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Precorneal drainage of polyvinyl alcohol solutions in the rabbit assessed by gamma scintigraphy

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A method for monitoring the ocular distribution of solutions for ophthalmic use was developed using the technique of gamma scintigraphy. This method was used to monitor the effects of polyvinyl alcohol on the precorneal drainage of [^{99m}Tc]pertechnetate in the rabbit. The rate of drainage of solutions decreased on addition of PVA and precorneal residence of the marker was significantly prolonged, compared with saline controls, at a PVA concentration of 5% w/v. Increases in the activity in the inner canthal region mirrored the decrease in corneal counts. The method has a more general application for the study of ophthalmic formulations and devices in larger mammals and in man.

It is generally supposed that the action of drugs in liquid ophthalmic formulations can be prolonged by incorporating polymers into the formulations. These polymers have the effect both of increasing the thickness of the precorneal tear film and of decreasing the rate of drainage of the formulations (Benedetto et al 1975). This approach is based on the concept that viscosity is the major factor influencing the ocular retention of an active constituent. However, the contributions of the various physicochemical parameters altered on addition of polymer to the formulation such as density, surface tension and hydrogen ion concentration along with changes in physiological processes such as lacrimation, tear drainage and tear film thickness must be considered. The interaction of these parameters makes it difficult to predict the optimum formulation.

Fluorimetric techniques, tear sampling (Lee & Robinson 1979) and the use of non-contact radiation probes (Chrai et al 1973) have been used previously to follow the precorneal dynamics of instilled solutions. These techniques however, do not allow the local variations of drug in the tear film to be followed with ease. There is an obvious need for a method which will allow discrimination between drug remaining in a depot or ocular device and that released on to the corneal surface. The techniques described previously do not allow for such precision.

The monitoring of lacrimal drainage by gamma scintigraphy has become an established technique in nuclear medicine for the evaluation of obstructive disease of the nasolacrimal system (Brown et al 1981). It is apparent that this technique could be adapted to facilitate the study of the regional deposition of radiolabelled ophthalmic formulations in the eye and to monitor the clearance of liquid, particulate and oily preparations. To evaluate the technique in the rabbit, the clearance of ^{99m}Tc-labelled albumin microspheres from the corneal surface was investigated in a pilot experiment. The procedure was then applied to the assessment of the precorneal kinetics of solutions containing polyvinyl alcohol (PVA) as a thickener and [^{99m}Tc]-pertechnetate as the tracer.

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MATERIALS AND METHODS

Materials

Polyvinyl alcohol, grade W40/140 was obtained from the Wacker Chemical Company (Walton-on-Thames, Surrey, UK) and used without further purification. Solutions of PVA in 0·9% NaCl (saline) were prepared at 1·0, 3·5 and 5·0% w/v and sterilized by filtration through Millipore filters before use.

Technetium-99 m pertechnetate in 2 ml saline was obtained by elution from a sterile generator (C.I.S. (UK), London) using saline. The ^{99m}Tc content was assayed and the solution diluted to obtain an activity of 140 MBq ml^{-1} ^{99m}Tc solution. Solutions containing 1·0, 3·5 and 5·0% PVA and 750 kBq of [^{99m}Tc]pertechnetate were sterilized by passing the solutions through a 10 μm Millipore filter.

A commercial human serum albumin microsphere kit (C.I.S. (UK) Ltd, London) was used for the preparation of ^{99m}Tc-labelled particles. The kit contained 90% of particles in the range 0·2–0·5 μm diameter.

Methods

Viscometry measurements

The kinetic viscosity was measured using a U-tube viscometer (British Pharmacopoeia 1980) at both 33 °C (corneal surface temperature) and 37 °C for all solutions used.

Animal experiments

In a pilot experiment a female NZW rabbit received a suspension of ^{99m}Tc-labelled microspheres in 50 μl saline into one eye. The rabbit was imaged using an I.G.E. 'MaxiCamera' gamma camera fitted with a 3 mm pinhole collimator positioned 4 cm from the rabbit eye with the aid of a plastic cone. A series of 35 × 5 second images was then taken in the dynamic acquisition mode and the data recorded by a computer for subsequent analysis.

For the studies involving the PVA solutions, two groups of rabbits, each containing four animals, were studied on two occasions one week apart. The eye contralateral to that imaged on the first occasion, was used for the second study. A small volume (50 μl) was applied to the test eye at the margin of the upper eyelid, and the dispersion and clearance of the tracer was monitored as a dynamic study comprising 40 images each of 15 s duration. The PVA formulations were administered in a random sequence and no adverse reactions were detected following the experiments. Data were stored on computer and analysed by defining regions of interest around the cornea, inner canthus and lacrimal sac. The count rates from each frame were determined for each region.

RESULTS AND DISCUSSION

The results of the microsphere experiment are shown in Fig. 1. By combining several images it was possible to identify three main regions: the corneal surface, the inner canthus and the lacrimal sac. Initially the corneal surface was well-defined, but the particles cleared rapidly and accumulated in the corner of the eye. The inner canthus gradually drained, and a concentration of activity was seen in the nasolacrimal duct at the level of the lacrimal sac.

By defining regions of interest around the cornea, inner canthus and lacrimal sac areas (Fig. 1 insert), the movement of isotope through these areas could be readily quantified. As may be seen from Fig. 1, 50% of the activity had cleared from the cornea by 10 s, and the activity in the inner canthus reached a maximum at 15 s. There was a steady drainage of the microspheres from the inner canthus, with a corresponding accumulation in the lacrimal sac. Thus the

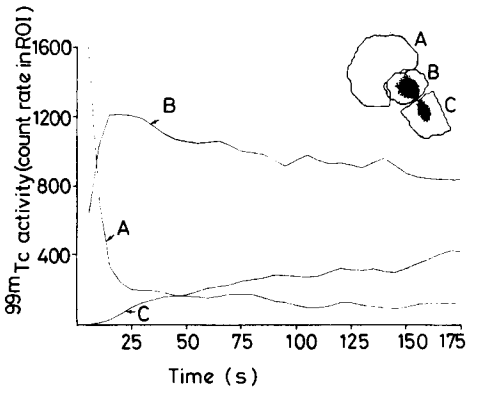

FIG. 1. Clearance of ^{99m}Tc-labelled albumin microspheres from the rabbit eye, typical results from one rabbit. Inset shows the regions of interest; A = Cornea, B = Inner Canthus, C = Lacrimal Sac.

method is capable of monitoring the movement of tracer through these three areas in the rabbit eye and appears suitable for measuring precorneal drainage.

The activity in the lacrimal sac reflects drainage of material down the nasolacrimal duct and is not so relevant to precorneal drainage (unless the nasolacrimal duct is blocked). It was therefore, considered sufficient for the subsequent study to measure the changes in activity in the cornea and inner canthus alone. The results for the experiments using the PVA solutions are shown in Figs 2–4. The clearance rate of [^{99m}Tc]pertechnetate solutions from the corneal surface was found to decrease markedly on addition of polyvinyl alcohol (Fig. 2). In the early phases, the clearance was approximately exponential with time. There was prolongation of the corneal residence with increasing PVA concentrations. This difference became statistically significant from saline controls at a PVA concentration of 5% ($P < 0{\cdot}05$, unpaired t-test). These differences have been summarized in a previous communication relating to this work (Olejnik et al 1982). Inner canthal Tc-99m concentrations in the same experiments are shown in Fig. 3. At PVA concentrations above 3·5%, the peak activity in the canthal region was lower, and the activity plateaued for longer, reflecting the slower corneal clearance of 3·5 and 5% PVA solutions.

The viscosities of the solutions are shown in Table 1. A curvilinear relationship was found when the corneal residence over the first 10 min following administration, was plotted against solution viscosity (Fig. 4). The greatest effects of changes in concentration were observed at low PVA concentrations.

It is noteworthy that although the addition of PVA to the solution increased the corneal contact time,

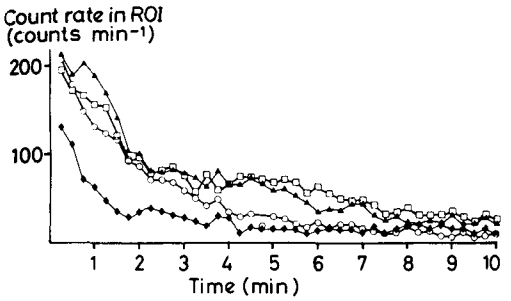

FIG. 2. Precorneal drainage of [^{99m}Tc]pertechnetate incorporated into polyvinyl alcohol (PVA) solutions. Key: (◆) 0% PVA, (○) 1% PVA, (▲) 3·5% PVA, (□) 5·0% PVA. Results are the mean from each batch of rabbits (n = 4 per group).

the period over which the eye was exposed to high concentrations of the marker was only a few minutes. It is not surprising therefore, to find that there has been much controversy concerning the benefits of viscolysers in ophthalmic solutions. Adler et al (1971) were unable to demonstrate an increase in contact time of fluorescein with the cornea on addition of 1·4% PVA. A higher initial concentration was found, however, which was attributed to a greater initial saturation of the tear film.

Davies et al (1977) have demonstrated an increase in the pharmacodynamic action of pilocarpine administered to glaucomatous patients in 3·75% PVA. The decrease in intraocular pressure was more marked and the period of efficacy increased from 3 to 7 h. Patton & Robinson (1975) have found that the relationship between the viscosity of solution, the contact time with the eye and the availability of drug to the anterior chamber is complex. There was little

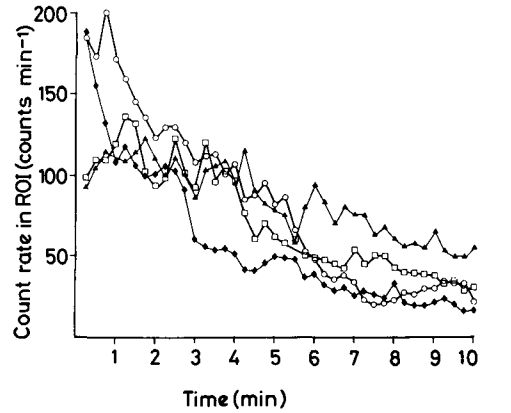

FIG. 3. Kinetics in the inner canthal region of [^{99m}Tc]pertechnetate incorporated into polyvinyl alcohol (PVA) solutions. Key: (◆) 0% PVA, (○) 1% PVA, (▲) 3·5% PVA, (□) 5·0% PVA. Results are the mean from each batch of rabbits (n = 4 per group).

increase in the penetration of pilocarpine into the anterior chamber at PVA concentrations of less than 2·25%. At PVA concentrations above 10%, the solutions become sticky and the tear ducts block. It has been noted that temporary blockage of the tear ducts in the rabbit results in only a two-fold increase

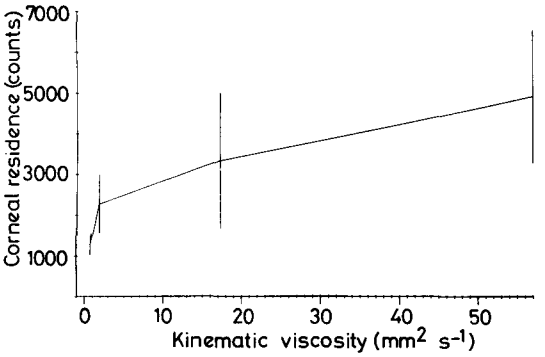

FIG. 4. Corneal residence as a function of solution viscosity. The residence is expressed in terms of the total counts detected in the corneal region during the 10 min following administration (mean ± s.d.).

in bioavailability (Patton & Robinson 1976). It is apparent therefore, that there is an optimum viscosity, at least for the rabbit eye, which Patton & Robinson have determined to be about 12–15 cP (1·2–1·5 m Pa s). Further increases in viscosity above this level did not appear to increase proportionally the drug concentration in the anterior chamber. In the present study, Fig. 4 illustrates why this should be so. In agreement with the findings of Patton & Robinson (1975), the present study demonstrated that there was only a very gradual increase in corneal residence with increasing PVA-concentrations above 3·5%.

Table 1. Kinematic viscosity determinations of polyvinyl alcohol vehicles. (mean ± s.d.; n = 6 per group).

PVA Concentration %	Kinematic viscosity (mm² s⁻¹) at 33 °C	at 37 °C
0	0·80 ± 0·01	0·72 ± 0·01
1	1·86 ± 0·01	1·70 ± 0·02
3·5	17·32 ± 1·10	13·22 ± 0·59
5	56·95 ± 1·91	46·23 ± 0·44

One of the causes of the controversy as to the benefit of viscosity-enhancers has been ascribed to the fact that attempts have been made to extrapolate results from the rabbit, the usual test animal in such studies, to man. Such studies therefore, have been criticized for attempting to determine the ideal

viscosity and to quantify a viscosity-retention time-bioavailability relationship (Saettone et al 1981); the rabbit having been described as being 'less sensitive to the effects of viscolysers' than man. There are obvious marked anatomical and physiological differences relating to the eye in the two species. Factors such as the rate of blinking might be expected to influence precorneal clearance of the solutions, particularly if the solutions are non-Newtonian and sensitive to rates of shear. However, over the range of PVA concentrations in this study the behaviour of the solutions is approximately Newtonian with viscosity independent of shear force (Patton & Robinson 1975).

Distinct differences have been observed in the drainage of ophthalmic vehicles when compared in rabbit and man (Saettone et al 1981). However, from their results, there is a similar rank order correlation between various pharmacodynamic parameters derived for pilocarpine in the two species. Thus, accepting that the behaviour of ophthalmic solutions in the two species is unlikely to be identical, the rabbit still provides a useful model for ophthalmic pharmacokinetics. Following preliminary screening experiments in the animal model, studies could readily be undertaken in man using the same techniques. The monitoring procedure is non-invasive and results in minimal disturbance to normal physiological function. The method has a wider application to the design of new formulations and delivery devices for ophthalmic therapy.

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