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Bioadhesive microspheres as a potential nasal drug delivery system

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Summary

The rapid mucociliary clearance mechanism in the nasal cavity can be considered as an important factor when low bioavailabilities are obtained for drugs given intranasally. A nasal delivery system in the form of bioadhesive microspheres has been developed. Studies in human volunteers using gamma scintigraphy showed great differences in clearance times between 3 microsphere systems and two controls. The half life of clearance for starch microspheres was found to be in the order of 240 min as compared to 15 min for the liquid and powder control formulations. The microspheres form a gel-like layer in contact with the nasal mucosa that is cleared slowly from the nasal cavity. In vitro studies using model compounds (cromoglycate and Rose bengal) showed high degrees of loading capacities for the various microsphere systems. Using various physical and chemical approaches, it was possible to a certain degree to control the release of the compounds from the microsphere systems.

Introduction

In recent years growing attention has been paid to the nose as an alternative route of administration for systemically active drugs such as peptides and proteins that are poorly absorbed orally and are extensively metabolised in the gastrointestinal tract itself or are subjected to first pass metabolism in the liver. The nose is well suited for the absorption of drugs since it has a large epithelial surface area available due to numerous microvilli. Furthermore, the subepithelial layer is highly vascularized and the venous blood from the nose passes directly into the systemic circulation short-cutting the liver.

Some drugs such as propranolol (Hussain et al., 1979, 1980a and b), progesterone (Hussain et al., 1981, David et al., 1981) and enkephalins (Su et al., 1985) appear to be absorbed effectively via the nasal route with bioavailabilities comparable to those for the intravenous route of administration. However, many drugs such as insulin (Moses et al., 1983; Salzman et al., 1985), analogues of luteinizing hormone releasing hormone (Berquist et al., 1979), growth hormone releasing factor (Evans et al., 1983) and calcitonin (Hanson et al., 1987) show much lower absorption efficiencies when administered intranasally.

Various approaches have been attempted in order to increase the absorption and thus the

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bioavailability of drugs administered intranasally. Substances such as bile salts (e.g. sodium glycocholate) and surfactants (e.g. polyoxyethylene-9-lauryl ether) in combination with the drug will modify the properties of the nasal mucosa, thereby enhancing absorption efficiency.

The absorption promoting effect of these enhancers has been shown generally to be due to their ability to increase membrane fluidity for example by extracting proteins from the nasal membrane. For bile salts there is also the ability of these materials to inhibit enzyme activity in the membrane (Hirai et al., 1981) and to reduce the viscosity of the mucus and thereby allow for an easier diffusion of the drug through this layer (Lee, 1987). It has been shown that in most cases absorption enhancers can increase the absorption efficiency of drugs. Thus, using a surfactant absorption enhancer, Hanson et al. (1987) showed a 10-fold increase in the area under the blood level curve for the intranasal administration of salmon calcitonin as compared to the administration of the drug alone. However, very little is known about the impact of these enhancer systems on the nasal membrane and especially about the long term toxicological consequences.

An alternative strategy to using absorption enhancers is to prevent the rapid clearance of the delivery system from the nasal cavity and thereby prolong the contact between the drug and the nasal mucosa. Due to the mucociliary functions of the nasal mucosa, material applied intranasally, for example in the form of a drug solution, will normally be cleared from the nasal cavity into the nasopharynx with an average speed of 5 mm/min (Mygind, 1978). Thus, when technetium-99m-labelled human serum albumin was applied to human volunteers as nasal sprays or drops the time for 50% clearance was found to be of the order of 20–30 min depending on the method of application (Aoki and Crawley, 1976; Hardy et al., 1985).

In the present work we have investigated a nasal delivery system in the form of bioadhesive microspheres that, by forming gel like structures in contact with the mucus, should be cleared slowly from the nasal cavity thereby prolonging the contact between the delivery system and the mucosa. Such systems should have the potential of releasing the drug from the microspheres in a sustained and controlled manner, thereby possibly increasing the absorption efficiency of the drug.

This paper describes the evaluation of selected microsphere systems in the form of albumin, starch (Spherex) and DEAE-dextran (DEAE-Sephadex) microspheres for the controlled release delivery of drugs via the nasal route. Using Rose bengal and sodium cromoglycate as model drugs the loading capacities and release characteristics for these microsphere systems have been studied.

Furthermore, the clearance of the technetium-99m labelled microsphere systems from the nasal cavity of human volunteers as compared to nasal powder and nasal spray systems, has been investigated using gamma scintigraphy.

Materials and Methods

Materials

Rose bengal (tetraiodotetrachlorofluorescein, sodium salt) was purchased from the Sigma Chemical Company Ltd. (Dorset, U.K.). Sodium cromoglycate (disodium 4,4′-dioxo-5,5′-(2-hydroxytrimethylenedioxy)di(4H-cromene-2-carboxylate) was a gift from Fisons Pharmaceuticals (Loughborough, U.K.). All other chemicals were of reagent grade.

Microspheres and control systems

Albumin microspheres were produced by an emulsification technique slightly modified to that described by Tomlinson et al. (1984). 50 ml of highly purified olive oil was mixed with 75 ml of petroleum ether and prestirred for 5–10 min in a 125 ml beaker using a Heidolph mixer. To this mixture 0.4 ml of a 25% w/v aqueous solution of rabbit serum albumin (RSA) in phosphate buffer (pH 7.4) was added dropwise and stirring was continued at 700 rpm for 15 min. The microspheres were stabilised by adding dropwise 0.1 ml of a 25% w/v 1.5-glutaraldehyde solution under continual stirring for 15 min. The microspheres were isolated by centrifugation, washed with petroleum ether, filtered through a Millipore filter, washed again with petroleum ether and then

ethanol and freeze-dried overnight.

The size of the microspheres was in the range of 40–60 μm.

Since the size of the microspheres was found to increase when drug was incorporated the manufacturing procedure was adjusted using a stirring speed of 900 rpm in order to obtain microspheres of the desired size range.

Starch microspheres (Spherex) of mean diameter 48 μm (swelled size) were obtained as a gift from Pharmacia AB (Uppsala, Sweden) freeze-dried and used as obtained.

DEAE-dextran microspheres (DEAE-Sephadex) were obtained from Pharmacia AB (Uppsala, Sweden). DEAE-Sephadex comprised particles of 40–150 μm in diameter prepared by cross-linking of DEAE-dextran with epichlorhydrine. This material is used for ion-exchange procedures. The microspheres were fractionated by microsieving to obtain a size range of 40–60 μm (swelled size) particles.

The control systems Lomudal nasal powder and Lomudal nasal nebulogenum both containing sodium cromoglycate either in powder or solution form, were obtained from Fisons Pharmaceuticals (Loughborough, U.K.).

Particle size analysis

It is important that the size of the microspheres for nasal delivery is above 10 μm since particles below this size would be able to be carried with the airstream down into the tracheobronchial region (Proctor and Anderson, 1982). Larger particles will mainly deposit in the anterior unciliated portion of the nose and thus a size range of 40–60 μm was chosen for the microsphere systems.

The microspheres were sized in normal saline containing 0.1% w/v Tween 80 using a Coulter Counter model TA_{II} (Coulter Counter Electronics Ltd., Herts, U.K.). The particle sizes were expressed as a mean volume diameter. The mean particle diameters of the swollen microsphere systems were found to be about 40 μm, 48 μm and 50 μm for the albumin, the starch and the DEAE-dextran microspheres, respectively.

Analytical measurements

The determination of the loading capacity and release of drug from the microspheres was performed using UV-spectroscopy (LKB Ultrospec 4050). The absorption maximum for Rose bengal is dependent on pH and the presence of proteinaceous materials. Furthermore, the molar absorptivity (ϵ) is dependent on the amount of protein present due to an interaction with Rose bengal. During the release studies, small amounts of protein material were released from the albumin microspheres into the medium. Therefore, in this case protein was added to the medium to ensure that the amount of protein being released from the microspheres would be small compared to the added amount and hence would not influence the determination of Rose bengal. 100 μl of a 3% w/v rabbit serum albumin solution were added to the withdrawn sample of 3.0 ml giving a final concentration of protein of 0.1%. The measurements were performed in a phosphate buffer of pH 7.4 at a wavelength of 568.6 nm.

For sodium cromoglycate the absorption spectrum was not influenced by the presence of protein and changes in pH in the range of interest. The measurements were performed in a phosphate buffer of pH 7.4. Since the peak of interest was present on an "obliquely" sloping baseline, a method employing measurements at 3 wavelengths (300, 330 and 390 nm) was used.

Drug incorporation

Albumin microspheres

Rose bengal and sodium cromoglycate were used as model drugs for incorporation into the albumin microspheres. The microspheres were prepared as described above with a stirring speed of 900 rpm and the model drug dissolved in the albumin solution at various concentrations.

Concentrations of 0.5, 2, 4 and 5% w/v Rose bengal were used. Above 5% w/v the aqueous phase became too viscous and microspheres did not form. Due to the solution characteristics of sodium cromoglycate in water the highest concentration that could be used was 8% w/v. Thus, microspheres were manufactured from solutions containing 0.5, 1, 2 and 4% w/v sodium cromoglycate.

The maximum loading capacities for these compounds were found to be 170 μg Rose bengal and 137 μg sodium cromoglycate per mg albumin microspheres.

DEAE-dextran. Particles made from DEAE-dextran are commercially available as ion-exchange materials. They will bind strongly negatively charged ions as for example carboxyl groups. Therefore ion-binding properties should be achieved by incorporating DEAE-dextran into the albumin microspheres, thereby decreasing the release rates of incorporated Rose bengal and sodium cromoglycate that both contain carboxylic groups. Microspheres containing 8 mg Rose bengal or sodium cromoglycate per 100 mg rabbit serum albumin were produced with different amounts of DEAE-dextran incorporated into the aqueous phase of the microspheres during production i.e. 2, 8, 16 and 20 mg (equivalent to concentrations in the aqueous phase of 0.5, 2, 4 and 5% w/v, respectively).

Heat denaturation. In order to decrease the rate of release of drug by increasing the density of the core protein the albumin microspheres were exposed to an additional heat denaturation treatment. The microspheres were produced as usual with 2 mg Rose bengal or sodium cromoglycate per 100 mg of rabbit serum albumin, and after isolation from the oil phase they were transferred to a beaker containing pure olive oil. The oil was heated to 170 °C (±5 °C) under continual stirring and then the temperature was kept constant for the desired period of time. At appropriate time intervals (3, 8 and 24 h) samples were taken and the microspheres washed and freeze-dried.

Precipitation. A method exploiting the possibility of precipitating the sodium cromoglycate within the microspheres by formation of a less soluble salt to decrease the rate of release of sodium cromoglycate from the albumin microspheres was evaluated. Stock solutions of $MgCl_2$ and sodium cromoglycate were mixed with the albumin solution immediately before the mixture was added to the prestirred oil phase. In this way the formation of large crystals would be avoided. The amount of $MgCl_2$ to be added was calculated on an equimolar basis. The microspheres were then produced in the normal way.

Starch microspheres (Spherex)

The loading of the starch microspheres with drug was performed using absorption of the solute into the matrix of ready-made microspheres. The loading capacity of the starch microspheres was found to be 160 μg sodium cromoglycate per mg Spherex. 200 mg of Spherex microspheres (freeze-dried) were added to 4 ml of sodium cromoglycate solution (80 mg/ml) and left for ca. half an hour to swell. The microspheres were separated from the solution by centrifugation and washed with water to remove excess of drug and then freeze-dried. In one study the freeze-dried microspheres containing the sodium cromoglycate were added to a solution containing $MgCl_2$ in order to precipitate the drug inside the matrix. They were left for half an hour, washed and freeze-dried.

DEAE-dextran microspheres (DEAE-Sephadex)

The loading of the DEAE-Sephadex microspheres with drug was performed by letting 100 mg DEAE-Sephadex swell in solutions of Rose bengal (40 mg/ml) or sodium cromoglycate (80 mg/ml) for at least 3 h, after which the particles were collected on a Millipore filter (pore size 5.0 μm) and freeze-dried.

Release of drugs from the microsphere systems

The in vitro release of drug from the different types of microspheres was evaluated using a phosphate buffer at pH = 7.4 for the release of Rose bengal, and deionized water at pH = 6.0 for the release of sodium cromoglycate. Between 20 and 40 mg (accurately weighed) of microspheres loaded with solute was suspended in 400 ml of the appropriate medium (sink conditions) contained in a beaker and kept at 37 °C under continual stirring. At selected time intervals samples of 5 ml were taken from the suspension, filtered through a Millipore filter (pore size 0.2 μm) and the solute content determined as described above. 5 ml of buffer were added to the suspension after each sampling to maintain a constant volume.

Labelling procedures

Albumin, Spherex and DEAE-Sephadex microspheres were all labelled as follows.

100 mg of microspheres were suspended in 5 ml

normal saline and 1 ml 5 mg/ml $SnCl_2$, $2H_2O$ (in 1 N HCl), 5 ml NaAc 100 mg/ml and 1 ml technetium-99m pertechnetate eluate containing about 20 MBq of activity were added. The suspension was left under continual stirring for 5 min and then centrifuged. The microspheres were freeze-dried overnight and appeared as free flowing powders. The labelling efficiency was found to be not less than 97%. The stability of the label in vitro was found to be high. Less than 2.5% of the activity was released into normal saline in 60 min.

The Lomudal spray and powder formulations were labelled by adding a technetium-99m labelled tracer DTPA (diethylenetriaminepenta-acetic acid) as a solution or as a freeze dried powder, respectively, giving final activities of 1.9 MBq per dose (i.e. 0.13 ml of solution and 10 mg of powder, respectively). It has been shown by Dudley et al. (1980) that DTPA in solution is absorbed readily from the nasal mucosa, thus this material can be used as a good model for an absorbable drug.

The labelled microspheres and the labelled Lomudal nasal powder were filled into gelatin capsules (10 mg dose, about 1.9 MBq of activity).

Nasal clearance studies

In order to study the clearance characteristics the nasal delivery systems were administered to a group of 6 healthy human volunteers (20–30 years of age) according to a cross-over design with at least two days between each treatment. The study was conducted in accordance with the declaration of Helsinki guide lines and was approved by the regional ethical committee. The 3 microsphere systems (without added solute) and the Lomudal nasal powder in gelatin capsules were applied using a Lomudal nasal insufflator. The total content of one capsule (10 mg–1.9 MBq) was applied evenly to the mucosal surface of the right half of the nose. Puffs were given during inhalation to the upper and to the lower part of the nasal cavity until the capsule was empty. The Lomudal spray was applied in one puff (0.13 ml) using the normal pump spray, to the right half of the nose during inhalation.

The deposition and subsequent clearance of the different nasal delivery systems was followed by gamma scintigraphy (Maxi Camera II, General Electric). Dynamic lateral views of the head were recorded for 15 min (60-s frames) and static views (3 min duration) were taken at appropriate time intervals up to 180 min after application. The position of the nose of the volunteer was fixed on the collimator of the gamma camera using a specially designed template. The images were recorded for subsequent quantification. Regions of interest were drawn around the site of deposition of the delivery system and the total activity in this area followed with time and corrected for decay.

Results and Discussion

Release of drugs from microspheres in vitro

Albumin microspheres

The release profiles obtained for the release of Rose bengal (in different concentrations) from albumin microspheres showed a biphasic release with an initial fast release phase followed by a second slower release phase. Typical release profiles are shown in Fig. 1. After ca. 5 h, the release of Rose bengal reached a plateau level for all concentrations tested. The rate and extent of release of Rose bengal was dependent on the amount of Rose bengal incorporated, with the higher rate and extent of release obtained for the highest drug loading. However, it should be taken into account that everything else being equal, a linear correlation exists between the amount of drug incorporated and the mean particle size as shown in Table 1. These results are in accordance with results obtained by Tomlinson et al. (1984) who also observed the initial so-called burst effect of drug from albumin microspheres. This effect is considered to be due to the fast release of surface-adsorbed material.

For the release of sodium cromoglycate from the albumin microspheres it was found that for the various microspheres with different amounts of drug incorporated, most of the drug was released within the first 5 min. This was surprising since some degree of binding of sodium cromoglycate to serum albumin has been reported in the literature (Sjöholm et al., 1979) and this would be expected to delay release. However, sim-

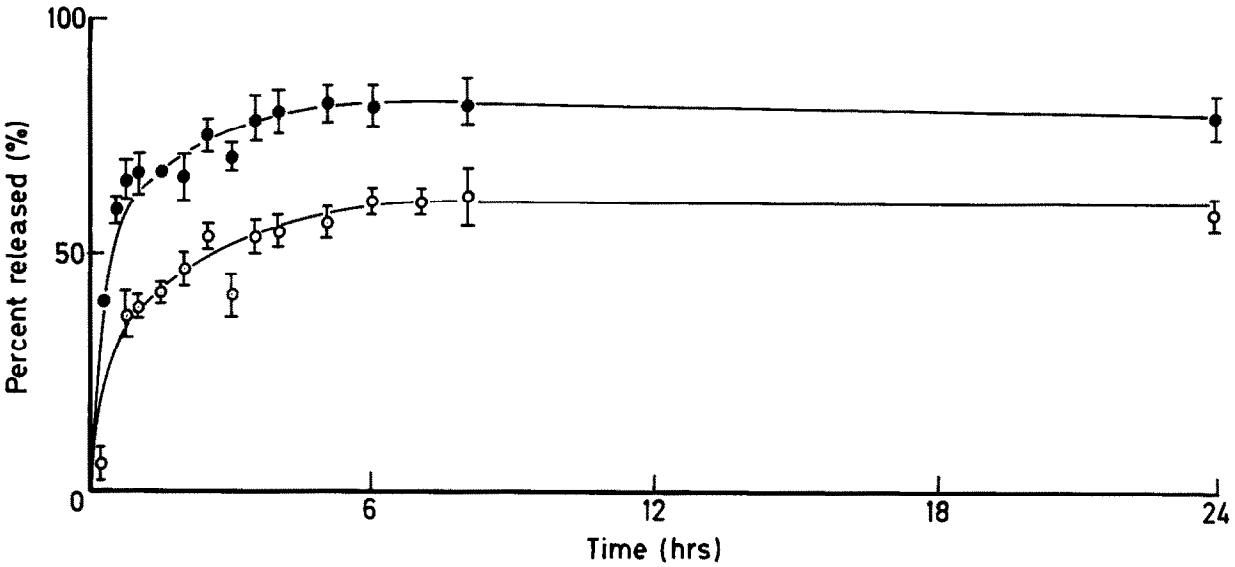

Fig. 1. Release of Rose bengal from albumin microspheres, ●, 16 mg RB/100 mg RSA; ○, 8 mg RB/100 mg RSA.

ilar release rates to those found in the present study have been reported for sodium cromoglycate by Tomlinson et al. (1984) and Mills (1983).

In an attempt to change the release characteristics of drugs from albumin microspheres the anionic exchange material DEAE-dextran was incorporated into the albumin matrix. The maximum amount of DEAE-dextran that could be incorporated into these microspheres was found to be 4% w/v. For higher concentrations the aqueous phase became too viscous and microspheres did not form. The mean particle size of the albumin microspheres was found to be dependent on the amount of DEAE-dextran incorporated (Table 2).

The release profiles for microspheres containing DEAE-dextran and Rose bengal had the same format as the profiles previously obtained for the albumin microspheres not containing DEAE-dextran. As expected, the quantity of DEAE-dextran incorporated had a profound influence on the rate and extent of Rose bengal released i.e. the higher the concentration of DEAE-dextran in the microspheres the lower the rate and extent of release of Rose bengal (Fig. 2). This effect is considered to be due to an ionic binding of Rose bengal to DEAE-dextran. Thus, by incorporation of 4% DEAE-dextran the release of Rose bengal after 5 h from the microspheres was decreased from 58% (as found for the similar microspheres not containing DEAE-dextran) to 14% of the total amount incorporated. However, it should be borne in mind that the mean particle sizes for the microspheres containing DEAE-dextran are relatively larger than that for microspheres containing the same

TABLE 1

Mean particle size of albumin microspheres containing Rose bengal

mg Rose bengal/ 100 mg RSA	Mean diameter (μm) (±S.E.M.)
2	34.5 (±4.0)
8	38.5 (±6.5)
16	44.0 (±6.0)
20	51.0 (±11.0)

TABLE 2

Mean particle size of albumin microspheres containing DEAE-dextran

mg DEAE-dextran/ 8 mg Rose bengal/ 100 mg RSA	Mean diameter (μm) (±S.E.M.)
2	60.3 (±1.8)
8	62.0 (±0.1)
16	80.0 (±0.1)

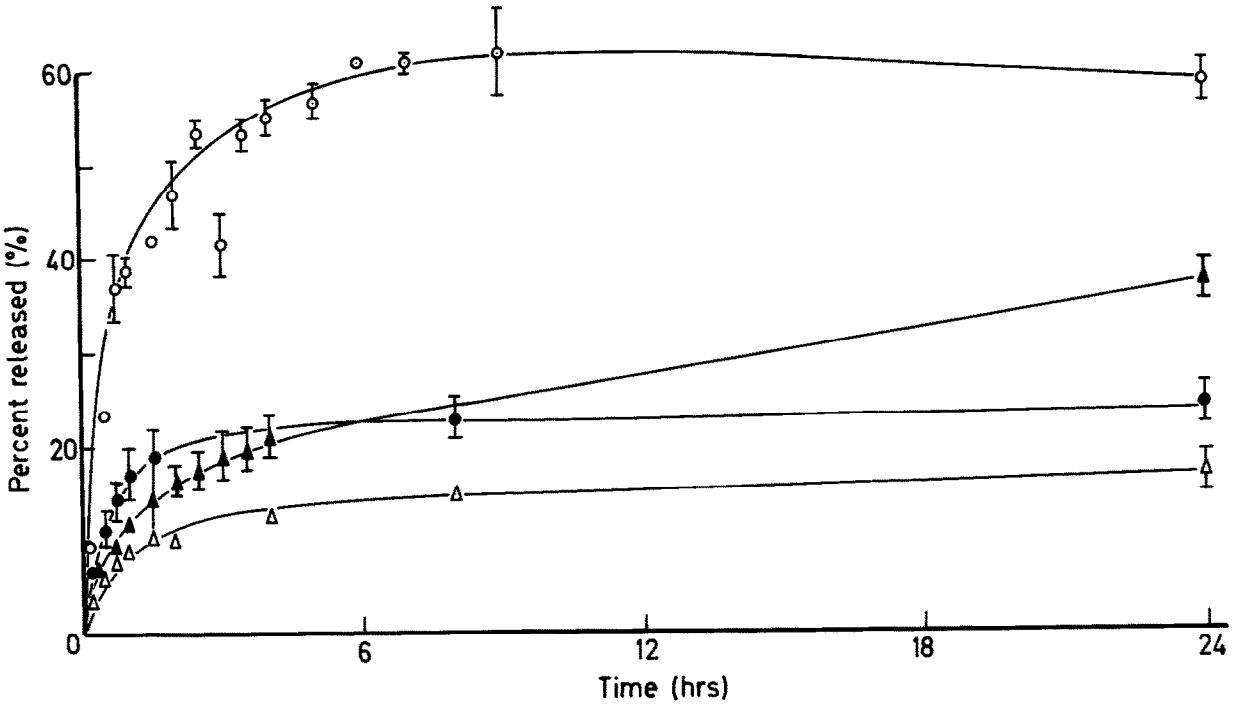

Fig. 2. Release of Rose bengal from albumin microspheres, containing DEAE-dextran; △, 16 mg DEAE-dextran/8 mg RB/100 mg RSA; ●, 8 mg DEAE-dextran/8 mg RB/100 mg RSA; ▲, 2 mg DEAE-dextran/8 mg RB/100 mg RSA; ○, 0 mg DEAE-dextran/8 mg RB/100 mg RSA.

amount of Rose bengal but with no DEAE-dextran are smaller. Thus, some of the differences in the release profiles could be due to the effect of size (i.e. the smaller the particles the faster the release of solute, Tomlinson et al., 1984).

Several attempts were also made to decrease the very rapid release of sodium cromoglycate from the albumin microspheres. The first approach was to incorporate DEAE-dextran into the matrix. Surprisingly, the rate of release of sodium cromoglycate from the microspheres did not differ significantly from that of the microspheres produced without DEAE-dextran. It would have been expected that this anionic drug (two carboxyl groups) would bind strongly to the cationic groups on DEAE-dextran. A possible explanation of this phenomenon could be the occurrence of a competitive ionic binding between albumin and DEAE-dextran.

The second approach to changing the release characteristic of compounds from albumin microspheres was the exposure of the microspheres containing solutes to an additional heat treatment so as to increase the density of the core protein. A slower release of drug should then be expected. Release experiments with heat-treated albumin microspheres containing Rose bengal showed that no solute was released from the microspheres for a period of 24 h. This is probably due to the increased density of the albumin and the consequent closer trapping of the large molecules (1018 Da). The decomposition of the Rose bengal after 24 h at 170 °C was negligible.

The release profiles for the microspheres containing 2 mg sodium cromoglycate per 100 mg rabbit serum albumin showed that the rate and extent of release was significantly decreased after 3 h heat treatment and further decreased after 8 h of treatment, as compared to the microspheres which had not been heat denaturated (Fig. 3). When the heating process continued for more than 8 h no further decrease in release rate was obtained. The amount of drug released from the microspheres after 24 h (at plateau level) were

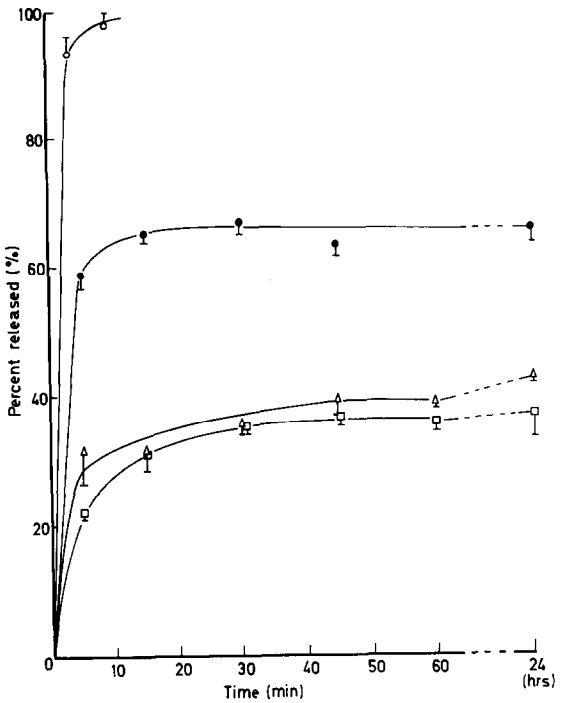

Fig. 3. Release of sodium cromoglycate from albumin microspheres after additional heat denaturation; ○, no heat denaturation; ●, 3 h heat denaturation; △, 8 h heat denaturation; □, 24 h heat denaturation.

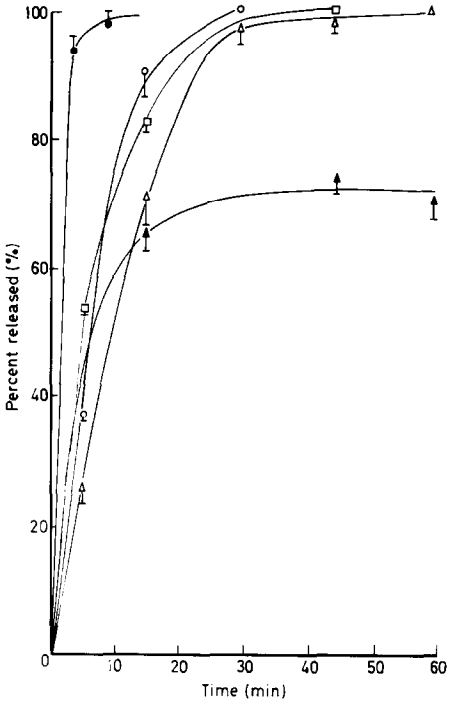

Fig. 4. Release of sodium cromoglycate from albumin microspheres after precipitation with $MgCl_2$; ●, 0 mg $MgCl_2$/100 mg RSA; ○, 2.4 mg $MgCl_2$/100 mg RSA; △, 4.8 mg $MgCl_2$/100 mg RSA; □, 7.3 mg $MgCl_2$/100 mg RSA; ▲, 45.3 mg $MgCl_2$/100 mg RSA.

65%, 45% and 35% of total amount of incorporated drug for microspheres that had been heat-treated at 170 °C for 3, 8 and 24 h, respectively. At 170 °C decomposition of sodium cromoglycate was found to take place but the extent of this degradation (i.e. 6.8% at 3 h, 11.7% at 10 h) would not account for the results obtained in the release studies.

In a similar attempt to decrease the fast rate of release of sodium cromoglycate from albumin microspheres Tomlinson et al. (1984) increased the time of stabilisation with glutaraldehyde. This resulted in a reduction in the initial burst release of drug but in an increased release rate in the second release phase.

The third approach to obtaining a decreased release of drug from the albumin microspheres was performed using microspheres containing 2 mg sodium cromoglycate per 100 mg rabbit serum albumin and by adding various amounts of $MgCl_2$ to the microsphere suspension.

The release profiles obtained were of similar format as before and showed a relatively good correlation between the amount of $MgCl_2$ added per mg of rabbit serum albumin and the rate of release (Fig. 4). The rate of release decreased with an increasing amount of $MgCl_2$ added, but the

TABLE 3

Bioadhesive delivery systems for the nose

Microsphere system	Swelled size (μm)	Degree of swelling	Bioadhesive properties	Binding
Albumin	40–60	ca. 40%	good	hydrogen bonding
Starch-Spherex	48	0.6–8.4 ml/g	good	hydrogen bonding
DEAE-Sephadex	40–60	swells readily	good	ionic bonding

decrease in release was not so pronounced as for the heat denaturation approach. Again, differences in the sizes of the microspheres should be taken into account.

Starch microspheres

Starch microspheres (Spherex) were loaded with sodium cromoglycate either by swelling the freeze-dried microspheres in a solution of the drug or by adding a solution of $MgCl_2$ to freeze-dried microspheres containing the drug.

Using the first method of loading the microspheres were found to release the total content of drug within 5 min whereas using the second (precipitation) method no detectable amount of drug was released within 4 h. These results show that drug does diffuse into the matrix of the spheres probably as a result of the very fast swelling of the microspheres when these are dispersed in the drug solution. The rapid release of sodium cromoglycate from the former system was not surprising since the Spherex microspheres are similar to albumin microspheres in terms of matrix structure.

DEAE-dextran microspheres

Finally, the DEAE-dextran microspheres (DEAE-Sephadex) were investigated as potential drug carrier systems. Release experiments were performed for these microspheres that were swelled in a solution of Rose bengal (40 mg/ml) or sodium cromoglycate (80 mg/ml) for at least 3 h. No detectable amount of Rose bengal was released from the microspheres within 24 h indicating a very strong binding to the cationic binding sites in the microsphere matrix. For the sodium cromoglycate, the release of drug seemed to be highly dependent on the degree of the ionic strength of the water as indicated by this and later investigations (Stevens, 1987).

The results from these in vitro studies show that it is possible to a certain degree to control the release of compounds from the various microsphere systems using the described approaches. Thus, it should be possible to develop controlled release systems individually suited for the drugs to be delivered via the nasal route.

Human studies

In order to increase the total absorption of drugs through the nasal mucosa and thereby the bioavailability we have explored the possibility of obtaining slow nasal clearance times for delivery systems in the form of bioadhesive microspheres. The microsphere delivery systems investigated have been produced from materials that are known to show a high degree of swelling in contact with an aqueous medium and to form a gel-like structure after swelling. The bioadhesive properties and the physical characteristics of the microsphere systems are given in Table 3.

The mechanism of adhesion of bioadhesive polymers to soft tissues has been discussed by Peppas and Buri (1985) and Park and Robinson (1984) and involves both chemical and physical binding. Both weak and strong interactions (i.e. van der Waals interaction, hydrogen bonding and ionic bonding) can develop between certain types of chemical groups on the polymer (e.g. hydroxyl or carboxyl groups) and the glycoprotein network of the mucus layer or the glycoprotein chains attached to the epithelial cells in for example the nose.

In order for strong adhesive bonds to develop the establishment of intimate molecular contact between the polymer and glycoprotein chains is essential (Peppas and Buri, 1985). Thus, an important requirement for bioadhesive polymers is their ability to swell by absorbing water (here from the mucous layer in the nasal cavity) thereby forming a gel-like layer in which environment the interpenetration of polymers and glycoprotein chains can take place and the bondings can form rapidly.

The applicability of these proposals for bioadhesive systems was investigated in vivo measuring the rate of clearance of the albumin, starch and DEAE-dextran microsphere systems and the two controls in human volunteers by means of gamma scintigraphy.

Upon administration to the nose of the human volunteers by means of the nasal insufflator the microsphere formulations and the Lomudal powder were mainly deposited in the anterior part of the nasal cavity with little of the dose reaching the turbinates initially. In contrast, the Lomudal

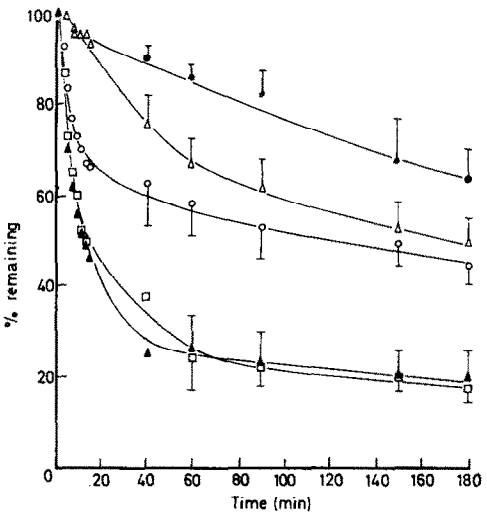

Fig. 5. Clearance of different microsphere systems and of two control systems from the nasal cavity; ●, DEAE-dextran microspheres; △, starch microspheres; ○, albumin microspheres; ▲, lomudal nasal solution; □, lomudal nasal powder.

solution was dispersed from the atrium to the nasopharynx after application by spray.

The activity–time profiles giving the activity remaining at the site of deposition show that both the Lomudal solution and powder formulations were cleared from the site of deposition quite rapidly (as indicated by the clearance of the gamma emitter) with a half time of clearance of the order of 15 min, whereas the microsphere systems had much longer clearance times (Fig. 5). After 3 h 50% of the initial activity combined with the albumin and starch microspheres and 60% combined with the DEAE-Sephadex was still at the site of deposition. By extrapolation, the half time of clearance from the initial deposition site of the last system was estimated to be about 4 h.

The apparent rapid clearance of the control solution and powder formulations was not surprising since similar results for albumin solutions have been reported earlier (Hardy et al., 1985). The slow clearance of the microsphere systems can most probably be attributed to the fact that the microspheres undergo a process of taking up water and swelling, thereby forming a mucoadhesive system that is cleared slowly. Furthermore, the deposition of the microsphere system in the anterior part of the nose where the number of epithelia cells bearing cilia is less than in the main part of the nose, could also be a factor of importance to the increase in clearance time. The observed variations in clearance time between the different microsphere systems can probably be related to the differences in type of bondings (hydrogen and ionic bondings) formed between the gel and mucus. Furthermore, differences in swelling characteristics could also be of importance. Work is now in progress to clarify these matters.

It is interesting to note, that similar bioadhesive systems have been used for the improvement of the absorption of drugs given via the nasal route. Nagai et al. (1984) have enhanced the absorption of insulin after nasal application in dogs using a powder formulation containing hydroxypropylcellulose and neutralized polyacrylic acid (Carbopol 934) that would form a gel in contact with the nasal mucosa. This insulin formulation produced hypoglycaemia of one-third the extent of that reached by intravenous administration for the same dose of insulin administered. Similarly, Morimoto et al. (1985) have used a polyacrylic acid gel base to enhance the absorption of both insulin and calcitonin in rats after intranasal administration. However, the rate of clearance of these delivery systems from the nose was not reported in either case.

It can be concluded from the present studies that by selecting a delivery system in the form of microspheres that have good bioadhesive characteristics and that swell easily in contact with the nasal mucosa, it is possible to control the rate of clearance of the delivery system from the nose and thereby provide potential for increasing the bioavailability of drugs incorporated into the microspheres. The microspheres should also be able to protect the drug against enzymatic degradation in the nasal cavity. Preliminary studies on sheep have indeed shown that gentamicin applied to the nose using the starch microsphere delivery system gave a 30 times higher blood concentration than when applied in a simple solution (Illum et al., unpublished results). Work is now being conducted to further evaluate and improve these nasal delivery systems.

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