.43 mCi/g was synthesized by Amersham (Amersham, U.K.). One ml of this preparation was dissolved in a solution of 200 mg Pluronic F 68 (Serva, Heidelberg, F.R.G.) and 1.0 g Dextran 70 (Pharmacia, Uppsala, Sweden) in 100.0 ml of 0.05 N HCl and stirred for 4 days at room temperature with a magnetic stirrer at about 400 rpm. The resulting polymer suspension was then filtered over a G1 fritte and stored in the refrigerator (4°C) until used. The suspension was neutralized with 0.1 N NaOH just prior to the experiments.

In vitro degradation

A 20-μl aliquot of the poly-hexyl-2-cyano-[3-^{14}C]acrylate nanoparticle suspension (Amersham, U.K.) was added to 100 μl of freshly collected albino rabbit tears in a holding vial. Tears were collected using 3 μl capillary tubes (American Hospital Supply, FL, U.S.A.). The holding vial containing nanoparticles in the tear fluid were then shaken in a constant temperature water bath at 37°C for 6 h. Two 5-μl samples were removed at the beginning of the experiment, and 5 μl samples were subsequently removed at 10, 30, 60, 180 and 360 min after initiating the experiment. These samples were then diluted with 155 μl of 0.1 N HCl in centrifuge tubes and centrifuged (Airfuge, Beckman, CA, U.S.A.) at 100,000 *g* for 15 h. Eighty μl of the

supernatant was pipetted into scintillation vials, a commercial liquid scintillation solution (Aquasol, New England Nuclear, MA, U.S.A.) was added, and samples were counted using a scintillation spectrometer (Packard Instruments, IL, U.S.A.). Two 5-μl aliquots of the original nanoparticle suspension were also removed, one of which was added directly to scintillation vials and counted. The other was diluted with 155 μl of 0.1 N HCl, centrifuged, and then counted.

In vivo disposition studies

In preparation for the in vivo disposition studies, the nanoparticle suspension was sonicated for 2–10 min to disperse the suspension. Prior to dosing, the suspension was neutralized with 0.1 N NaOH. Fully awake albino rabbits, 2–2.5 kg, (Klubertanz, WI, U.S.A.) were dosed with 25 μl of 0.385% (w/v) nanoparticle suspension by topical application onto the cornea. For instillation, the lower lid was gently pulled away from the globe of the eye to form a pocket but was immediately returned to the normal position after dosing. Animals were maintained in an upright position using restraining boxes. At periodic intervals, post-instillation, rabbits were sacrificed by rapid injection of sodium pentobarbital. Approximately 200 μl of aqueous humor was aspirated from the anterior chamber, and a 150-μl aliquot was quantitatively transferred to a liquid scintillation counting vial. Scintillation cocktail was added to the vial and the counts determined using a scintillation spectrophotometer. The cornea and part of the conjunctiva were then excised using a scalpel, gently rinsed with normal saline, carefully blotted with tissue paper, and weighed. These tissue samples were then placed in scintillation vials and 1.0 ml of tissue solubilizer (Protosol, New England Nuclear, MA, U.S.A.) added to each vial and left overnight in an oven at 55°C. An aliquot (100 μl) of a 30% solution of hydrogen peroxide was added to each vial and then left to cool for approximately 30 min. Scintillation fluid was then added to each vial and the samples counted.

In order to assess the concentration of nanoparticles remaining in the precorneal area of the eye, albino rabbits were dosed with nanoparticles as previously described. A tear sample was withdrawn from the center of the lower marginal tear strip using a disposable 1-μl glass pipet (Drummond Scientific, PA, U.S.A.). No more than 3 tear samples were withdrawn from the same eye postdosing. Pipets containing tear samples were then transferred to minivials (RPI, IL, U.S.A.) containing 0.5 ml of doubly-distilled water. 4.5 ml of scintillation cocktail were added 1 h later. Tear samples were stored in the dark for at least 24 h prior to counting. The presence of glass pipets in the scintillation fluid did not alter the counting efficiency or affect the results in any way.

Effect of predosing with a mucolytic agent

Albino rabbits were dosed with 25 μl of a 20% (w/v) solution of N-acetyl-L-cysteine (U.S. Biochem., OH, U.S.A.) as previously described. After 10 min, the animals were dosed with 25 μl of the nanoparticle suspension. Five minutes later, the animals were sacrificed by rapid injection of sodium pentobarbital into a marginal ear vein. Aqueous humor was aspirated, the cornea excised, and a portion of the conjunctiva also removed. The various tissues were weighed and prepared for counting as previously described.

Results and Discussion

Fig. 1 illustrates the results obtained from the in vitro degradation studies. At zero time, approximately 3–4% of the nanoparticles appeared to be in a degraded form. This suggests that the nanoparticle suspension contained impurities probably due to unpolymerized monomer or possibly aqueous degradation products. Degradation of the nanoparticles in tears occurred at a relatively rapid rate for the first hour with approximately 19% degradation followed by a general levelling off over the next 5 h. The degradation of alkyl-2-cyanoacrylates have been investigated by previous workers. Leonard et al. (1966) report the degradation of poly-alkyl-cyanoacrylates in aqueous solution as following the route shown in Scheme I. Lenaerts et al. (1984) investigated the degradation of polyisobutylcyanoacrylate in the presence of rat liver microsomes and identified isobutanol as the major degradation product (Scheme II). Either of these degradation mechanisms may be operating in the tear fluid. It has long been known there is considerable lysosomal activity in the precorneal area (Cummings, 1980), and it is possible that this enzyme is involved in degradation of the nanoparticles. The fact that this polymer is susceptible to biodegradation may be advantageous from a drug delivery point of view, since drug dispersed within the polymeric matrix will be released and available for absorption as the nanoparticles degrade.

Concentration of nanoparticles in the tear film versus time profile following topical application of the nanoparticle suspension is shown in Fig. 2. Similar to aqueous drug solutions, nanoparticles were rapidly removed from the precorneal area as a result of drainage. However, nanoparticles were better retained than drug solutions, possibly due to binding to mucin or buoyancy of the nanoparticles, whose density is approximately 1 g/ml (Kreuter, 1983). Both factors are expected to slow removal of nanoparticles by bulk movement of tears.

Profiles of corneal and conjunctival concentration of nanoparticles for up to 360 min following topical administration are shown in Fig. 3. The conjunctival concentration of nanoparticles remains essentially constant over the time frame for which this tissue was sampled. Moreover, the corneal concentration of nanoparticles exhibits only a modest decline during this same time period.

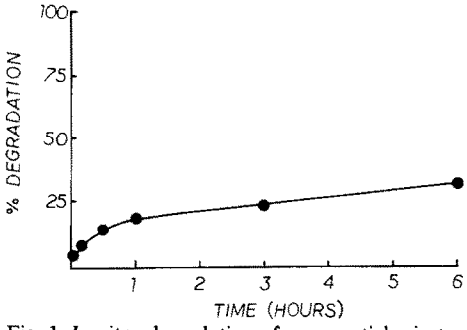
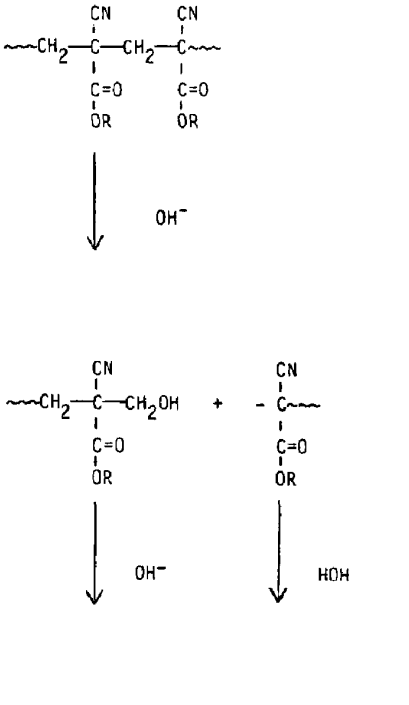


Fig. 1. In vitro degradation of nanoparticles in tears of the albino rabbit.

Scheme 1



Scheme 2

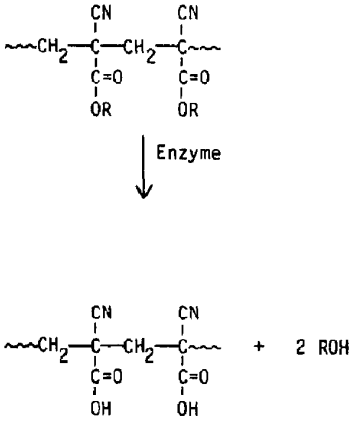


Fig. 2.

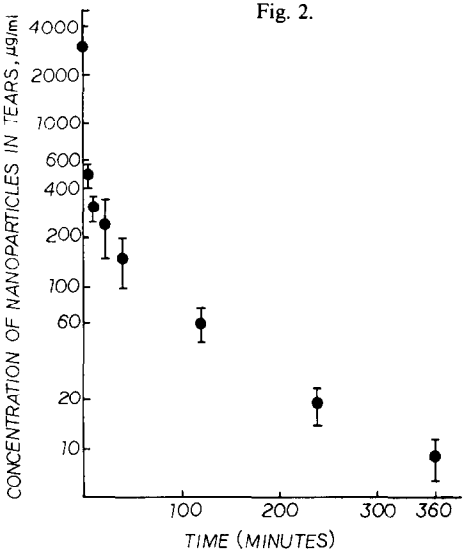


Fig. 3.

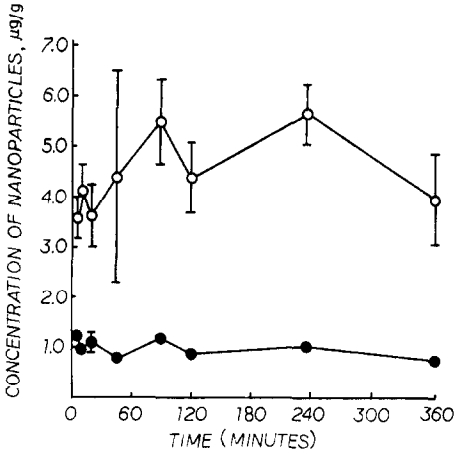


Fig. 2. Concentration versus time profile of nanoparticles in the tear film. The zero time point was calculated based on a resident tear fluid volume of 7 μl. (Error bars represent standard error of the mean, $n \geq 8$.)

Fig. 3. Concentration versus time profile of nanoparticles in the cornea (●) and in the conjunctiva (○). (Error bars represent standard error of the mean, $n \geq 8$.)

It was found that approximately 0.1% of the initial amount of nanoparticles remained on the cornea after 360 min based on comparison of number of counts detected in this tissue with the number of counts obtained with the 25 μl volume of nanoparticles used for dosing. Similarly, approximately 0.1% of the dose was found in the conjunctival sample for up to 360 min post-instillation. The surface area of the conjunctiva has been reported to be six times larger than the cornea (Cummings, 1980). If we assume the binding affinity of the nanoparticles is similar for both tissues and further assume that the amount taken up is directly proportional to surface area, then it can be estimated that approximately 0.6% of the initial dose is retained by the conjunctiva for up to 360 min post-instillation. Therefore, the fraction of initial dose retained by the combined cornea and conjunctiva, at 360 min post-instillation, approaches 1%.

Although endocytosis of particles of colloidal dimensions by ocular tissue has been demonstrated (Kaye and Pappas, 1962), it is unlikely that this process is occurring in the case of these nanoparticles. However, since the concentration of nanoparticles in the conjunctiva and cornea is relatively constant for up to 360 min post-instillation, it is likely that adhesion of the nanoparticles to the mucin/epithelial surface of the cornea and conjunctiva is occurring. The structural features of the polymer (see Schemes I and II), i.e. charge density and hydrophobicity, are similar to those of polymers that have been previously shown to possess bioadhesive properties (Ch'ng et al., 1984).

One of the existing theories regarding the mechanism of bioadhesion is that bioadhesive polymer adheres at the mucin–epithelial surface of cells. To determine the effect of mucin on the interaction between the nanoparticles and conjunctival and corneal tissue, animals were predosed with N-acetyl-L-cysteine, a known mucolytic agent (Swingard and Pathak, 1980) before instilling the nanoparticle suspension. The results of this experiment are shown in Table 1. There was no significant difference between treatments for the cornea and aqueous humor suggesting that the nanoparticles are able to adhere directly to corneal tissue. The results shown in Table 1 also indicate that the mucin layer does not appear to represent a barrier to permeation of the cornea by degradative products of the polymer. However, there is a significantly higher conjunctival concentration of nanoparticles when the eye was treated with the mucolytic agent. Upon treatment with the mucolytic agent, it was observed that quantities of mucin collected in the cul-de-sac forming a gel-like substance. It is probable that nanoparticles became entrapped in this substance, and this may account for the higher conjunctival concentration with N-acetyl-L-cysteine treatment.

For drug solutions applied topically to the eye, it has been shown that only the first 5 min are important for corneal absorption (Lee and Robinson, 1979). After 5 min, due to rapid loss of drug from the tear film, negligible amounts of drug are absorbed by the cornea. Therefore, if 1% of the nanoparticle dose is retained in the precorneal area by adhesion to the cornea and conjunctival tissue, contact time between the dosage form and the absorbing tissue is increased.

A profile of ^{14}C-label found in the aqueous humor as a function of time following topical application of the nanoparticle suspension is shown in Fig. 4. It is assumed that intact nanoparticles are unable to permeate the cornea and enter the anterior segment. Since it is known that there were ^{14}C-labelled impurities initially present in the dosage form and that the nanoparticles biodegrade upon instillation into the tear film, the measured chemical in the aqueous humor is probably not nanoparticles but rather a degradation product.

TABLE 1

EFFECT OF PREDOSING WITH N-ACETYL-L-CYSTEINE ON CONCENTRATION OF NANOPARTICLES IN OCULAR TISSUES [a]

Tissue	Concentration (μg/g or dpm/ml) of nanoparticles without N-acetyl-L-cysteine pretreatment	Concentration (μg/g or dpm/ml) of nanoparticles with N-acetyl-L-cysteine pretreatment
Cornea	1.25 (0.057) [b]	2.42 (0.597)
Conjunctiva	3.64 (0.622)	6.02 (0.513) *
Aqueous humor [c]	6.80×10^5 (4.22×10^4)	9.28×10^5 (2.45×10^5)

[a] Tissues were analyzed for ^{14}C 5 min following topical dose of nanoparticles.
[b] Numbers in parentheses are standard error of the mean, n = 8.
[c] Disintegrations per min per ml of aqueous humor.
* Significant difference at $P < 0.025$.

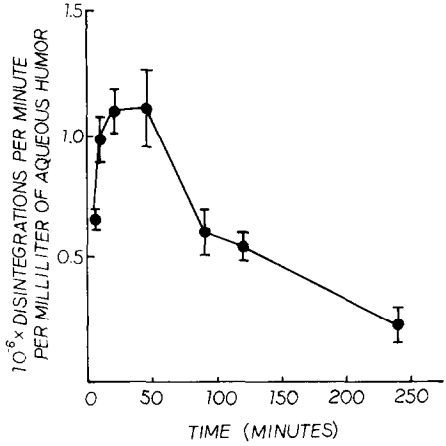


Fig. 4. Disintegration ($\times 10^{-6}$) per min per ml of aqueous humor versus time. (Error bars represent standard error of the mean, $n \geq 8$.)

In summary, poly-hexyl-2-cyanoacrylate nanoparticles are degradable in tear fluid and able to adhere to corneal and conjunctival surfaces. Although this preliminary study on precorneal ocular retention of poly-hexyl-2-cyanoacrylate gives less than ideal rate of degradation and percent of dose retained, it is likely that a different cyanoacrylate ester would generate a more favorable profile. The potential of nanoparticles as ocular drug delivery systems will be discussed in a subsequent publication.

Acknowledgement

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