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Disposition of topically applied sodium cromoglycate in the albino rabbit eye

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The influence of pH, tonicity, preservatives, polymers and instilled drop size on the disposition of sodium cromoglycate, an agent used in the prophylaxis of vernal keratoconjunctivitis, in the tear chamber of the albino rabbit eye has been examined. Radiotracer techniques were used throughout. The initial decline in concentration in the tear chamber was found to be unaffected by the presence of preservatives, pH, and tonicity over the ranges studied. However, significant increases in the residence time of sodium cromoglycate in the precorneal area were noted when a smaller instilled drop size was used and when 5% polyvinyl alcohol (PVA) was added to the aqueous vehicle. Tissue uptake was found to be greatest in the conjunctiva, followed by the cornea, the iris-ciliary body and the aqueous humor. In both the conjunctiva and the cornea, the addition of 5% PVA produced an elevation in the peak concentration of sodium cromoglycate and an increase in the time at which the peak concentration was achieved, indicating improved drug delivery to these sites.

Sodium cromoglycate has been found to be effective in the treatment of a variety of conditions in which an allergic component may be involved. These conditions include asthma (Altounyan & Howell 1969), rhinitis (Smith 1971; Holopainen et al 1971) and proctitis (Heatley et al 1974). In ophthalmology, sodium cromoglycate in the form of 2% drops (Opticrom, Fisons, plc) has proved to be of value in the management of mild to moderate cases of allergic and vernal keratoconjunctivitis (Easty et al 1972; Greenbaun et al 1977; Kazdan et al 1976; Tabbara & Arafat 1977; El Hennawi 1980; Leino & Tuovinen 1980; Nemoto 1980). Although its mechanism of action is not fully understood, sodium cromoglycate is thought to act by inhibiting the release of histamine and other mediators of inflammation which occurs when antigen combines with IgE antibody on mast cells (Foreman & Garland 1976).

We have evaluated several formulation variables and dosing parameters that could influence the retention of sodium cromoglycate in the tear chamber and its distribution within the albino rabbit eye. Thus the effect of preservatives, polymers, drug concentration, drop size, pH and tonicity has been investigated. It is hoped that such information will be useful in the rational design of a strategy to prolong the presence of sodium cromoglycate in the tear chamber of patients with vernal and allergic keratoconjunctivitis.

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MATERIALS AND METHODS

Materials

Sodium cromoglycate and ^{14}C-labelled sodium cromoglycate (spec. act. 7·685 mCi mmol^{-1} and of greater than 99% purity) were obtained from Fisons plc, Pharmaceutical Division (Loughborough, England) and used as received. All other chemicals were either analytical or reagent grade and were also used as received. Male, albino rabbits (ABS Rabbitry, Pomona, California), 2·2–2·5 kg, were used. They were fed a regular diet with no restriction on food or water.

Solution preparation

The dosing solutions containing sodium cromoglycate are listed in Table 1. All solutions were prepared fresh and were spiked with ^{14}C-labelled sodium cromoglycate immediately before each experiment. It was determined that 3·3 and 10 μCi of the labelled material ml^{-1} of final solution was sufficient to insure good counting efficiency in the tears and ocular studies, respectively.

The tonicity and pH of each dosing solution, shown in Table 1, were determined using a Model 2007 Osmette (Precision Systems, Sudbury, Massachusetts) and a Model 3500 Beckman pH meter (Beckman Instruments, Irvine, California), respectively. Most of the solutions were hypotonic and slightly acidic or alkaline.

Table 1. Effect of formulation of sodium cromoglycate solutions on rate of disappearance from tear chamber of albino rabbit eyes.

Solution number	Sodium cromoglycate concn % w/v	Preservatives present[a]	Vehicle	Volume instilled µl	pH	Tonicity (mOsmol kg^{-1})	Number of eyes used	Disappearance rate constant $\bar{x} \pm s_m$ (min^{-1})
I	2·0	yes	water[b]	50	5·65	111·3	13	0·499 ± 0·033
II	2·0	yes	water[b]	25	5·65	111·3	12	0·368 ± 0·030
III	2·0	no	water[b]	50	7·89	68·0	12	0·620 ± 0·043
IV	2·0	yes	isotonic phosphate buffer	50	7·41	290·0	12	0·435 ± 0·033
V	2·0	yes	5% PVA in water[b]	50	5·90	120·3	9	0·252 ± 0·014[d]
VI	4·0	yes	water[b]	50	7·66	123·0	14	0·463 ± 0·032
VII	4·0	no	water[b]	50	8·02	82·0	12	0·527 ± 0·045
VIII	10·0	no	water[b]	50	8·04	113·0	12	0·654 ± 0·048
IX	—	yes	water[b]	c	7·05	36.0	—	—
X	—	no	5% PVA in water[b]	c	5·80	15·5	—	—

[a] 0·01% benzalkonium chloride plus 0·4% phenylethyl alcohol.
[b] Double deionized.
[c] Solution not instilled.
[d] Tear film profile showed one compartment behaviour with concentration increasing to a peak between 1 and 2 min. Value reported represents the terminal slope.

Sodium cromoglycate concentration-time profile in the tear film

The disappearance of sodium cromoglycate from the tear film of the albino rabbit was monitored for 10 min as reported by Lee & Robinson (1979). Unanaesthetized rabbits were used and unless otherwise specified, a 50 µl dose was instilled.

During the experiments, all test animals were kept in restraining boxes in a normal upright posture. Both eyes of the rabbit were employed. Sodium cromoglycate solution was instilled directly onto the cornea of the test animal, collecting in the cul-de-sac. During instillation, the upper lid was slightly raised and the lower lid was pulled slightly away from the globe. The lids were immediately returned to their normal position after instillation.

One-µl tear samples were removed at 0·25, 1, 2, 3, 4, 5 and 10 min post-instillation using 1 µl disposable glass capillary pipettes (Curtin Matheson Scientific, Fountain Valley, California). It was assumed that uniform mixing occurred at or before the time at which the decline in concentration was observed and that sample removal had a negligible effect on the overall rate constant governing the decline in drug concentration. This approach has been used by Lee & Robinson (1979) with studies involving pilocarpine and glycerol. Even if such assumptions are not totally correct, any relative differences observed between formulations will remain significant.

During sampling, care was exercised to avoid eye irritation. Thus, all samples were withdrawn from the centre of the marginal tear strip without touching any eye tissue. On those rare occasions when the entire pipette was not filled with tear fluid, the volume of sample withdrawn was estimated from the height to which the capillary pipette was filled. Pipettes containing tear samples were transferred to vials (BioVials, Beckman, Irvine, California) containing 4 ml of prerefrigerated scintillation cocktail (Aquasol-2, New England Nuclear, Boston, Massachusetts) and counted in a liquid scintillation spectrometer (Beckman Model 7500, Irvine, California) after 24 h of storage in the dark. The presence of glass capillaries in the scintillation cocktail did not alter the counting efficiency or affect the results.

After correcting for background and quenching, the data expressed as sodium cromoglycate in µg g^{-1} of tear fluid were plotted semilogarithmically as a function of time and subjected to linear regression analysis. First order rate constants were obtained for the decline of the sodium cromoglycate concentration in the precorneal area of each rabbit eye.

Sodium cromoglycate concentration-time profiles in ocular tissues

The effect of 5% polyvinyl alcohol (PVA) on the concentration-time profiles of sodium cromoglycate in conjunctiva, cornea, aqueous humour and iris-ciliary body was evaluated. Two formulations were used; these contained sodium cromoglycate (2% w/v)

and the preservatives benzalkonium chloride and phenylethyl alcohol in (i) an aqueous solution or (ii) a 5% w/v polyvinyl alcohol (PVA) solution.

The basic experimental techniques used for instilling solutions and for monitoring ocular tissue/fluid drug concentrations after topical dosing have been described previously (Sieg & Robinson 1975). Unanaesthetized albino rabbits were used and a standard 25 μl dose was instilled. Both eyes were used, but the dosing time was staggered so that one animal could be used for two time points. Approximately 5 s before the rabbit was killed by marginal ear vein injection of a 30% sodium phenobarbitone solution, 1 μl of tears was collected using a disposable glass capillary pipette. Following death, the corneal and conjunctival surfaces were thoroughly rinsed with saline and blotted dry. The anterior segment samples, namely, conjunctiva, aqueous humour, cornea and iris-ciliary body, were obtained in that order.

The aqueous humour samples were transferred to vials containing 4 ml of pre-refrigerated scintillation cocktail. Each of the tissue samples was digested at 55 °C for 18 h in 1·5 ml of a tissue solubilizer (Protosol, New England Nuclear, Boston, Massachusetts) contained in a glass scintillation vial and decolourized by the addition of 100 μl H_2O_2. Ten ml of a scintillation cocktail (Econofluor, New England Nuclear, Boston, Massachusetts) was then added. All samples were stored in the dark for 24 h before counting in a liquid scintillation spectrometer. After correcting for background and quenching effects, the data in counts min^{-1} were converted to μg of sodium cromoglycate g^{-1} of tissue through the use of standards. The density of aqueous humour was assumed to equal 1·0. It was also assumed that, as with systemic administration of the drug (Ashton et al 1973), none of the sodium cromoglycate was metabolized in the ocular tissues.

Throughout the studies, the surgical procedures on each eye were completed within 5 min of death so that any errors due to redistribution of drug during the time required to obtain samples were minimized.

RESULTS AND DISCUSSION

Effect of formulation variables on the precorneal loss of sodium cromoglycate

It is now known that the duration of activity of ophthalmic preparations can be influenced by such factors as drop size, concentration of solution used, viscosity, pH, tonicity and the presence of compounds which modify tear turnover and corneal integrity (Patton 1980). The present study was undertaken to evaluate certain of these factors on the precorneal loss of sodium cromoglycate in the albino rabbit.

Fig. 1 is a typical plot showing the changes in concentration in the tear chamber of a single rabbit for 10 min after the instillation of 50 μl of 2% sodium cromoglycate solutions in (i) water and (ii) 5% PVA solution. The first order disappearance rate constant was derived from the slope of such a plot for each eye. The mean values and standard errors of the mean are listed in Table 1 for each of the solutions studied.

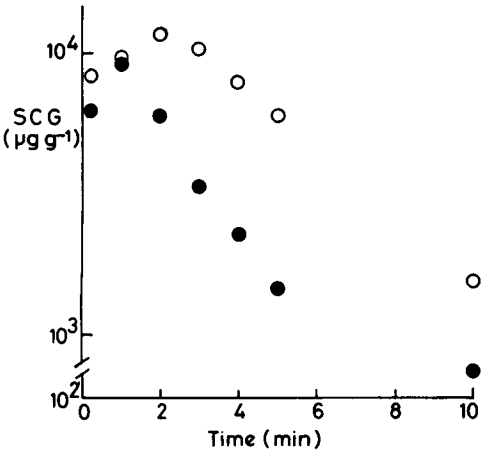

FIG. 1. Change in sodium cromoglycate concentration (μg g^{-1} of tear fluid) in the tear film following topical instillation of 50 μl of 2% sodium cromoglycate in (●) aqueous solution and (○) 5% PVA aqueous solution.

Of the two preservatives present in the commercial solution (Opticrom), phenylethyl alcohol has been implicated as the cause of transient stinging at the time of drop instillation (Dawson 1979). This could induce lacrimation, thereby leading to accelerated drug loss. The results obtained from the present study do not support this hypothesis. Rather the data in Table 1 indicate that with 2% solutions the presence of preservatives reduces the rate of drug loss. This trend also exists with the 4% solutions, although the difference between the mean rate constants is not statistically significant. While a 10% sodium cromoglycate solution containing preservatives was not studied, the disappearance rate constant for 10% drug without preservatives is similar to values calculated for the 2 and 4% solutions. Taken as a whole, the results indicate that at least in the rabbit, the combined presence of benzalkonium chloride and phenylethyl alcohol lowers the rate at which drug is lost from the tear pool. The mechanism by which this occurs is unclear. It does not appear to

be related either to the pH or the tonicity of the various solutions used (Table 1). Thus, there is no significant difference in the rate of loss of drug between solution I and solution IV, even though there are differences in pH and tonicity. Presumably the buffer capacity of solution I (and II) is such that the tears can quickly modify the pH and tonicity of the solution once instilled (Holly & Lamberts 1981). Ion pair formation can occur between sodium cromoglycate and benzalkonium chloride and enhances the corneal permeability of both species (Wilson et al 1981). However, this could be expected to further deplete the tear pool of drug.

Polymer solutions, by virtue of their high viscosity relative to aqueous solutions, tend to prolong the residence time of an instilled dose in the tear chamber (Patton & Robinson 1975). In the case of sodium cromoglycate, its rheological properties are such that it was thought that they may provide the same effect on precorneal retention as polymer solutions. The binary sodium cromoglycate-water phase diagram has been reported by Cox et al (1971); liquid crystalline mesophases are evident over much of the diagram. In subsequent work, Champion & Meeten (1973) showed that sodium cromoglycate had a planar configuration at low concentrations in solution. Preliminary viscosity studies apparently showed aggregation or strong solute-solute interactions at concentrations greater than 0·5%. Our viscosity data (Fig. 2) show that, while the viscosity of these solutions increases significantly at 25 °C at concentrations above 4%, this effect virtually disappears at 37 °C. The average corneal temperature in man has been shown to be 34·8 °C (Mapstone 1968). As seen in Table 1, a 10% solution (solution VIII) was lost from the tear chamber with a rate constant statistically indistinguishable from those obtained with the 2 and 4% solutions (solutions III and IV, respectively). This suggests that the dosing solution rapidly achieves thermal equilibrium with the tear pool, the cornea and the extraocular tissues so as to nullify any viscosity effect that might have been expected when the temperature of the instilled drop was 25 °C.

Viscosity studies with PVA at 25 and 37 °C (Fig. 2) showed that a 5% PVA solution has approximately the same apparent viscosity at 25 °C as a 10% sodium cromoglycate solution. However, it is far less affected by temperature. The increased viscosity at the temperature of the corneal area is undoubtedly one reason why the rate of loss of drug from the tear pool is reduced from 0·499 to 0·252 min^{-1} when 5% PVA is added to the formulation. This two-fold reduction

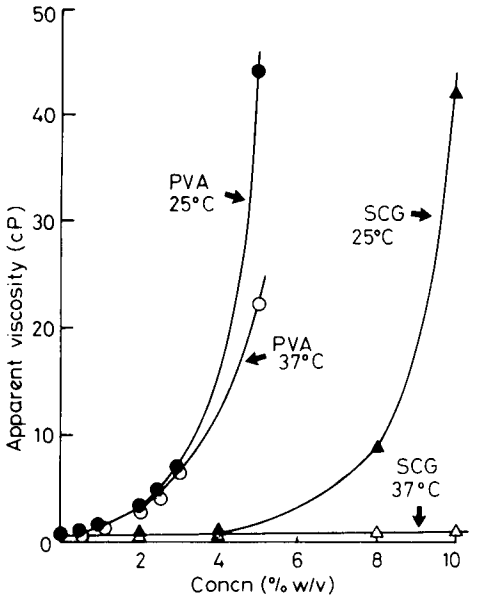

FIG. 2. Effect of concentration on the apparent viscosity of aqueous solutions of sodium cromoglycate and PVA at 25 and 37 °C.

contrasts with the four-fold reduction reported for 25 μl drops of technetium solution containing 5% PVA reported by Patton & Robinson (1975).

Drop size was found to have an effect on rate loss, being less with a 25 μl drop than a 50 μl drop at 0·368 and 0·499 min^{-1}, respectively (Table 1). These values are less than those reported by Chrai et al (1973) for similar drop sizes. Comparison of the rate constant governing the loss of 25 μl of an aqueous 2% drug solution (0·368 min^{-1}) with that governing the loss of 50 μl of sodium cromoglycate solution in 5% PVA (0·252 min^{-1}) suggests that in this instance, viscosity is more effective than a smaller drop size in prolonging the presence of drug in the precorneal area. However, the two effects would be expected to be additive.

Vehicle influence on disposition of sodium cromoglycate in rabbit eye

Based on the results described previously, it was decided that the distribution of drug in the conjunctiva and other ocular tissues be evaluated following the instillation of 25 μl doses of two 2% sodium cromoglycate solutions, one in deionized water with preservatives and the other in a 5% PVA solution containing preservatives. Fig. 3 shows the time course of drug concentration in the tear film, conjunctiva, cornea, iris-ciliary body and aqueous humour from 5 to 120 min following instillation of

these two solutions. After 30 min there is no significant difference between the concentration of sodium cromoglycate in the tear film from the aqueous solution alone and that containing 5% PVA (Fig. 3A).

Regardless of the viscosity of the vehicle, the concentration of sodium cromoglycate is highest in the conjunctiva followed by the cornea, iris-ciliary body and aqueous humour in that order (Fig. 3B, C, D, E). As is typical with other drugs administered as aqueous solutions (Sieg & Robinson 1976, 1981), peak concentration is attained in the conjunctiva and cornea within 5 min of solution instillation. However, with the more viscous 5% PVA solution as the vehicle, the time taken to reach the peak is increased to approximately 15 min. This prolongation is most likely due to an increase in residence time of the dose in the precorneal area. An additional effect could be a slowing in the release of drug from the vehicle, as its planar molecular structure suggests that it may complex with the hydrophobic backbone of the PVA polymer.

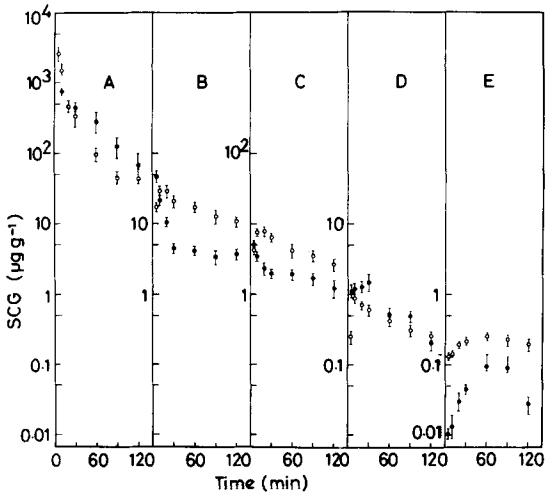

FIG. 3. Sodium cromoglycate concentration (μg g^{-1}) in the tear film (A), conjunctiva (B), cornea (C), iris-ciliary body (D), and aqueous humor (E) following topical instillation of 25 μl of 2% sodium cromoglycate in (●) aqueous solution and (○) 5% PVA aqueous solution. Between 10 and 12 eyes were used for each time point. Error bars represent standard error of the mean.

Because the target site of sodium cromoglycate is the mast cells in the conjunctiva (Jones & Dwyer 1979), its concentration in this tissue is of particular importance. The results indicate that sustained level of drug can be achieved in the conjuctiva as well as the cornea from a topically applied aqueous solution. A more viscous 5% PVA solution further increases the level attained by a factor of two to four (Fig. 3B, C). Based on a Student's *t*-test, there is no statistical difference between the terminal slopes observed with the aqueous and the PVA formulations in both the conjunctiva and the cornea. The slow rate at which drug is removed from the vascularized conjunctiva suggests binding of drug to this tissue. Because of its low pK_a's (1·1 and 1·9), essentially all of the sodium cromoglycate will exist in the non-absorbable ionized form at the pH of tears. However, our results indicate that the drug can overcome the lipophilic corneal epithelial barrier and gain entry to the intraocular tissues, an effect which has also been observed with the water-soluble, high molecular weight inulin molecule (Lee & Stratford, unpublished data). It is possible that this process may be facilitated by a detergent effect on the epithelium by the benzalkonium chloride preservative or formation of an ion pair with the same compound (Wilson et al 1981).

While most workers opine that the normal route for entry into the anterior chamber is through the cornea directly into the aqueous humour (Patton 1980), the results obtained in the present study suggest that a possible additional route for entry of drug into the anterior chamber is laterally from the cornea into the iris-ciliary body. Thus the concentrations of sodium cromoglycate in the iris-ciliary body during the first 30 or 60 min following instillation exceed those in the aqueous humour (Fig. 3D, E). This implies that drug which appears in the aqueous humour has come from the iris-ciliary body and/or endothelial surface of the cornea. Such a pathway does not appear to have been reported for this or any other compound. Based on the results on topically applied prostaglandins (Bito & Baroody 1982), it is unlikely that the sodium cromoglycate found in the iris-ciliary body comes from material which has been absorbed systemically. Paradoxically, the 5% PVA solution does not promote higher drug concentrations in the iris-ciliary body. In fact, during the first 30 min, higher concentrations are attained with the aqueous solution without added PVA. The reason for this is unclear, particularly since the concentrations in the aqueous humour are elevated following instillation of the drug in 5% PVA (Fig 3E).

Because ophthalmic solutions of sodium cromoglycate are intended for conditions involving the exterior of the eye, its prolonged presence in the precorneal area is important to its therapeutic effectiveness. Taken overall, there is good reason to increase the viscosity and reduce the instilled drop

size of aqueous solutions of sodium cromoglycate, since by so doing the rate at which drug is removed from the tear chamber is reduced while uptake into the conjunctiva (and cornea and aqueous humour) is increased. The results of this study indicate that most of this enhancement occurs within the first 30 min following solution instillation. After that time it appears that the incoming tears and the shearing effect that accompanies blinking will have reduced the viscosity of the instilled polymer solution to such an extent that it is readily removed from the tear chamber. Because the rabbit blinks at a lower rate than man, prolonged effects observed in rabbits might be thought to be exaggerated when compared to humans. However, studies with tropicamide (Saettone et al 1982) suggest that effects noted in the rabbit eye may be of at least equal significance in man. Overall, it is reasonable to assume that relative changes in residence time, and therefore duration of the drug's prophylactic activity, due to formulation changes will carry over from the rabbit into man. This has been shown to be the case for pilocarpine (Sugaya & Nagataki 1978). Finally, it should be remembered that the protein content of tears increases in patients with conjunctivitis (Sen & Sarin 1979). This could result in reduced conjunctival availability, when compared to healthy eyes.

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