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Intranasal mucoadhesive buspirone formulation: *in vitro* characterization and nasal clearance studies

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Oral administration is unsuitable for drugs prone to extensive first-pass metabolism, like buspirone. Thus, in the present study an attempt has been made to develop a mucoadhesive intranasal formulation improving permeation characteristics of buspirone HCl. Nasal formulations containing different concentrations of chitosan HCl and hydroxypropyl-β-cyclodextrins (HP-β-CD) were prepared and compared with control buspirone HCl solution regarding permeability, *in vitro* duration of mucoadhesion, *in vivo* nasal clearance in rats and *in vitro* cytotoxicity on cell culture. Nearly two fold increase in buspirone permeation was observed with 1% chitosan HCl and a 3.5 fold increase with 1% chitosan HCl and 5% HP-β-CD. Nasal clearance studies showed retention of 50% radioactivity up to about 3.5 h for formulation F7 containing 1% chitosan HCl compared to 1.5 h for control buspirone solution (F1). Results conclusively demonstrated enhancement in permeation with no cytotoxicity. Thus formulations can be used to improve bioavailability of buspirone HCl.

1. Introduction

For systemic delivery, drugs are traditionally administered orally. However in many instances, oral administration is unsuitable when the drug undergoes significant degradation in the gastrointestinal tract or is metabolized to a high degree via first-pass effect in the liver. In recent years, intranasal administration has been extensively evaluated because the large surface area of the nasal mucosa affords a rapid onset of therapeutic effect, no first-pass metabolism, and non-invasiveness; all of which may maximize patient convenience, comfort, and compliance.

The nasal dosage forms include solutions, sprays, microspheres, gels and liposomes (Moa et al. 2004).

Although solutions are easy to use, they achieve a poor bioavailability due to their short residence time in the nasal mucus. The incorporation of substances in a nasal formulation which increase the residence time of the drug at the absorption site can help the formulator in obtaining preparations with good biopharmaceutical performances. For that reason the use of bioadhesive systems is a solution, prolonging the contact time between the formulation and the absorption membrane and as a consequence improves onset and duration of pharmacological effects (Harris and Robinson 1990).

Chitosan has been shown to have mucoadhesive properties because of its viscosity and interaction of the positively charged amino group with the negatively charged sites on the mucosa surface (Artursson et al. 1994). Studies also indicated that chitosan could enhance absorption of poorly absorbable drugs such as peptides and proteins (Illum et al. 2000). Thus chitosan delivery systems have two effects, the mucoadhesive properties of the polymer can reduce the clearance rate of drugs from the nasal cavity, thereby prolonging the contact time of the delivery system with nasal epithelium and the interaction of the positively charged amino group of chitosan with the negatively charged sialic acid residues in mucus causes the transient opening of the tight junctions and allows large hydrophilic compounds to be transported across the epithelium. The opening mechanism of tight junctions has been demonstrated by a decrease in ZO-I proteins and the change in the cytoskeletal protein F-actin from a filamentous to a globular structure (Schipper et al. 1997).

The nonbenzodiazepine anxiolytic, buspirone HCl seems to be good candidate for nasal delivery owing to its extensive first-pass metabolism (very poor oral bioavailability ~4%) (Galichet 2004), short half-life and poor absorption through the lower gastrointestinal tract. Therefore buspirone HCl was selected for the present studys and an attempt has been made to prepare a mucoadhesive formulation of buspirone hydrochloride using chitosan HCl having the capability to improve permeability as well.

2. Investigations and results

The *in vitro* duration of mucoadhesion was found to increase with the increase in concentration of chitosan HCl. Further increase in concentration above 1% did not enhance significantly ($P < 0.1$) the duration. There was no significant effect of HP-β-CD on the duration of mucoadhesion (Table 1). Figure 1 shows the percent radioactivity remaining into the nose of rats at different time intervals for formulation F7 and control formulation F1. Formulation F7 containing 1% chitosan HCl showed greater ability to retain the formulation into the nostrils with retention

Table 1: Duration of mucoadhesion, permeability coefficient and enhancement ratio of different formulations, n = 5

Formulation	Duration of mucoadhesion (h)	Permeability coefficient (cm/s)	Transport enhancement ratio
1	$<0.5 \pm 0.75$	$4.6 \pm 0.28 \times 10^{-6}$	1
2	3.0 ± 0.5	$6.1 \pm 1.02 \times 10^{-6}$	1.32
3	3.8 ± 0.45	$8.7 \pm 0.89 \times 10^{-6}$	1.89
4	4.2 ± 0.52	$8.6 \pm 1.5 \times 10^{-6}$	1.86
5	4.0 ± 0.45	$12.1 \pm 0.95 \times 10^{-6}$	2.63
6	4.0 ± 0.5	$14.1 \pm 1.89 \times 10^{-6}$	3.06
7	4.0 ± 0.35	$17 \pm 0.56 \times 10^{-6}$	3.69

of about 50% radioactivity for about 3.5 hrs whereas the same amount could be retained upto 1.5 hrs only for control formulation.

Effect of varying concentrations of chitosan HCl on permeation of buspirone is shown in Fig. 2. Effect of different concentrations of HP-β-CD with 1% chitosan HCl was also studied. The control buspirone HCl solution (F1) showed 27.5% cumulative drug permeation after 6 h. Addition of chitosan HCl to the formulation showed enhancement in drug permeation. The percentage drug permeation rose to 49.21% when concentration of chitosan HCl was increased to 1%. However, further increase in concentration to 2% did not improve the drug permeation. There was nearly two fold increase in permeation of buspirone with 1% chitosan HCl (enhancement ratio 1.89). Permeability coefficient and enhancement ratio of different formulations is given in Table 1. Figure 2 shows a marked improvement in the permeation of drug after addition of HP-β-CD to the formulations containing 1% chitosan HCl. Increase in concentration of HP-β-CD showed simultaneous increase in permeation. Maximum (96.8%) percent drug permeation was observed for formulation F7 containing 1% chitosan HCl and 5% HP-β-CD. There was about 3.5 fold increase in permeation of buspirone with this formulation. The permeability coefficient of buspirone increased significantly ($P < 0.0001$) from 4.6×10^{-6} cm/s (control formulation F1) to 17×10^{-6} cm/s (F7).

All formulations were noncytotoxic to fibroblast cells as they were scored 0 on a cytotoxicity scale where as the positive control was scored as 3. The fibroblast cells around formulation F7 containing maximum (5%) HP-β-CD is shown in Fig. 3.

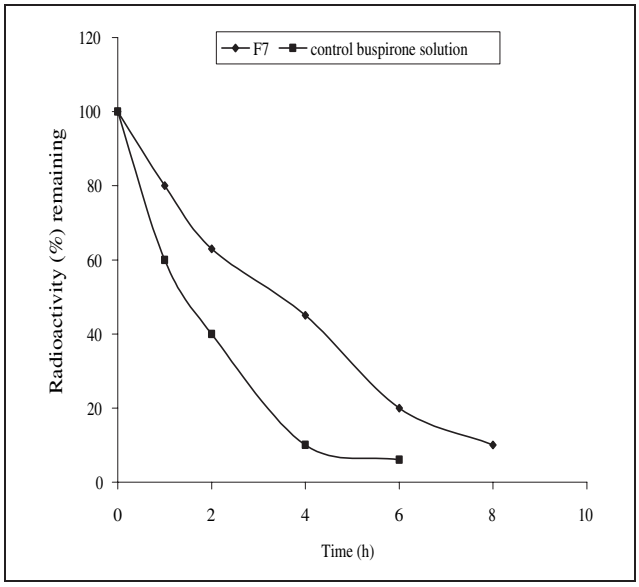

Fig. 1: Percent radioactivity remaining into the nose after different time intervals for nasal formulation F7 and control buspirone solution (F1), n = 6, S.D. < ±5, radiolabeling efficiency was greater than 93%

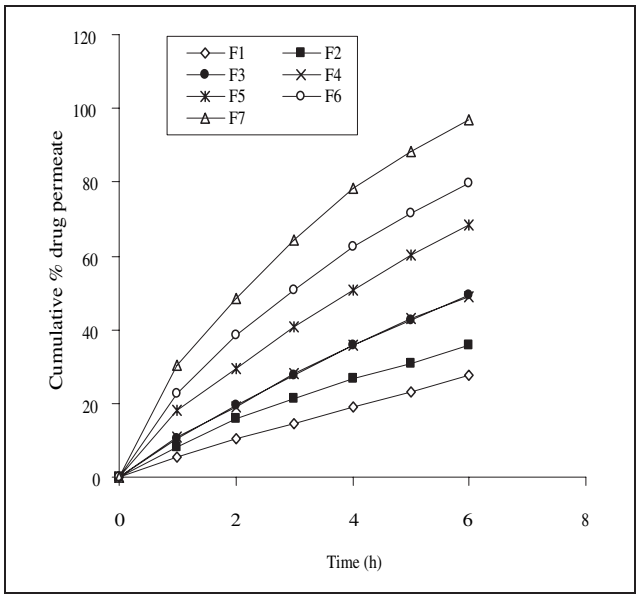

Fig. 2: Permeation of buspirone HCl from different formulations, n = 3, S.D. < ±2.5%

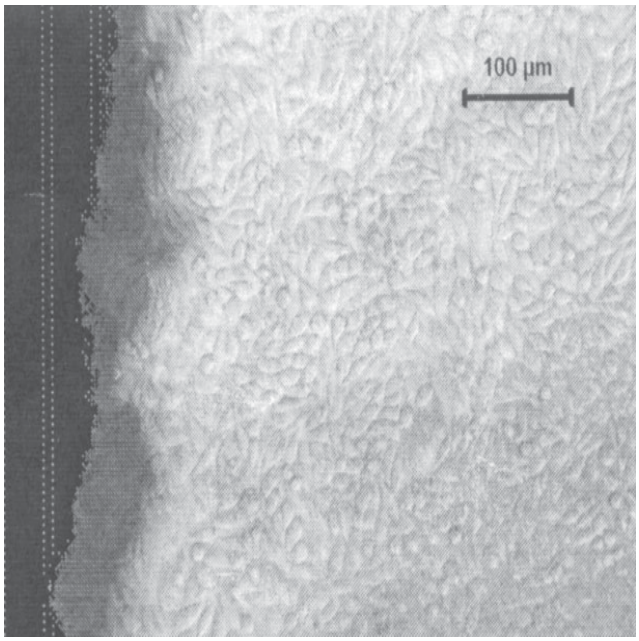

Fig. 3: Fibroblast cell around formulation F7

Table 2: Composition of formulations, amount of buspirone HCl is for 5 ml formulation, 0.05%w/v benzalkonium chloride added as preservative

Ingredients	Formulations						
	1	2	3	4	5	6	7
Buspirone HCl (mg)	165	165	165	165	165	165	165
Chitosan HCl (%w/v)	–	0.5	1	2	1	1	1
HP-β-CD (%w/v)	–	–	–	–	1	3	5
Sterile sodium chloride solution (0.5%w/v)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

3. Discussion

The increase in duration of mucoadhesion of formulations F2 to F7 was due to chitosan which is a mucoadhesive material. Because of the high density of the positive charges on the molecule, it adheres strongly to negative sites on the nasal membrane such as sialic acid residues in mucin glycoproteins (Leung and Robinson 1988; Ahuja et al. 1997; Illum 1998). This mucoadhesive property resulted in an increased clearance time in the nasally administered chitosan formulations (at least doubled, 2.3 fold increase in 50% retention of activity at nose for formulation F7). Less duration of mucoadhesion determined by an *in vitro* method with respect to the gammascintigraphic method may be due to more stress than it is actually observed due to ciliary beat under *in vivo* conditions.

The increase in permeation of buspirone by the formulations containing chitosan HCl (F2-F3) could be attributed to an interaction of a positively charged amino group on the C-2 position of chitosan with negatively charged sites on the cell membranes and tight junctions of the mucosal epithelial cells to allow opening of the tight junctions. It has been demonstrated that chitosan when applied to confluent cell cultures, is able to transiently open the tight junctions between the cells (Artursson et al. 1994; Borchard et al. 1996; Dodane et al. 1999). Permeability coefficient increased when concentration of chitosan HCl increased from 0.5% to 1% but after that there was no further increase in permeation. Thus effect on tight junction seems to be saturable. This is in agreement with the findings of a previous study, in which plateau levels were reached between 0.25 and 0.5% concentration of chitosan glutamate (Artursson et al. 1994). Formulations containing 1% chitosan HCl and 5% HP-β-CD showed highly significant improvement in permeation as compared to the control buspirone nasal solution. This might be due to synergistic effect of HP-β-CD and chitosan hydrochloride on the permeation of drug because chitosan HCl opens up the tight junctions and HP-β-CD extracts the phospholipids and proteins from membrane by forming a new inclusion compartment in the aqueous phase.

Buspirone is a dibasic compound with P_{ka} values of 4.12 and 7.32; hence between pH 5.5 to 6.0 (nasal formulation as well as nasal mucosa have the same pH) about 90% of buspirone remains as monoprotonated species as reported by Birudaraj et al. (2005). Results therefore show substantial contribution of paracellular transport through nasal mucosa and the substantial enhancement demonstrated by the permeation enhancer for the non-polar pathway.

In conclusion, formulations showed enhanced permeation as compared to control buspirone solution. A formulation containing both chitosan and HP-β-CD was more effective in improving permeation due to a synergistic effect of the excipients. Formulations containing chitosan HCl cleared slowly from rat nose compared to control buspirone solution.

4. Experimental

4.1. Materials

Buspirone HCl was purchased from Sigma Aldrich Chemical Pvt. Ltd., (Bangalore, India), chitosan hydrochloride (85% deacetylated) was obtained as gift from Mahtani chitosan Pvt. Ltd., (Veraval, India), hydroxy propyl β-cyclodextrin was gifted by Roquette Freres, (Lestrem, France). All other reagents were of analytical grade.

4.2. Methods

4.2.1. Formulations

The nasal buspirone control solution (formulation 1) was prepared by dissolving 166.5 mg of buspirone hydrochloride in 4 ml of 0.5% sterile sodium chloride solution. Benzalkonium chloride (0.05%) was added as preservative and pH was adjusted to 5.5. A final volume of 5 ml was achieved with 0.5% sodium chloride solution and filtered through a sterile (0.2 μm) membrane filter. The final solution had a buspirone concentration of 33.3 mg/ml.

The nasal buspirone chitosan solutions were prepared by dissolving different concentrations of chitosan hydrochloride (weight adjusted for hydrochloride) in 0.5% sterile sodium chloride solution and benzalkonium chloride added as preservative. The final solutions had a pH of 5.5 and a buspirone hydrochloride concentration 33.3 mg/ml.

The nasal buspirone chitosan solutions with hydroxyl propyl-β-cyclodextrins (HP-β-CD) were prepared by dissolving different concentrations of HP-β-CD to 1% chitosan HCl in 0.5% sodium chloride solution. The remaining procedure was the same as with the nasal chitosan solutions and all the formulations were passed through a sterile (0.2 μm) membrane filter (Ultipor®N66® membrane, PALL Life Sciences, Mumbai, India).

4.2.2. In vitro duration of mucoadhesion

Duration of mucoadhesion was assessed (Galichet et al. 1996; Mortazavi and Smart 1994) by applying 2 g of the formulation containing 0.1% blue lake on the mucosal surface which was attached over a polyethylene plate fixed in an angle of 40° relative to the horizontal plane. A phosphate buffer pH 7.4 warmed to 37 °C was peristaltically pumped over the tissue at a rate of 5 ml/min. The duration for complete washing of the formulation detected on the basis of the presence of color was recorded. Formulations were compared with control buspirone solutions.

4.2.3. Nasal clearance ctudies

4.2.3.1. Radiolabeling procedure (Illum et al. 1987)

Radiolabeling was done with 99m-technetium using stannous chloride as reducing agent. To 1 ml formulation 100 μg of stannous chloride in 100 μl of 0.01 N HCl was added and pH adjusted to 5.5 using 50 mM sodium bicarbonate solution. To the resultant mixture (filtered through 0.2 μm membrane filter) 1 ml of sterile ^{99m}Tc-pertechnate (75–400 MBq) was added over a period of 60 s and incubated at 30 ± 5 ºC for 30 min.

4.2.3.2. Determination of the labeling efficiency of formulations

The labeling efficiency was determined by paper chromatography using acetone: water (60 : 40) as the mobile phase. After labeling of formulations, samples were placed on chromatographic paper. In this system, free pertechnetate migrates to the top of the paper, while formulation-attached material remains at the application point. The labeling yield was expressed as a percentage of the total amount of radioactivity applied in the testing system.

4.2.3.3. Animal study

Animals care and procedures were conducted according to the "Guide to the Care and Use of Experimental Animal Care". Nasal clearance study was performed at Department of Clinical Veterinary Medicine, Nuclear Medicine Centre, Bombay Veterinary College, Parel, Mumbai, India. The study was approved by the regional animal ethical committee. The deposition and subsequent clearance of the drug from the rat nose was investigated by gamma scintigraphy.

Six healthy albino rats of either sex weighing between 200–250 g were selected for the clearance studies. All of them were anaesthetized by 25 mg/kg ketamine. First of all 10 μl of ^{99m}Tc labeled (100 μCi/50 μl, containing buspirone HCl equivalent to 0.2 mg/kg) formulation was placed in a microinjector equipped with soft polyethylene tube. The tube was carefully inserted 6 mm into the nasal cavity and the solution was administered. Same procedure was followed for plain nasal formulation which was used as control. Immediately after administration the animals were laid on their backs under the monitor of Spect system (Model Millenium MPS, Elqems, Israel) and nasal clearance of formulations were followed by gamma scintigraphy. Regions of interest were drawn around the site of deposition and the total activity in this area was characterized (Moa et al. 2004).

4.2.4. In vitro permeation studies (Wadell et al. 1999; Maitani et al. 1992)

Porcine nasal mucosa obtained from the local slaughterhouse was used as model membrane. The skin around the nasal region was removed and snout was separated from the animal and opened up to expose the conchae. The mucosa covering the ventral nasal conchae was carefully removed using forceps and a scalpel. After being rinsed in saline solution and then distilled water, a piece of nasal mucosa was mounted as flat sheet in a two chamber diffusion cell (area, 0.785 cm^2) maintained at 37 ± 0.5 °C

with the mucosa side facing the donor compartment. Three hundred µl formulation (contains 10 mg buspirone HCl) was placed on mucosal surface in the donor while phosphate buffer saline (PBS) pH 7.4 was placed in the receiver compartment. An aliquot of 0.5 ml was withdrawn after a fixed time interval and replaced with same amount of drug free buffer. Aliquots so withdrawn were suitably diluted and analyzed spectrophotometrically at 304 nm (Shimadzu-2401 PC, Singapore). Permeability coefficient was determined using the following equation:

$$P = \frac{dQ/dt}{CoA} \quad (1)$$

Where dQ/dt represents the permeability rate and Co the initial concentration in the donor chamber, while A is the effective surface area of the mucosa.

4.2.5. In vitro cytotoxicity test

An *in vitro* cytotoxicity test using the direct contact method was performed. Liquid test materials were deposited on filter discs and these discs were taken as test samples. Test samples, negative controls (ultra high molecular weight polyethylene) and positive controls (PVC) in triplicate were placed on subconfluent monolayer of L-929 mouse fibroblast cells. After incubation of cells with test samples at 37 ± 2 °C for 24 ± 1 h, cell culture was examined microscopically for cellular response around test samples. Cellular responses were scored as 0, 1, 2 and 3 according to non-cytotoxic, moderately cytotoxic and severely cytotoxic.

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