Chitosan microspheres of carvedilol for nasal delivery: in vivo characterization

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INTRODUCTION

The nasal route has gained tremendous attention for systemic drug delivery by many researchers within the last few decades due to its great potential utility for drug delivery. It offers an attractive alternative for drugs that have limited oral bioavailability, are destroyed by gastrointestinal fluids, or are highly susceptible to hepatic first pass or gut wall metabolism. Nasal drug delivery also offers the convenience and safety of being noninvasive. In addition, nasal drug administration results in quick onset of action as compared to oral, sublingual and transdermal administrations (Mygind N. et. al. 1998).

The objective of the present study was preparation of mucoadhesive chitosan microspheres for nasal administration of carvedilol to avoid first pass metabolism and to improve therapeutic efficacy in treatment of hypertension and angina pectoris. The microspheres were prepared by emulsification-cross linking method and characterized in terms of particle size, morphology. In vivo studies were performed on rabbit which is one of the preferred animal models for pharmacokinetic studies for nasal drug delivery.

MATERIAL AND METHODS

Chitosan (Molecular weight <600,000 Daltons, Degree of deacetylation > 85%) was a gift sample from Ample Effect Sdn Bhd, Selangor (Malaysia). Carvedilol was a gift sample from Torrent Research Centre, India. Stannous chloride dihydrate were purchased from Sigma Chemical Company, St. Louis, MO. Sodium pertechnetate, separated from molybdenum-99 (99m) was provided by Regional Center for Radiopharmaceutical Division (Northern Region), India. All other chemicals and reagents used in the study were of analytical grade.

Preparation of chitosan microspheres

Chitosan microspheres were prepared by simple w/o emulsification-cross linking process using liquid paraffin (heavy and light, 1:1) as external phase (Thanoo B.C. et al. 1992). Briefly, chitosan (0.2 g) was dissolved in 2% aqueous acetic acid solution (10 mL) by continuously stirring until a homogeneous solution was obtained. The drug (0.1 g) was added in chitosan solution and the dispersion was added slowly through syringe to 100 mL of liquid paraffin (heavy and light, 1:1) containing 0.2% w/v of DOSS as stabilizer under constant stirring at 1200 rpm for 15 min using a Eurostar (IKA Labortechnik, Germany) high speed stirrer. To this W/O emulsion, 1 mL of glutaraldehyde (25% solution) was added slowly and stirring was continued for 2 h. The hardened microspheres were separated by vacuum filtration and washed several times with hexane to remove oil. Finally, microspheres were washed with distilled water and air dried for 24 h and then stored in vacuum desiccator until further use.

Radiolabeling of microspheres and carvedilol

Microspheres were labeled with technetium-99m (^{99m}Tc) by direct labeling method (Tafaghodi M. et al. 2004) Ten milligrams of microspheres were suspended in the labeling medium containing 0.5 mL of normal saline, 50 µL stannous chloride (5 mg/mL) and 1 mL technetium–99m pertechnetate eluate containing about 3 MCi of activity and pH was adjusted to 6.5 using 0.5 M sodium bicarbonate. The mixture was left under continuous stirring for about 10 min and separated by centrifugation. Microspheres were washed with 2×5ml sterile distilled water and supernatants were collected. The labeled microspheres were washed with acetone (2 × 5 ml). Microspheres were separated by centrifugation and dried by incubation at 60 0C for 30 min.

Carvedilol was labeled with technetium-99m (^{99m}Tc) by direct labeling method. Briefly, to 1 mL of drug solution (2 mg/mL) mixture containing 40 µL stannous chloride (5 mg/mL) and 0.5 mL technetium–99m pertechnetate eluate containing about 1 MCi of activity was added and pH was adjusted to 7.4 using 0.5 M sodium bicarbonate. Then mixture was incubated for 20 min at room temperature.

Determination of labeling efficiency of carvedilol and microspheres

The labeling efficiency was determined by ascending instant thin layer chromatography (ITLC) using silica gel (SG) coated fiber sheets (Gelman Sciences Inc, Ann Arbor, MI). The ITLC was performed using 100% acetone or 0.9% saline as the mobile phase. Around 2 to 3 µL of the radiolabeled complex (in case of microspheres before washing step) was applied at a point 1 cm from the end of an ITLC-SG strip. The strip was developed in acetone or 0.9% saline, and the solvent front was allowed to reach at the top. The strip was cut into two halves, and the radioactivity in each segment was measured in a shielded well-type gamma scintillation counter (Caprac-R, Capintec, USA). The radiolabeling efficiency was evaluated with ITLC-SG strips as stationary phase and acetone 100% as the mobile phase.

$$\% \text{ Radiolabeling} = \frac{\text{Radioactivity (counts) retained in the lower half of the strip}}{\text{Initial radioactivity associated (total count present) with the strip}} \times 100$$

In vivo studies

The in vivo studies were performed following the guidelines approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animal protocol was duly approved by the Institutional Animal Ethics Committee. Eight New Zealand white rabbits weighing 2.8±0.4 kg were divided in two groups. The animals were fasted overnight prior to the experiment, with free access to water. To one group of rabbits, radiolabeled microspheres (approximately 5-7 mg) were administered intranasally using monodose insufflator (Miat®, Milano, Italy). To other group, radiolabeled carvedilol was administered intravenously (0.8 mL). At selected time intervals, blood samples were withdrawn from the marginal ear vein of the rabbits. The radioactivity in terms of counts per minute (KCPM/gm) was measured in a well-type gamma scintillation counter. The animals were conscious during the whole experiment and between each blood sampling they were allowed to move freely within an enclosed area.

The noncompartmental pharmacokinetic analysis was performed by Kinetica 5.0 (Thermo Fisher Scientific, USA) and maximum plasma concentration (C_{max}), its time of occurrence (T_{max}) and the area under the curve (AUC) were determined from the individual time versus radioactivity profiles. The bioavailability (F) of the intranasal (IN) dose of microsphere formulation was calculated with the following equation:

$$F = \frac{AUC_{IN} \times Dose_{IV}}{AUC_{IV} \times Dose_{IN}} \times 100\%$$

Here AUC_{IN} and AUC_{IV} are the individual areas under radioactivity time curves of each rabbit administered microspheres containing carvedilol ($Dose_{IN}$) intranasally and that of the free carvedilol solution administered intravenously (IV), respectively.

The deposition, distribution and subsequent clearance of microspheres were studied by gamma scintigraphy and imaging was performed immediately at 0 min and 4 h post administration of microspheres to nasal cavity of rabbits using a Single Photon Emission Computerized Tomography (SPECT, LC 75–005, Diacam, Siemens AG, Earlangan, Germany) gamma camera. The quantification of the data was made defining region of interest (ROI) around the desirable area of the nasal cavity. The highest count rate at 0 min after dosing was assigned a 100% value, which was then used to calculate the percentage remaining for the other time point.

RESULTS AND DISCUSSION

The particle size of the microspheres was in the range of 20.82-49.26 µm which is favorable for intranasal administration. The microspheres were non aggregated, free flowing powders with spherical shape and smooth surface (Figure 1).

Microspheres and carvedilol were labeled by the direct labeling method using reduced ^{99m}Tc. Results for radiolabeling efficiency and stability of the ^{99m}Tc labeled complex were obtained by ITLC using silica gel (SG) coated fiber sheets using saline or 100% acetone as the solvent. Acceptably high labeling efficiency was found for microspheres and carvedilol (96.36±2.05 and 98.45±0.97 respectively). The relevant pharmacokinetic parameters including maximum concentration (C_{max}), time of maximum plasma concentration (T_{max}), the area under the curve (AUC) and relative bioavailability are shown in Table 1. The nasal bioavailability (F) was found to be 72.29% indicating that nasal administration results in improved absorption of carvedilol from chitosan microspheres in rabbits. It is reported in previous studies (Gavini E. et al. 2005; Alpar, H.O. et al. 2005) that chitosan has absorption enhancing effect, as it improves the paracellular transport by opening the tight junctions. The high carvedilol absorption through nasal mucosa may be attributed to the combined effects of bioadhesion and absorption enhancement due to chitosan. It has been demonstrated that the mucoadhesive microparticles have a significant effect on the mucosal uptake of drugs. Hence, both the higher local drug concentration and the increased paracellular transport are likely to play an important role in absorption process.

Formulation	T_{max} (h)	C_{max} (KCPM/gm)	AUC_{0-8h} (KCPM.h/gm)*	Bioavailability (F, %)
IV (carvedilol) (0.17 mg kg $^{-1}$)	--	--	54.06±6.45	100
IN (Microspheres) (1 mg kg $^{-1}$)	1	78.79	236.59±21.68	72.29

* Mean ± SD (n = 4)

Table 1: Pharmacokinetic parameters following intranasal (IN) and intravenous administration (IV)

The gamma scintigraphy images showed that the microsphere powder was spread over a wide area within the nasal cavity of rabbits (Figure 2). The activity determined for ROI at 0 min after administration was considered 100%. The activity after 4 h post administration was found 38.45%. These results indicated that the microspheres cleared slowly and were retained for extended periods in the nasal cavity, thereby providing sustained and enhanced drug absorption from the nasal mucosa, as confirmed from pharmacokinetic studies.

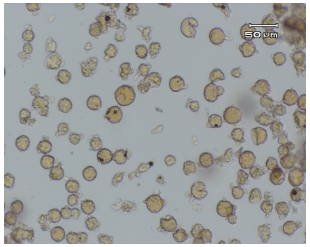

Figure 1: Photomicrograph of chitosan microspheres

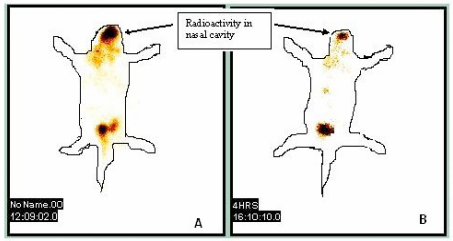

Figure 2: Scintigraphy image of rabbit at 0 min (A) and 4 h (B) post administration of microspheres to nasal cavity.

CONCLUSIONS

Free flowing chitosan microspheres of carvedilol with a smooth surface and spherical shape were prepared by emulsification cross linking method. The size of the microspheres was in the range of 20-50 µm, which is favorable for intranasal absorption. The pharmacokinetics of chitosan microspheres after nasal administration in rabbits showed that the microspheres were able to promote enhanced drug absorption through the nasal mucosa and to remarkably improve the bioavailability of carvedilol.

REFERENCES

- Alpar, H.O. et al. (2005) *Biodegradable mucoadhesive particulates for nasal and pulmonary antigen and DNA delivery.* Adv. Drug Deliv. Rev. 57, 411–430.
- Gavini E. et al. (2005) *Mucoadhesive microspheres for nasal administration of an antiemetic drug, metoclopramide: in-vitro/ex-vivo studies.* J. Pharm. Pharmacol. 57, 287-294.
- Mygind, N. et. al. (1998) *Anatomy, physiology and function of the nasal cavity.* Adv. Drug Del. Rev. 29, 3-12.
- Tafaghodi M. et al. (2004) *Evaluation of the clearance characteristics of various microspheres in the human nose by gamma-scintigraphy.* Int. J. Pharm. 280, 125–135.
- Thanoo, B.C. et al. (1992) *Cross-linked chitosan microspheres: preparation and evaluation as a matrix for the controlled release of pharmaceuticals.* J. Pharm. Pharmacol. 44, 283-286.