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Preparation and characterization of HSA-propranolol microspheres for nasal administration

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Summary

Human serum albumin based microspheres bearing propranolol hydrochloride were prepared by an emulsion internal phase stabilization technique. The prepared microspheres were tested for release characteristics, bioadhesion and controlled in vivo absorption following nasal administration. The factors, which include HSA concentration and stabilization temperature in the case of heat stabilized microspheres and density, were studied for their effect on release characteristics and bioadhesion. The albumin microspheres were noted to possess good bioadhesion, and to release the contents slowly. However, the release rate was related to the period of heat treatment given for stabilization which in turn was related to the density of microspheres. In vivo experiments revealed that the nasal administration of an albumin based system eliminated first pass metabolism and could maintain an effective drug concentration for 10–12 h with improved bioavailability as compared to that following intravenous drug administration. Moreover, magnetite containing microspheres on application of an external magnetic field of 8 kOe strength exhibited additional pulse dosing at the magnet application point. This latter effect could be exploited for drug plasma level monitoring.

Introduction

The nasal route has been restricted for local action of drugs and it is only recently that it has been used for systemic bioavailability. The nasal absorption of some β-blocking adrenergic agents, alprenolol, metapronol and propranolol has been shown to be as effective as their intravenous administration (Hussain et al., 1979; Duchateau et al., 1986).

Other drugs that have been studied for nasal application include progestational steroids (Chein, 1988), testosterone (Hussain et al., 1984), clofilium (Champanale and Gries, 1984), desmopressin (Harris et al., 1986), sodium cromoglycate (Fisher et al., 1985), progesterone (Hussain et al., 1981), enkephalin (Su et al., 1985), and insulin (Morimoto et al., 1985; Nagai and Machida, 1985).

An attempt towards improved bioavailability of gentamicin following nasal administration has been made using starch microspheres as drug bearing delivery system (Illum et al., 1988). These bioadhesive microspheres were effective in con-

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trolling the drug release over a prolonged period of time with increased bioavailability (Illum et al., 1987).

Various factors which influence the nasal absorption of drug through mucosa and delivery of drug from the dosage form include surfactants (Hirai et al., 1981; Duchateau et al., 1986), viscosity, particle size, nasal clearance (Harris et al., 1988), dose, pH and osmolarity (Ohwaki et al., 1987), and structural drug requirement (McMartin et al., 1987).

Considering the mucociliary clearance of applied material, attempts have been made either to reduce the viscosity of nasal mucosa and thereby to enhance absorption or to increase applied drug versus nasal mucosa contact (Illum et al., 1987).

Propranolol hydrochloride, a β-receptor-blocking drug widely used in the treatment and management of angina pectoris, has been studied for its absorption via the nasal route. It has been reported to be absorbed and to reach systemic circulation following nasal application. The nasal route eliminates the intersubject variation normally associated with the oral route (Hussain et al., 1980).

The need for controlled systemic delivery of propranolol by some convenient route is well established and hence it was selected as a model drug. It is administered in 40 mg doses three to four times a day and is reported to be absorbed fairly through nasal mucosa. The present study was aimed at developing human serum albumin (HSA) based bioadhesive microspheres bearing propranolol for nasal delivery and exploring the possibility of dose delivery modulation using magnetic HSA-propranolol microspheres and an external magnetic field.

Materials and Methods

Materials

Propranolol HCl (Lupin Labs Pvt. Ltd, Aurangabad, India), human serum albumin (Sigma, St. Louis, U.S.A.), magnetite (Ferrofluidics Corp., Burlington, MA, U.S.A.) and glutaraldehyde (Fluka, Germany) were used. All other ingredients were of A.R. grade (B.D.H.) unless otherwise stated.

Preparation of albumin microspheres

Magnetic and plain HSA based microspheres bearing propranolol hydrochloride were prepared by an emulsification technique; 75.0 ml of cotton seed oil was mixed with 25.0 ml of petroleum ether and stirred for 10 min in a 200.0 ml beaker using a magnetic stirrer. Propranolol hydrochloride was dissolved in the HSA solution (2, 3 or 5% w/v), to obtain drug solution (2% w/v) in aqueous phase. The aqueous phase containing HSA and propranolol was added to the ethereal solution of cotton seed oil dropwise with continuous stirring using a mechanical stirrer at 1000 rpm for 15 min. The microspheres were stabilized by adding 0.1 ml of 25% w/v glutaraldehyde solution with continuous stirring for 15 min or by adding the emulsion system to the preheated cotton seed oil (100.0 ml) at 120 °C dropwise with continuous stirring. The microspheres were separated by centrifugation at $3000 \times g$ for 15 min and washed with petroleum ether three times for complete removal of oil adhering to the microsphere surface. The microspheres were filtered using Millipore filter (pore size 10 μm), and again washed with petroleum ether and ethanol. Preparations were freeze-dried and stored frozen until used in further studies.

Magnetic HSA propranolol microspheres

The magnetic HSA propranolol bearing microspheres were prepared using 0.5, 1.0 and 2.0% w/v concentrations of magnetite in the aqueous phase using the above-described procedure.

Characterization of microspheres

Determination of propranolol An accurately weighed quantity of microspheres (100 mg) was centrifuged with freshly prepared distilled water at $3000 \times g$ three times for 5 min each. The supernatants after centrifugation of each washing were separated and estimated for drug concentration spectrophotometrically at 216 nm (Shimadzu DB Spectrophotometer UV 150-02, Japan) (Clarke, 1969). The sum of drug quantity in all the washings was recorded as surfacial drug.

The settled microspheres were hydrolysed in 0.1 M glacial acetic acid with the help of gentle heat and after appropriate dilution the absorbance was

measured spectrophotometrically at 216 nm to determine drug content.

Determination of bioadhesion The albumin microspheres stabilized using glutaraldehyde as cross-linking agent and those stabilized at high temperature were tested for bioadhesion using the method described by Ranga Rao and Bari (1989). The suspension containing 100 mg albumin microspheres was poured dropwise at the mucosal site of a 5 cm long piece of rabbit small intestine. The intestine with the microspheres was placed in a desiccator maintained at 80% R.H. at room temperature ($28 \pm 2°C$) to allow hydration of microspheres for 20 min. The mucosal lumen was thoroughly washed with distilled water and the concentration of drug in the collected perfusate was determined. The ratio of applied and adhered microspheres was computed as per cent adhesion.

Microscopic analysis of microspheres

The microspheres were sized in normal saline containing 0.1% w/v Tween 80. The microspheres swollen system so prepared was observed under a microscope (Wild Leitz, Germany) fitted with an ocular micrometer. The microspheres had mean diameter between 30 and 50 μm (Table 1).

Determination of microsphere density

The density of freeze-dried microspheres was determined at 25 °C using a specific gravity bottle and benzene (density 0.874 g/ml) as the medium in which practically no swelling of HSA microspheres was noted.

Isolation of magnetic HSA microspheres

The magnetic HSA microspheres were isolated using a reported apparatus and following the methods described by Malaiya and Vyas (1988) and Senyei et al. (1978).

Magnetic responsiveness

Magnetic responsiveness of the prepared microspheres was determined using the same apparatus that was used in the isolation of microspheres. The drug content in microspheres retained at the top of the apparatus was determined spectrophotometrically after treatment with Triton X-100 (1% v/v).

Dissolution study

In vitro propranolol · HCl dissolution profiles from HSA microspheres were determined by a manual method (Illum et al., 1987). The dissolution assembly consisted of a beaker containing microspheres suspension prepared freshly in phosphate buffer (pH 6.8) maintained at $37 \pm 1°C$. The contents of the beaker were continuously stirred at 100 rpm. Samples were withdrawn at regular time intervals through a hypodermic syringe fitted with a 0.4 μm Millipore filter and replaced by fresh buffer after each sampling. Ab-

TABLE 1

Characterization of propranolol bearing HSA microspheres

Microsphere system	Stabilized chemically/ heat treatment	Drug content per 150 mg microspheres	Magnetite content (%)	Surface drug (% w/w) (based on weight of incorporated drug)	Density (g/ml)	Swell size (μm)	Bio-adhesion (%)
MPC (2.5% w/w)	glutaraldehyde	25.00	–	25.00	1.28	40	89.00
MPt_1	at 120 °C, 30 min	24.50	–	24.00	1.35	50	87.00
MPt_2	at 120 °C, 60 min	26.00	–	18.50	1.40	44	85.50
MPt_3	at 120 °C, 90 min	26.50	–	20.00	1.42	40	83.00
MPt_4	at 120 °C, 120 min	26.00	–	15.00	1.48	30	80.00
Mmt_3P_1	at 120 °C, 90 min	14.50	0.5	18.00	1.52	41	82.50
Mmt_3P_2	at 120 °C, 90 min	13.00	1.0	15.00	1.54	42	82.00
Mmt_3P_3	at 120 °C, 90 min	12.00	2.0	12.50	1.60	40	82.60

MPC, propranolol microspheres chemically stabilized; MPt, propranolol microspheres heat stabilized; Mmt_3P, magnetic propranolol microsphere heat stabilized at 120 °C for 90 min.

sorbance of the diluted sample was measured spectrophotometrically at 216 nm.

In vitro diffusion studies

Diffusion of propranolol · HCl from HSA microspheres was studied, across rabbit intestine. A duodenum segment (2 cm^2) from rabbit intestine was cut and washed with saline to remove any debris. Accurately weighed quantity of magnetic HSA microspheres was placed on the mucosal side of the intestine segment and kept for 30 min to allow bioadhesion. A segment of intestine bearing adhered microspheres was interposed between the donor and receptor compartments of the Franz diffusion cell (Crown Glass Co., NJ, U.S.A.). The receptor and donor compartments contained 25 and 10 ml phosphate buffer (pH 6.8), respectively. The diffusion cell was maintained at ambient temperature ($37 \pm 1°$ C). The content of the receptor compartment was stirred continuously using a magnetic stirrer. After assemblage of the diffusion cell a magnet of 8 kOe strength was placed at 1, 3, 5, 7 and 9 h at a distance of 5 cm above the mucosal side of the interposed intestine which was continuously bathed with the phosphate buffer of the receptor compartment (Fig. 1). The magnet was applied for 2 min every time and 1 min post magnet removal, a 0.5 ml sample was withdrawn with the help of a hypodermic syringe. The experiment was also conducted without the application of an external magnet. The drug content in withdrawn samples was estimated spectrophotometrically. Release profiles for drug from the magnetic microspheres with and without application of magnet were constructed (Fig. 3).

In vivo performance evaluation

In vivo performance evaluation of prepared plain/magnetic HSA microsphere based propranolol · HCl was carried out in Indian street dogs weighing about 10 kg. Three dogs were used for the study in a cross-over design. The dogs were anaesthetized with 30 mg pentobarbitone i.v./kg body weight prior to intravenous administration of 20 mg propranolol · HCl solution through the cubital vein.

HSA based plain/magnetic microsphere (M-Pt_3/Mmt_3P_3) equivalent to 20 mg of propranolol · HCl were administered nasally. The dogs were anaesthetized and microsphere suspension in buffer pH 6.8 was administered through the nostril with a micropipette. Blood samples were collected periodically from the cubital vein. After centrifugation, 0.5 ml of the plasma was separated and analysed fluorometrically (Systronics, India) following the method described by Trivedi et al. (1986). Fluorescence excitation and emission wavelengths for propranolol are 295 and 340 nm, respectively. Pentobarbitone did not interfere with the estimation procedure.

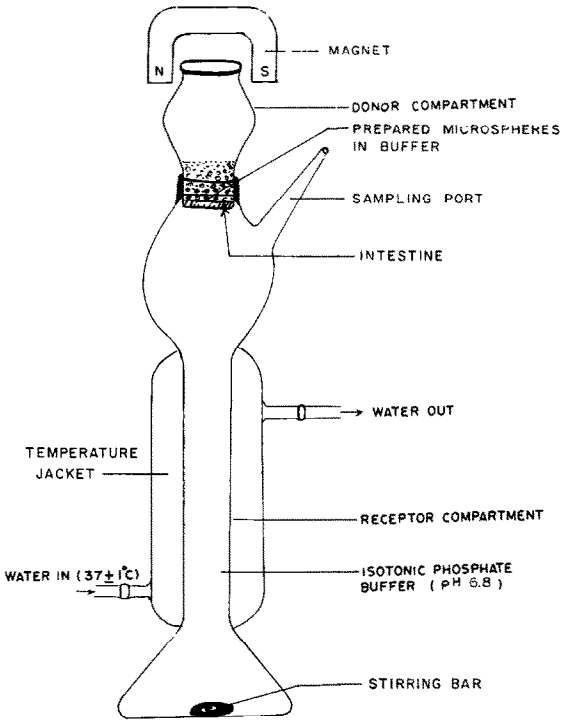

Fig. 1. Schematic representation of Franz diffusion cell. Top is open to an external magnetic field 8 kOe.

Results and Discussion

HSA microspheres bearing propranolol were prepared by an emulsification method wherein microspheres were stabilized chemically using glutaraldehyde solution as well as by the heat stabilization technique. Additional heat treatment was

given to prepared microspheres by placing them at 120 °C for 30, 60, 90 and 120 min.

The density of microspheres stabilized at 120 °C for 120 min was maximum whilst those stabilized for 30 min possessed minimum density (Table 1). The period of heat treatment did not affect bioadhesion significantly. However, the swollen volume was remarkably different and was noted to be minimum in the case of microspheres treated at 120 °C for 120 min. Swellability could be attributed to the tortuosity of the microspheres whilst the latter could be accounted for by the degree of cross-linking or magnitude of denaturation that results in reorientation of albumin macromolecules.

It was noticed that under the same set of preparative conditions, with increasing amount of HSA in the internal phase the size of the microspheres was increased. The larger microspheres required relatively longer times for complete swelling. Magnetite incorporation affected the density of microspheres vis-a-vis their swollen size. With 2% w/v magnetite content 1.60 g/ml density was obtained whereas swollen microsphere size was 35 μm. At 2% w/v magnetite concentration the microspheres exhibited good magnetic response, as on application of magnet of 8 kOe magnetic strength more than 90% of microspheres could be retained at the tap vent of the apparatus described by Malaiya and Vyas (1988).

Release experiments with heat and chemically stabilized microspheres showed that with increasing density of microspheres due to heat treatment for varied time the release rate decreased. This is probably due to the closer trapping of solute molecules in the denatured albumin structure of high tortuosity. The decrease in release of drug from microspheres stabilized at 120 °C for 120 min was significant as compared to those which had not been heat-denatured but chemically stabilized. The amount of drug released from microsphere after 8 h was 85, 76, 70 and 65% of the total amount of incorporated drug from the microspheres that had been heat treated at 120 °C for 30, 60, 90 and 120 min, respectively (Fig. 2).

In vitro diffusion study across the intestine (duodenum) revealed that in the case of magnetic microspheres, on application of an external mag-

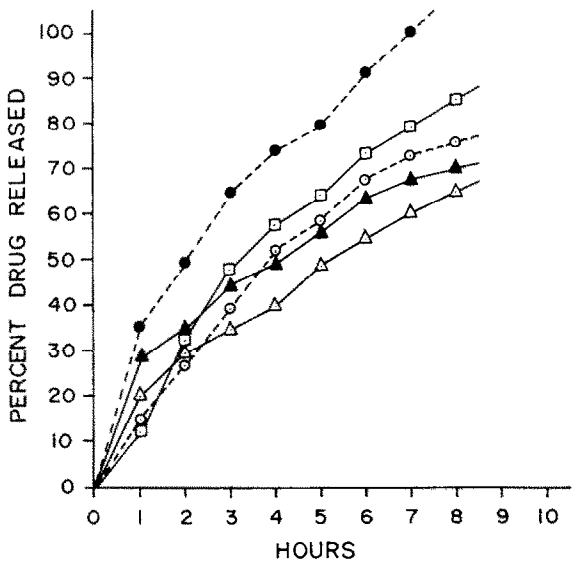

Fig. 2. In vitro release profile of propranolol hydrochloride from HSA microspheres. (△———△) MPt_4, (▲———▲) MPt_3, (○- - - - -○) MPt_2, (□———□) MPt_1, (●- - - - -●) MPC.

netic field of 8 kOe, the pulse release could be affected. This could have been due to the attraction of magnetite bound to albumin, under magnetic effect that may have resulted in lowering of the viscosity of mucus by pulling it along the adhered microspheres. On withdrawal the process of restoration may be attributed to a 'pull-push'-like effect which may promote the penetration of eluting fluid during the pull phase and squeezing of solubilized drug during the push-back phase (Fig. 3). The study was conducted in triplicate. A significant difference in the amount released was recorded ($p < 0.5$) demanding an appropriate and sensible method to study the effect of an external magnet on drug release from HSA-drug microspheres bearing magnetite. No initial burst release has been recorded except for HSA propranolol microspheres stabilized using 2.5% w/w glutaraldehyde as cross-linking agent. The findings are suggestive of effective washing of surfacial drug of microspheres after their stabilization. The greater amount of HSA resulted in monolithic systems (microspheres); as a result, the surfacial drug which is associated with conventionally described preparation was estimated to be low.

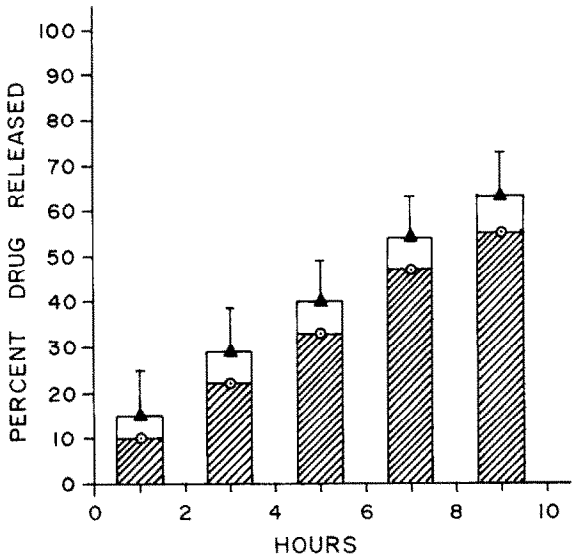

Fig. 3. Diffusion profile of propranolol hydrochloride across duodenum from magnetic HSA microspheres. (▨) Without application and (□) with application of external magnet of 8 kOe strength.

In vivo study was conducted on two groups of three dogs each, in a cross-over design. The dogs of the first group were administered propranolol · HCl solution intravenously (treatment I) whilst the second group received an equivalent amount of drug in the form of microspheres (MPt_3) nasally (treatment II). After a gap of 7 days the animals and the treatments were crossed over. After a further washout period of 7 days the animals of both groups received magnetic HSA-microspheres in an amount equivalent to the intravenously administered drug. To animals of one group an external magnetic field of 8 kOe strength was applied at 1, 3, 5, 7 and 9 h post-nasal administration (Mmt_3P_3) (treatment III) whilst the blood samples from the other group were collected periodically without the application of magnet (treatment IV).

After 7 days treatments III and IV were repeated by exchanging the animals. Fig. 4 shows the mean plasma levels of drug following the treatments. The maximum blood drug concentration was determined (800 ng/ml) after 1 h of treatment I that tended to decline gradually. However, the inter-subject variation in drug levels was recorded as non-significant ($p > 0.5$).

The maximum systemic level determined in the case of treatment II (nasal administration of plain HSA propranolol microspheres) was 180 ng/ml and remained around the C_{max} level for 10–12 h. The sustained and controlled drug delivery from microspheres may be ascribed to the controlled drug release profile.

No primary and secondary maxima that are reported to be associated with oral administration (Dvornik et al., 1983; Jain et al., 1986) were noted, indicating the protection of drug from first-pass metabolism. Results were noted to be fairly consistent as inter-subject variation was found to be insignificant ($p > 0.5$). Like plain (non magnetic) microspheres, magnetic microspheres also exhibited in vivo absorption with nearly constant plasma levels at or around C_{max}.

Plasma levels following nasal administration of magnetic HSA microspheres were similar to plain HSA drug microspheres. However, on application of a magnetic field of 8 kOe strength over nostrils at 1, 3, 5, 7 and 9 h post-administration jump

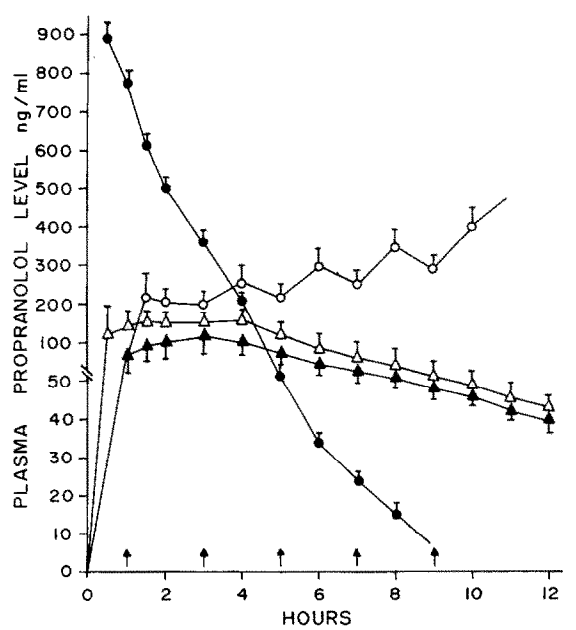

Fig. 4. Mean blood levels of propranolol in dogs ($n = 6$). Following treatment I (●———●), treatment II (△———△), treatment III (○———○), and treatment IV (▲———▲). Bar at data point indicates one side ±S.D. and ↑ indicates the application point of magnet of 8 kOe.

TABLE 2

The C_{max}, t_{max} and AUC_{0-12} [a] values after each propranolol nasal treatment

Treatment	$AUC_{0-12} \pm SE$ (ng h ml^{-1})	$t_{max} \pm SE$ (min)	$C_{max} \pm SE$ (ng ml^{-1})
I. Propranolol i.v.	3835 ± 150	15 ± 5	900
II. Propranolol-HSA microsphere, plain	3205 ± 218	90 ± 8	165
III. Propranolol-HSA microsphere, magnetic under 8 kOe magnetic field	3940 ± 350	90 ± 20	–
IV. Magnetic propranolol-HSA microspheres without application of magnetic field	3150 ± 240	90 ± 14	135

[a] Calculated by trapezoidal rule.

release/absorption resulted as indicated by the plasma profile. The absorption modulation under the influence of an external magnetic field could be ascribed to a pull-push effect that may decrease the drug saturated mucin layer and trap density (tortuosity) of denatured albumin to affect the drug release. However, to establish the actual mechanism working for drug release modification, an extensive study needs to be undertaken.

Derived pharmacokinetic parameters from drug-plasma profiles of each treatment (Table 2) reveal that plain and magnetic microspheres performed comparably when tested in vivo. However, on application of an external magnetic field pulse, absorption in the case of magnetic microspheres was recorded that resulted in relatively higher availability. The C_{max} noted in the case of plain HSA microspheres was comparatively higher than magnetic microspheres (when no external field applied). This finding was expected on the basis of in vitro studies. The higher density could be accounted for by the relatively low degree of release of drug from magnetic microspheres when no external field was applied for release modulation.

It is concluded from the present study that propranolol hydrochloride could be successfully administered via the nasal route that it is as effective as intravenous injection. Moreover, controlled drug release following nasal administration of bioadhesive human serum albumin microspheres resulted in sustained and controlled drug absorption and elimination of hepatic first-pass metabolism. Furthermore, magnetic microspheres could be exploited for burst release at desired times to affect any required modulation in drug plasma level.

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