JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 1199–1205

Received January 9, 2007
Accepted June 1, 2007
DOI 10.1211/jpp.59.9.0003
ISSN 0022-3573

Nose-to-brain delivery of tacrine

Viral V. Jogani, Pranav J. Shah, Pushpa Mishra, Anil Kumar Mishra and Ambikanandan R. Misra

Abstract

In the treatment of Alzheimer's disease tacrine, a cholinesterase inhibitor, is not the drug of choice due to its low oral bioavailability, extensive hepatic first-pass effect, rapid clearance from the systemic circulation, pronounced hepatotoxicity, and the availability of drugs better than tacrine in the same pharmacological class. Hence, the aim of this investigation was to ascertain the possibility of direct nose-to-brain delivery of tacrine to improve bioavailability, to avoid the first-pass effect and to minimize hepatotoxicity. Tacrine solution (TS) in propylene glycol was radiolabelled with ^{99m}Tc (technetium) and administered in BALB/c mice intranasally (i.n.) and intravenously (i.v.). Drug concentrations in blood and brain were determined at predetermined time intervals post dosing. Drug targeting efficiency (DTE %) and the brain drug direct transport percentage (DTP %) were calculated to evaluate the brain targeting efficiency. Brain scintigraphy imaging in rabbits was performed to ascertain the uptake of the drug into the brain. Tacrine solution was effectively labelled with ^{99m}Tc and was found to be stable and suitable for in-vivo studies. Following intranasal administration tacrine was delivered quickly (T_{max} 60 min) to the brain compared with intravenous administration (T_{max} 120 min). The brain/blood ratios of the drug were found to be higher for [^{99m}Tc]$TS_{i.n.}$ compared with [^{99m}Tc]$TS_{i.v.}$ at all time points. The DTE (207.23%) and DTP (51.75%) following intranasal administration suggested that part of tacrine was directly transported to brain from the nasal cavity. Rabbit brain scintigraphy imaging showed higher uptake of the drug into the brain following intranasal administration compared with intravenous administration. The results showed that tacrine could be directly transported into the brain from the nasal cavity and intranasal administration resulted in higher bioavailability of drug with reduced distribution into non-targeted tissues. This selective localization of tacrine in the brain may be helpful in reducing dose, frequency of dosing and dose-dependent side effects, and may prove an interesting new approach in delivery of the drug to the brain for the treatment of Alzheimer's disease.

Department of Pharmacy, Faculty of Technology & Engineering, The Maharaja Sayajirao University of Baroda, Post Box No. 51, Kalabhavan, Vadodara – 390 001, India

Viral V. Jogani, Pranav J. Shah, Ambikanandan R. Misra

Division of Radiopharmaceuticals and Radiation Biology, Institute of Nuclear Medicine and Allied Sciences, Brig S. K. Mazumdar Road, New Delhi-110 054, India

Pushpa Mishra, Anil Kumar Mishra

Correspondence: A. R. Misra, Department of Pharmacy, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Post Box No. 51, Kalabhavan, Vadodara – 390 001, Gujarat state, India. E-mail: misraan@hotmail.com or misraan@satyam.net.in

Acknowledgements: The authors thank Brig. (Retd.) Dr R. P. Tripathi, Director, Institute of Nuclear Medicine and Allied Sciences (INMAS), Ministry of Defence, New Delhi, India, for providing the necessary facilities to carry out the radiolabelling experiments. The authors also thank Ms Ankur Kaul, INMAS, New Delhi, India, for her assistance with the animal experiments.

Introduction

In the past decade, the use of the nasal cavity as a route for drug delivery has been an area of great interest and targeting the brain/central nervous system (CNS) via the nasal administration of drugs has been studied frequently (Frey 2002; Illum 2004; Vyas et al 2005). The direct anatomical connection between the nasal cavity and the CNS makes it possible to deliver many substances, including tracer materials, heavy metals, low molecular weight drugs and peptides into the CNS by circumventing the blood–brain barrier (BBB), which provides the basis for the development of therapeutic agents for intranasal administration (Illum 2000). Drugs have been shown to reach the CNS from the nasal cavity by direct transport across the olfactory and trigeminal neural pathways (Frey 2002; Misra et al 2003; Thorne et al 2004). It is the only site in the human body where the nervous system is in direct contact with the surrounding environment. Drugs administered by the intranasal route not only circumvent the BBB but also avoid the hepatic first-pass effect. Previous studies have demonstrated that intranasal administration offers a simple, practical, non-invasive, convenient, cost effective, and an alternative route for rapid drug delivery to the brain/CNS (Dragphia et al 1995; Liu et al 2001; Wermling et al 2001; Dorman et al 2002; Vyas et al 2005). Thus, direct transport of drugs to the brain/CNS circumventing the BBB following intranasal administration provides a unique feature and

a better option to target drugs to the brain/CNS (Illum 2000, 2004; Frey 2002; Graff & Pollack 2005; Vyas et al 2005).

Alzheimer's disease is a highly disabling neuropsychiatric disorder characterized by an irreversible deterioration of memory and intellectual behaviour. While the aetiology of Alzheimer's disease remains unknown, evidence has been presented that the hippocampus (an essential brain structure for memory and learning) is one of the principal areas affected by the disease (Marx 1991). A specific loss of cholinergic neurons and deficits of choline acetyltransferase have been suggested to play a major role in the primary cognitive symptoms of Alzheimer's disease. Decreased central cholinergic activity has received major attention from investigators in search of a biochemical approach that supports a pharmacotherapy for the disease. Inhibition of acetylcholinesterase is a promising approach and the most common method under investigation for the treatment of Alzheimer's disease (Giacobini 1993).

Tacrine (1,2,3,4-tetrahydro-9-aminoacridine), a potent, centrally active, reversible cholinesterase inhibitor, was the first drug approved by the US Food and Drug Administration in 1993 for treating the symptoms of mild to moderate Alzheimer's disease (Small 1992; Davis & Powchik 1995; Giacobini 1998) and is used perorally. However, peroral administration of tacrine is associated with low bioavailability, extensive hepatic first-pass effect, rapid clearance from the systemic circulation, a short elimination half-life (Telting-Diaz & Lunte 1993), large interindividual differences (Hartvig et al 1990; Lou et al 1996), a reversible dose-dependent hepatotoxicity and peripheral cholinergic side effects (O'Brien et al 1991; Farlow et al 1992; Sathyan et al 1995). Its clinical uses have been limited due to associated cholinergic, hepatic and gastrointestinal adverse reactions (Abramowicz 1993; Qizilbash et al 2000; Yang et al 2001). A recent study showed that gastrointestinal side effects, such as diarrhoea, anorexia, dyspepsia and abdominal pain, and raised serum liver enzymes were the major reasons for its withdrawal (Qizilbash et al 2000). Previous experience with the nasal delivery of neuropeptides (Gozes et al 1996) and neurotropic factors (Frey et al 1997; Chen et al 1998; Capsoni et al 2002; Thorne et al 2004; De Rosa et al 2005), and monosialoganglioside (GM1) (Kumbale et al 1999) to rats has shown that the nose could be a possible administration route for these potential drugs in treating Alzheimer's disease. Therefore, the nasal route for delivering tacrine to the brain appears to be an attractive alternative to the conventional administration route for the management of this disease. Hence, the aim of this investigation was to ascertain the possibility of direct nose-to-brain delivery of tacrine following intranasal administration to avoid first-pass effect and to minimize hepatotoxicity. The concentration profiles of tacrine in blood and brain after intranasal and intravenous administration in mice were determined to find out whether the nasal route could be used to transport tacrine directly from the nasal cavity to the brain. It was hypothesized that selective localization of tacrine in brain was likely to minimize dose-dependent side effects, dose and frequency of dosing, and probably provide a new life to an otherwise abandoned drug.

Materials and Methods

Materials

Tacrine HCl (log P 2.71, pK_a 9.95, MW 234.7, freely soluble in water (Clarke's Analysis of Drugs and Poisons 2003)) was purchased from Sigma (St Louis, MO). Propylene glycol (AR Grade) was purchased from ISP Technologies (Mumbai, India). Diethylene triamine penta acetic acid (DTPA) and stannous chloride dihydrate ($SnCl_2.2H_2O$) were purchased from Sigma (St Louis, MO). Sodium pertechnetate, separated from molybdenum-99 (99 m) using a solvent extraction method, was provided by the Regional Center for Radiopharmaceutical Division (Northern Region), Board of Radiation and Isotope Technology (BRIT, Delhi, India). All other chemicals and solvents were of analytical reagent grade and were used without further purification.

Radiolabelling of tacrine

Tacrine (obtained from HCl salt, solubility in water and propylene glycol 0.25 and 252 mg mL^{-1}, respectively (Sathyan et al 1995)), was dissolved in propylene glycol (33 mg mL^{-1}). The resultant tacrine solution was radiolabelled using ^{99m}Tc by a direct labelling method (Eckelman et al 1995; Babbar et al 2000; Mishra et al 2004). $SnCl_2.2H_2O$ solution (200 μg in 100 μL of 10%, v/v, acetic acid) was added to 1 mL tacrine solution. The pH was adjusted to 6.50 ± 0.20 using 0.5 M sodium bicarbonate solution (200 μL). To the resultant mixture, 1 mL sterile ^{99m}Tc-pertechnetate (75–400 MBq) was added gradually over a period of 60 s with continuous mixing. The mixture was incubated at room temperature for 30 min with continuous nitrogen purging. The final volume was made up to 2.50 mL using 0.90% (w/v) sodium chloride (normal saline) solution. The radiochemical purity (Saha 1993, 2005) of [^{99m}Tc]tacrine solution was assessed using ascending instant thin-layer chromatography. Silica gel-coated fibre glass sheets (Gelman Sciences, Inc., Ann Arbor, MI) were used as the stationary phase and dual solvent systems consisting of acetone and pyridine:acetic acid:water (3: 5: 1.5, v/v) were used as the mobile phases. The effects of incubation time, pH, and $SnCl_2.2H_2O$ concentrations on radiolabelling efficiency were studied to achieve optimum reaction conditions (Saha 1993, 2005; Babbar et al 2000). The radiolabelled formulations were challenged for bonding strength using DTPA (Theobald 1990). The optimized radiolabelled formulations were assessed for in-vitro stability in normal saline solution and in mouse serum (Garron et al 1991). Consequently, the optimized stable radiolabelled formulations were used for in-vivo studies.

Biodistribution studies

All experiments conducted on animals were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India, New Delhi, India.

BALB/c mice (30–40 g) were selected for the study on the basis of a randomized technique. Three mice for each

formulation per time point were used. The radiolabelled complex of [^{99m}Tc]tacrine solution (100 μCi/50 μL) containing 0.039–0.052 mg tacrine (equivalent to 1.3 mg kg^{-1} body weight) was injected intravenously through the tail vein. Similarly, the radiolabelled complex of [^{99m}Tc]tacrine solution (100 μCi/10 μL) containing 0.039–0.052 mg tacrine (equivalent to 1.3 mg kg^{-1}) was administered (5 μL) in each nostril. Before nasal administration, the mice were partially anaesthetized by diethyl ether and the formulations were instilled into the nostrils with the help of a micropipette (10 μL) attached with low density polyethylene tube (0.1-mm i.d.) at the delivery site and inserted 0.25-cm deep in the nostrils. The mice were held from the back in a slanted position during nasal administration of the formulations. Blood was collected using cardiac puncture at predetermined time intervals (15, 30, 60, 120, 240, and 480 min). After collecting the blood, the mice were killed by exposure to diethyl ether. Subsequently, different tissues/organs including the brain were dissected, washed twice using normal saline solution, and made free from adhering tissue/fluid and weighed. The radioactivity present in each tissue/organ was measured using a shielded well-type γ-scintillation counter (Capintec Inc., New Jersey). The radiopharmaceutical uptake per gram in each tissue/organ was calculated as a fraction of administered dose (Babbar et al 2000). The results of the radioactivity in blood and brain are recorded in Table 1. The pharmacokinetic parameters were derived from Table 1 and Figure 1 using WinNonlin software (version 5.0.1, Pharsight Corporation, NC) and recorded in Table 2. To evaluate the brain targeting efficiency, two indices (DTE % and DTP %) were adopted as mentioned below (Chow et al 1999; Zhang et al 2004; Vyas et al 2006).

Drug targeting efficiency (DTE %)

DTE (%) represents the time average partitioning ratio:

$$DTE\ (\%) = \frac{(AUC_{brain}/AUC_{blood})_{i.n.}}{(AUC_{brain}/AUC_{blood})_{i.v.}} \times 100 \quad (1)$$

The brain drug direct transport percentage (DTP %)

To define nose-to-brain direct transport clearly, DTP (%), which was derived from equations 2 and 3 was calculated:

$$DTP\ (\%) = \frac{B_{i.n.} - B_X}{B_{i.n.}} \times 100 \quad (2)$$

$$B_x = (B_{i.v.}/P_{i.v.}) \times P_{i.n.} \quad (3)$$

Where, B_x = brain AUC fraction contributed by systemic circulation through the BBB following intranasal administration; $B_{i.v.} = AUC_{0\Rightarrow480}$ (brain) following intravenous administration; $P_{i.v.} = AUC_{0\Rightarrow480}$ (blood) following intravenous administration; $B_{i.n.} = AUC_{0\Rightarrow480}$ (brain) following intranasal administration; $P_{i.n.} = AUC_{0\Rightarrow480}$ (blood) following intranasal administration; and AUC = area under the curve for blood/brain concentration vs time.

Reports in the literature revealed that the drug uptake into the brain from the nasal mucosa mainly occurred via three different pathways (Thorne et al 2004; Vyas et al 2005). The first was the systemic pathway by which some of the drug was absorbed into the systemic circulation and subsequently reached the brain by crossing the BBB. The other two were the olfactory and the trigeminal neural pathways by which part of the drug was transported directly from the nasal cavity to CSF and brain tissue (Illum 2000; Thorne et al 2004). We could deduce that the amount of drug that reached the brain tissue after nasal administration was attributed to these two parts. Since the amount of drug in blood was proportional to the AUC, we could assume that the brain AUC fraction contributed by systemic circulation through the BBB (represented by B_x), divided by blood AUC from the nasal route was equal to the same ratio of that of the intravenous route (see equation 3). Therefore, DTP (%) represented the percentage of drug directly transported to the brain via the olfactory pathway and the trigeminal neural pathway. DTP (%) and DTE (%) were calculated using tissue/organ distribution data following intranasal and intravenous administration.

Table 1 Distribution of [^{99m}Tc]tacrine solution following intranasal ([^{99m}Tc]TS$_{i.n.}$) and intravenous ([^{99m}Tc]TS$_{i.v.}$) administration in BALB/c mice* at predetermined time intervals

Sampling time points (min)	[^{99m}Tc]TS$_{i.v.}$			[^{99m}Tc]TS$_{i.n.}$		
	Blood	Brain	Brain/blood ratio	Blood	Brain	Brain/blood ratio
15	4.17 ± 0.47	0.28 ± 0.08	0.07 ± 0.02	0.71 ± 0.05	0.46 ± 0.07	0.65 ± 0.13
30	3.58 ± 0.36	0.33 ± 0.10	0.09 ± 0.03	0.82 ± 0.04	0.48 ± 0.06	0.59 ± 0.10
60	2.45 ± 0.32	0.36 ± 0.08	0.15 ± 0.04	1.13 ± 0.12	0.53 ± 0.07	0.47 ± 0.01
120	1.75 ± 0.25	0.41 ± 0.05	0.24 ± 0.05	1.23 ± 0.18	0.50 ± 0.06	0.41 ± 0.01
240	0.84 ± 0.14	0.29 ± 0.05	0.35 ± 0.09	0.80 ± 0.12	0.38 ± 0.06	0.48 ± 0.07
480	0.37 ± 0.04	0.11 ± 0.02	0.30 ± 0.06	0.43 ± 0.06	0.14 ± 0.03	0.33 ± 0.09

*The mice were administered 100 μCi [^{99m}Tc]tacrine solution and the radioactivity was measured in percent per gram of tissue of the administered dose. Radioactivity was measured at 0 min and all the measurements were performed using 0 min sample of corresponding tissue/organ as blank sample. Each value is the mean ± s.d. (n = 3).

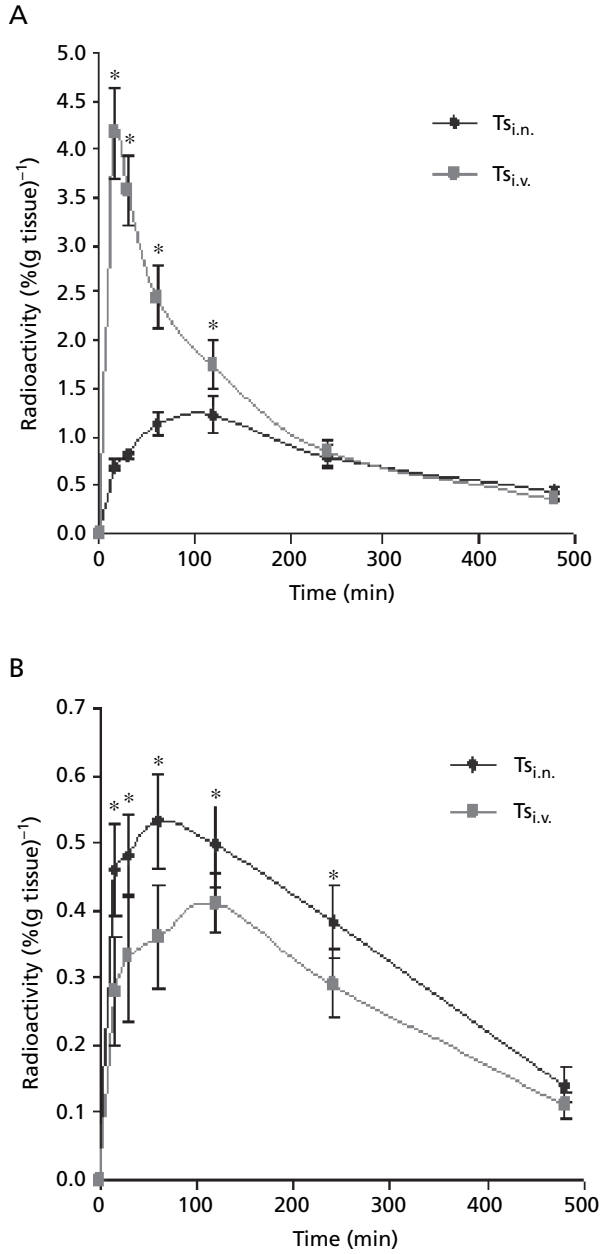

Figure 1 A. Tacrine concentration in blood at different time intervals following [^{99m}Tc]$TS_{i.v.}$ or [^{99m}Tc]$TS_{i.n.}$ administration in mice (n = 3). *$P < 0.05$ compared with $TS_{i.n.}$. B. Tacrine concentration in brain at different time intervals following [^{99m}Tc]$TS_{i.v.}$ or [^{99m}Tc]$TS_{i.n.}$ administration in mice (n = 3). *$P < 0.05$ compared with $TS_{i.v.}$. Mice were administered 100 μCi radioactivity.

γ-Scintigraphy imaging

New Zealand rabbits (2.00–2.50 kg) were selected and the radiolabelled complex of [^{99m}Tc]tacrine solution (100 μCi/100 μL) containing 0.94–1.18 mg tacrine (equivalent to 0.47 mg kg^{-1}) was intravenously injected through the ear vein. Similarly, the radiolabelled complex of [^{99m}Tc]tacrine solution (100 μCi/100 μL) containing 0.94–1.18 mg tacrine (equivalent to 0.47 mg kg^{-1}) was administered intranasally (50 μL in each nostril). The formulation was administered with the help of a micropipette (100 μL) attached with a low density polyethylene tube (0.1-mm i.d.) at the delivery site and inserted 1-cm deep in the nostrils. The rabbits were held from the back in a slanted position during nasal administration. The rabbits were anaesthetized with 1 mL ketamine hydrochloride (50 mg mL^{-1}, i.m.) and placed on the imaging platform. Imaging was performed using single photon emission computerized tomography (SPECT, LC 75–005, Diacam, Siemens AG; Erlanger, Germany) gamma camera (Capala et al 1997; Babbar et al 2000). The scintigraphy images 15-min post intranasal and intravenous administration of [^{99m}Tc]tacrine solution are shown in Figure 2.

Statistical analysis

All data are reported as mean ± s.d. The differences between the groups were tested using analysis of variance and $P < 0.05$ was considered statistically significant.

Results and Discussion

Radiolabelling of tacrine

Tacrine solution was effectively radiolabelled with ^{99m}Tc and optimized for maximum labelling efficiency and stability. Radiochemical purity achieved was 96.35% when evaluated for reduced/hydrolysed ^{99m}Tc and free ^{99m}Tc. The optimal $SnCl_2.2H_2O$ concentration was found to be 200 μg mL^{-1} at pH 6.50 with an incubation time of 30 min. [^{99m}Tc]Tacrine solution ([^{99m}Tc]TS) was found to be stable in normal saline solution and in mouse serum up to 24 h (degradation < 5%, w/w). Bonding strength of [^{99m}Tc]tacrine solution was investigated by the DTPA challenge test, and the percent transchelation of the labelled complex was 1.58%, w/w, at 25 mM DTPA, while at 100 mM, it increased to 4.78%, w/w. The results suggested high bonding strength and stability of [^{99m}Tc]tacrine solution. Thus, this complex was found suitable for biodistribution studies of the drug in-vivo.

Biodistribution studies

Biodistribution studies of [^{99m}Tc]tacrine solution following intravenous and intranasal administration in BALB/c mice were performed and the radioactivity was estimated at predetermined time intervals up to 480 min. The results obtained are recorded in Table 1. The brain/blood ratio of the drug at all time points was also calculated and recorded in Table 1. The pharmacokinetic parameters were calculated from Figure 1 and recorded in Table 2. Following intravenous administration, tacrine attained the peak blood level (C_{max} 4.17 ± 0.47% radioactivity (g tissue)$^{-1}$) within 15 min. Comparatively, after nasal administration the C_{max} (1.23 ± 0.18% radioactivity (g tissue)$^{-1}$) was attained at 120 min, far lower than that after intravenous administration. During the period of 15–480 min, drug concentrations in blood after intranasal administration were all significantly ($P < 0.05$) lower than those after intravenous injection except the last two time points (240 and

Table 2 Pharmacokinetics of [^{99m}Tc]$TS_{i.v.}$ and [^{99m}Tc]$TS_{i.n.}$ in BALB/c mice*

Pharmacokinetic parameter	[^{99m}Tc]$TS_{i.v.}$		[^{99m}Tc]$TS_{i.n.}$	
	Blood	Brain	Blood	Brain
C_{max} (% radioactivity g^{-1})	4.17 ± 0.47	0.41 ± 0.03	1.23 ± 0.18	0.53 ± 0.10
T_{max} (min)	15 ± 0	120 ± 0	120 ± 0	60 ± 0
$AUC_{0\Rightarrow480}$ (min % radioactivity g^{-1})	606.45 ± 75.66	130.13 ± 21.13	386.25 ± 46.17	171.75 ± 12.16
$AUC_{0\Rightarrow\infty}$ (min % radioactivity g^{-1})	683.29 ± 87.06	160.37 ± 28.93	533.44 ± 64.77	211.63 ± 4.33
K_{el} (L min^{-1})	0.0045 ± 0.0003	0.0037 ± 0.0003	0.0029 ± 0.0002	0.0036 ± 0.0009
t½ (min)	155.03 ± 11.90	186.85 ± 16.57	241.36 ± 14.49	191.20 ± 43.57
Bioavailability (%)	–	–	63.69 ± 1.98	131.99 ± 20.69

*The mice were administered 100 μCi [^{99m}Tc]tacrine solution and the radioactivity was measured in percent per gram of tissue of the administered dose. Each value is the mean ± s.d. (n = 3).

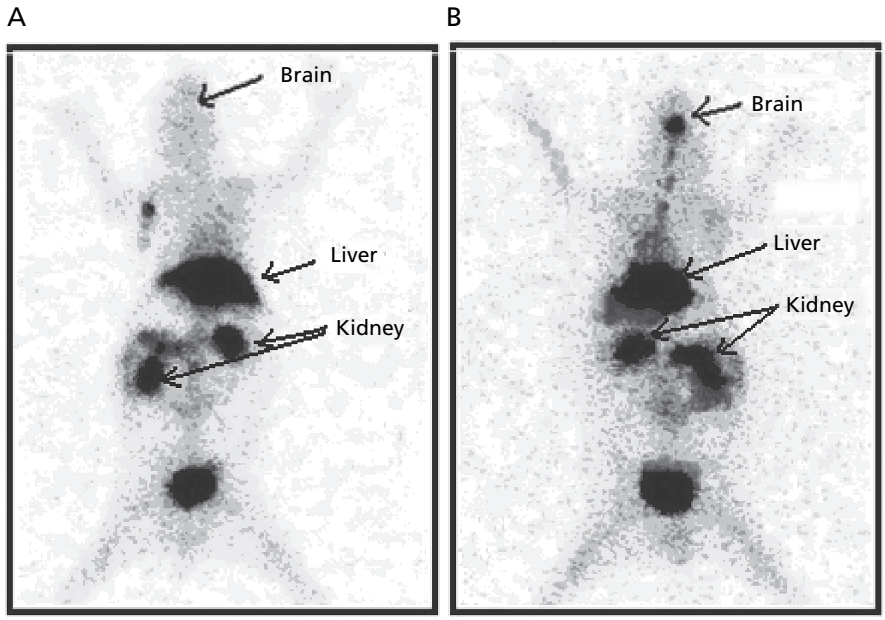

Figure 2 γ-Scintigraphy images of rabbit showing the presence of radioactivity in the brain after intravenous (A; [^{99m}Tc]$TS_{i.v}$ 100 μCi) or intranasal (B; [^{99m}Tc]$TS_{i.n.}$ 100 μCi) administration of [^{99m}Tc]tacrine solution.

480 min). At 240 and 480 min the difference in blood concentrations of the drug following intranasal and intravenous administration was insignificant. The AUC of drug concentration curves in blood were calculated, and the absolute bioavailability of tacrine obtained following intranasal administration was 63.69 ± 1.98% (Table 2) compared with the reported oral and regular route of administration bioavailability of 17% (Drug Facts and Comparisons 1997).

After nasal administration of [^{99m}Tc]tacrine solution, tacrine was delivered to the brain quickly (T_{max} 60 min vs 120 min for intravenous administration). These results were suggestive of preferential nose-to-brain transport of tacrine following nasal administration. The brain/blood ratios of the drug were found to be higher for intranasally administered tacrine compared with intravenous administration at all time points. This further confirmed direct nose-to-brain transport of tacrine (Pietrowky et al 1996; Chow et al 1999). The concentrations of the drug in brain following intranasal administration were found to be significantly higher at all sampling time points compared with intravenous administration up to 480 min, and the bioavailability in brain after intranasal administration was 131.99 ± 20.69% (Table 2). The substantially higher uptake in the brain with intranasal administration suggested a larger extent of selective transport of tacrine from nose-to-brain. Many researchers (Fehm 2000; Quay 2001) have reported a unique connection between the nose and the brain and intranasal delivery of drugs to the brain bypassing the blood–brain barrier (Illum 2000; Thorne et al 2004; Graff & Pollack 2005; Vyas et al 2005). The half-life (t½) and K_{el} of drug in blood was found to be significantly different for

intranasal and intravenous administration, but insignificant differences in these values were observed in brain for both routes of administration (Table 2). These differences in the results may have been due to more selective distribution of the drug to the brain after intranasal administration.

To evaluate the brain targeting efficiency, DTE (%) and DTP (%) were calculated. Following intranasal administration, the DTE (%) of tacrine was 207.23, far greater than 1, which suggested there was a great portion of tacrine targeting to the brain following intranasal administration. The results of DTP (%) calculation further demonstrated that approximately 51.75% of tacrine content within 480 min in the brain was transported directly from nose to brain. These data showed the existence of an alternative transport pathway for tacrine to the brain other than penetration across the blood–brain barrier from the systemic circulation. Thus, tacrine nasal administration might have the characteristic of brain targeting.

Mechanisms responsible for the direct nose-to-brain transport of nasally applied substances are not clear. One suggested anatomical pathway for the direct transport of foreign compounds from the nasal cavity to the brain theorizes that foreign compounds enter into the olfactory sensory neurons by endocytosis or by binding to surface receptors and subsequently undergoing adsorptive endocytosis. The compounds could thereafter be transported within the olfactory sensory neurons by the axoplasmic flow. Neuronal transport is generally believed to be a slow process (Thorne & Frey 2001). However, this pathway is not likely to explain the observed rapid delivery of tacrine to the brain following nasal administration. Another plausible explanation is that foreign substances could diffuse into the nasal submucosa and subsequently travel into the olfactory perineuronal channels to reach the brain parenchymal tissues (Thorne & Frey 2001). Being a highly lipophilic drug (log P 2.71) with a low molecular weight (234.7 kDa), tacrine is expected to travel by this extraneuronal epithelial pathway for direct nose-to-brain delivery. Kumbale et al (1999) reported the direct nose-to-brain delivery of intranasally administered low molecular weight, lipophilic molecules, such as GM1 monomers, via extracellular olfactory pathway. However, more studies are needed to define the exact mechanism for direct nose-to-brain transport of tacrine.

γ-Scintigraphy imaging

γ-Scintigraphy images of rabbit at 15-min post-dosing are shown in Figure 2. Significantly higher radioactivity was noticed in the rabbit brain following intranasal administration of tacrine compared with intravenous administration. Scintigraphy images were consistent with the biodistribution data shown in Table 1.

Conclusion

Nasal administration of tacrine could provide direct nose-to-brain transport resulting in higher bioavailability with reduced distribution into non-targeted tissues. This investigation demonstrated that nasal administration could provide selective localization of tacrine in brain and hence, may be helpful in reducing the dose, frequency of dosing, and the dose-dependent systemic side effects. Hence, the nasal route could be a viable alternative to the currently used oral route. To conclude, nose-to-brain delivery of tacrine may be a promising and attractive new approach for delivery of tacrine, which otherwise finds limited clinical use due to its low bioavailability and pronounced side effects, for the treatment of Alzheimer's disease.

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