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Distribution of poly-hexyl-2-cyano-[3-^{14}C]acrylate nanoparticles in healthy and chronically inflamed rabbit eyes

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Summary

The distribution of poly-hexyl-2-cyanoacrylate nanoparticles in aqueous humor, cornea, conjunctiva and nictitating membrane of albino rabbits in healthy and chronically inflamed eyes was studied using radiotracer techniques. Results indicate that the nanoparticles adhere to these tissues for several hours after instillation. The residence time in inflamed tissues was about 4 times higher than in healthy tissues. The low radioactivity observed in the aqueous humor was presumably due to degradation of the nanoparticles rather than to endocytosis of the intact nanoparticles.

Introduction

Topical application of a drug to the eye is the most common route of ocular drug treatment. Only a small amount of the instilled dose (1–10%) penetrates across the cornea and reaches the internal target areas. 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 colloidal drug carriers (Harmia et al., 1986; Fitzgerald et al., 1987). Polycyanoacrylate colloidal carriers were eliminated from the tears with a half-life of approximately 20 min (Wood et al., 1985). However, they prolonged the intraocular pressure-reducing effect of pilocarpine in rabbits for more than 9 h, using the betamethasone-model (Diepold et al., 1989). One possible explanation of the prolonged retention of these nanoparticulate polycyanoacrylate drug carriers is that they are slightly bioadhesive, consistent with the usual properties of acrylic acid polmers. If this explanation is valid, it is possible that their retention in inflamed ocular tissue would be even greater than in normal tissue. Such a finding would suggest that these systems would be good carriers for anti-inflammatory and other drugs, and would be considered targeted delivery systems for certain ocular pathologies.

For this reason the present work investigated the ocular distribution of poly-hexyl-2-cyanoacrylate nanoparticles in healthy and inflamed albino rabbit eyes.

Materials and Methods

Preparation of the nanoparticles

Hexyl-2-cyano[3-^{14}C]acrylate with spec. act. 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 F68 (Serva, Heidelberg, F.R.G.) and 1.0 g Dextran 70

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(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 vivo distribution studies

New Zealand albino rabbits were dosed topically with 50 µl clove oil in one eye after local anesthesia (25 µl oxybuprocain 0.4%). At 24 h after clove oil application, fully awake rabbits, 2.5–3.0 kg, were dosed in both eyes with 30 µl of the poly-hexyl-2-cyanoacrylate nanoparticle suspension by topical application to the cornea. Activity of one dose was 0.304 µCi, relating to 1.91 mg polymer/30 µl. Prior to dosing, this suspension was adjusted to pH 5.7. Tonicity was 310 mOsm. For instillation, the lower lid was gently pulled away from 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, the rabbits were sacrificed by rapid injection of sodium pentobarbital (2 ml/kg). Aqueous humor was aspirated from the anterior chamber. The conjunctiva, the nictitating membrane and the cornea 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 (Soluene-350, Packard) was added to each vial. The vials were shaken in a waterbath at 55° C for 4 h. An aliquot (100 µl) of a 30% solution of hydrogen peroxide was then added to each vial and left to cool for approximately 30 min. After this, 1.0 ml HCl (6.0 N) and 10 ml scintillation fluid (ACS II, Amersham) were added and the samples counted in a liquid scintillation counter (Betamatic, Kontron).

Results and Discussion

A variety of chemical and mechanical insults, including known inflammatory agents, have been used to create a standardized ocular inflammation. Chronic inflammation by intracorneal injection of clove oil is a well established animal model (Leibowitz and Kupfermann, 1974). In our study topical clove oil application was used.

Figs. 1–4 show the distribution profiles of poly-hexyl-2-cyano[3-^{14}C]acrylate nanoparticles in the conjunctiva, cornea, nictitating membrane and aqueous humor in healthy and chronically inflamed eyes due to topical clove oil pretreatment. The concentration of labelled nanoparticles in tissues of the precorneal area, as well as the aqueous humor level versus time profile, is shown in Figs. 1–4. Tissue concentrations were expressed as µg polymer/g tissue in healthy (open circles) and inflamed eyes (closed circles).

Fig. 1 illustrates the results obtained for the conjunctiva. Within 5–10 min post dosing a maximum peak concentration of nanoparticles on healthy conjunctiva was observed, with a rapid decrease during the first hour. The concentration in the healthy conjunctiva 10 min post instillation corresponds to 7% of the initial dose. During the first hour, this value dropped to about 0.3% and then stayed approximately constant for up to 240 min post instillation. This level was very consistent with previous work (Wood et al., 1985).

In the inflamed conjunctiva, the radioactivity was almost half of that in the healthy conjunctiva at early time points. Later, it showed a much less significant decrease and remained constant up to 4 h post dosing at a level of approximately 2% of

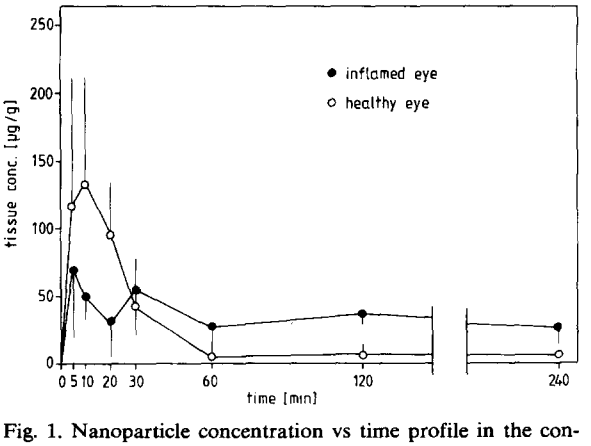

Fig. 1. Nanoparticle concentration vs time profile in the conjunctiva. Mean ± S.D., $n = 10$.

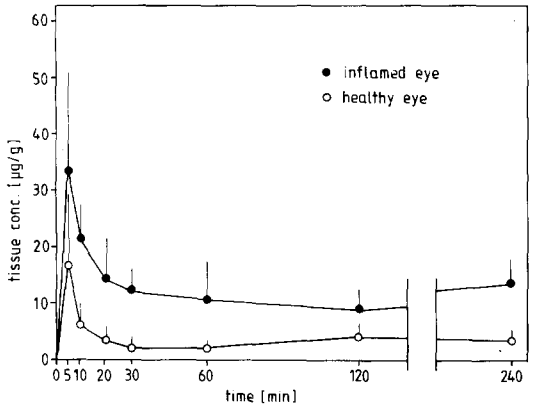

Fig. 2. Nanoparticle concentration vs time profile in the cornea. Mean ± S.D., $n = 10$.

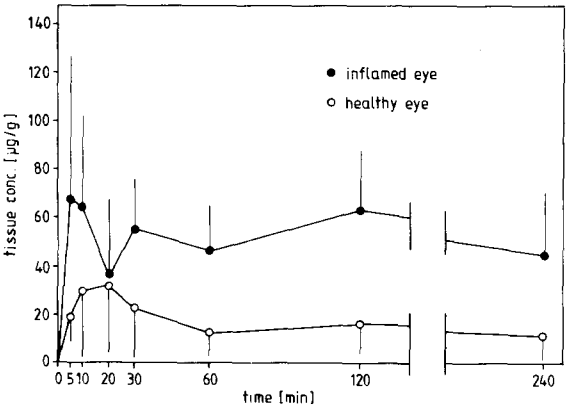

Fig. 3. Nanoparticle concentration vs time profile in the nictitating membrane. Mean ± S.D., $n = 10$.

the initial dose. The lower nanoparticle levels at early times, compared to healthy eyes, may tentatively be ascribed to binding and loss of the colloid to inflammatory fluids and increased proteins in tears.

Fig. 2 shows the concentration profile in healthy and inflamed cornea. The peak in both cases was found at about 5 min post instillation. In the healthy cornea, approximately 1% of the initial dose remained on the cornea after 5 min with a rapid drop 10 min after instillation down to about 0.15%. This value was maintained for 4 h which was again consistent with earlier work (Wood et al., 1985). Inflamed cornea concentrations of nanoparticles remained essentially constant from 30 until 240 min. Compared to healthy cornea, the nanoparticle concentration was increased by a factor of 4.

Nanoparticle content in the nictitating membrane is shown in Fig. 3. In normal eyes, the concentrations were about three times lower than in inflamed eyes with a peak time of 5–30 min. In inflamed eyes, the peak appeared to be reached much faster, after 5 min, and the decrease was much less pronounced. The drop at the 20 min time point, in the case of the inflamed conjunctiva, was probably caused by biological variations and was statistically insignificant in any case.

The aqueous humor concentrations (Fig. 4) were much lower than in the other tissue samples. The concentration vs time profile in normal eyes again was very similar to previous work (Wood et al., 1985). Concentrations in the inflamed eyes were about 5 times higher up to 60 min. These higher concentrations were probably caused by degradation products of the nanoparticles. As previously shown by Wood et al. (1985), nanoparticles biodegrade rapidly in tear fluid and it is expected that the degradation would be even more pronounced in inflamed tissues, due in part to an increase in various repair and protective enzymes. In addition, a minor amount of low-molecular-weight components may have been present in the sample prior to dosing. These low-molecular-weight components are expected to diffuse much faster

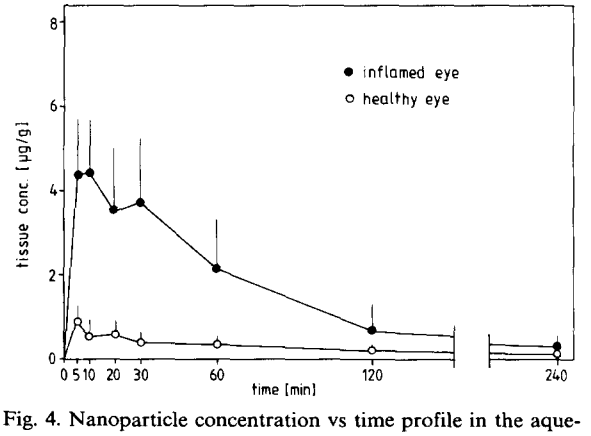

Fig. 4. Nanoparticle concentration vs time profile in the aqueous humor. Mean ± S.D., $n = 10$.

through inflamed than through normal cornea. Although endocytosis of particles of colloidal dimensions by ocular tissues has been demonstrated (Kaye and Pappas, 1962), the concentration vs time profile makes it unlikely that this process occurred with nanoparticles: the peak aqueous humor concentrations were obtained after 5–10 min. Endocytosis is a much slower process. If this process would have been the dominant factor governing radioactivity transport into the aqueous humor, a much later peak and a much less rapid aqueous humor concentration drop would have been expected.

Because of the higher aqueous humor concentration in the clove oil-treated eyes, an additional experiment was performed: rabbit eyes were obtained with and without clove oil treatment. After dual staining with trypan blue and Alizarin red S (Taylor and Hunt, 1981) endothelial photomicrographs were taken. No cell loss or junctional separations were detectable, demonstrating that the normal endothelial array had been maintained.

These results show very clearly that there is a significantly higher concentration of polyhexyl-2-cyano[3-^{14}C]acrylate nanoparticles in all inflamed tissues. In the inflamed state, the albumin concentration in the precorneal area is increased (Woodward and Ledgard, 1985) along with many other proteins, including fibrin. It is probable that nanoparticles are better retained in precorneal fluid and tissues due to specific and non-specific protein binding. The increased residence time of the nanoparticles in the inflamed eye might also be influenced by a partial blockade of the nasolacrimal duct due to the swollen conjunctival tissue (Primbs et al., 1961). In addition, inflammation also caused swelling of the tissues, accompanied by a higher degree of hydration and by secretion of substances, that may be adhesive towards polymeric materials, e.g. fibrin. The structural features of the polymer, i.e. charge density and hydrophobicity, are similar to those polymers that have been previously shown to possess bioadhesive properties (Ch'ng et al., 1985).

The results of this study have further implications. Bioadhesion has to date been examined primarily from the viewpoint of artificial bioadhesive polymers. It has largely been neglected that the tissue and the state of the tissue, i.e. inflamed vs normal, may also influence the degree of adhesiveness and hence binding. An example for this is the indomethacin–Oros system. This product may travel through a normal gut at a constant rate without adhesion to the gut wall. However, if the gut wall of patients was inflamed, due to previous medication with non-steroidal antirheumatic drugs, the gut wall may have become bioadhesive for the Oros-system. As a consequence, the system would have adhered to the previously inflamed sites and thus been responsible for some of the reported gut damage by the combined action of the indomethacin and the osmotically active substances. Note that this scenario is pure speculation but it is consistent with findings in the present study.

Conclusions

The concentration of ^{14}C-labelled nanoparticles in the cornea, conjunctiva, nictitating membrane and aqueous humor was 3–5 times higher in eyes in which a chronic inflammation was induced by clove oil administration 24 h prior to nanoparticle dosing, than in normal eyes. This demonstrates the enhanced bioadhesiveness of inflamed tissue to this type of polymer and this delivery system. Moreover, the ratio of nanoparticles between inflamed and normal tissue was higher in the conjuctiva (Fig. 1) than in the cornea (Fig. 2) between 60 and 240 min. Both of these findings demonstrate that polycyanoacrylate nanoparticles hold promise as drug carrier systems for ophthalmic delivery of anti-inflammatory or anti-allergic drugs. The increase in the ratio conjunctiva vs cornea is especially favourable since a number of anti-inflammatory drugs are used against conjunctival inflammations but exhibit side effects after permeation through the cornea into the aqueous humor. This latter aspect is especially important since our results indicate that the permeability of the cornea is enhanced due to inflammation.

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