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A comparison of the effect of viscosity on the precorneal residence of solutions in rabbit and man

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The precorneal drainage of radiolabelled solutions containing polyvinyl alcohol (PVA) or hydroxypropyl methylcellulose (HPMC) have been measured in rabbit and man by gamma scintigraphy. Concentration of the polymers was varied to produce solutions with a viscosity range between 10·2 and 102 mPas. Solution drainage was faster in man than in the rabbit with a more pronounced effect of viscosity. Significant retardation of drainage in man was noted at the higher polymer concentrations (0·9% HPMC or 5·85% PVA).

Recent studies which have compared the bioavailability of tropicamide and pilocarpine from various vehicles in man and rabbit (Saettone et al 1982a, b, 1984), have called into question the relevance of the rabbit as a predictive species in the development of ophthalmic formulations. Despite these observations, the rabbit remains the species of choice in the testing of new formulations for use in man, and researchers derive schemes for medication based on such animal experiments (e.g. Lambrecht & Packer 1985).

The differences between the anatomy of the eye in man and the rabbit probably contribute to differences in drug absorption. It is unclear how physiological variables such as blink rate and tear turnover influence the precorneal residence of ophthalmic formulations. Blink rate is much slower in the rabbit than in man (Mishima 1965). This in addition to a slower turnover in lachrymal fluid, should have marked effects on the behaviour of viscous solutions in the eye. Saettone et al (1982a) have commented that within each species, solutions of the same viscosity but prepared with different polymers should behave identically. To test this hypothesis, the effect on precorneal drainage of two widely used thickening polymers, polyvinyl alcohol and hydroxypropyl methylcellulose, have been compared in man and in rabbit using gamma scintigraphy.

Materials

Polyvinyl alcohol, Polyviol, grade W40/140, 86–89 mol % degree of hydrolysis was used as supplied from Wacker-Chemie GmbH, FDR. Hydroxypropyl methylcellulose (Methocel, 28–30% methoxyl and 7–12% hydroxypropyl groups) was supplied by Dow Chemical Company. Both materials were used without further purification.

Polyvinyl alcohol solutions (PVA) were prepared in citrate buffer pH 6 to a final osmolality of 300 mOsm kg^{-1} at concentrations of 3·0, 5·0, 6·0 and 6·5% w/v.

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Isotonic solutions of hydroxypropyl methylcellulose (HPMC) were prepared in the same buffer at concentrations of 0·45, 0·70, 0·85 and 1·0% w/v. Solutions were transferred in 4·5 ml amounts into sealed glass vials and autoclaved for 25 min to sterilize them.

On the day of the study, each vial of polymer solution was radiolabelled by the addition of 0·5 ml solution containing 300 MBq ^{99m}Tc-labelled diethylenetriaminepentaacetic acid ([^{99m}Tc]DTPA) (C.I.S. (UK), London).

Methods

Viscosity measurements. Viscosity measurements were made using a Rheomat 30 rheometer. Determinations were made before and after dilution with the radioactive label, at 24 °C and a shear rate of 263 s^{-1}.

Animal experiments. Twelve New Zealand White rabbits, 2·0–3·5 kg, were used in all experiments and randomly divided into groups of four on the trial days. Each formulation was tested in either the left or right eye of the rabbit in the group, the other eye being untreated. The precorneal clearance was measured using a gamma camera tuned to detect the 140 keV radiation of technetium-99m and fitted with a 3 mm pinhole collimator. The procedure used was the same as that described by Wilson et al (1983). The rabbit was positioned on a table, with its eye 5 cm from the collimator aperture. The eyedrop, 30 µl of polymer or saline solution radiolabelled with approximately 1·5 MBq technetium-99m, was instilled into the lower fornix and the eye manually blinked three times. A total of 60 images (36 × 5 s then 24 × 15 s) were recorded by computer for later analysis.

Human experiments. Sixteen healthy volunteers with no evidence of eye infection or nasal pathology participated. They comprised 4 males and 12 females, with an age range 20–23 years. The study was approved by the local ethical committee and written, informed consent was obtained from all the subjects. Subjects were acclimatized to the room conditions for 30 min before the study and none of the subjects wore eye cosmetics on the day of the trial.

Each subject was positioned at a distance of 5 cm from the collimator, with the head supported by a modified ophthalmic table. The subject was then instructed to remain in this position throughout the

monitoring period. The eyedrop, containing the saline or polymer solution, was instilled by gently pulling down the lower eyelid and placing 30 μl of solution delivered from a micropipette into the lateral quarter of the lower conjunctival sac. Following instillation of the eyedrop the subject was instructed to blink three times in quick succession and imaging commenced to the same protocol as that used in the rabbit experiments.

The procedure was repeated on the contralateral eye 1 h later, with a different test solution. Two days later, the experiment was repeated with a different batch of solutions. Each formulation was tested in six volunteers in a random order.

Image analysis. The subject was positioned so that the test eye was positioned 5 cm from the centre of the collimator, since initial experiments with radioactive phantoms indicated that the image could be contained within the central 50% of the field of view. It was noted that instillation of a 30 μl drop into the human eye caused a reflex blinking, which splashed a proportion of the dose onto the eyelashes. This peripheral radioactivity was excluded from the analysis by constructing area-activity profiles horizontally and vertically across the centre of the image of the eye. This allowed the definition of a region of interest encompassing the centre of the cornea, avoiding this artefact, but yielding a region of interest comparable with that defined for the rabbit studies. Towards the periphery of an image recorded with a pinhole collimator, a marked reduction in sensitivity occurs. The sensitivity within the central 50% of the diameter of the field of view, however, varied by less than 10%.

Results and discussion

The objective in selecting the concentrations of HPMC and PVA was to obtain four sets of solutions of comparable but increasing viscosity (Table 1). As can be seen, the viscosity of the four pairs of solutions were approximately equivalent. The PVA solutions showed a linear increase in viscosities with concentration, but the HPMC showed pseudoplastic behaviour particularly at the higher concentrations.

The precorneal clearance of saline in both man and rabbit was found to follow a multiexponential pattern, with half-times of approximately 15 s and 1 min, respectively (Fig. 1). The rates of clearance over the first 30 s

Table 1. Viscosity measurements of PVA and HPMC solutions used for instillation, after addition of [^{99m}Tc]DTPA, measured at 24 °C.

HPMC		PVA	
Concn (% w/v)	Viscosity (mPa s)	Concn (% w/v)	Viscosity (mPa s)
0·41	10·2	2·7	10·2
0·63	37·4	4·5	37·4
0·77	68·0	5·4	71·4
0·90	102·0	5·9	93·5

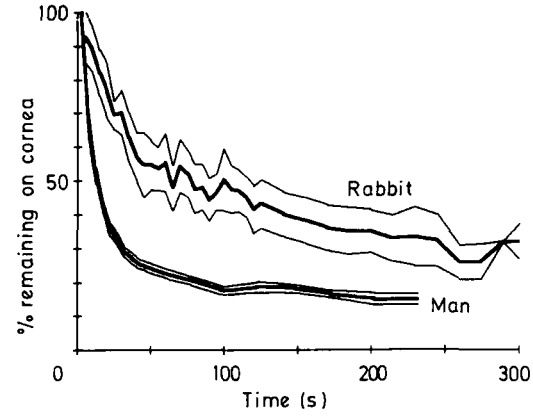

FIG. 1. Precorneal clearance of saline containing [^{99m}Tc]DTPA in man and in rabbit. Means ± standard deviation indicated for both species (n = 6 for both groups).

were compared by analysis of covariance (log% remaining versus time) and shown to be significantly different ($P < 0{\cdot}05$). After the first minute there was no significant difference between the rates of disappearance for man and rabbit.

The conjunctival sac holds about 25–30 μl, the rate of drainage of instilled solution being proportional to the volume instilled (Chrai et al 1974). The administration of drops is associated with reflex tearing, since tear flow increases in response to stimulation. The present study has demonstrated that the drainage apparatus of man is much more efficient than that of the rabbit.

The instillation of solution into the eye caused a reflex blinking in man with a considerable proportion (20–30%) splashed onto the eyelashes. This effect was not observed in the rabbit and may be an important additional factor limiting bioavailability in man. It is well established that rabbits blink less frequently than man, about 4 h^{-1}, compared with man who blinks 6–12 min^{-1} (Mishima 1965). The later phase of clearance from the cornea would be expected to correlate with lachrymal volume and its rate of turnover. Chrai et al (1973) calculated that the turnover rate of lachrymal fluid was considerably faster in man (16% min^{-1}) compared with 7% min^{-1} in the rabbit. The clearance rates measured between 1 and 4 min in the present study were the same for both rabbits and man, although there were greater variation in the animal data.

It is intuitively accepted that factors increasing corneal contact time, will lead to an increase in ophthalmic bioavailability for most drugs. For this reason viscosity enhancers such as polyvinyl alcohol are included in a number of formulations to increase ocular retention. Previous studies in this laboratory using gamma scintigraphy have shown that the precorneal drainage of an instilled radioactive marker [^{99m}Tc]pertechnetate was significantly decreased in the rabbit eye in the presence of 5% w/v polyvinyl alcohol compared to saline (Wilson et al 1983).

Table 2. Clearance of solutions containing [^{99m}Tc]DTPA plus PVA or HPMC from the rabbit cornea. Each value represents the mean value ± standard deviation from a group of 6 rabbits.

Time (s)	Saline	PVA 2·7%	HPMC 0·41%	PVA 4·5%	HPMC 0·63%	PVA 5·4%	HPMC 0·77%	PVA 5·9%	HPMC 0·90%
10	62 ± 3	98 ± 3	92 ± 11	89 ± 16	100 ± 0	64 ± 46	75 ± 43	56 ± 24	69 ± 41
20	73 ± 39	72 ± 30	76 ± 14	78 ± 30	72 ± 4	78 ± 14	76 ± 10	74 ± 40	87 ± 13
40	66 ± 10	48 ± 23	64 ± 21	62 ± 30	66 ± 13	66 ± 12	74 ± 25	71 ± 20	78 ± 21
80	50 ± 13	38 ± 28	53 ± 27	39 ± 14	48 ± 8	35 ± 9	56 ± 27	64 ± 24	51 ± 14
150	42 ± 15	39 ± 31	47 ± 22	27 ± 17	33 ± 18	29 ± 6	45 ± 30	53 ± 25	31 ± 2
250	32 ± 21	22 ± 15	42 ± 24	21 ± 23	22 ± 12	19 ± 13	36 ± 20	40 ± 14	22 ± 5

Table 3. Clearance of solutions containing [^{99m}Tc]DTPA plus PVA or HPMC from the cornea in human volunteers. Each value represents the mean value ± standard deviation of 6 subjects.

Time (s)	Saline	PVA 2·7%	HPMC 0·41%	PVA 4·5%	HPMC 0·63%	PVA 5·4%	HPMC 0·77%	PVA 5·9%	HPMC 0·90%
10	66 ± 10	66 ± 10	65 ± 11	82 ± 8**	73 ± 15	78 ± 10	78 ± 5	85 ± 4**	84 ± 11**
20	42 ± 4	42 ± 14	37 ± 15	53 ± 13	56 ± 11**	64 ± 11**	60 ± 20*	65 ± 9**	64 ± 16**
40	27 ± 4	26 ± 11	18 ± 7	27 ± 8	37 ± 16	45 ± 20*	39 ± 17	38 ± 8**	50 ± 23*
80	20 ± 3	22 ± 7	14 ± 5	16 ± 4	32 ± 17	33 ± 12*	24 ± 15	27 ± 9	36 ± 18
150	18 ± 3	15 ± 5	12 ± 3	14 ± 3	26 ± 9	27 ± 12	15 ± 8	26 ± 7	27 ± 18
250	15 ± 4	13 ± 4	13 ± 6	12 ± 3	21 ± 9	22 ± 10	15 ± 9	23 ± 7	22 ± 14

Significant differences from the saline group were tested using an unpaired t-test (*$P < 0.05$; **$P < 0.01$).

In the present study, a different type of analysis has been applied to enable calculation of the activity remaining on the corneal surface alone. The results are shown in Tables 2 and 3. As can be seen from the Tables, increasing viscosity had little effect on the retention on the rabbit cornea. Both polymers caused an increase in retention on the corneal surface, compared with the saline controls. In man equal viscosity solutions of the two polymers provided the same degree of precorneal retention. This is in agreement with the prediction of Patton & Robinson (1975). Thus shear-thinning of the viscous HPMC solutions on blinking does not significantly alter precorneal drainage.

Shell (1982) commented that despite numerous claims of enhancement of drug contact time using polymers such as methylcellulose and PVA, the effects on corneal absorption were modest. As can be seen from Tables 2 and 3, significant retention of the marker occurred only for the initial 20–50 s after which any benefit due to viscosity effects was lost. However, Davies et al (1977) investigated the effect of PVA on the miotic response to pilocarpine in man and concluded that the pharmacodynamic effects would be a function of the concentration on initial contact.

Physicochemical parameters other than those related to viscosity effects, however, may also be important. Benedetto et al (1975) examined an in-vitro model of the corneal surface, and suggested that PVA but not HPMC would significantly increase the thickness of the corneal tear film. The results obtained in the present study indicate that this effect must be small, since the corneal activity-time profiles for both polymers show remarkable concordance.

Many published studies have tried to relate the pharmacodynamic effects of mydriatic drugs or aqueous humour concentrations of antibiotics to the viscosity of the polymer solution used. The results have been variable and disappointing. This is probably not surprising in view of the modest improvement in bioavailability seen after experimental blockage of the nasolachrymal drainage apparatus (Patton & Robinson 1976). Green & Downs (1975) compared aqueous humour concentrations of pilocarpine after instillation of the drug in 1·67% polyvinylpyrrolidine (PVP) (viscosity 20 centistokes), 1·4% PVA (3 centistokes), 0·5% HPMC (13 centistokes), 1·0% HPMC (113 centistokes) and saline. Only 1% HPMC and the polyvinylpyrrolidone produced any elevation of aqueous humour concentrations of pilocarpine compared with control. The authors suggested that a minimum viscosity of 20 centistokes is needed to produce enhanced corneal absorption. Saettone et al (1984) also compared in rabbit and man the mydriatic effect of tropicamide in solutions of PVA, HPMC or PVP at concentrations yielding viscosities of 20 centistokes. PVA increased the maximum response and duration of action, whereas the other polymers were not significantly different from controls. This suggests that the other physicochemical properties of the viscosity enhancer may be more important than considerations of viscosity.

The present study has demonstrated that the rabbit eye is much less sensitive to the effects of viscosity enhancers than the human eye. Caution should therefore be taken in extrapolating results from this species to therapeutic situations in man.

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The effect of auranofin on the colonic transport of Na^+ and fluid in the rat

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Auranofin in the mucosal fluid caused a dose-dependent inhibition of fluid and Na^+ absorption by everted sacs of rat colon. Serosal auranofin was without effect. (Na^+ + K^+)ATPase activity of homogenates of mucosal scrapes of rat colon was inhibited by auranofin in a dose-related manner, while Mg^{2+}-ATPase activity was little affected. These actions of the drug on colonic transport mechanisms could contribute to the diarrhoea associated with auranofin therapy.

Auranofin (*S*-(triethylphosphoranediylaurio)-1-thio-β-D-glucopyranose 2,3,4,6-tetraacetate) is a gold compound that can be administered orally in the treatment of rheumatoid arthritis. One of the common side-effects experienced by patients receiving this compound is a disturbance of bowel function, ranging from loose stools to diarrhoea (Heuer & Morris 1982). It has been shown that auranofin inhibits the absorption of nutrients, Na^+ and fluid by the small intestine (Hardcastle et al 1984) and this could contribute to the bowel symptoms described. However, disturbances in small intestinal function do not necessarily lead to diarrhoea, since the colon has a considerable capacity for compensation (Binder 1979). This aspect of colonic activity could be compromised if, in addition to its effects in the small intestine, auranofin also inhibited absorption in the colon. The present investigation was therefore designed to assess the effects of auranofin on the absorption of Na^+ and fluid by rat colon.

Methods

Experiments were carried out on male albino rats (Sheffield strain, 230–250 g). These were allowed free access to food (diet 86, Oxoid, London) and water. They were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.).

Measurement of intestinal transport. The transport of fluid and Na^+ was determined by the everted sac technique (Wilson & Wiseman 1954), using the entire colon. Each sac was filled with 0·75 ml fluid (serosal fluid) and incubated for 30 min at 37 °C in 25 ml fluid (mucosal fluid). The incubation fluid was Krebs bicarbonate saline (Krebs & Henseleit 1932) equilibrated with 95% O_2/5% CO_2 and containing additions as indicated. Auranofin was initially dissolved in ethanol and control sacs were exposed to an equivalent volume of this vehicle. Fluid transport was measured gravimetrically and the volume of fluid taken up by the sac, the mucosal fluid transport (MFT), is expressed as ml g^{-1} initial wet weight in 30 min. At the end of the incubation period the serosal fluid was collected and the intestinal sac was deproteinized with 5% trichloroacetic acid and homogenized. The Na^+ content of the final serosal fluid and gut homogenate was analysed using a Corning flame photometer (Model 430). Na^+ uptake was determined by subtracting from the total Na^+ content of the serosal fluid plus gut homogenate at the end of the incubation, an estimate of the initial Na^+ content of the sac (gut + serosal fluid). The initial Na^+ content of the serosal fluid was calculated from the initial serosal volume (0·75 ml) and its Na^+ concentration. The initial Na^+ content of the gut wall was determined in separate experiments and the mean value (75·3 ± 2·4 (10) μmol g^{-1} initial wet weight) used. Na^+ uptake is expressed as μmol g^{-1} initial wet weight in 30 min.

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