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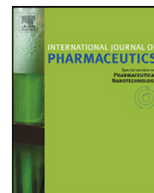

Pharmaceutical Nanotechnology

Intranasal nanoemulsion based brain targeting drug delivery system of risperidone

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A B S T R A C T

The objective of investigation was to prepare nanoemulsion containing risperidone (RSP) to accomplish the delivery of drug to the brain via nose. Risperidone nanoemulsion (RNE) and mucoadhesive nanoemulsion (RMNE) were characterized for drug content, pH, percentage transmittance, globule size and zeta potential. Biodistribution of RNE, RMNE, and risperidone solution (RS) in the brain and blood of Swiss albino rats following intranasal (i.n.) and intravenous (i.v.) administration was examined using optimized technetium labeled (^{99m}Tc-labeled) RSP formulations. Gamma scintigraphy imaging of rat brain following i.v. and i.n. administrations were performed to ascertain the localization of drug in brain. The brain/blood uptake ratio of 0.617, 0.754, 0.948, and 0.054 for RS (i.n.), RNE (i.n.), RMNE (i.n.), and RNE (i.v.), respectively, at 0.5 h are indicative of direct nose to brain transport bypassing the blood–brain barrier. Higher drug transport efficiency (DTE%) and direct nose to brain drug transport (direct transport percentage, DTP%) for mucoadhesive nanoemulsions indicated more effective and best brain targeting of RSP amongst the prepared nanoemulsions. Studies conclusively demonstrated rapid and larger extent of transport of RSP by RMNE (i.n.) when compared to RS (i.n.), RNE (i.n.) and RNE (i.v.) into the rat brain.

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1. Introduction

The site-specific targeted drug delivery negotiates an exclusive delivery to specific pre-identified compartment with maximum intrinsic activity of drugs and concomitantly reduced access of the drug to irrelevant non-target cells. A number of strategies are followed to target various body tissue/organs. The brain is a delicate organ with many vital functions and formidable mechanisms isolate and protect it from the outside world. Unfortunately, the same mechanisms that prevent intrusive environmental chemicals accessing the brain also prevent the access of therapeutic chemicals. Hence, a number of strategies like invasive approach (blood–brain barrier (BBB) disruption, intracerebral implants), physiological approach (pseudonutrients, ligand binding proteins, chimeric peptides), pharmacological approach (liposomes, nanoparticles, nano-conjugates, chemical drug delivery) are used for targeting drug molecules to brain (Soni et al., 2004; Chien et al., 1989). The olfactory region of nasal mucosa that provides a direct connection between nose and brain can be exploited for targeting of CNS acting drug molecules used in conditions like Alzheimer's disease, depression, migraine, schizophrenia, etc.

Risperidone,3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-*a*] pyrimidin-4-one is an approved antipsychotic drug belonging to the chemical class of benzisoxazole derivative and is available as tablet, oral liquid (Risperidal®) and orally disintegrating tablet (Risperidal® M-TAB).These dosage forms exhibit low bioavailability due to extensive first pass metabolism and non targeted delivery results in numerous side effects (http://www.drugs.com). Since the target site of the risperidone is brain, thereby a strategy is desirable that not only improves the bioavailability by preventing first pass metabolism but also provides targeting to the receptor site and bypasses the blood–brain barrier, so as to achieve desired drug concentration at the site of action, hence preventing availability of drug at non-targeting sites and reducing the side effects.

Earlier studies (Sakane et al., 1999; Li et al., 2002; Vyas et al., 2006a,b) have demonstrated that intranasal administration offers a practical, non-invasive, and an alternative route of administration for rapid drug delivery to brain. It also offers the advantages of being administered simply, cost effectively and conveniently. Additionally, direct transport of drugs to brain, circumventing the brain

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barriers following intranasal administration provides a unique feature and better option for targeting drugs to brain (Behl et al., 1998; Candace and Pollock, 2005; Illum, 2002). However, few formulation factors should be considered while designing the drug delivery system for intranasal administration. The formulation should be designed so as to provide a rapid transport of drug across nasal mucosa and a longer residence time in nasal cavity to overcome the nasal mucociliary clearance (Ugwoke et al., 2001). Microemulsions by virtue of their lipophilic nature and low globule size are widely explored as a delivery system to enhance uptake across nasal mucosa (Lawrence and Rees, 2000). Addition of mucoadhesive agents such as polyelectrolyte polymer helps in retention of the formulation on the nasal mucosa (Sinswat and Tengamuay, 2003).

The objective of this investigation was to prepare, characterize RNE/RMNE and evaluate their performance in animal model. It is proposed that nanoemulsion/mucoadhesive nanoemulsion based drug delivery system will result in rapid nose to brain transport of risperidone and greater transport and distribution into and within the brain. This can reduce the side effects, decrease the dose and frequency of administration, and perhaps even the cost of the therapy.

2. Materials and methods

2.1. Drugs and reagents

Risperidone (RSP) was received as a gift from Sun Pharmaceuticals, India. Capmul MCM was received as a Gift sample from Abitech Corporation Limited, Columbus, OH. Tween 80 and polyethylene glycol 400 were purchased from S.D. Fine chemicals (Mumbai, India). Polycarbophil (AA-1, pharmagrade, molecular weight approximately 3.50 billion) was purchased from Noveon Polymers, India. Transcutol was a gift sample received from Colorcon Asia Ltd., India. Diethylene triamine penta acetic acid (DTPA) and stannous chloride dihydrate ($SnCl_2{\cdot}2H_2O$) were purchased from Sigma Chemical Company, St. Louis, MO. Sodium pertechnetate, separated from molybdenum-99 (99m) by solvent extraction method, was provided by Regional Center for Radiopharmaceutical Division (Northern Region), Board of Radiation and Isotope Technology, New Delhi, India. All other chemicals and solvents were of analytical reagent grade and were used without further purification (Fig. 1).

2.2. Animals

Mice of either sex with average weight of 25 g were selected for the measurement of locomotor activity and Swiss albino

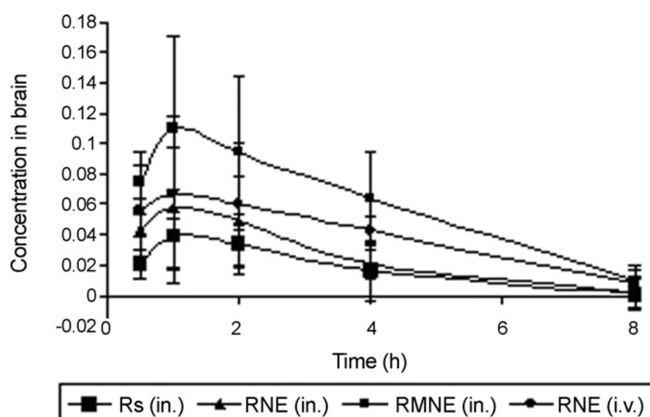

Fig. 1. RSP concentration in rat blood at different time intervals following RS (i.n.), RNE (i.n.), RMNE (i.n.) and RNE (i.v.).

rats (aged 4–5 months) weighing between 200 and 250 g were selected for paw test and biodistribution studies. Three animals for each formulation per time point were used in the studies.

2.3. Experiments

2.3.1. Preparation and characterization of RSP nanoemulsions

Risperidone solution (RS, 3.5 mg/ml RSP) was prepared by addition of RSP (35 mg) to a mixture of 1 ml ethanol and 2 ml propylene glycol, and the volume made to 10 ml with distilled water with continuous stirring until a clear solution was obtained. Risperidone nanoemulsion (3.5 mg/ml RSP) was prepared using capmul MCM as the oily phase (8%, w/w) and tween 80 as surfactant (S, 29.33%, w/w). A mixture of transcutol and propylene glycol (1:1, w/w) was used as co-surfactant (CoS, 14.66%, w/w) and distilled water (48%, w/w) as the aqueous phase. Formulation was prepared by dissolving RSP in the oil phase, followed by the addition of S, CoS and finally (%transmittance at 630 nm, 99.32%) distilled water was added with continuous stirring, which resulted in transparent and homogenous risperidone nanoemulsion (RNE). Risperidone mucoadhesive nanoemulsion (RMNE) was prepared by addition of chitosan (0.50%, w/w) to RNE and the dispersion stirred for 1 h.

RSP content in the formulations was determined using a UV–vis spectrometric method, since the excipients interfered in the estimation of drug, hence second order derivatization of zero order spectra was done and the wavelength of 300 nm was selected for the estimation of drug in the formulations in methanol. The globule size determination was performed using photon correlation spectroscopy with in-built Zetasizer (model: Nano ZS, Malvern Instruments, UK) at 633 nm. Helium–neon gas laser (intensity of 4 mW) was the light source. The equipment was programmed to provide 18 mm laser width. Electrophoretic mobility (mm/s) was measured using small volume disposable zeta cell and converted to zeta potential (Rolan et al., 2003) by in-built software using Helmholtz–Smoluchowski equation.

2.3.2. Pharmacodynamic studies

2.3.2.1. Paw test. Swiss albino rats (aged 4–5 months) weighing between 200 and 250 g were selected for the study. Three rats for each formulation per time point were used in the study. The drug formulation, RNE containing 0.011–0.021 mg RSP (equivalent to 0.09 mg/kg body weight) was injected (50 μl) through the tail vein of Swiss albino rats. The formulations RS/RNE/RMNE containing 0.011–0.021 mg RSP (equivalent to 0.09 mg/kg body weight) were administered (10 μl) in each nostril using micropipette (10–100 μl) attached with low-density polyethylene (LDPE) tubing, having 0.1 mm internal diameter at the delivery site. Haloperidol (standard) was given in a dose of 0.5 mg/kg of body weight intraperitoneally. The paw test was performed using Perspex platform measuring 30 cm × 30 cm, with a height of 20 cm. The top of the platform had two holes of 4 cm diameter for the forelimbs and two larger holes of 5 cm diameter for hind limbs and a slit for the tail. The test was performed 30 min after the intranasal administration of the saline (control group) or the drug formulations by carefully lowering the hind limbs of the rats in the holes, followed by fore limbs. The forelimb retraction time (FRT) was defined as the time it took the rat to withdraw either of the forelimb. Likewise, the hind limb retraction time (HRT) was defined as the time it took the rat to withdraw either of the hind limb. For both FRT and HRT the minimum was set to 1 s and maximum to 30 s. The paw test was repeated at 40 and 50 min after the administration of the formulations (Braso et al., 2003; Powell and Miyakawa, 2006; Ellenbroek et al., 2006).

2.3.2.2. Locomotor activity. Mice of either sex with average weight of 25 g were taken and deprived of food and water for 24 h before the test. To avoid any influence of the circadian rhythm the experimentation was performed only between 8.00 and 12.00 a.m. Three mice were taken per time point. The intranasal formulations were administered in mice using micropipette (5 μl/nostril) in a dose of 0.325 mg/kg of body weight followed by intraperitoneal administration of L-dopa (13 mg/kg of body weight) and carbidopa (3.25 mg/kg of body weight) after 30 min. The locomotor activity was measured for 10 min by placing the animals in digital photoactometer (Jindal Scientific Industries, India). For the formulation RME, given by intravenous route, the locomotor activity was measured after 2 min for a period of 10 min (Mohr et al., 2001; Powell and Miyakawa, 2006; Carey et al., 1995).

2.3.3. Preparation of radiolabeled-drug formulation of RS, RNE and RMNE

The formulations RS, RNE, RMNE were radiolabeled using technetium-99 (^{99m}Tc) by direct-labeling method (Saha, 1993; Eckelman, 1995; Babbar et al., 2000; Koziara et al., 2003). To 0.321 ml of RSP formulations 200 μl of stannous chloride dihydrate (2 mg/ml in 10% acetic acid) was added, and pH was adjusted to 6.0–6.5 using 50 mM sodium bicarbonate solution. To the resultant mixture (filtered through 0.22 μm nylon 66 membrane), required volume of sterile ^{99m}Tc-pertechnetate (5 mCi) was added with continuous mixing such that the resultant solution had a radioactivity of 5 mCi/ml and was incubated at 30 ± 5 °C for 30 min. The final volume was made up to 1.0 ml using 0.9% (w/v) sterile sodium chloride solution. The resultant formulations obtained had 100 μCi/20 μl activity.

The radiochemical purity of ^{99m}Tc-RS (^{99m}Tc-labeled RS), ^{99m}Tc-RNE (^{99m}Tc-labeled RNE), ^{99m}Tc-RMNE (^{99m}Tc-labeled RMNE) was determined by ascending instant thin layer chromatography (TLC) using silica gel-coated fiberglass sheets and acetone as the mobile phase. The effects of incubation time, pH, and stannous chloride concentration on labeling were studied to achieve optimum reaction conditions. The in vitro stability of radiolabeled formulation was evaluated in 0.9% (w/v) sodium chloride and the optimized, stable radiolabeled-drug formulations of RSP were used for biodistribution study in rats.

2.3.4. Biodistribution studies

Swiss albino rats (male, aged 4–5 months) weighing between 200 and 250 g were selected for the study. Three rats for each formulation per time point were used in the study. Radiolabeled drug formulation, ^{99m}Tc-RNE (100 μCi/50 μl) containing 0.011–0.021 mg RSP (equivalent to 0.09 mg/kg body weight) was injected through the tail vein of Swiss albino rats. The radiolabeled complex of ^{99m}Tc-RS/RNE/RMNE (100 μCi/20 μl) containing 0.011–0.021 mg RSP (equivalent to 0.09 mg/kg body weight) was administered (10 μl) in each nostril. Formulations were instilled into the nostrils with the help of micropipette (10–100 μl) attached with LDPE tubing, having 0.1 mm internal diameter at the delivery site. The rats were held from the back in slanted position during nasal administration. The rats were killed humanely at different time intervals and the blood was collected using cardiac puncture. Subsequently, brain and other tissues (liver, spleen, intestine, kidney and tail) were dissected, washed twice using normal saline, made free from adhering tissue/fluid, and weighed. Radioactivity present in each tissue/organ was measured using shielded well-type gamma scintillation counter. Radio pharmaceutical uptake per gram in each tissue/organ was calculated as a fraction of administered dose using

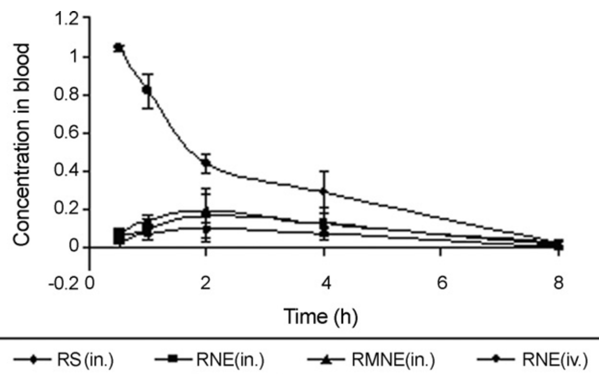

Fig. 2. RSP concentration in rat brain at different time intervals following RS (i.n.), RNE (i.n.), RMNE (i.n.) and RME (i.v.) administrations.

Eq. (1) (Saha, 1993).

$$\%\text{Radioactivity/g of tissue} = \frac{\text{counts in sample} \times 100}{\text{wt of sample} \times \text{total counts injected}} \quad (1)$$

Pharmacokinetic parameters (Keck and Elray, 2002) for RSP formulations were calculated from Figs. 2 and 3 using Quickcal software (developed by Dr. Shivprakash, Plexus Supporting Services, India). Brain targeting efficiency was calculated using two equations mentioned below (Vyas et al., 2005, 2006a,b) Drug targeting efficiency (DTE%) that represents time average partitioning ratio was calculated as follows,

$$\text{DTE\%} = \left[\frac{(\text{AUC}_{\text{brain}}/\text{AUC}_{\text{blood}})_{\text{i.n.}}}{(\text{AUC}_{\text{brain}}/\text{AUC}_{\text{blood}})_{\text{i.v.}}}\right] \times 100 \quad (2)$$

Nose to brain direct transport percentage (DTP%) was calculated using equation,

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

where $B_x = (B_{\text{i.v.}}/P_{\text{i.v.}}) \times P_{\text{i.n.}}$, B_x is the brain AUC fraction contributed by systemic circulation through the BBB following intranasal administration, $B_{\text{i.v.}}$ is the $\text{AUC}_{0\text{–}480}$ (brain) following intravenous administration, $P_{\text{i.v.}}$ is the $\text{AUC}_{0\text{–}480}$ (blood) following intravenous administration, $B_{\text{i.n.}}$ is the $\text{AUC}_{0\text{–}480}$ (brain) following intranasal administration, $P_{\text{i.n.}}$ is the $\text{AUC}_{0\text{–}480}$ (blood) following intranasal administration, AUC is the area under the curve.

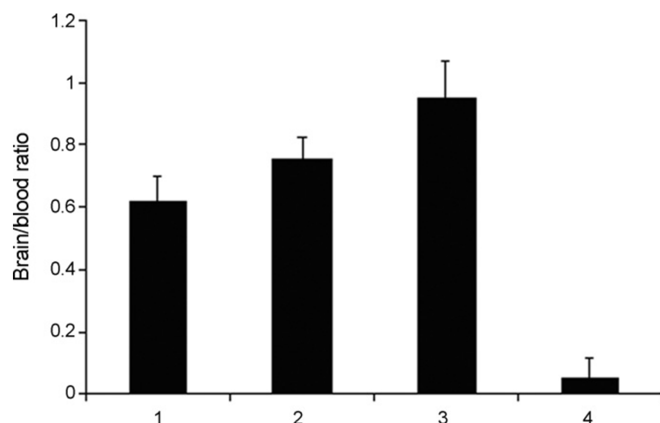

Fig. 3. Brain/blood ratio after intranasal administration of (1) RS, (2) RNE, (3) RMNE and (4) intravenous administration of RNE at 0.5 h post administrations in Swiss albino rats.

2.3.5. Gamma scintigraphy imaging

The Swiss albino rats (male, aged 4–5 months) weighing between 200 and 250 g were selected for the study. Radiolabeled drug formulation, ^{99m}Tc-RNE (100 μCi/50 μl) containing 0.018–0.023 mg RSP (equivalent to 0.09 mg/kg body weight) was injected through the tail vein of Swiss albino rats. Similarly, radiolabeled-drug formulations ^{99m}Tc-RS/RNE/RMNE (100 μCi/20 μl) containing 0.018–0.023 mg RSP (equivalent to 0.09 mg/kg body weight) were administered (10 μl) in each nostril. The rats were anaesthetized using 0.25 ml diazepam intramuscular injection (10 mg/ml) and placed on the imaging board. Imaging was performed using Single Photon Emission Computerized Tomography (SPECT, LC 75-005, Diacam, Siemens AG, Erlanger, Germany) gamma camera (Koziara et al., 2003; Pietrowky et al., 1996). The scintigraphy images following intravenous administration of RNE and intranasal administration of RMNE were recorded.

2.3.6. Statistical analysis

All data are reported as mean ± S.E.M. and the difference between the groups were tested using Student's *t*-test at the level of $P < 0.05$. More than two groups were compared using ANOVA and difference greater at $P < 0.05$ were considered significant.

3. Results and discussion

RS, RNE and RMNE were prepared and characterized for drug content, pH, globule size and distribution, zeta potential, conductivity and viscosity (Table 1). The RSP content was found to be 98.86%, 99.12% and 99.04% for RMNE, RNE and RS respectively. The pH of all the RSP formulations was within the nasal pH range (3.5–6.4). The globule size range (15.5–16.7 nm) and polydispersibility indices of 0.210 and 0.191 for RNE and RMNE indicated that the nanoemulsions approached a monodisperse stable system and could deliver the drug effectively owing to large surface area. The presence of zeta potential to the tune of −12.0 and −9.15 mV on the globules of RNE and RMNE, respectively, conferred physical stability to the system (significant different, $P < 0.5$). The conductivity measurements indicated the nanoemulsions to be of oil-in-water type and the viscosity measurements supported hassle free administration of formulations for biodistribution studies.

All the animals administered RSP formulations (Table 2) by either route exhibited no significance difference in FRT ($P < 0.05$) when compared to the control group. However, a significant ($P < 0.05$) rise in HRT was observed for all the RSP formulations when compared to the control group, administered saline intranasally. Further RMNE administered intranasally showed higher HRT values as compared to RNE (i.n.) and RNE (i.v.) indicating the superiority of intranasal mucoadhesive nanoemulsion over the intranasal and intravenous administration of RNE (Ugwoke et al., 1999).

Evaluation of locomotor activity showed maximum activity in animals not administered RSP formulation (control group) due to the D2 receptor activation by L-dopa and carbidopa. While the groups administered RSP formulations exhibited significant reduction ($P < 0.05$) in locomotor activity. Moreover, RNE (i.n.) and RMNE (i.n.) groups showed significant reduction in locomotor activity when compared to RNE (i.v.) group indicating better performance of these formulations (Table 2) when administered by intranasal route. RMNE (i.n.) displayed much lower locomotor counts as compared to RNE (i.n.) proving the former to be superior in terms of brain uptake.

The pharmacodynamic results of better brain uptake of RSP by nasal route were confirmed by biodistribution studies. RSP was

Table 1
Composition and characterization of risperidone formulations

Formulation	O (%)	S (%)	CoS (%)	AQ (%)	C (%)	Drug content (%)	pH	Globule size (nm)	PDI	Zeta potential (mV)	Viscosity (cp)	Conductivity (mS)	Radiolabeld complex (%)
RMNE	8	29.33	14.66	48	0.5	98.86 ± 1.21	5.15 ± 0.23	16.7 ± 1.21	0.191 ± 0.04	−9.15 ± 2.14	244 ± 12.0	0.163 ± 0.1	97.71 ± 1.2
RNE	8	29.33	14.66	48	–	99.12 ± 0.98	4.62 ± 0.34	15.5 ± 0.92	0.210 ± 0.06	−12.0 ± 1.43	231 ± 10.2	0.156 ± 0.04	98.23 ± 0.9
RS	–	–	–	–	–	99.04 ± 0.64	4.52 ± 0.52	–	–	–	–	–	98.02 ± 1.1

The results are mean values ± S.E.M. derived from three different experimental batches. O is denoted for Oily Phase (Capmul MCM), S for surfactant (Tween 80), CoS for cosurfactant (a mixture of transcutol and propylene glycol, 1:1), AQ is denoted for aqueous phase (distilled water), C for chitosan and PDI for Polydispersibility index. The formulations (RS, RNE, RMNE) mentioned in the table contain risperidone 3.5 mg/ml. All values were determined for $n = 3$ ($P < 0.05$).

Table 2
Pharmacodynamic studies of risperidone formulations in normal Swiss albino rats (paw test) and mice (locomotor activity)

Parameter	Control group	RS (i.n.)	RNE (i.n.)	RMNE (i.n.)	RNE (i.v.)	Haloperidol (i.p.)
FRT (s)	5 ± 2.0	4 ± 2.0	3 ± 2.0	3 ± 3.0	5 ± 2.0	20 ± 7.0
HRT (s)	2 ± 3.0	10 ± 3.0	11 ± 4.0	29 ± 5.0	12 ± 3.0	35 ± 6.0
Locomotor counts	223 ± 9.0	138 ± 5.0	96 ± 6.0	52 ± 5.0	102 ± 12	–

The rats and mice were administered with 10 and 5 μl of risperidone formulations per nostril, respectively.

Table 3
Stability of radiolabeled complexes of RSP formulations (^{99}Tc-RS, ^{99}Tc-RNE, ^{99}Tc-RMNE) in saline

Time (h)	Radiolabeling efficiency (%)		
	^{99}Tc-RS	^{99}Tc-RNE	^{99}Tc-RMNE
2	99.23	99.72	98.53
4	98.47	99.21	98.12
6	97.59	98.57	97.52
8	96.72	97.48	96.64

effectively labeled using ^{99m}Tc and radiolabeled-drug formulations of RS, RNE, RMNE were optimized for maximum labeling efficiency and stability. The radiochemical purity achieved for RS, RNE, RMNE were found to be 98.02%, 98.23% and 97.71% and respectively when evaluated for reduced/ hydrolyzed ^{99m}Tc and free ^{99m}Tc. The pH range of 6.0–6.5 and 400 μg of stannous chloride with incubation time of 60 min for RMNE and 30 min for RS, RNE were selected as conditions for the optimum radiolabeling. The radiolabeled complexes of RSP formulations were found to be stable in saline (Table 3) and in rat serum up to 8 h (degradation < 5%, w/w). Bonding strength of the ^{99m}Tc-RS/RNE/RMNE were also investigated using DTPA challenging test and the percent transchelation of the labeled complex was 1.64% (w/w) at 25 mM DTPA concentration, while at 100 mM it increased to 2.11% (w/w). The results suggested high bonding strength and stability ^{99m}Tc-RS/RNE/RMNE and hence, were used to study biodistribution of the drug in rats.

Biodistribution studies of ^{99m}Tc-RSP formulations following intravenous administration (RNE) and intranasal administrations (RS, RNE and RMNE) on Swiss albino rats were performed and the radioactivity was estimated at different intervals up to 8 h (Table 4). The brain–blood ratio of the drug at all sampling time points for different formulations was also calculated and is recorded in Table 4. The RSP concentration in brain following the intranasal (i.n.) of RMNE (Fig. 1) were found to be significantly higher at all the time points compared to both RNE (i.n.) and RNE (i.v.). While the brain concentration of RSP after i.n. administration of RNE was comparable to that of i.v. administration of RNE at all the time points. The brain/blood ratios of 0.617, 0.754, 0.948, and 0.054 of RS (i.n), RME (i.n), RMME (i.n) and RME (i.v), respectively, at 0.5 h are indicative of direct nose to brain transport bypassing the blood–brain barrier, hence prove the superiority of nose to brain delivery of RSP by microemulsion (Qizhi et al., 2004). Table 5 shows the calculated pharmacokinetic parameters for the RSP formulations using Figs. 2 and 3.

The lower T_{max} values for brain (1 h) when compared to blood (2 h) may also be attributed to preferential nose to brain transport following i.n. administration. When the C_{max} and AUC of brain concentration of RS (i.n.), RNE (i.n.) and RMNE (i.n.) were compared, the C_{max} (0.11%/g) and AUC (0.48 h %/g) of RMME were found to be significantly higher because the addition of mucoadhesive agent decreased the mucociliary clearance, which under normal circumstances rapidly clears the instilled formulation.

Reports in the literature (Illum, 2000, 2003; Vyas et al., 2005; Mathison et al., 1998; Chow et al., 1999) reveal that the drug uptake into the brain from the nasal mucosa mainly occurs via two different pathways. One is the systemic pathway by which some of the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing the BBB. The other is the olfactory pathway by which the drug partly travels from the nasal cavity to CSF and/or brain tissue. It can be concluded, that the amount of drug in the brain tissue after nasal administration is attributed to these two pathways. The DTP% and DTE% represent the percentage of drug directly transported to the brain via the olfactory pathway. DTP% and DTE% were calculated using tissue/organ distribution data following intranasal and intravenous administration and are recorded in Table 6. RMNE showed the highest DTE% (476) and DTP% (78) amongst the three tested formulations, followed by the RS and then by RNE. Nearly two-fold higher DTP% of RMNE as compared to RS and RNE showed the benefit of mucoadhesive nanoemulsion formulation. The higher DTE% and DTP% suggest that RMNE has better

Table 4
Compartmental distribution of ^{99m}Tc-RS (i.n.), ^{99m}Tc-RNE (i.n.), ^{99m}Tc-RMNE (i.n.) and ^{99m}Tc-RNE (i.v.) at different time interval in normal Swiss albino rats

Formulation	Organ/tissue	0.5 h	1.0 h	2.0 h	4.0 h	8.0 h
RS (i.n.)	Blood	0.034 ± 0.01	0.073 ± 0.03	0.096 ± 0.04	0.079 ± 0.02	0.008 ± 0.003
	Brain	0.021 ± 0.01	0.039 ± 0.03	0.034 ± 0.02	0.016 ± 0.02	0.0009 ± 0.01
RNE (i.n.)	Blood	0.057 ± 0.03	0.098 ± 0.05	0.172 ± 0.14	0.129 ± 0.08	0.017 ± 0.02
	Brain	0.043 ± 0.02	0.058 ± 0.04	0.049 ± 0.03	0.021 ± 0.01	0.002 ± 0.01
RMNE (i.n.)	Blood	0.078 ± 0.02	0.140 ± 0.03	0.191 ± 0.09	0.131 ± 0.05	0.022 ± 0.01
	Brain	0.074 ± 0.02	0.110 ± 0.06	0.094 ± 0.05	0.064 ± 0.03	0.01 ± 0.01
RNE (i.v.)	Blood	1.04 ± 0.02	0.820 ± 0.09	0.440 ± 0.05	0.291 ± 0.11	0.027 ± 0.01
	Brain	0.056 ± 0.03	0.067 ± 0.05	0.060 ± 0.04	0.043 ± 0.01	0.007 ± 0.01
RS (i.n.)	Brain/blood	0.617 ± 0.08	0.534 ± 0.06	0.354 ± 0.09	0.203 ± 0.06	0.113 ± 0.08
RNE (i.n.)	Brain/blood	0.754 ± 0.07	0.592 ± 0.05	0.285 ± 0.03	0.163 ± 0.08	0.118 ± 0.07
RMNE (i.n.)	Brain/blood	0.948 ± 0.12	0.786 ± 0.14	0.492 ± 0.21	0.489 ± 0.09	0.318 ± 0.14
RNE (i.v.)	Brain/blood	0.054 ± 0.06	0.081 ± 0.08	0.136 ± 0.09	0.148 ± 0.05	0.296 ± 0.013

The rats were administered with 100 μCi ^{99m}Tc-risperidone and the radioactivity was measured in percent per gram of tissue of the administered dose. Each value is the mean ± S.E.M. of three estimations. Radioactivity was measured at 0 h and all the measurements were performed using 0 h sample of corresponding tissue/organ as blank sample.

Table 5
Pharmacokinetics of ^{99m}Tc-RS (i.n.), ^{99m}Tc-RNE (i.n.), ^{99m}Tc-RMNE (i.n.) and ^{99m}Tc-RNE (i.v.) at different time interval in normal Swiss albino rats

Formulation	Organ/tissue	C_{max} (%/g)	T_{max} (h)	$AUC_{0\to480\,min}$ (h%/g)	$AUC_{0\to\infty}$ (h%/g)	K_{el} (l/h)	$T_{1/2}$ (h)
RS (i.n.)	Blood	0.096 ± 0.04	2.0 ± 0.15	0.46 ± 0.16	0.48 ± 0.18	0.44 ± 0.11	1.58 ± 0.5
	Brain	0.039 ± 0.03	1.0 ± 0.1	0.14 ± 0.11	0.15 ± 0.12	0.62 ± 0.21	1.12 ± 0.52
RNE (i.n.)	Blood	0.172 ± 0.14	2.0 ± 0.20	0.78 ± 0.14	0.82 ± 0.22	0.40 ± 0.16	1.72 ± 0.46
	Brain	0.058 ± 0.04	1.0 ± 0.1	0.21 ± 0.12	0.21 ± 0.13	0.54 ± 0.18	1.28 ± 0.26
RMNE (i.n.)	Blood	0.191 ± 0.09	2.0 ± 0.15	0.87 ± 0.21	0.93 ± 0.43	0.37 ± 0.14	1.86 ± 0.47
	Brain	0.11 ± 0.06	1.0 ± 0.1	0.47 ± 0.13	0.48 ± 0.15	0.45 ± 0.23	1.54 ± 0.62
RNE (i.v.)	Blood	1.04 ± 0.03	0.5 ± 0.11	2.86 ± 0.25	2.91 ± 0.26	0.47 ± 0.15	1.47 ± 0.84
	Brain	0.067 ± 0.05	1.0 ± 0.1	0.32 ± 0.11	0.45 ± 0.12	0.28 ± 0.16	2.52 ± 0.63

The rats were administered with 100 μCi ^{99m}Tc-risperidone and the radioactivity was measured in per percent gram of tissue of the administered dose. Each value is the mean ± S.E.M. of three estimations.

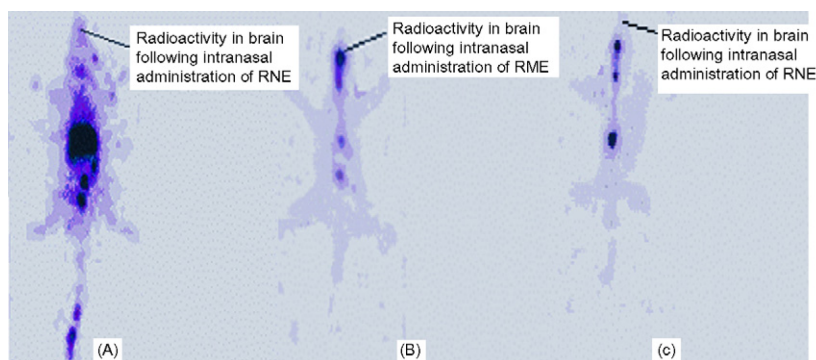

Fig. 4. Gamma scintigraphy images of rat (A/P view) showing the presence of radioactivity (A) RNE (i.v.), (B) RMNE (i.n.) and (C) RNE (i.n.).

Table 6
Drug targeting efficiency and direct nose to brain transport following intranasal administration of ^{99}Tc-RS, ^{99}Tc-RNE, ^{99}Tc-RMNE

Formulation and route of administration	Drug targeting efficiency (DTE (%))	Direct nose to brain transport (DTP (%))
RS (i.n.)	265 ± 2.51	62 ± 1.23
RNE (i.n.)	232 ± 1.92	57 ± 1.56
RMNE (i.n.)	476 ± 2.14	78 ± 1.31

Parameters derived using values of three different estimations and each value is mean ± S.E.M.

brain targeting efficiency and the findings are in consequence with reports of Qizhi et al. (2004) that mucoadhesive microemulsion increase nose to brain uptake of drugs.

In order to visualize brain uptake following intranasal and intravenous administrations of ^{99m}Tc RSP formulations, gamma scintigraphy was performed and the scintigrams of rats 0.5 h post i.v. administration of RNE and i.n. administration of RMNE. The scintigrams (Fig. 4A and B) clearly demonstrate the accumulation of formulations in brain administered via respective routes. Major radioactivity accumulation was seen in brain following intranasal administration of RMNE as compared to intravenous administration of RNE. Additionally, a part of activity was also noticed in oesophagus and in the abdominal region, which is in conformity with the results of biodistribution studies.

4. Conclusions

Significant quantity of risperidone was quickly and effectively delivered to the brain by intranasal administration of formulated mucoadhesive nanoemulsion of risperidone. The study conducted in rats clearly demonstrated effectiveness of intranasal delivery of risperidone as an antipsychotic agent, however clinical data is needed to evaluate the risk vs. benefit ratio.

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