Society For Biomaterials

In vivo evaluation of alginate microspheres of carvedilol for nasal delivery

Sanjay B. Patil,[1] Ankur Kaul,[2] Anil Babbar,[2] Rashi Mathur,[2] Anil Mishra,[2] Krutika K. Sawant[3]

[1]Shri Neminath Jain Brahmacharyashram's Shriman Sureshdada Jain College of Pharmacy, Nashik 423 101, Maharashtra, India
[2]Ministry of Defence, Government of India, Institute of Nuclear Medicine and Allied Sciences, Delhi 110054, India
[3]New Drug Delivery Systems Laboratory, Pharmacy Department, TIFAC Center of Relevance and Excellence in NDDS, The M. S. University of Baroda, Vadodara 390002, Gujarat, India

Received 28 March 2011; revised 21 June 2011; accepted 20 July 2011
Published online 24 November 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.b.31947

Abstract: Mucoadhesive alginate microspheres of carvedilol (CRV) for nasal administration intended to avoid first pass metabolism and to improve bioavailability were prepared and evaluated. The microspheres were prepared by emulsification cross-linking method. Radiolabeling of CRV and its microspheres was performed by direct labeling with reduced technetium-99m (^{99m}Tc). *In vivo* studies were performed on New Zealand white rabbits by administering the microspheres intranasally using monodose nasal insufflator. The radioactivity was measured in a well-type gamma scintillation counter. The noncompartmental pharmacokinetic analysis was performed. The pattern of deposition and clearance of the microspheres were evaluated using a radioactive tracer and the noninvasive technique of gamma scintigraphy. The clearance of alginate microsphere was compared with that of control lactose. The microspheres were nonaggregated, free flowing powders with spherical shape, and smooth surface. Pharmacokinetics study displayed an increase in area under the curve and hence in relative bioavailability when compared with intravenous administration of drug. The nasal bioavailability was 67.87% which indicates that nasal administration results in improved absorption of CRV. The results of gamma scintigraphy showed that the alginate microspheres had significantly reduced rates of clearance from the rabbit nasal cavity when compared with the control lactose.
 J Biomed Mater Res Part B: Appl Biomater 100B: 249–255, 2012.

Key Words: alginate microspheres, carvedilol, nasal administration, pharmacokinetics

How to cite this article: Patil SB, Kaul A, Babbar A, Mathur R, Mishra A, Sawant KK. 2012. In vivo evaluation of alginate microspheres of carvedilol for nasal deliveryConflict of Interest: None.The authors alone are responsible for the content and writing of the paper.. J Biomed Mater Res Part B 2012:100B:249–255.

INTRODUCTION

The nasal route for systemic drug delivery is of interest because it provides several advantages over other routes of drug administrations. These have been suggested as follows: rapid absorption, avoidance of the intestinal and hepatic presystemic disposition, fast onset of therapeutic action, avoidance of irritation of the gastrointestinal membrane, noninvasive administration, ease of convenience, and self-medication, improved patient compliance.[1–3]

Nasal mucociliary clearance is one of the most important limiting factors for nasal drug delivery. It severely limits the time allowed for drug absorption to occur and effectively prevents sustained nasal drug administration. Thus, to retain the drug in the nasal cavity, a particle formulation would be preferable to solution.[4] In addition, mucoadhesive polymers have been introduced to prepare microparticles, which could further overcome problems of poor bioavailability by increasing the residence time at the applied site. Mucoadhesion requires a highly expanded and hydrated polymer network, which could promote an intimate contact between microspheres and the mucus layer.[5] Thus, mucoadhesive microspheres have been developed to decrease the effect of mucociliary clearance.[6–15] The microparticles form a gel-like layer, which is cleared slowly from the nasal cavity, resulting in a prolonged residence time of the drug formulation. Mucoadhesive microspheres significantly increase the systemic absorption of conventional drugs as well as polypeptides across the nasal membrane without the use of absorption enhancing agents that have the potential for irritation or damage.[16]

Alginate is an anionic mucoadhesive polymer, which is known for its ability to create hydrogen bonds with mucin-type glycoproteins through carboxyl–hydroxyl interactions.[17] This anionic biopolymer is used in many pharmaceutical

Conflict of Interest: None.
The authors alone are responsible for the content and writing of the paper.
Correspondence to: S. B. Patil; e-mail: sbpatil_99@rediffmail.com

and biotechnological applications.[18] Sodium alginate is a water-soluble, natural, linear polysaccharide that is most widely used as a polymer matrix due to its nontoxicity, biocompatibility, and gel formation ability.[19] It has been reported that polyanion polymers are more effective bioadhesives than polycation polymers or nonionic polymers.[20] Sodium alginate develops a simple and rapid gelation with divalent metal ions such as Ca^{2+}. Therefore, it has been used frequently as the matrix to prepare microparticles. It can be cross-linked with divalent or polyvalent cations to form an insoluble meshwork. Ca^{2+} and Zn^{2+} have been reported for cross-linking of acid groups of alginate.[21]

The present study aimed to prepare and evaluate mucoadhesive alginate microspheres of carvedilol (CRV) for nasal administration, which would avert first pass metabolism and to improve bioavailability for treatment of hypertension and angina pectoris. The microspheres were prepared by emulsification cross-linking. The radiolabeling of alginate microspheres is described. The clearance characteristics of the microspheres after nasal administration to rabbits were investigated using the noninvasive technique of gamma scintigraphy. *In vivo* studies were performed on rabbit, which is one of the preferred animal models for pharmacokinetic studies in nasal drug delivery.

MATERIALS AND METHODS

Materials

CRV, a water-insoluble drug, was a gift from Torrent Research Centre (Ahmedabad, India). Sodium alginate (molecular weight 3.2×10^5, determined by viscosity), *n*-octanol, calcium chloride, and Span 80 were procured from S. D. Fine Chemicals (Mumbai, India). Stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$) was 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), Board of Radiation and Isotope Technology, New Delhi, India. All other chemicals and reagents used in the study were of analytical grade.

Preparation of alginate microspheres

Carvedilol was dispersed in an aqueous solution containing 3%, w/v sodium alginate. The solution was dispersed in *n*-octanol containing 2%, v/v Span 80 using a mechanical stirrer (Remi Stirrer, Mumbai, India) at 1800 rpm. The ratio of the aqueous to *n*-octanol phase used was 1:20. The resultant w/o emulsion was stirred for 30 min. Calcium chloride solution was added drop wise and the dispersion was stirred for another 5 min. The microspheres were collected by vacuum filtration, washed thrice with isopropyl alcohol, and dried in air at room temperature.[13]

Radiolabeling of alginate microspheres

Alginate microspheres containing carvedilol (ALCRV) were labeled with technetium-99m (^{99m}Tc) by direct labeling method as described by Tafaghodi et al.[22] The radiolabeling procedure was performed in the presence of the powerful reducing agent, stannous chloride. The stannous ion reduces 99m-technetium from the +7 oxidation state to the more reactive +5 oxidation state to promote binding. 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 2×5 mL 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°C for 30 min.

Radiolabeling of carvedilol

Carvedilol was labeled with technetium-99m (^{99m}Tc) by direct labeling method. A 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 to 1 mL of drug solution (2 mg/mL) and pH was adjusted to 7.4 using 0.5*M* sodium bicarbonate. The mixture was incubated for 30 min at room temperature.

Radiolabeling of lactose

Lactose powder was labeled as above and used in animal studies as a negative control. Lactose powder (20 mg) was dissolved in the above-mentioned labeling media and incubated for 10 min, followed by addition of 10 mL acetone. The labeled lactose was desolvated and precipitated in the presence of acetone. Supernatant was decanted and powder was washed with acetone and dried in 60°C for 30 min.

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. About 2–3 μL of the radiolabeled complex (in the 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.

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

Stability study of ^{99m}Tc–CRV/microsphere complexes

The *in vitro* stability study of ^{99m}Tc–CRV/Microsphere complexes was determined using 0.9% sodium chloride and rabbit serum by ascending thin layer chromatography. The complex (0.1 mL) was mixed with 1.9 mL of normal saline (0.9% sodium chloride) or rabbit serum and incubated at 37°C. ITLC was performed at different time intervals up to 24 h to assess the stability of the complex.

Histopathological studies

Histopathological studies were performed using isolated sheep nasal mucosa. The nasal mucosa tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissues were immediately used after separation. Histopathological evaluation of tissue incubated in phosphate buffer (pH 6.2) was compared with tissue incubated in the diffusion chamber of Franz cell with microsphere formulations. After treatment for 4 h, tissue was fixed in 10% buffered formalin (pH 6.2), routinely processed, and embedded in paraffin. Paraffin sections were cut on glass slides and stained with hematoxylin and eosin. The sections were examined by optical microscopy, to examine the morphological changes to the tissue by a pathologist blinded to the study.[23]

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, Ministry of Social Justice and Empowerment, Government of India. The animal protocol was duly approved by the Institutional Animal Ethics Committee.

A total of 12 New Zealand white rabbits weighing 2.5–3.5 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 5–10 mg) were administered intranasally using monodose insufflator (Miat, Milano, Italy). Radiolabeled CRV was administered intravenously to the other group (0.5 mL). Blood samples were withdrawn from the marginal ear vein of the rabbits at selected time intervals. The radioactivity in terms of counts per minute (cpm) was measured in a well-type gamma scintillation counter (Caprac-R, Capintec, USA). The animals were conscious during the whole experiment and between each blood sampling they were allowed to move freely within an enclosed area.

Pharmacokinetics analysis

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:

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

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

Gamma scintigraphy

The deposition, distribution, and subsequent clearance of microspheres and lactose were studied by gamma scintigraphy and imaging was performed immediately at 0, 1, 2, 3, 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 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.

Statistical data analysis

Statistical tests of significance were performed using Student's *t*-test and differences were considered to be statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Radiolabeling of carvedilol and its microspheres

$^{99m}TcO^{4-}$ was used because of easy availability, cost effectiveness, and low radiation dose. Since half-life of $^{99m}TcO^{4-}$ is 6 h as compared to 60 days for ^{125}I, it presents less radiation burden. ^{99m}Tc has been used to directly label the prepared microspheres based on using stannous chloride as a reducing agent. Chemically, $^{99m}TcO^{4-}$ is a nonreactive species and does not label any compounds by direct addition. In ^{99m}Tc-labeling of many compounds, prior reduction of $^{99m}TcO^{4-}$ from 7+ state to a lower oxidation state (4+) is required.

The alginate microspheres (ALCR) and CRV were labeled with high efficiency by the direct labeling technique using reduced ^{99m}Tc. Table I depicts the effect of pH on labeling efficiency. As the pH increased from 6 to 6.5, the radiolabeling efficiency increased from 91.45 to 97.25% for ALCRV. Further increase in the pH upto 8 led to reduction in the labeling efficiency. As the pH increased from 6 to 7, the radiolabeling efficiency also increased from 94.37 to 98.84% for CRV. Further increase in the pH upto 8 led to reduction in the labeling efficiency of 90.47%.

Table II shows the effect of incubation time on labeling efficiency. The incubation time required for high labeling efficiency was 10 min for ALCR and 30 min for CRV. Further increase in incubation time did not increase the labeling efficiency considerably. The amount of stannous chloride (reducing agent) used for labeling plays a very decisive role in determining labeling efficiency. A large amount of stannous chloride led to the formation of radiocolloids (reduced/hydrolyzed $^{99m}TcO^{4-}$), which is undesirable. Conversely, smaller amounts of stannous chloride result in poor labeling.[24,25] Table III shows the effect of various

TABLE I. Effect of pH on the % Radiolabeling Efficiency of Microspheres and Carvedilol

pH	Radiolabeled[a](%) ALCR	CRV
6	91.45 ± 1.94	94.37 ± 1.65
6.5	97.25 ± 1.31	96.22 ± 1.15
7	94.37 ± 1.68	98.84 ± 1.34
7.5	92.64 ± 1.48	93.78 ± 1.38
8	90.34 ± 1.32	90.47 ± 1.54

[a] Mean ± SD, $n = 3$.

concentrations of stannous chloride ($SnCl_2 \cdot 2H_2O$) on labeling efficiency. By varying the amount of stannous chloride from 50 to 300 μg, but keeping the other factors like pH and incubation time constant, the influence on labeling efficiency was studied. With increase in stannous chloride amount from 50 to 250 μg, the labeling efficiency was increased from 86.42 to 97.84% for ALCR. Further increase in the amount of stannous chloride led to a reduction in labeling efficiency. With increase in stannous chloride amount from 50 to 200 μg, the labeling efficiency was increased from 91.45 to 98.64% for CRV. Further increase in the amount of stannous chloride upto 300 μg led to a reduction in labeling efficiency.

Stability study of ^{99m}Tc–CRV/microsphere complexes

The *in vitro* stability of the labeled formulations (^{99m}Tc–CRV/microsphere complexes) was evaluated in saline and in rabbit serum at 37°C for 24 h. All formulations exhibited good *in vitro* stability as shown in Table IV. It is evident from the results that there is insignificant detachment of the radioisotope from the complex. There was no significant reduction in the radiolabeling efficiency upto 24 h, which indicates its stability and suitability for *in vivo* use.

Histology studies

The morphologic changes in the nasal mucosa caused by drugs, enhancers, or other formulation additives, may result in damage to the ability of the nasal mucosa to carry out its normal defense functions. In addition, chronic infection may occur when recovery or regeneration of the normal epithelium cannot be achieved.[26] Thus, it is important to study the histology of the nasal mucosa with the formulation. The histology of control and treated nasal mucosa is shown in

TABLE II. Effect of Incubation Time on the % Radiolabeling Efficiency of Microspheres and Carvedilol

Incubation Time (min)	Radiolabeled[a](%) ALCR	CRV
0	94.68 ± 1.73	95.22 ± 2.48
10	97.86 ± 1.84	95.38 ± 1.78
20	96.74 ± 2.14	96.34 ± 0.86
30	96.12 ± 1.32	98.55 ± 1.94
40	95.36 ± 2.36	98.26 ± 1.46

[a] Mean ± SD, $n = 3$.

TABLE III. Effect of Stannous Chloride Concentration on the % Radiolabeling Efficiency of Microspheres and Carvedilol

Amount of Stannous Chloride (μg)	Radiolabeled[a](%) ALCR	CRV
50	86.42 ± 1.85	91.45 ± 1.68
100	90.56 ± 1.67	95.35 ± 1.25
150	93.14 ± 1.24	96.34 ± 0.86
200	95.67 ± 0.96	98.64 ± 1.42
250	97.84 ± 1.14	88.36 ± 2.16
300	92.45 ± 2.15	84.46 ± 1.98

[a] Mean ± SD, $n = 3$.

Figure 1. The microscopic observations indicated that the formulation has no significant effect on the microscopic structure of sheep nasal mucosa. The surface epithelium lining and the granular cellular structure of the nasal mucosa were totally intact. No major changes in the ultrastructure of mucosa morphology could be seen and the epithelial cells appeared mostly unchanged. Neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after diffusion study as compared with control mucosa treated with phosphate buffer pH 6.2. Thus, the microsphere formulation seems to be safe with respect to nasal administration.

Pharmacokinetics studies

The blood kinetics data (blood radioactivity time profiles) of CRV solution injected intravenously and the microsphere formulations (ALCR) administered intranasally to rabbits at various time intervals is shown in Figure 2. The relevant pharmacokinetic parameters including maximum concentration (C_{max}), time of maximum plasma concentration (T_{max}), the AUC, half-life ($T_{1/2}$), mean residence time (MRT), elimination rate constant (K_{el}), and relative bioavailability (F) are shown in Table V.

The C_{max} values observed after intranasal administration of ALCR was 64.85 ± 4.15 kcpm/g. The AUC after intranasal administration of ALCR and CRV solution intravenously were about 215.83 ± 18.56 and 54.06 ± 6.45 kcpm h/g, respectively, which was statistically significant ($p > 0.05$; Student's t-test). The average $T_{1/2}$ values were 2.30 ± 0.98 and 1.99 ± 0.32 h for ALCR nasally and following IV

TABLE IV. *In vitro* Stability of the ^{99m}Tc–CRV and ^{99m}Tc–Microspheres in Physiological Saline and Serum at 37°C

Time (h)	Radiolabeling (%) Efficiency in Saline[a] ALCR	CRV	Radiolabeling (%) Efficiency in Serum[a] ALCR	CRV
0.5	97.28 ± 1.24	98.35 ± 1.48	97.14 ± 1.31	98.22 ± 1.25
1	97.11 ± 1.36	98.22 ± 1.46	96.92 ± 1.61	97.88 ± 1.29
2	96.86 ± 1.89	97.78 ± 1.21	96.35 ± 2.12	97.64 ± 2.74
4	96.42 ± 2.19	97.55 ± 1.65	96.12 ± 1.54	97.03 ± 1.86
6	95.84 ± 1.98	97.34 ± 2.47	95.72 ± 2.94	96.67 ± 2.12
24	95.56 ± 2.15	96.88 ± 1.22	95.32 ± 2.81	96.14 ± 1.36

[a] Mean ± SD, $n = 3$.

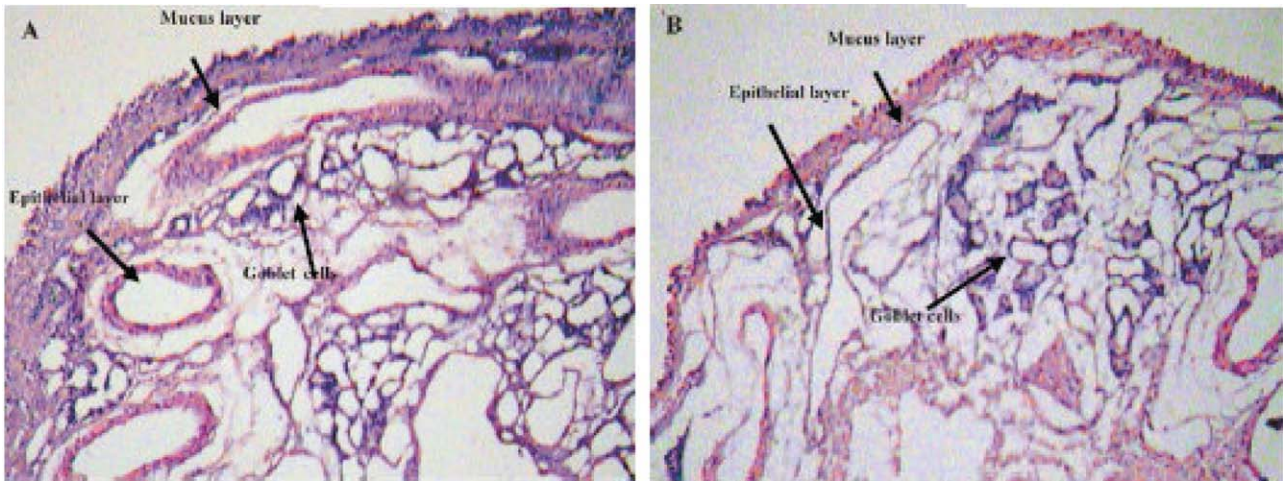

FIGURE 1. Histology evaluations of sections of sheep nasal mucosa. (A) Control mucosa after treatment with phosphate buffer pH 6.2; (B) Mucosa after treatment with microsphere formulation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

administration of CRV, respectively. The MRT was considerably increased following nasal administration of the mucoadhesive formulation of carvedilol (ALCR) when compared with IV administration. The average MRT values after nasal administration of ALCR was 4.37 ± 1.31 h when compared with 2.18 ± 0.78 h after IV administration of CRV, which were significantly different ($p < 0.05$, ANOVA followed by Dunnett's multiple comparison test). The relative bioavailability (F) for ALCR was 67.87%, which indicate that nasal administration results in improved absorption of CRV from alginate microspheres in rabbits. Illum et al.[27] described bioadhesive starch microspheres for nasal administration, which were retained in the nasal cavity for an extended time period owing to their bioadhesive nature and from which, after gelling, a local high drug concentration was reached. The high CRV absorption through nasal mucosa may be attributed to the combined effects of mucoadhesion and absorption enhancement due to mucoadhesive polymer. It was demonstrated that the mucoadhesive microparticles had a significant effect on the mucosal uptake of drugs.[4,28] Hence, both the higher local drug concentration and the increased paracellular transport are likely to play an important role in absorption process.

TABLE V. Pharmacokinetic Parameters of Carvedilol After IV and of ALCR After Intranasal Administration in Rabbits

Parameters[a]	CRV IV	ALCR Intranasal
C_{max} (kcpm/g)	–	64.85 ± 4.15
T_{max} (h)	–	2.0
$AUC_{0-8\ h}$ (kcpm h/g)	54.06 ± 6.45	215.83 ± 18.56
$T_{1/2}$ (h)	1.99 ± 0.32	2.30 ± 0.98
MRT (h)	2.18 ± 0.78	4.37 ± 1.31
K_{el} (h^{-1})	0.346 ± 0.092	0.300 ± 0.084
F (%)	100	67.87

[a] Each value represents the mean ± SD ($n = 6$).

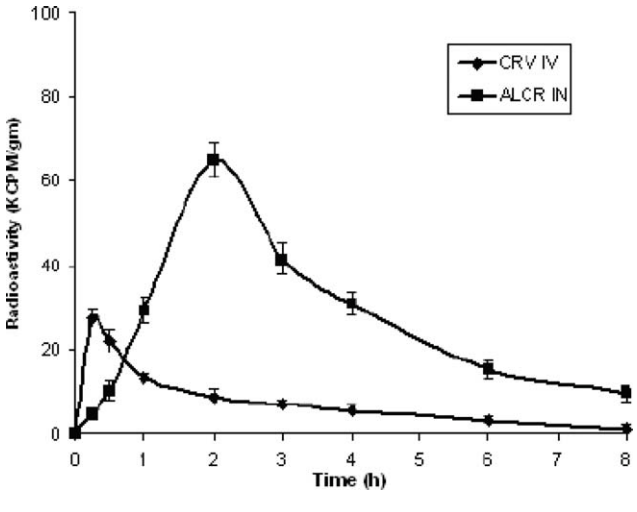

FIGURE 2. Blood radioactivity time profiles of CRV after administration of microspheres intranasally (IN) (1 mg/kg) and CRV solution intravenously (IV) (0.17 mg/kg) in rabbits (Mean ± SD, $n = 6$).

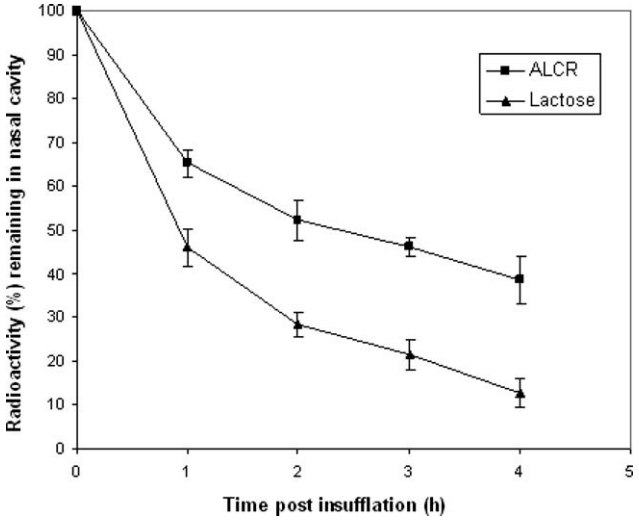

FIGURE 3. The clearance characteristics of radiolabeled alginate microspheres (ALCR) from the rabbit nasal cavity when compared with lactose powder a control (mean ± SD, $n = 3$).

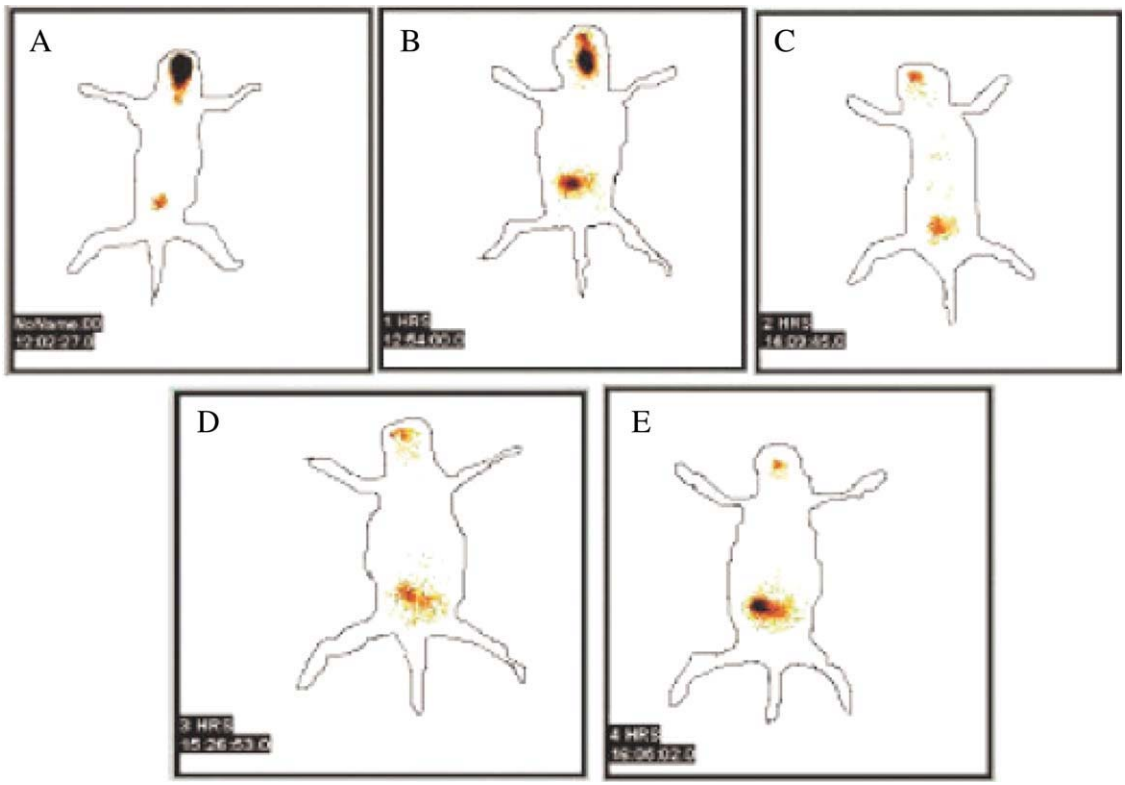

FIGURE 4. Scintigraphic rabbit whole body images showing radioactivity in the nasal cavity after administration of ^{99m}Tc-labeled alginate microspheres of carvedilol (ALCR) at different times of 0 h (A), 1 h (B), 2 h (C), 3 h (D), and 4 h (E) postinsufflation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Gamma scintigraphy

Several methods, both *in vitro* and *in vivo*, were used to evaluate mucociliary transport rates. Advantages of the gamma scintigraphy lies in its ability to noninvasively monitor the deposition and clearance of drug formulations, allowing both quantitative and photographic illustrations of distribution and clearance of the radiolabeled formulation. Using this technique to evaluate the nasal clearance of mucoadhesive preparations requires a radiotracer, which is stable and nondiffusible to prevent absorption into the vascular compartment. ^{99m}Tc tracer is reported as technically easy to perform and more representative of ciliary function since it investigates a large surface of the mucosa as a whole and not the fastest flow rate.[29] Therefore, ^{99m}Tc was used in present study.

The nasal clearance characteristics of alginate microspheres were studied. Lactose powder was used as negative control. The clearance data for the formulation and lactose from the nasal cavity is shown in Figure 3. This data shows that the control lactose powder was cleared rapidly (half-life of nasal clearance was $<$1.0 h), whereas the mucoadhesive delivery systems were retained within the nasal cavity

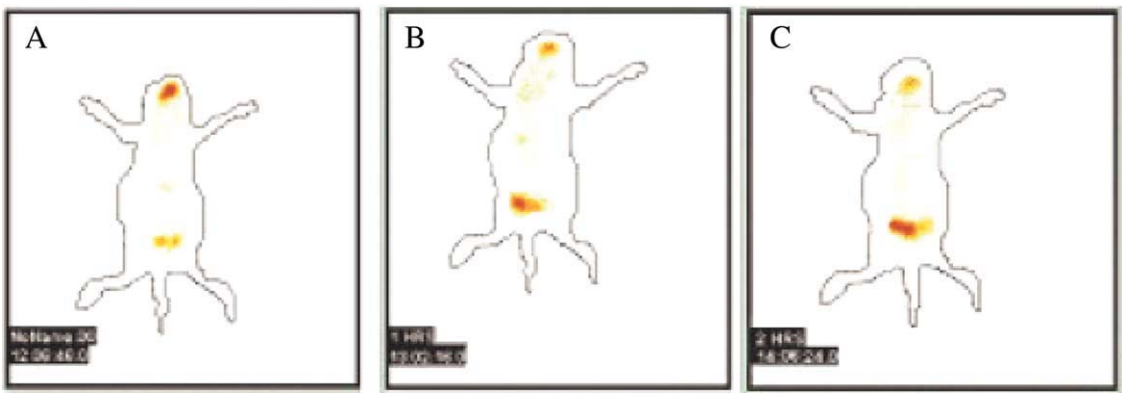

FIGURE 5. Scintigraphic rabbit whole body images showing radioactivity in the nasal cavity after administration of ^{99m}Tc-labeled lactose powder (control) at different times of 0 h (A), 1 h (B), and 2 h (C) postinsufflation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

for longer time (half-lives of nasal clearance were >2.5 h). It has been reported that the normal half-life of nasal clearance in man is about 20 min.[30] The nasal clearance half-lives of microspheres were higher than normal clearance half-life of human nose (at least fourfold higher), which is representative of high mucoadhesive strength of these particulate systems. After 4 h, 61.55% of alginate microspheres were cleared from nasal cavity while in the same time 87.36% lactose powder was cleared. The gamma scintigraphy images (Figures 4 and 5) showed that the microsphere powder was spread over a wide area within the nasal cavity of rabbits. The results indicated that the microspheres cleared slowly when compared with lactose powder 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.

CONCLUSION

In this article, the alginate microspheres of CRV were prepared by emulsification cross-linking method. The CRV and its microsphere formulations were then successfully radiolabeled using reduced ^{99m}Tc with the labeling efficiency of above 97%. The radiolabeled complexes were proved for their stability in both saline and serum up to 24 h. The pharmacokinetics of the microspheres after nasal administration in rabbits showed that the microspheres were able to promote enhanced drug absorption through the nasal mucosa and to improve the bioavailability of CRV. The gamma scintigraphy indicated that the microspheres cleared slowly when compared with lactose powder 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.

ACKNOWLEDGMENTS

The authors are thankful to Miat, Milano, Italy for providing gift sample of monodose powder nasal insufflator.

REFERENCES

1. Behl CR, Pimplaskar HK, Sileno AP, deMeireles J, Romeo VD. Effects of physicochemical properties and other factors on systemic nasal drug delivery. Adv Drug Deliv Rev 1998;29:89–116.
2. Mygind N, Dahl R. Anatomy, physiology and function of the nasal cavity in health and disease. Adv Drug Deliv Rev 1998;29:3–12.
3. Illum L. Nasal drug delivery-possibilities, problems and solutions. J Control Release 2003;87:187–198.
4. Ugwoke MI, Verbeke N, Kinget R. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. J Pharm Pharmacol 2001; 53:3–21.
5. Fundueanu G, Constantin M, Dalpiaz A, Bortolotti F, Cortesi R, Ascenzi P, Menegatti E. Preparation and characterization of starch/cyclodextrin bioadhesive microspheres as platform for nasal administration of Gabexate Mesylate (Foy®) in allergic rhinitis treatment. Biomaterials 2004;25:159–170.
6. Illum L, Jorgensen H, Bisgaard H, Krogsgaard O, Rossing N. Bioadhesive microspheres as a potential nasal drug delivery system. Int J Pharm 1987;39:189–199.
7. Sachetti C, Artusi M, Santi P, Colombo P. Caffeine microparticles for nasal administration obtained by spray drying. Int J Pharm 2002;242:335–339.
8. Mao S, Chen J, Wei Z, Liu H, Bi D. Intranasal administration of melatonin starch microspheres. Int J Pharm 2003;272:37–43.
9. Gavini E, Rassa G, Vanna S, Massimo C, Giunchedi P. Mucoadhesive microspheres for nasal administration of an antiemetic drug metaclopramide: In vitro/ex-vivo studies. J Pharm Pharmacol 2004; 57:287–294.
10. Martinac A, Filipovic-Grcic J, Voinovich D, Perissutti B, Franceschinis E. Development and bioadhesive properties of chitosan-ethylcellulose microspheres for nasal delivery. Int J Pharm 2005; 291:69–77.
11. Patil SB, Murthy RSR. Preparation and in vitro evaluation of mucoadhesive chitosan microspheres of amlodipine besylate for nasal administration. Indian J Pharm Sci 2006;8:64–67.
12. Gavini E, Hegge AB, Rassa G, Vanna S, Testa C, Pirisino G, Karlsen J, Giunchedi P. Nasal administration of carbamazepine using chitosan microspheres: In vitro/in vivo studies,Int J Pharm 2006;307:9–15.
13. Patil SB, Sawant KK. Development, optimization and in vitro evaluation of alginate mucoadhesive microspheres of carvedilol for nasal delivery. J Microencapsul 2009;26:432–443.
14. Patil SB, Babbar AK, Mathur R, Mishra AK, Sawant KK. Mucoadhesive chitosan microspheres of carvedilol for nasal administration. J Drug Target 2010;18:321–331.
15. Patil SB, Sawant KK. Chitosan microspheres as a delivery system for nasal insufflation. Colloids Surf B Biointerfaces 2011;84: 384–389.
16. Illum L. Bioadhesive formulations for nasal peptide delivery. In: Mathiowitz E, Lehr CM, Chickering D, editors. Bioadhesion in Drug Delivery-Issues in Fundamentals, Novel Approaches and Development. New York: Marcel Decker; 1998. pp 507–539.
17. Bernkop-Schnurch A. Mucoadhesive polymers. In: Dumitriu S, editor. Polymer Biomaterial. New York: Marcel Decker; 2002. pp 147–165.
18. Stokke BT, Draget KI, Smidsrod O, Yuguchi Y, Urakawa H, Kajiwara K. Small angle X-ray scattering and rheological characterization of alginate gels. 1. Calcium alginate gels. Macromolecules 2000;33:1853–1863.
19. Emmerichs N, Wingender J, Flemming HC, Mayer C. Interaction between alginates and manganese cations: Identification of preferred cation binding sites. Int J Biol Macromol 2004;34:73–79.
20. Serp D, Cantana E, Heinzen C, Stockar UV, Marison IW. Characterization of an encapsulation device for the production of monodisperse alginate beads for cell immobilization. Biotechnol Bioeng 2000;70:41–53.
21. Chan LW, Jin Y, Heng PWS. Cross-linking mechanisms of calcium and zinc in production of alginate microspheres. Int J Pharm 2002;242:255–258.
22. Tafaghodi M, Tabassi SAS, Jaafari MR, Zakavi SR, Momen-Nejad M. Evaluation of the clearance characteristics of various microspheres in the human nose by gamma-scintigraphy. Int J Pharm 2004;280:125–135.
23. Majithiya RJ, Ghosh PK, Umrethia ML, Murthy RSR. Thermoreversible-mucoadhesive gel for nasal delivery of sumatriptan. AAPS Pharm Sci Technol 2006;7:Article 67.
24. Arulsudar N, Subramanian N, Mishra P, Sharma RK, Murthy RSR. Preparation, characterization and biodistribution of ^{99m}Tc-labeled liposome encapsulated cyclosporine. J Drug Target 2003;11: 187–196.
25. Thakkar H, Sharma RK, Mishra AK, Chuttani K, Murthy RS. Celecoxib incorporated chitosan microspheres: In vitro and in vivo evaluation. J Drug Target 2004;12:549–557.
26. Boling LR. Regeneration of nasal mucosa. Arch Otolaryngol 1935; 22:689–742.
27. Illum L, Fisher AN, Jabbal-Gill I, Davis SS. Bioadhesive starch microspheres and absorption enhancing agents act synergistically to enhance the nasal absorption of polypeptides. Int J Pharm 2001;222:109–119.
28. Pereswetoff-Morath,L. Microspheres as nasal drug delivery systems. Adv Drug Deliv Rev 1998;29:185–194.
29. Ingels K, Van Hoom, V, Obrie E, Osmanagaoglu K. A modified technetium-99m isotope test to measure nasal mucocillary transport: Comparison with the saccharine-dye test. Eur Arch Otorhinolaryngol 1995;252:340–343.
30. Schipper NGM, Verhoef JC, Merkus FWHM. The nasal mucociliary clearance: Relevance to nasal drug delivery. Pharm Res 1991;8: 807–814.