Brain targeting studies on buspirone hydrochloride after intranasal administration of mucoadhesive formulation in rats

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Abstract

Objectives The purpose of this study was to find out whether nasal application of buspirone could increase its bioavailability and directly transport the drug from nose to brain.

Methods A nasal formulation (Bus-chitosan) was prepared by dissolving 15.5 mg buspirone hydrochloride, 1% w/v chitosan hydrochloride and 5% w/v hydroxypropyl β-cyclodextrin (HP-β-CD) in 5 ml of 0.5% sodium chloride solution. The formulation was nasally administered to rats and the plasma and brain concentration compared with that for buspirone hydrochloride solution after intravenous and intranasal (Bus-plain) administration. The brain drug uptake was also confirmed by gamma scintigraphic study.

Key findings The nasal Bus-chitosan formulation improved the absolute bioavailability to 61% and the plasma concentration peaked at 30 min whereas the peak for nasal Bus-plain formulation was 60 min. The AUC_{0-480} in brain after nasal administration of Bus-chitosan formulation was 2.5 times that obtained by intravenous administration (711 ± 252 ng/g vs 282 ± 110 ng/g); this was also considerably higher than that obtained with the intranasal Bus-plain formulation (354 ± 80 ng/g). The high percentage of direct drug transport to the brain (75.77%) and high drug targeting index (>1) confirmed the direct nose to brain transport of buspirone following nasal administration of Bus-chitosan formulation.

Conclusions These results conclusively demonstrate increased access of buspirone to the blood and brain from intranasal solution formulated with chitosan and HP-β-CD.

Keywords brain uptake; buspirone hydrochloride; intranasal administration; pharmacokinetics; rat

Introduction

Nasal delivery has long been used to administer topically acting drugs to treat localized ailments such as nasal symptoms of common cold and allergy.[1] Advantages of using the nasal route to treat local symptoms include the immediate targeting of relatively high drug concentrations, the simplicity of administration, reduced systemic exposure and good patient acceptability.[2]

Recently, drugs with systemic actions have been marketed for nasal delivery in preference to oral delivery or injection. This takes advantage of the potentially rapid and high systemic availability of nasally administered compounds.[2] Diseases or conditions for which nasal administration has been used to achieve such delivery include hormone replacement therapy (estradiol),[3] osteoporosis (calcitonin),[4] pain management (butorphanol, sumatriptan and zolmitriptan),[5–7] enuresis (desmopressin),[8] endometriosis (nafarelin)[9] and motion sickness (metoclopromide).[10]

The possibilities for central nervous system (CNS) delivery via nasal administration are currently being investigated for the delivery of polar drugs to treat chronic CNS conditions such as Parkinson's disease or Alzheimer's disease.[11] It has been reported that the olfactory neurons connect the brain and surrounding cerebrospinal fluid (CSF) with the open air that is inhaled via the nasal cavity. This anatomical observation raised the hypothesis that drugs may have a direct access to the CNS following intranasal administration, thereby circumventing the blood–brain barrier.[12,13]

In past decades, this potential transport route has been investigated extensively.[14,15] This transport is generally separated into transfer within the nerve axon targeting the

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olfactory bulbs and then from there to the rest of the brain, or different transfer mechanisms outside the nerve, reaching the olfactory bulbs, the CSF or the brain. These olfactory pathways offer potential for bypassing the blood–brain barrier, which prevents some CNS-active drugs from reaching the brain.

Although the olfactory route has not been investigated widely in humans,[16] several studies in animals have been published – the olfactory transfer of dopamine and picolinic acid in mice[17,18] and a 3-kDa fluorescein dextran in rats.[19]

Buspirone hydrochloride (8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4,5]decane-7,9-dione monohydrochloride) is an azaspirodecanedione derivative anxiolytic agent.[20] It is used to treat generalized anxiety disorder and anxiety caused by alcohol craving or smoking cessation, as well as attention deficit hyperactivity disorder in children.[21] Buspirone undergoes extensive first-pass metabolism, which leads to low oral bioavailability (absolute bioavailability ~4%), a short half-life of 2–3 h and a low amount reaching the brain.[20] Consequently, treatment with buspirone hydrochloride requires three to four daily doses of 20 mg and so, because of the chronic nature of the treatment, a reduction in daily doses would be advantageous.[22]

Chitosan is a linear polysaccharide comprised of two monosaccharides – *N*-acetyl-D-glucosamine and D-glucosamine – linked together by glucosidic bonds. Chitosan is produced by alkaline hydrolysis (deacetylation) of chitin obtained from crustacean shells and forms positively charged salts when dissolved in inorganic or organic acids. Chitosan is available in a wide range of molecular weights and degrees of deacetylation. The clearance of chitosan formulations from the nasal cavity has been shown to be significantly slower than that of simple aqueous solutions.[23,24] Hence, nasal chitosan drug formulations provide a longer time for drug transport across the nasal membrane before the formulation is cleared by the mucociliary clearance mechanism. Furthermore, chitosan has also been shown in Caco-2 cell culture studies to open transiently the tight junctions between cells, which enable hydrophilic drugs to pass through the membrane by the paracellular route.[25]

To improve patient compliance and for greater success of therapy, nasal delivery of buspirone is a good alternative as it is expected to increase bioavailability and, with the possibility of nose to brain delivery, greater therapeutic efficacy is anticipated particularly in attention deficit hyperactivity disorder in children.

The purpose of this study was to find out whether the nasal route could increase the bioavailability of buspirone and could provide direct transport from nose to brain.

Materials and Methods

Drugs and reagents

Buspirone hydrochloride 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), and hydroxypropyl β-cyclodextrin (HP-β-CD) was gifted by Roquette Freres, (Lestrem, France). All the reagents used for analysis were of HPLC or analytical grade.

Formulations for animal studies

The buspirone hydrochloride solution for intravenous injection (Bus-i.v.) was prepared by dissolving 15.5 mg of buspirone hydrochloride in 5 ml of sterile isotonic saline and filtering through a sterile (0.2 μm) membrane filter. The final pH of the formulation was adjusted to 7.4.

Control buspirone hydrochloride solution for nasal administration (Bus-plain) was prepared by dissolving 15.5 mg of buspirone hydrochloride in 5 ml of sterile 0.5% sodium chloride solution and filtering through a sterile (0.2 μm) membrane filter. The final pH of the formulation was 5.5.

The nasal buspirone formulation containing chitosan hydrochloride (Bus-chitosan) was prepared by dissolving 15.5 mg of buspirone hydrochloide, 387 mg chitosan hydrochloride (1% w/v, weight adjusted for hydrochloride salt) and 625 mg HP-β-CD (5% w/v) in 4 ml of 0.5% sodium chloride solution, pH adjusted to 5.5, and the final volume was made to 5 ml with 0.5% sodium chloride solution. The final concentration was 3.1 mg/ml. This formulation is optimized on the basis of our previous in-vitro studies.[26]

The dose of the formulation was 20 μl, which contained buspirone hydrochloride equivalent to 0.25 mg/kg.

Animal experiments

Male Wistar rats, 250–300 g, were selected for the study. Animal care and procedures were conducted according to the Principles of Laboratory Animal Care. The animal study was approved by the Animal Ethical Committee, Institute of Pharmaceutical Education and Research, Wardha, India. The animals were housed four per cage at 20–25°C with free access to food and water with a 12-h light–dark cycle.

Seventy six rats were divided into three groups, one for control buspirone hydrochloride solution, the second for the buspirone formulation containing chitosan hydrochloride and the third for the intravenous buspirone formulation. There were 24 rats in each group for intranasal administration and the intravenous group had 28 rats. The rats were anaesthetized using an intramuscular injection of ketamine (25 mg/kg). This dose was sufficient to keep the rats sedated for a short period of 3 min during instillation of formulations to prevent sneezing. Nasal formulations (10 μl) at a buspirone hydrochloride dose level of 0.25 mg/kg were instilled into each nostril with the help of a microlitre syringe attached to polyethylene tubing (i.d. 0.1 mm). The polyethylene tubing was inserted 7 mm inside the nostril. The rats were euthanized at scheduled times by exposure to gaseous CO_2 to collect blood and brain tissue. The skull was cut open and brain was carefully excised. It was quickly rinsed with saline and blotted with filter paper to get rid of blood-taint and macroscopic blood vessels as much as possible. After weighing, the brain was homogenized with one volume of saline in a tissue homogenizer at 8000 rev/min (RQ 127A; REMI Instruments Ltd, Mumbai, India). Blood samples were anticoagulated with heparin and centrifuged at $6238g$ for 10 min to obtain plasma. Samples were stored in a deep freezer (−70°C) until analysis. For rats receiving nasal

formulations, blood and brain samples were removed at 10, 30, 60, 120, 240 and 480 min whereas for rats which received the intravenous formulation, blood and brain samples were removed at 5, 10, 30, 60, 120, 240 and 480 min. Four rats at each time point were used.

Analytical procedures

Blood and brain homogenate samples were analysed by the GCMS method reported by Gammans.[27] GCMS data were obtained on a Shimadzu QP-2000 instrument. Briefly, to a 2-ml volume of each sample (plasma/brain homogenate), 0.2 ml of borate buffer was added and immediately mixed. The borate buffer, pH 8.5, was prepared by dissolving 12.37 g boric acid and 14.91 g potassium chloride in water and diluting to 1000 ml. Fifty millilitres of boric acid and potassium hydrochloride solution was placed in a 200-ml volumetric flask and 50 ml 0.2 M NaOH solution was added and the volume made up with water. The aqueous phase was extracted with 10 ml of n-butyl chloride. The layers were separated and the organic layer was decanted into a new tube and extracted with 2.0 ml of 0.01 M hydrochloric acid for 10 min on a rotator. The organic layer was decanted and discarded. The aqueous layer was washed with 10 ml of diethyl ether for 10 min on a rotator. The layers were separated after centrifugation and the pH of the aqueous phase was adjusted to 8.5 by adding 2 ml of borate buffer and then extracted with 10 ml of n-butyl chloride on the rotator. The organic layer was transferred to a screw-cap tube and dried under nitrogen. The dried residue was rinsed to the bottom of the tube with 500 μl of methanol, which was again removed under nitrogen. The extract was stored overnight at −17°C in 100 μl of methanol. During analysis the methanol was removed under nitrogen and the sample was re-dissolved in 20 μl of toluene for estimation of buspirone hydrochloride by GCMS.

Standard calibration curves of buspirone hydrochloride were prepared with plasma and brain homogenate spiked with known amounts of drug (0.2–10 ng/ml and 0.2–10 ng/g, respectively).

Chromatographic conditions

Three microlitres of sample was injected into the gas chromatograph (GC). The GC column (ULBON HR-1) was fused silica capillary 0.25 mm × 30 m with film thickness 0.25 μm. The initial temperature was 100°C for 6 min and then heated at a rate of 10°C/min to 250°C. Helium carrier gas was used at a velocity of 2 ml/s. The mass spectrometer was set to monitor mass 277 only. The 70 eV electron impact mass spectra of buspirone were recorded.

Gamma scintigraphy imaging

The radiolabelling procedure was done according to the method reported in our previous study on nasal clearance.[26] Eighteen Wistar rats, 250–300 g, were divided into three groups, one for control buspirone hydrochloride solution, the second for the buspirone formulation containing chitosan hydrochloride and the third for the intravenous buspirone formulation. The study was approved by the regional animal ethical committee of the Department of Clinical Veterinary College, Parel, Mumbai, India. To the rats in the intravenous group, 20 μl of

^{99m}Tc-labelled formulation (100 μCi/50 μl, containing buspirone hydrochloride equivalent to 0.25 mg/kg) was intravenously injected through the tail vein of the rat. Rats in the intranasal group were first anaesthetized by intramuscular injection of ketamine hydrochloride at a dose of 25 mg/kg and thereafter anaesthesia was maintained using local anaesthetic chloroform during the instillation of the nasal formulations. Ten microlitres of ^{99m}Tc-labelled formulation (100 μCi/50 μl, containing buspirone hydrochloride equivalent to 0.25 mg/kg) was instilled in each nostril of the rat with the help of a microinjector equipped with a soft polyethylene tube. The tube was carefully inserted 7 mm into the nasal cavity during administration. The rats were placed on a board and images were monitored using a Spect system (Model Millenium MPS, Elquems, Israel).

Data analysis

All concentration data were normalized for dose and weight. The pharmacokinetic data were determined using non-compartmental analysis. The area under the concentration-time profile (AUC_{0-t}) was calculated using the trapezoidal method. To evaluate the brain targeting after nasal administration two indices were adopted (DTI and DTP, as described below).

To evaluate the brain-targeting after nasal dosing, the drug targeting index (DTI)[28,29] was calculated as the ratio of the value of AUC_{brain}/AUC_{plasma} following intranasal administration to that following intravenous injection:

$$DTI = (AUC_{brain}/AUC_{plasma})_{i.n.}/(AUC_{brain}/AUC_{plasma})_{i.v.} \quad (1)$$

To clarify nose–brain direct transport, brain drug direct transport percentage (DTP)[30] was calculated from equations 2 and 3.

$$B_{i.v.}/P_{i.v.} = B_x/P_{i.n.} \quad (2)$$

$$DTP\% = [(B_{i.n.} - B_x)/B_{i.n.}] \times 100 \quad (3)$$

where $P_{i.v.}$, $B_{i.v.}$, $P_{i.n.}$ and $B_{i.n.}$ respectively denote the AUC_{0-t} of buspirone in plasma and brain obtained after intravenous and intranasal administration. B_x represents the brain AUC fraction contributed by systemic circulation through the blood–brain barrier after nasal dosing.

Statistical methods

Statistical analysis of the effect of formulation type and time on the plasma and brain concentration of buspirone was performed using a repeated measures analysis of variance. Tukey–Kramer multiple comparisons post-test was performed only when $P < 0.05$. $P < 0.05$ denoted significance in all cases. Comparison of data from the two nasal groups was performed using unpaired (two-tailed) *t*-test. Experiments were performed in replicates of four.

Results

To assess the improvement in bioavailability of buspirone and its brain distribution on nasal administration, the concentration of buspirone in blood and brain was determined. Figures 1 and 2 represent the mean plasma and brain concentration–time profile of buspirone after the intravenous
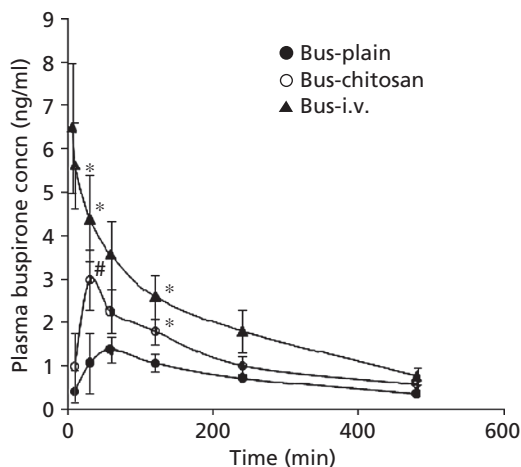
Figure 1 Plasma concentration–time profile of buspirone after intravenous and intranasal administration of a 0.25 mg/kg dose in rats. Intranasal control buspirone solution (Bus-plain); intranasal formulation of buspirone with chitosan hydrochloride and HP-β-CD (Bus-chitosan); intravenous buspirone hydrochloride (Bus-i.v.). Data represent the mean ± SD, $n = 4$. [#]$P < 0.05$, compared with Bus-Plain; *$P < 0.05$, comparing all formulations (Tukey–Kramer comparisons test following analysis of variance done for all formulations).
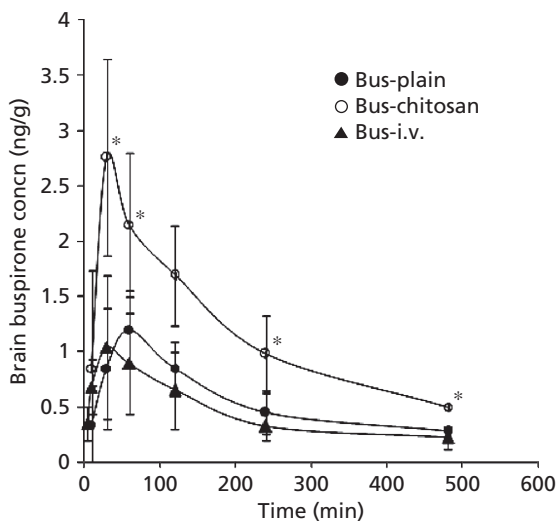

Figure 2 Brain concentration–time profile of buspirone after intravenous and intranasal administration of a 0.25 mg/kg dose in rats. Intranasal control buspirone solution (Bus-plain); intranasal formulation of buspirone with chitosan hydrochloride and HP-β-CD (Bus-chitosan); intravenous buspirone hydrochloride (Bus-i.v.). Data represent the mean ± SD, $n = 4$. *$P < 0.05$, comparing all formulations (Tukey–Kramer comparisons test following analysis of variance done for all formulations).

administration of buspirone hydrochloride solution (Bus-i.v.) and intranasal administration of control buspirone hydrochloride solution (Bus-plain) and buspirone formulation containing chitosan hydrochloride and HP-β-CD (Bus-chitosan). Pharmacokinetic parameters were calculated by

a non-compartmental model using the software Kinetica 4.4.1 (Thermo Electron Corporation, MA, USA). The non-compartmental pharmacokinetic parameters are given in Table 1. The maximum plasma concentration was achieved at 30 min for nasal buspirone formulation containing chitosan hyrochloride (1% w/v) and HP-β-CD (5% w/v) whereas it took 60 min to reach the peak concentration for control buspirone hydrochloride solution. The nasal absorption of buspirone hydrochloride from the control formulation was relatively slow.

The absolute bioavailability of buspirone from Bus-chitosan nasal formulation was 61%, which was markedly superior to the reported oral bioavailability of <5%.[31] The AUC_{0-480} of buspirone in plasma following intranasal administration of the formulation containing chitosan hydrochloride and HP-β-CD (762 ± 218 ng min/ml) was significantly ($P < 0.05$) higher than that following administration of control buspirone solution (463 ± 210 ng min/ml).

The time–drug concentration profile for the uptake of drug into brain showed notable differences between buspirone in chitosan and HP-β-CD solution and control buspirone solution.

There was an increased uptake of the drug into the brain after nasal administration compared with that obtained after intravenous injection. The AUC_{0-480} value in brain after nasal administration of buspirone formulation containing chitosan hydrochloride and HP-β-CD was 2.5 times that obtained after intravenous administration (711 ± 252 ng min/g vs 282 ± 110 ng min/g); this value was also considerably higher than that obtained with intranasal buspirone control solution (711 ± 252 ng min/g vs 354 ± 180 ng min/g). These differences were statistically significant ($P < 0.05$).

Brain uptake was significantly ($P < 0.05$) increased (in terms of C_{max} and AUC) for the mucoadhesive formulation as compared with a simple nasal solution. The brain drug direct transport percentage (% DTP) of control nasal buspirone formulation (Bus-plain) and nasal formulation containing chitosan (Bus-chitosan) was 70.41 ± 30.5% and 75.77 ± 22.5%, respectively. The drug targeting index was 3.38 for Bus-plain and 4.13 for Bus-chitosan. High % DTP and DTI > 1 confirm the direct pathway from nose to brain.[32]

To visualize brain uptake of drug, gamma scintigraphic images were taken at different time points after administration of ^{99m}Tc-labelled Bus-i.v. (intravenous), Bus-plain (intranasal) and Bus-chitosan (intranasal) formulations. The gamma scintigraphic images of rats 30 min post-administration (Figure 3) clearly show higher brain uptake on intranasal administration of Bus-chitosan (nasal formulation containing chitosan and HP-β-CD).

Discussion

The rapid absorption when 1% chitosan hydrochloride and 5% HP-β-CD was added to the formulation may be due to rapid and greater permeation of buspirone from the formulation.

Administration of buspirone in chitosan and HP-β-CD solution improved the bioavailability and also decreased the t_{max} to 30 min. Improvement in nasal absorption is caused firstly by mucoadhesion due to chitosan. The high density

Table 1 Pharmacokinetic parameters following intravenous and nasal administration of buspirone formulations

Formulation	Route	Parameter	Plasma	Brain
Bus-plain	Intranasal	C_{max} (ng/ml or ng/g)	1.35 ± 0.5	1.2 ± 0.7
		t_{max} (min)	60 ± 15	60 ± 10
		AUC_{0-480} (ng min/ml or ng min/g)	463 ± 210	354 ± 180
		AUC_{in}/AUC_{iv} (%)	37.2^{a}	125.4
		$t\frac{1}{2}$ (min)	220 ± 20	205 ± 25
Bus-chitosan	Intranasal	C_{max} (ng/ml or ng/g)	$3.0 \pm 0.5^{\#}$	2.76 ± 1.0
		t_{max} (min)	30 ± 10	30 ± 15
		AUC_{0-480} (ng min/ml or ng min/g)	762 ± 218	$711 \pm 252^{*}$
		AUC_{in}/AUC_{iv} (%)	61^{a}	251.8
		$t\frac{1}{2}$ (min)	206 ± 28.9	200.13 ± 32.5
Bus-i.v.	Intravenous	C_{max} (ng/ml or ng/g)	6.15 ± 1.23^{b}	1.45 ± 0.32
		AUC_{0-480} (ng min/ml or ng min/g)^{c}	$1248 \pm 302^{*}$	282 ± 110
		$t\frac{1}{2}$ (min)	199.11 ± 20	197 ± 23.5

C_{max}, maximum plasma/brain concentration of buspirone; t_{max}, time taken to reach C_{max}; AUC_{0-480}, area under the plasma/brain concentration–time curve from 0 to 480 min; $t\frac{1}{2}$, half-life of buspirone in plasma. [a]absolute bioavailability; [b]non-extrapolated, observed at first time point; [c]calculated considering extrapolated C for $t = 0$ using Kinetica. *$P < 0.05$, comparisons made of all formulations, Tukey–Kramer comparisons test following analysis of variance done for all formulations; [#]$P < 0.05$, comparisons made between the two nasal formulations using unpaired (two-tailed) *t*-test. Data represent mean $\pm$ SD, $n = 4$.
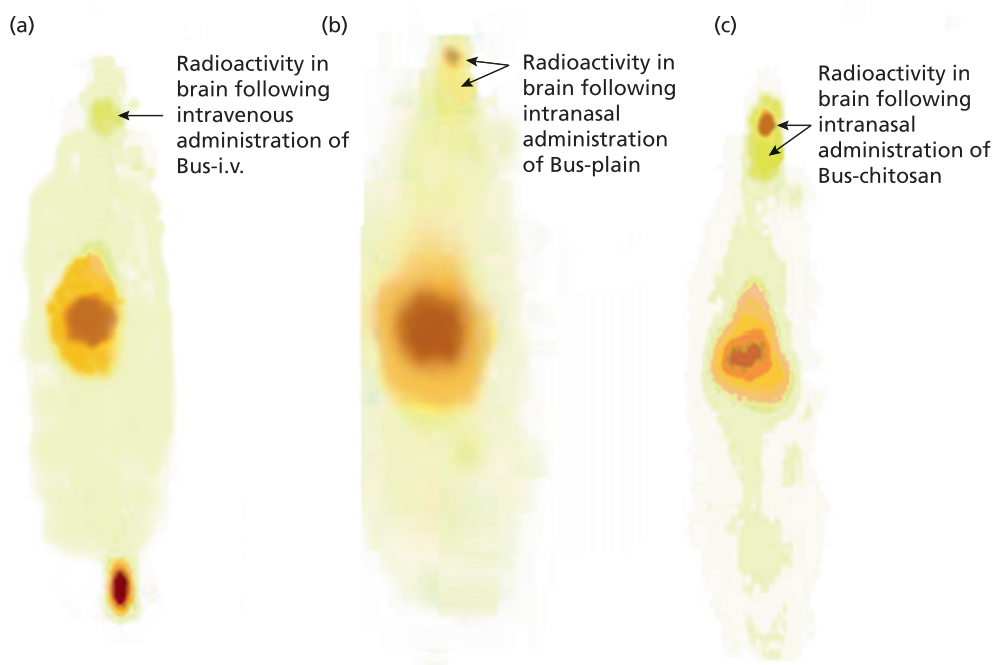

Figure 3 Gamma scintigraphy images of rats (A/P view) showing the presence of radioactivity in the brain 30 min post administration of intravenous and intranasal buspirone formulations. Radioactivity is indicated by arrows. (a) Bus-i.v. (intravenous); (b) Bus-plain (intranasal) and (c) Bus-chitosan (intranasal).

positive charges on the molecule adhere strongly to negative sites on the nasal membrane, such as sialic acid residues in mucin glycoproteins. This mucoadhesive property results in the nasally administered chitosan formulation having an increased clearance time,[26] thereby promoting nasal absorption of the drug. Secondly it has been demonstrated that chitosan, when applied to confluent cells, is able to transiently open the tight junctions between the cells and that HP-β-CD can precipitate the protein.[33]

Thus due to this synergistic effect there was increased nasal absorption.

Buspirone is a dibasic compound with a pK_a value of 4.12–7.32; hence, at pH 5.5–6.0 (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.*[34] Their report also states that transcellular transport of monoprotonated species is very low and they are preferably transported via the apparent paracellular pathway. Our results

therefore show a substantial contribution of paracellular transport through the nasal mucosa.

There was increased access of the drug to the blood and brain following intranasal administration as a solution of drug in chitosan and HP-β-CD. This effect might be attributed to two factors: a systemic effect and a direct nose-to-brain effect. It is believed that drug uptake into the brain from the nasal mucosa can be achieved via two different pathways: a systemic pathway of absorption into the circulation then into the brain across the blood–brain barrier and an olfactory pathway of partial direct drug delivery to the brain through the CSF.

It is assumed that if drug concentration in the brain is significantly higher after intranasal administration than that after intravenous administration, or DTI > 1, a direct pathway from the nasal olfactory region to the brain exists. The DTI of buspirone after intranasal administration of Bus-chitosan formulation was 4.13, showing direct transport to the brain.

A drug can cross the olfactory pathway by one or a combination of pathways.[1] Firstly, the drug can be delivered by a transcellular pathway, which is especially suited to small lipophilic molecules or large molecules. Secondly, the drug can be transported through the paracellular pathway by passing through the tight junctions or through open clefts in the membrane, which is especially suited to smaller hydrophilic molecules. Thirdly, the drug can be transported through the olfactory neuron cells by intracellular axonal transport primarily to the olfactory bulb. For buspirone, which is a small (MW 422) water-soluble molecule,[20] the second pathway would have played a major role in its transport. In addition, the presence of chitosan in the formulation would have facilitated the paracellular transport by opening the tight junctions.

Conclusions

It was concluded that nasal administration of buspirone formulation containing chitosan and HP-β-CD could avoid first-pass metabolism in the liver and markedly improve the bioavailability. Uptake of drug into the brain via the direct nose–brain pathway after nasal delivery was also confirmed.

Thus nasal delivery is a viable alternative to other routes of administration to improve the therapeutic efficacy of buspirone hydrochloride.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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