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Altered ocular absorption and disposition of sodium cromoglycate upon ion-pair and complex coacervate formation with dodecylbenzyldimethylammonium chloride

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Ion-pair formation between the dianionic drug sodium cromoglycate and dodecylbenzyldimethylammonium chloride has been found to alter the extent and rate of corneal penetration of both large ions upon their coadministration. Additionally, using a complex coacervate of the two large ions as the applied dose form (in which the complex is in equilibrium with the ion pairs), a change in the overall disposition of both large ions to the various components of the eye is observed.

The classical concepts of drug absorption into and through biological membranes are that a priori only uncharged forms will have sufficient lipid solubilities to interact favourably with the lipid bilayer. However, ionized solutes, such as paraquat, suxamethonium and the phenothiazines, are well absorbed from, for example, the gut. Schanker (1964), in developing the pH-partition hypothesis of absorption, suggested that organic ions might penetrate in the form of less polar complexes formed with some material normally present in the lumen. Passage of an ionized drug through a membrane must involve (Selwyn & Dawson 1977) either the existence of an aqueous pore, or the transport of the ion from an aqueous environment to a lipid environment, movement across the lipid (lipoprotein) and then transfer into the aqueous phase on the other side of the membrane. Membrane transport of ions (ionophoresis) is generally discussed in terms of either endogenous ionophoric material present in the membrane which causes ion-ionophore shunt transport to occur, or exogenous material such as detergents [sic] or some antibiotics (e.g. filipin) which can form membrane disrupting complexes with cholesterol so leading to membrane lysis or permeability. Thus, when the action of a synthetic surfactant on ion transport is considered there are the possibilities that the surfactant can act either on the drug ion, (i.e. by acting as an ionophore), or on an existing membrane system such that the membrane may be modified to permit passage of the drug ion.

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We wish to report here on the involvement of ion pairing between a large anionic drug, (sodium cromoglycate), and a cationic surfactant, (dodecylbenzyldimethylammonium chloride), in the altered corneal penetration of these ions when coadministrated as a solution of ion pairs in equilibrium with free ions. In addition, the use of a complex coacervate as the applied dose form, (in which the complex is in equilibrium with the ion pairs and free ions), has been shown to alter the ocular disposition of both of these large ions.

MATERIALS AND METHODS

Materials

Sodium cromoglycate, (SCG), and dodecylbenzyldimethylammonium chloride (DBDAC), were as previously described, (Tomlinson & Davis 1978), and were at least 98% pure. Samples of ^{14}C-labelled SCG were gifts from Fisons Limited, Loughborough, U.K., and were described as pure with radioactivity as described under *Procedures*. ^{3}H-labelled DBDAC was prepared by The Radiochemical Centre, Amersham, U.K., from the above described cold material by catalytic exchange in a tritiated aqueous medium and as supplied had a radioactivity of approx. 1 mCi mg^{-1}. This preparation was purified using a semi-preparative t.l.c. system of pre-coated silica gel plates and an eluent of butan-1-ol–glacial acetic acid–water, (3:1:1); [^{3}H] DBDAC was identified using standards. The lability of radioactive label in a 1×10^{-3} mol dm^{-3} aqueous solution of purified [^{3}H] DBDAC was 7%, as found by distillation. All radioactive solids and solutions were stored at +2 °C.

Procedures

Transcorneal penetration. Two methods were used to examine the transcorneal penetration of large organic ions. (i), 50 μl of a sterile 0·9% NaCl (saline) solution containing either [^{14}C]SCG (1×10^{-3} mol dm^{-3}; specific activity 0·54 Ci mol^{-1}), or [^{3}H]DBDAC (1×10^{-3} mol dm^{-3}; specific activity 118 Ci mol^{-1}) or a mixture of both large ions at these same concentrations, were instilled into one eye of a New Zealand White (NZW) female rabbit, (six animals per solution). Thirty minutes after dosing, animals were killed and the anterior chambers of both eyes drained completely into preweighed syringes. The concentration(s) of large ion(s) in the aqueous humour were then measured using a dual-isotope scintillation counting technique.

(ii) 50 μl of a sterile saline solution of the two large ions at 1×10^{-3} mol dm^{-3} were instilled, alone and together, into a separate group of 6 NZW rabbits, using a single cross over random allocation sequence, (SCG activity 0·54 Ci mol^{-1}; DBDAC activity 118 Ci mol^{-1}). At 0·25, 0·5, 1, 2, and 4 h post-instillation, 50 μl of aqueous humour were removed by paracentesis under local anaesthesia, (5 min topical application of a 1% amethocaine solution). Thus each animal was used three times, but sampled only once in each experiment. Animals were instilled at three week intervals, and in no animal was there occurrence of scarring or persistent inflammation after paracentesis. One sample from each experiment was taken as the control. The concentrations of the ion(s) were determined as before.

Disposition. 50 μl of sterile saline solutions containing 5×10^{-4} mol dm^{-3} [^{14}C]SCG (2·7 Ci mol^{-1}), and 2×10^{-3} mol dm^{-3} [^{3}H]DBDAC, (1·35 Ci mol^{-1}) were administered, alone or together, to the right eye of NZW rabbits (n = 5 or 6). One hour after dosing the animals were killed, both eyes dissected and the cornea, iris, lens, vitreous humour and remainder separated quickly into tared vials and weighed. Aqueous humour was removed immediately before dissection. Samples were digested in NCS solubilizer, (7 ml g^{-1} tissue), for approximately 2 days at 43 °C to give complete solution and samples were then reweighed. 200 μl of this was transferred to Fisofluor liquid scintillation fluid, (Fisons Limited, Loughborough, U.K.) and after storage at 10 °C for 7 days counted as before. (Prolonged storage was found necessary to minimize chemiluminescence effects). After calculation of the radioactivity per unit weight of tissue, (d min^{-1} mg^{-1}), for both left and right eyes, the uptake(s) of istope(s) was found by subtraction of the control value.

RESULTS AND DISCUSSION

Sodium cromoglycate and dodecylbenzyldimethylammonium chloride form a complex coacervate when mixed in aqueous solution which is in equilibrium with the ion-pair and free ion(s) forms, (Scheme I). The coacervate can be identified by a white turbidity in solution (Tomlinson & Davis 1978), and is characterized by a solubility product having a 2:1 stoichiometry, (K_s), which in double distilled water at 25 °C and 37 °C is $1{\cdot}6 \times 10^{-10}$ (mol dm^{-3})^3 and $2{\cdot}2 \times 10^{-10}$ (mol dm^{-3})^3 respectively (Tomlinson et al 1979). The effect of saline on this coacervation can be estimated from other studies (Mukhayer & Davis 1976) to increase K_s by approximately one order of magnitude. Below the solubility product solutions of mixtures of SCG and DBDAC will contain free ions and 1:1 ion-pair species, (association constant 579 mol^{-1} dm^3; Tomlinson & Davis 1980). From liquid/liquid distribution studies (Tomlinson & Davis 1980), it can be demonstrated that the formation of such SCG-DBDAC ion pairs will cause both large ions to move to phases of lower polarity, that this movement is dependent upon the relative concentrations of both large ions, and that maximum movement occurs just below and above the K_s value.

FREE IONS ⇌ ION PAIRS ⇌ COMPLEX COACERVATE

SCHEME I. Large organic ion equilibria in aqueous solution

In an attempt to examine the feasibility of using ion-pair or coacervate systems as dose forms, we have examined in this study two delivery systems: (i) a solution containing both large ions at concentrations where maximum amounts of ion pair are present, i.e. just below the K_s point, (ii) higher concentrations of large ions where complex coacervation occurs. This latter system was examined to see if ion pairs were facilitating corneal penetration of the large ions, if this coacervate could act as a reservoir for the ion pairs (Scheme I). The critical micelle concentration (cmc) of tetradecylbenzyldimethylammonium chloride was determined at 30 °C (corneal temperature) in saline using a De Nouy tensiometer, and found to be 3·5 times smaller than when measured in double distilled water at 25 °C (Mukhayer et al 1975). By extrapolation, the cmc of DBDAC in saline at 30 °C (which in double distilled

water at 25 °C is found to be $1{\cdot}2 \times 10^{-2}$ mol dm^{-3}; Mukhayer et al 1975), will be approximately $3{\cdot}4 \times 10^{-3}$ mol dm^{-3}. Hence all surfactant concentrations we used were below the cmc, so avoiding both solubilization of coacervate (Tomlinson & Davis 1978) and micellar effects on membranes (Gouda 1974).

For the ion-pair system it was observed that both large ions could be found in the aqueous humour at 30 min in an approximately 10:1 ratio of DBDAC to SCG, whereas neither ion could be demonstrated in this fluid after dosing with solutions containing a single large ion (Table 1). The values given in Table 1 indicate that approximately 0.07% of the total administered dose of SCG and 0·7% of the DBDAC ion were to found in the aqueous humour at 30 min. These data suggest that either the extent and/or the rate of large ion penetration is altered, or the ocular clearance of both large ions, (due possibly to altered disposition), is affected by ion-pair formation. To examine this further, the time course of appearance of both large ions in the aqueous humour was determined for single ion and ion-pair systems.

Fig. 1 (a, b) illustrates the percentage amount of applied dose of large ion in the aqueous humour versus time for SCG and DBDAC when given as single large ion(s) or ion-pair systems. For the latter, both large ions are found in the aqueous humour up to 4 h after administration, with the first sampling time (15 min) being too late to demonstrate the absorption phase. Such profiles are consistent with maximal concentration peak times of between 5 and 20 min reported for neutral molecules (e.g. Sieg & Robinson 1976). At these concentrations, and using the paracentesis technique, neither large ion could be detected in the aqueous humour after single large ion dosing. Since both large ions could be found in the aqueous humour when given as a mixture (Table 1, Fig. 1), it is unlikely that a disruptive effect on the membrane by the surfactant (Selwyn & Dawson 1977) was responsible for the presence of the large ions, as this would be evident in the experiment using surfactant alone. However, the literature suggests that low concentrations of ionic surfactants can give rise to an increased absorption of drug across membranes (e.g. Gouda 1974), although Whitmore et al (1978) have argued that at low concentrations cationic surfactants (in contradistinction to anionic and non-ionic surfactants) do not appear to interfere with the structure of the mucosal membrane. Certainly it can be argued from Fig. 1 and Table 1 that the clearance of these ions from the aqueous humour is different after administration of the ion-pair

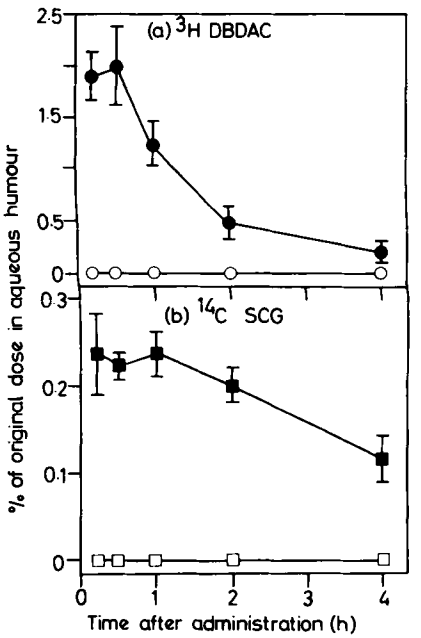

FIG. 1(a) and (b). Kinetics of appearance and loss of each ion (a-DBDAC, b-SCG) in the aqueous humour when administered either alone (○□), or together as the ion pair (●■), at concentrations of 1×10^{-8} mol of each ion in a 50 µl dose. (Data are given as means ± s.e.m. For other conditions see *Procedures*).

Table 1. Amounts of labelled sodium cromoglycate and dodecylbenzyldimethylammonium chloride found in the aqueous humour at 30 min after single ion or ion pair dosing. For conditions see *Procedures*.

Amount (mol) of each ion in 50 µl dose	Amount (mol) recovered in aqueous humour at 30 min (mean with s.d.)		Anterior chamber volume (µl) (mean with s.d.)
	SCG	DBDAC	
5×10^{-8} SCG	0	—	380 (s.d. 22)
5×10^{-8} DBDAC	—	0	356 (s.d. 84)
5×10^{-8} SCG and 5×10^{-8} DBDAC	3.5×10^{-11} (s.d. $1{\cdot}8 \times 10^{-11}$)	3.7×10^{-10} (s.d. 0.8×10^{-10})	346 (s.d. 77)

system, suggesting that the two ions are not cleared as the ion-pair species. (In the paracentesis procedure 50 µl of aqueous humour was taken from the anterior chamber, in front of the iris. Since there is approximately 350 µl of aqueous humour in the rabbit eye, and a proportion of this is behind the iris and in front of the vitreous humour, it is reasonable to expect differences in concentration between the total aqueous humour and paracentesis samples, particularly considering the dynamics of aqueous humour flow).

From these experiments it would seem attractive to use ion pairs as dose forms. However, as the concentrations of both ions are increased, the complex coacervate phase state is reached and from

Scheme I it can be argued that this coacervate could act as a reservoir for ion-pair and free ion(s) species. Although it is not strictly possible to compare bioavailability of large ions from an ion-pair system with a coacervate (as the latter is a two-phase system, Tomlinson et al 1979), since the coacervate doses contain ion pairs it is useful to examine whether the coacervate system has an effect in the membrane penetration (and ocular disposition) of the large ions.

Table 2. Percentage of applied dose in eye component at 1 h after single ion or complex coacervate dosing, (SCG 5×10^{-4} mol dm^{-3}; DBDAC 2×10^{-3} mol dm^{-3}). For conditions see *Procedures*.

	Percentage of applied dose remaining			
	SCG		DBDAC	
Component	Single ion	Mixture	Single ion	Mixture
Cornea	0·026	0·048	0	0·231
Aqueous humour	0·011	0·005	0·233	0·030
Iris	0·021	0·014	0·021	0·113
Lens	0·033	0·006	0·014	0·163
Vitreous humour	0	0·107	0	0·526
Rest[a]	0·431	0·210	0	5·50
Total	0·522	0·390	0·268	6·56

(a) Sclera and eyelids.

Table 2 and Fig. 2 show that 1 h after administration the overall disposition and uptake of the large ions as a coacervate are different compared with single large ion dosing.

The histograms show that, except for the sclera and eyelids ('rest'), changes in the disposition of the dose are in the same direction (positive or negative) for both large ions when given as the coacervate compared with the administration of the single large ion. Thus, the amount of dose remaining in the cornea at 1 h, expressed as a percentage, is increased for both large ions, that remaining in the iris, lens and aqueous humour is decreased, and that remaining in the vitreous humour is very greatly increased. For the sclera and eyelids the uptake of DBDAC is increased greatly and for SCG it is reduced by approximately 30%.

In these disposition experiments it was found for single ion dosing that SCG and DBDAC were present in the aqueous humour. However the percent transfer is low and approaches the zero values found in the transcorneal penetration studies (Fig. 1). It is perhaps because of the different tissue treatment method, the dose, and the sampling time, that these ions are to be found at such low concentrations in the aqueous humour. What is remarkable however is that, when they are given as a coacervate, less of the large ions are to be found in the aqueous humour (which differs from the findings after administration of ion pair, Fig. 1). Also, DBDAC from the coacervate is found in the sclera and eyelids and, especially, in the vitreous humour, and large amounts of SCG are found in the vitreous humour compared with single ion dosing, although there is a fall in sclera and eyelid disposition.

Sieg & Robinson (1976) have shown that although the corneal epithelium is the rate-limiting tissue for

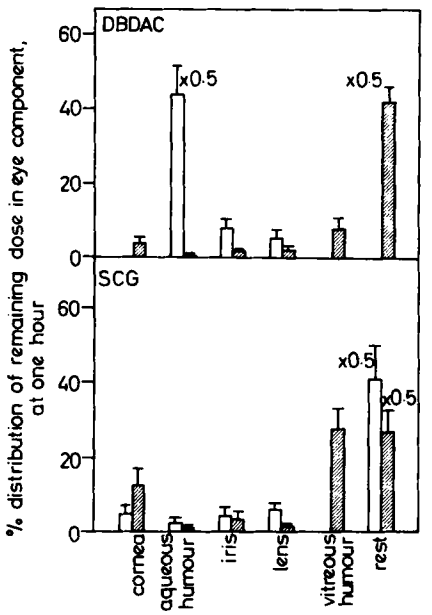

FIG. 2. Histograms showing the percent distribution of the dose remaining at 1 h after administration as single large ion or as the complex coacervate (shaded values). (Data are given as means ± s.e.m.). For conditions see *Procedures*.

ocular absorption of drugs, it acts as both barrier to penetration and as a drug reservoir. Drainage, vasodilation and non-conjunctival loss also exert major influences on drug loss before ocular absorption (Lee & Robinson 1979). The formation of a more lipid-soluble species, such as the ion pair, would be expected to result in a different disposition in the eye due to alterations in non-conjunctival loss and increased corneal uptake. The increase in corneal concentrations of large ions when given as the coacervate that we have found is consistent with the studies of Sieg & Robinson. Table 2 indicates that there is a different clearance of DBDAC compared with SCG from all components of the eye, significantly more DBDAC remaining in the eye at 1 h. Although the total amount of SCG found in the eye is reduced to approximately 75% of that found after a single ion dose, the total amount for DBDAC

is over 20 times higher than the single ion dose. Without knowing the pharmacokinetic profile of each ion in each eye component after single ion and coacervate dosing, it is not possible to state whether more or less large ion is delivered into the eye when given as a complex coacervate.

Conclusions

Whitmore et al (1978) have concluded that surfactants of all classes (ionic and non-ionic) are unlikely to enhance the permeability of membranes to solutes without causing membrane damage, unless at very low concentrations they are able to interact directly with a particular solute in such a way that its absorption is facilitated. Our findings here and elsewhere (Tomlinson & Davis 1978, 1980) indicate that sodium cromoglycate and dodecylbenzyldimethylammonium chloride do interact to form a species which in a physical in vitro model transfers to phases to lower polarity, and which in vivo causes marked alterations in the ocular penetration and disposition of both species. What has not been demonstrated is whether this occurs via transfer of an ion-pair species, or whether the formation of ion pairs merely increases the availability of both ions to the surface of the corneal tissue. Certainly the differences in percent disposition of both ions, when given as a coacervate, and the ratios of ion found in the aqueous humour after dosing as the ion-pair system, suggest that the large ions do not travel as the ion pair within the eye.

Gaginella et al (1973) have shown that although ion pairing with bile salts can increase the liquid/liquid distribution of the drug isopropamide, the pairing is unable to increase gastrointestinal absorption of the cation itself. Two comments can be made. First, oral route delivery of a solution of an ion pair, because of dilution, will tend to shift the physicochemical equilibrium towards there being a preponderance of unassociated ions, and second, much of the conflict in the literature (e.g. Kakemi et al 1969; Suzuki et al 1972; Gaginella et al 1973; Nakamara et al 1977; Bhuta et al 1980) on the role of ion pairs in drug delivery is due seemingly to the character of the ions forming the ion pair. We have shown (Tomlinson et al 1979) that above a combined anion-cation carbon number of approximately 25 for ions having hydrophobic integrity, it is likely that ion pairs produced by a water structure enforcement mechanism (as envisaged by Diamond 1963) can be formed in solely aqueous environments, whereas for a pair of ions where one or both are small (mol. wt less than 100), or are inorganic, ion pairs will form only in areas of low polarity or in the diffusion layers at interfaces. Our results here suggest that if ion pairing is to be used rationally to alter ion absorption and disposition, then the type of organic ion associations proposed by Diamond (1963) should be the preferred area of enterprise. What is also the case is that the use of complex coacervates of large organic ions alters the corneal penetration and disposition of these large organic ions in a manner different from that found when ion pair systems are used as the dose form.

We have reported briefly on aspects of this study previously (Davis et al 1978).

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