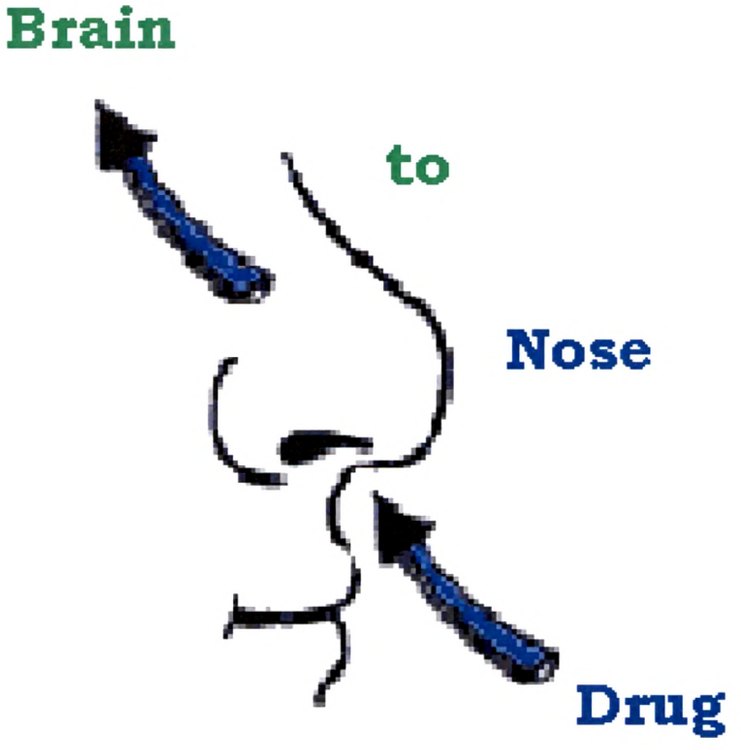

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Book Chapters

1. **Jogani V**, Shah P, Misra A, Role of Herbal Absorption Promoters in Oral Delivery of Therapeutics – In ***Recent Progress in Medicinal Plants***, Vol. 14-Biopharmaceuticals, Chapter 27. 503-536, **2006.**

Publications

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2. **Jogani V**, Shah P, Mishra A, Mishra P, Misra A, Intranasal Mucoadhesive Microemulsion of Tacrine to Improve Brain Targeting, ***Alzheimer's Disease and Associated Disorders*, Accepted.**
3. Shah P, **Jogani V**, Bagchi T, Mishra A, Mishra P, Misra A, Modulation of Ganciclovir Intestinal Absorption in Presence of Absorption Enhancers, ***Journal of Pharmaceutical Sciences*, In Press.**
4. Shah P, **Jogani V**, Bagchi T, Mishra A, Mishra P, Misra A, Role of ^{99m}Tc-Mannitol and ^{99m}Tc-PEG in Assessment of Paracellular Integrity of Cell Monolayers, ***Nuclear Medicine Communications*, In Press.**
5. Shah P, **Jogani V**, Bagchi T, and Misra A, Role of Caco-2 Cell Monolayers in Prediction of Intestinal Drug Absorption, ***Biotechnology Progress***, 22, 186-198, **2006.**

In communication

1. Recent Patents Review on Intranasal administration for CNS Drug Delivery, ***Recent Patents on Drug Delivery and Formulation.***
2. Shah P, **Jogani V**, Bagchi T, Mishra A, Mishra P, Misra A, In Vitro Assessment of Transepithelial Permeation of Acyclovir in presence of Absorption Enhancers, ***Drug Development and Industrial Pharmacy.***

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1. Role of Intranasal Delivery in Alzheimer's disease
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1. Influence of Permeation Enhancers on the Nasomucosal Delivery of Insulin, at *56th Indian Pharmaceutical Congress* ***2004***, Calcutta (Co-author).
2. Nanotechnology and Nose to Brain Drug Delivery: Myth or reality? At National Symposium on *Exploring Nanotechnology in Drug Delivery, 29th and 30th July,* ***2005***, Pharmacy Department, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Vadodara.
3. Intranasal Mucoadhesive Microemulsions of Sumatriptan: Investigations On Brain Targeting Efficiency, *Gujarat Council on Science and Technology (GUJCOST) sponsored State Level Pharmacy Paper Presentation Competition, 18th and 19th February,* ***2006***, at K. B. Institute of Pharmaceutical Education and Research, Gandhinagar **(First Prize).**
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Nose to Brain Delivery of Tacrine

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Abstract

Tacrine, cholinesterase inhibitor, is not drug of choice in the treatment of Alzheimer's disease (AD) due to low oral bioavailability, extensive hepatic first pass effect, rapid clearance from systemic circulation, pronounced hepatotoxicity and availability of better drugs of the same pharmacological class. Hence, the aim of the investigation was to ascertain possibility of direct nose to brain delivery of tacrine to improve bioavailability, to avoid first pass effect and to minimize hepatotoxicity. Tacrine solution (TS) in propylene glycol was radiolabeled 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 also performed to ascertain the uptake of the drug into the brain. TS was effectively labeled with ^{99m}Tc and was found to be stable and suitable for *in vivo* studies. Following i.n. administration tacrine was delivered quickly (T_{max} – 60min) to the brain compared to i.v. administration (T_{max} – 120min). The brain/blood ratios of the drug were found to be higher for ^{99m}Tc-$TS_{i.n.}$ compared to ^{99m}Tc-$TS_{i.v.}$ at all time points. The DTE (%) (207.23 %) and DTP (%) (51.75 %) following i.n. administration suggested that part of tacrine is directly transported to brain from nasal cavity. Rabbit brain scintigraphy imaging also showed higher uptake of the drug into the brain following i.n. administration compared to i.v. administration. The results of this investigation showed that tacrine can be directly transported into the brain from nasal cavity and i.n. administration results in higher bioavailability of drug with reduced distribution into non-targeted tissues. This selective localization of tacrine in brain may be helpful in reducing the dose, frequency of dosing and the dose dependent side effects and may prove an interesting new approach in delivery of the drug in brain for the treatment of AD.

Key Words: Nose to Brain, Tacrine, Blood Brain Barrier, Alzheimer's disease, Cholinesterase inhibitors

Introduction

In the last one 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 i.n. administration (Illum 2000). Drugs have been shown to reach the CNS from the nasal cavity by a direct transport across the olfactory and trigeminal neural pathways (Frey 2002; 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 i.n. route not only circumvent the BBB but also avoid the hepatic first pass effect. Previous studies have demonstrated that i.n. administration offers a simple, practical, noninvasive, convenient, cost effective, and an alternative route for rapid drug delivery to the brain/CNS (Dragphia et al 1995; Wermling et al 2001; Liu et al 2001; Dorman et al 2002; Vyas et al 2005). Thus, direct transport of drugs to the brain/CNS circumventing the BBB following i.n. administration provides a unique feature and better option to target drugs to brain/CNS (Illum 2000; Frey 2002; Illum 2004; Vyas et al 2005; Graff & Pollack 2005).

AD is a highly disabling neuropsychiatric disorder characterized by an irreversible deterioration of memory and intellectual behavior. While the etiology of AD 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 AD (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 the disease. Decreased central cholinergic activity has received major attention from investigators in search of 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 AD (Giacobini 1993).

Tacrine (1,2,3,4-tetrahydro-9-aminoacridine), a potent, centrally active, reversible cholinesterase inhibitor, was the first drug approved by the USFDA in 1993 for treating the symptoms of mild to moderate AD (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 inter individual 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 had shown that gastrointestinal side effects, such as diarrhea, 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 WH II 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 AD. Therefore, the nasal route for delivering tacrine to the brain appears to be an attractive alternative to conventional administration route for the management of AD. Hence, the aim of this investigation was to ascertain the possibility of direct nose to brain delivery of tacrine following i.n. administration to avoid first pass effect and to minimize hepatotoxicity. The concentration profiles of tacrine in blood and brain after i.n. and i.v. administration in mice were determined to find out whether the nasal route could be used to transport tacrine directly form nasal cavity to the brain. It was hypothesized that selective localization of tacrine in brain is likely to minimize dose dependent side effects, dose and frequency of dosing, and probably provide a new life to otherwise abandoned drug.

Materials and Methods

Materials

Tacrine HCl (log P – 2.71; pKa – 9.95; Mole. Wt. – 234.7; freely soluble in water (Clarke's Analysis of Drugs and Poisons 2003)) was purchased from Sigma (St. Louis, MO, USA). 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, USA). Sodium pertechnetate, separated from molybdenum-99 (99 m) using a solvent extraction method, was provided by 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.

Radiolabeling of Tacrine

Tacrine (obtained from HCl salt, solubility in water and propylene glycol 0.25 and 252 mg/mL respectively (Sathyan et al 1995)) was dissolved in propylene glycol (33 mg/mL). The resultant TS was radiolabeled using ^{99m}Tc by direct labeling method (Eckelman et al 1995; Babbar et al 2000; Mishra et al 2004). One milliliter of TS was taken and $SnCl_2.2H_2O$ solution (200 µg in 100 µL of 10% v/v Acetic Acid) was added. The pH was adjusted to 6.50 ± 0.20 using 0.5 M sodium bicarbonate solution (200 µL). To the resultant mixture, 1 mL of sterile ^{99m}Tc-pertechnetate (75 to 400 MBq) was added gradually over a period of 60s with continuous mixing. The mixture was incubated at room temperature for 30min 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; Saha 2005) of ^{99m}Tc-TS was assessed using ascending instant thin layer chromatography. Silica gel coated fiber glass sheets (Gelman Sciences, Inc., Ann Arbor, MI) were employed as stationary phase and dual solvent systems consisting of acetone and pyridine: acetic acid: water (3: 5: 1.5 v/v) were used as mobile phases. The effects of incubation time, pH, and $SnCl_2.2H_2O$ concentrations on radiolabeling efficiency were studied to achieve optimum reaction conditions (Saha 1993; Babbar et al 2000; Saha 2005). The radiolabeled formulations were challenged for bonding strength using DTPA (Theobald 1990). The optimized

radiolabeled formulations were assessed for *in vitro* stability in normal saline solution and in mice serum (Garron et al 1991). Consequently, the optimized stable radiolabeled 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 weighing between 30 and 40 g were selected for the study on the basis of randomization technique. Three mice for each formulation per time point were used in the study. The radiolabeled complex of ^{99m}Tc-TS (100 μCi/50 μL) containing 0.039–0.052 mg Tacrine (equivalent to 1.3 mg/ kg body weight (B.W.)) was injected i.v. through tail vein of mice. Similarly, radiolabeled complex of ^{99m}Tc-TS (100 μCi/10 μL) containing 0.039–0.052 mg Tacrine (equivalent to 1.3 mg/kg B.W.) was administered (5 μL) in each nostril. Prior to nasal administration of the formulations, the mice were partially anaesthetized by diethyl ether and the formulations were instilled into the nostrils with the help of micropipette (10 μL) attached with low density polyethylene tube having 0.1 mm internal diameter at the delivery site and inserted 0.25 cm deep in the nostrils. The mice were held from the back in slanted position during nasal administration of the formulations. Blood was collected using cardiac puncture at predetermined time intervals (15, 30, 60, 120, 180, 240, and 480min). After collecting the blood, the mice were sacrificed with mercy by exposure to diethyl ether. Subsequently, different tissues/organs including 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 shielded well-type gamma scintillation counter (Capintec Inc., New Jersey, USA). The radiopharmaceutical uptake per gram in each tissue/organ was calculated as a fraction of administered dose (Babbar et al 2000). The results of radioactivity in blood and brain are recorded in Table 1. The pharmacokinetic parameters were derived from Table 1 and Figure 1(A) and (B) using WinNonlin® software (version 5.0.1, Pharsight Corporation, North Carolina, USA) 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 time average partitioning ratio.

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

The brain drug direct transport percentage (DTP (%)): In order to define nose to brain direct transport clearly, DTP (%); which has been derived from equations (2) and (3) was calculated.

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

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

Where,

B_x = Brain AUC fraction contributed by systemic circulation through the BBB following i.n. administration.

$B_{i.v.}$ = $AUC_{0\rightarrow480}$ (brain) following i.v. administration.

$P_{i.v.}$ = $AUC_{0\rightarrow480}$ (blood) following i.v administration.

$B_{i.n.}$ = $AUC_{0\rightarrow480}$ (brain) following i.n. administration.

$P_{i.n.}$ = $AUC_{0\rightarrow480}$ (blood) following i.n. administration.

AUC = Area under the curve for blood/brain concentration vs. time.

Reports in the literature reveal that the drug uptake into the brain from the nasal mucosa mainly occurs via three different pathways (Thorne et al 2004; Vyas et al 2005). One is the systemic pathway by which some of the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing BBB. The others are the olfactory and the trigeminal neural pathway by which part of the drug is transported directly from the nasal cavity to CSF and brain tissue (Illum 2000; Thorne et al 2004). We can deduce that the amount of drug that reaches in the brain tissue after nasal administration is attributed to these two parts. Since, the amount of drug in blood is proportional to AUC, we can assume that the brain AUC fraction contributed by systemic circulation through BBB (represented by B_x), divided by blood AUC from the nasal route is equal to the same ratio of that of the i.v. route (see Equation (3)). Therefore, DTP (%) represents 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 i.n. and i.v. administrations.

Gamma Scintigraphy Imaging

The New Zealand rabbits (2.00–2.50 kg) were selected for the study. The radiolabeled complex of ^{99m}Tc-TS (100 μCi/100 μL) containing 0.94–1.18 mg Tacrine (equivalent to 0.47 mg/kg B.W.) was i.v. injected through the ear vein of the rabbit. Similarly, the radiolabeled complex of ^{99m}Tc-TS (100 μCi/100 μL) containing 0.94–1.18 mg Tacrine (equivalent to 0.47 mg/kg B.W.) was administered i.n. (50 μL in each nostril). Formulation were administered with the help of micropipette (100 μL) attached with low density polyethylene tube having 0.1 mm internal diameter at the delivery site and inserted 1 cm deep in nostrils. The rabbits were held from the back in slanted position during nasal administration of formulations. The rabbits were anaesthetized using 1 mL ketamine hydrochloride intramuscular injection (50 mg/mL) 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 minutes post i.n. and i.v. administrations of ^{99m}Tc-TS are shown in Figure 2 (A) and (B).

Statistical Analysis

All data are reported as mean ± SD and the difference between the groups were tested using ANOVA and value of $p<0.05$ was considered statistically significant.

Results and Discussion

Radiolabeling of Tacrine

TS was effectively radiolabeled with ^{99m}Tc and optimized for maximum labeling efficiency and stability. Radiochemical purity achieved was 96.35% when evaluated for reduced/hydrolyzed ^{99m}Tc and free ^{99m}Tc. The optimal $SnCl_2.2H_2O$ concentration was found to be 200 μg/mL at pH 6.50 with an incubation time of 30min. ^{99m}Tc-TS was found to be

stable in normal saline solution and in mice serum up to 24h (degradation <5% w/w). Bonding strength of ^{99m}Tc-TS was also investigated by the DTPA challenge test, and the percent transchelation of the labeled complex was 1.58 % w/w at 25mM DTPA concentration, while at 100mM, it increased to 4.78 % w/w. The results suggested high bonding strength and stability of ^{99m}Tc-TS. Thus, this complex was found suitable for biodistribution studies of the drug *in vivo*.

Biodistribution studies

Biodistribution studies of ^{99m}Tc-TS following i.v. and i.n. administration in BALB/c mice were performed and the radioactivity was estimated at predetermined time intervals up to 480min. 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 (A) and (B) and recorded in Table 2.

Following i.v. administration, tacrine attained the peak blood level (C_{max} – 4.17 ± 0.47 % radioactivity/g of tissue) within 15min. Comparatively, after nasal administration; the C_{max} (1.23 ± 0.18 % radioactivity/g of tissue) was attained at 120min, far lower than that of i.v. administration. During the period of 15 to 480min, drug concentrations in blood after i.n. administration were all significantly ($p<0.05$) lower than those after i.v. injection except the last two time points i.e. 240 and 480min. At 240 and 480min the difference in blood concentrations of the drug following i.n. and i.v. administration was insignificant. The AUC of drug concentration curves in blood were calculated, and the absolute bioavailability of tacrine obtained following i.n. administration was 63.69 ± 1.98% (Table 2) compared to reported oral and regular route of administration bioavailability of 17% (Drug Facts and Comparisons 1997).

After nasal administration of ^{99m}Tc-TS, tacrine was delivered to the brain quickly (T_{max} – 60min versus 120min for i.v. administration). These results are 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 i.n. administered tacrine compared to i.v. administration at all time points. This further confirms direct nose to brain transport of tacrine (Chow et al 1999; Pietrowky et al 1996). The concentrations of the drug in brain following i.n.

administration were found to be significantly higher at all sampling time points compared to i.v. administration up to 480min and the bioavailability in brain after i.n. administration was 131.99 ± 20.69% (Table 2). The substantially higher uptake in the brain with i.n. administration suggests 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 i.n. delivery of drugs to the brain bypassing the BBB (Illum 2000; Thorne et al 2004; Vyas et al 2005; Graff & Pollack 2005). The $T_{1/2}$ and K_{el} of drug in blood was found to be significantly different for i.n. and i.v. administrations, but insignificant differences in these values were observed in brain for both of the routes of administration (Table 2). These differences in the results may be due to more selective distribution of the drug to the brain after i.n. administration. Many researchers (Fehm 2000; Quay 2001) have reported a unique connection between the nose and the brain and i.n. delivery of drugs to the brain bypassing the BBB (Illum 2000; Thorne et al 2004; Vyas et al 2005; Graff & Pollack 2005).

To evaluate the brain targeting efficiency, DTE (%) and DTP (%) were calculated as mentioned above (Equation (1) and (2)). Following i.n. 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 i.n. administration. The results of DTP (%) calculation further demonstrated that about 51.75 % of tacrine content within 480min 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 the penetration across the BBB 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 and 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 can diffuse into the nasal submucosa and subsequently travel into the olfactory perineuronal channels to reach the brain parenchymal tissues (Thorne and Frey 2001). Being highly lipophilic (log P - 2.71) and low molecular weight (Mole. Wt.-234.7) drug; tacrine is expected to travel by this extraneuronal epithelial pathway for direct nose-to-brain delivery. Kumbale et al (1999) have also 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(s) for direct nose-to-brain transport of tacrine.

Gamma Scintigraphy Imaging

Gamma scintigraphy images of rabbit at 15min post dosing are shown in Figure 2 (A) and (B). Significantly higher radioactivity was noticed in the rabbit brain following i.n. administration of tacrine compared with i.v. administration. Scintigraphy images are consistent with the biodistribution data shown in Table 1.

Conclusion

Nasal administration of tacrine can provide direct nose to brain transport resulting in higher bioavailability with reduced distribution into non-targeted tissues. This investigation demonstrates that the nasal administration can 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, nasal route can be viable alternative to 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 low bioavailability and pronounced side effects, for treatment of AD.

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also thankful to Ms. Ankur Kaul, INMAS, New Delhi, India, for her assistance in animal experiments.

Table 1 Distribution of ^{99m}TC-TS following i.n. and 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 with 100 μCi ^{99m}Tc-Tacrine and the radioactivity was measured in percent per gram of tissue of the administered dose. Radio activity was measured at 0min and all the measurements were performed using 0min sample of corresponding tissue/organ as blank sample. Each value is the mean ± SD (n = 3).

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)	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)	606.45 ± 75.66	130.13 ± 21.13	386.25 ± 46.17	171.75 ± 12.16
$AUC_{0\rightarrow\infty}$ (min*% radioactivity/g)	683.29 ± 87.06	160.37 ± 28.93	533.44 ± 64.77	211.63 ± 4.33
K_{el} (L/min)	0.0045 ± 0.0003	0.0037 ± 0.0003	0.0029 ± 0.0002	0.0036 ± 0.0009
$T_{1/2}$ (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 with 100 μCi ^{99m}Tc-Tacrine and the radioactivity was measured in percent per gram of tissue of the administered dose.

[#] Each value is the mean ± SD (n = 3)

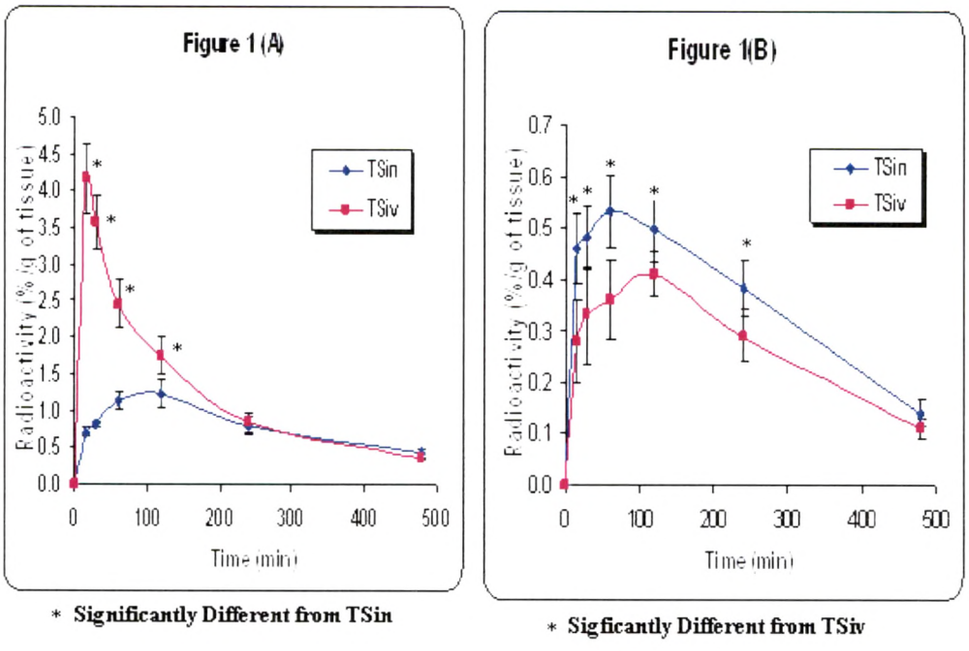
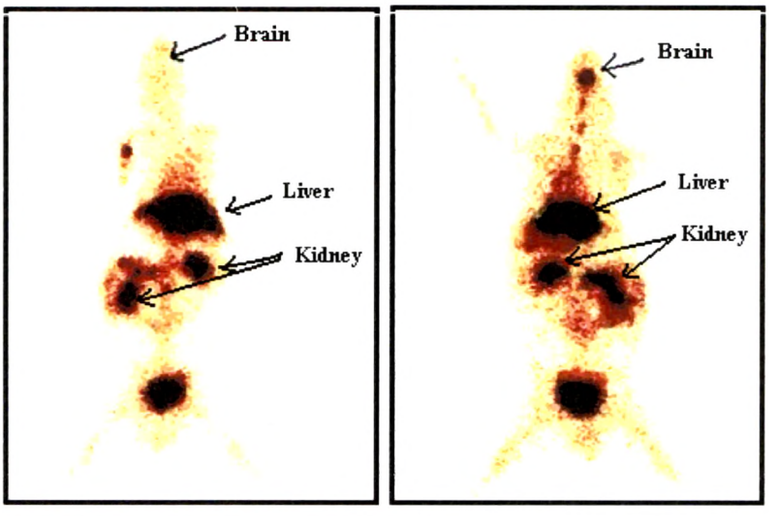


Figure 1 (A) Tacrine concentration in mice (n = 3) blood at different time intervals following ^{99m}TC-TS$_{i.v.}$ and ^{99m}TC-TS$_{i.n.}$ administrations. (B) Tacrine concentration in mice (n = 3) brain at different time intervals following ^{99m}TC-TS$_{i.v.}$ and ^{99m}TC-TS$_{i.n.}$ administrations. Mice were administered (100 μCi) radioactivity by i.v. and i.n. administration.



(A) (B)

Figure 2 Gamma scintigraphy images of rabbit showing the presence of radioactivity into the brain. (A) ^{99m}TC-TS$_{i.v.}$ (100 μCi) and (B) ^{99m}TC-TS$_{i.n.}$ (100 μCi).

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Intranasal Mucoadhesive Microemulsion of Tacrine to Improve Brain Targeting

Short Title: Intranasal Mucoadhesive Microemulsion of Tacrine for Brain Targeting

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Abstract

The aim of the investigation was to prepare and characterize microemulsion/ mucoadhesive microemulsion of tacrine (TME/TMME), assess its pharmacokinetic and pharmacodynamic performances for brain targeting and for improvement in memory in scopolamine induced amnesic mice. The TME was prepared by the titration method and characterized. Biodistribution of tacrine solution (TS) and formulations following intravenous and intranasal administrations were evaluated using ^{99m}Tc as marker. From the data, the pharmacokinetic parameters, drug targeting efficiency (DTE) and direct nose-to-brain drug transport (DTP) were calculated. To confirm drug localization in brain gamma scintigraphy in rabbits was performed. Lower T_{max} values (60 min) following intranasal compared to intravenous administration (120 min) suggested selective nose-to-brain transport. The brain bioavailability of tacrine following intranasal TMME compared to intranasal TS was found to be 2-fold higher indicating larger extent of distribution of the drug to brain with intranasal TMME. Rabbit brain scintigraphy also showed higher uptake of drug into the brain following intranasal administration. The results demonstrated rapid and larger extent of transport of tacrine into the mice brain and fastest regain of memory loss in scopolamine induced amnesic mice following intranasal TMME. Hence, results are suggestive of possible role of i.n. tacrine delivery in treating Alzheimer's patients.

Key Words: Intranasal; Mucoadhesive microemulsion; Tacrine; Brain targeting; Biodistribution; Alzheimer's disease; Morris water maze test

Introduction

Alzheimer's disease (AD) is a highly disabling neuropsychiatric disorder characterized by an irreversible deterioration of memory and intellectual behavior. While the etiology of AD 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 AD[1]. 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 the disease. Decreased central cholinergic activity has received major attention from investigators in search of 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 AD[2].

Tacrine (1,2,3,4-tetrahydro-9-aminoacridine), a potent, centrally active, reversible cholinesterase inhibitor, was the first drug approved by the USFDA in 1993 for treating the symptoms of mild to moderate AD[3-5]. Presently tacrine is available in the market as oral capsule dosage forms. 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[6], large inter individual differences[7, 8], a reversible dose dependent hepatotoxicity and peripheral cholinergic side effects[9-11]. Its clinical uses have been limited due to associated cholinergic, hepatic, and gastrointestinal adverse reactions[12-14]. A recent study had shown that gastrointestinal side effects, such as diarrhea, anorexia, dyspepsia, and abdominal pain, and raised serum liver enzymes were the major reasons for its withdrawal[13]. Hence, alternative route of tacrine delivery that selectively target tacrine directly to the various regions of brain is needed for the treatment of AD.

In the last one 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[15-17]. Drugs administered by intranasal (i.n.) route not only circumvent the BBB but also avoid the hepatic first pass effect. Previous studies have demonstrated that i.n. administration offers a simple, practical, noninvasive, convenient, cost effective, and an alternative route for rapid drug delivery to the brain/CNS[17-21]. Thus, direct transport of drugs to the brain/CNS circumventing the BBB following i.n. administration provides a unique feature and better option to target drugs to brain/CNS[15-17, 22-24]. Previous experience with the nasal delivery of Tacrine in solution form (our unpublished data) has shown that i.n. administration results in selective localization of drug in brain and hence, may be helpful in reducing the dose, frequency of dosing, and the dose dependent systemic side effects and thus may be explored as a promising and attractive new approach for delivery of tacrine for treatment of AD. However, to enhance effectiveness of the drug, a few issues should be considered by the formulator when designing i.n. drug delivery. Intranasal drug delivery system must be meticulously designed to provide rapid transport of drug across nasal mucosa and longer residence time in nasal cavity. Microemulsions have been explored widely as a delivery system by virtue of having considerable potential to enhance transport of a wide range of drug molecules across biological membranes[25]. The addition of a mucoadhesive agent such as a polyelectrolyte polymer helps in retention of formulation in nasal cavity[26, 27]. Evidences of i.n. drug delivery systems formulated using mucoadhesive agent and its benefits in enhancing nose-to-brain drug transport have been reported by our team as well as other scientists[28-32].

It was hypothesized that i.n. administration of tacrine microemulsion/mucoadhesive microemulsion (TME/TMME) will result in to selective and effective nose-to-brain tacrine transport, reduce drug distribution in other parts of the body, reduce side effects, and

rejuvenate its life in treatment of AD. Hence, the objective of this investigation was to prepare and characterize TME/TMME and to assess their pharmacokinetic performance for brain drug delivery in mice. It was an also objective to assess their role pharmacodynamically for improvement in memory in scopolamine induced amnesic mice.

Materials and Methods

Materials

Tacrine HCl received as gift sample from Pfizer, USA. Propylene glycol (AR Grade) was purchased from ISP Technologies (Mumbai, India). Diethylene triamine penta acetic acid (DTPA), stannous chloride dihydrate ($SnCl_2.2H_2O$), and scopolamine hydrochloride were purchased from Sigma (St. Louis, MO, USA). Sodium pertechnetate, separated from molybdenum-99 (99 m) using a solvent extraction method, was provided by Regional Center for Radiopharmaceutical Division (Northern Region), Board of Radiation and Isotope Technology (BRIT, Delhi, India). Labrafil M 1944 CS® and Transcutol P® were kindly gifted by Colorcon, Goa, India. Cremophor RH 40® was kindly gifted by BASF, Mumbai, India. All other chemicals and solvents were of analytical reagent grade and were used without further purification.

Preparation and characterization of microemulsions

Tacrine solution (TS, 33 mg/ml) was prepared by dissolving tacrine in propylene glycol. TME (33mg/ml) was prepared using Labrafil M 1944 CS® as an oil phase (15%), Cremophor RH 40® as a surfactant (S, 41.25%), Transcutol P® as a co-surfactant (CoS, 13.75%) and distilled water (30%) as an aqueous phase. Formulations were prepared by dissolving tacrine in S, CoS and oil mixture. Distilled water was added gradually with continuous stirring, which resulted in transparent and homogenous TME (% transmittance at 630nm > 99%). TMME was prepared by addition of Carbopol 934 P (0.50% w/w) to TME and the dispersion

was stirred for 30 min. Tacrine content in the formulations was determined using UV-Visible spectrophotometer at λ_{max} 326 nm using methanol as blank. The globule size determination[33] was performed using photon correlation spectroscopy with in-built Zetasizer (Model: Nano ZS, Malvern Instruments, Worcestershire, UK) at 633 nm. Helium–neon gas laser having intensity of 4mW was the light source. The equipment was programmed to provide 18mm laser width. Electrophoretic mobility (mm/s) was measured using small volume disposable zeta cell and converted to zeta potential[33] by in-built software using Helmholtz–Smoluchowski equation. Compositions, globule size, zeta potential, and radiolabeling efficiency of the formulations are recorded in Table 1.

Radiolabeling of Tacrine

TS, TME and TMME were radiolabeled using ^{99m}Tc by direct labeling method[34-36]. One milliliter of TS/TME/TMME was taken and $SnCl_2.2H_2O$ solution (200 µg in 100 µL of 10% v/v Acetic Acid) was added. The pH was adjusted to 6.50 ± 0.20 using 0.5 M sodium bicarbonate solution. To the resultant mixture, 1 mL of sterile ^{99m}Tc-pertechnetate (75 to 400 MBq) was added gradually over a period of 60s with continuous mixing. The mixture was incubated at room temperature for 30min 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[37, 38] of ^{99m}Tc-TS, ^{99m}Tc-TME, and ^{99m}Tc-TMME was assessed using ascending instant thin layer chromatography. Silica gel coated fiber glass sheets (Gelman Sciences, Inc., Ann Arbor, MI) were employed as stationary phase and dual solvent systems consisting of acetone and pyridine: acetic acid: water (3: 5: 1.5 v/v) were used as mobile phases. The effects of incubation time, pH, and $SnCl_2.2H_2O$ concentrations on radiolabeling efficiency were studied to achieve optimum reaction conditions [35, 37, 38]. The radiolabeled formulations were challenged for bonding strength using DTPA[39]. The optimized radiolabeled formulations were assessed for *in vitro* stability in normal saline solution and in

mice serum[40]. Consequently, the optimized stable radiolabeled 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 weighing between 30 and 40 g were selected for the study on the basis of randomization technique. Three mice for each formulation per time point were used in the study. The radiolabeled complex of ^{99m}Tc-TS (100 μCi/50 μL) containing 0.039–0.052 mg Tacrine (equivalent to 1.3 mg/ kg body weight (B.W.)) was injected intravenously (i.v.) through tail vein of mice. Similarly, radiolabeled complex of ^{99m}Tc-TS/^{99m}Tc-TME/^{99m}Tc-TMME (100 μCi/10 μL) containing 0.039–0.052 mg Tacrine (equivalent to 1.3 mg/kg B.W.) was administered (5 μL) in each nostril. Prior to nasal administration of the formulations, the mice were partially anaesthetized by diethyl ether and the formulations were instilled into the nostrils with the help of micropipette (10 μL) attached with low density polyethylene tube having 0.1 mm internal diameter at the delivery site. The mice were held from the back in slanted position during nasal administration of the formulations. Blood was collected using cardiac puncture at predetermined time intervals (15, 30, 60, 120, 240, and 480min). After collecting the blood, the mice were sacrificed with mercy by exposure to diethyl ether. Subsequently, different tissues/organs including 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 shielded well-type gamma scintillation counter (Capintec Inc., New Jersey, USA). The radiopharmaceutical uptake per gram in each tissue/organ was calculated as a fraction of administered dose[35]. The results of radioactivity in blood and brain are recorded in Table 2. The pharmacokinetic parameters were derived

from Table 2 and Figure 1(A) and (B) using WinNonlin® software (version 5.0.1, Pharsight Corporation, North Carolina, USA) and recorded in Table 3. To evaluate the brain targeting efficiency, two indices (DTE (%) and DTP (%)) were adopted as mentioned below [30-32, 41, 42].

Drug targeting efficiency (DTE (%)): DTE (%) represents time average partitioning ratio.

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

The brain drug direct transport percentage (DTP (%)): In order to define nose-to-brain direct transport clearly, DTP (%); which has been derived from equations (2) and (3) was calculated.

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

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

Where,

B_x = Brain AUC fraction contributed by systemic circulation through the BBB following i.n. administration.

$B_{i.v.}$ = $AUC_{0\rightarrow480}$ (brain) following i.v. administration.

$P_{i.v.}$ = $AUC_{0\rightarrow480}$ (blood) following i.v. administration.

$B_{i.n.}$ = $AUC_{0\rightarrow480}$ (brain) following i.n. administration.

$P_{i.n.}$ = $AUC_{0\rightarrow480}$ (blood) following i.n. administration.

AUC = Area under the curve for blood/brain concentration vs. time.

Reports in the literature reveal that the drug uptake into the brain from the nasal mucosa mainly occurs via three different pathways [17, 43]. One is the systemic pathway by which some of the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing BBB. The others are the olfactory and the trigeminal neural pathway by which part of the drug is transported directly from the nasal cavity to CSF and brain tissue[23, 43]. We can deduce that the amount of drug that reaches in the brain tissue after nasal administration is

attributed to these three pathways. Since, the amount of drug is proportional to AUC, we can assume that the brain AUC fraction contributed by systemic circulation through BBB (represented by B_x), divided by blood AUC from nasal route is equal to that of i.v. route (see Equation (3)). Therefore, DTP (%) represents 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 i.v. and i.n. administrations.

Gamma Scintigraphy Imaging

The New Zealand rabbits (2.00–2.50 kg) were selected for the study. The radiolabeled complex of ^{99m}Tc-TS (100 μCi/100 μL) containing 0.94–1.18 mg Tacrine (equivalent to 0.47 mg/kg B.W.) was i.v. injected through the ear vein of the rabbit. Similarly, the radiolabeled complex of ^{99m}Tc-TS/^{99m}Tc-TME/^{99m}Tc-TMME (100 μCi/100 μL) containing 0.94–1.18 mg Tacrine (equivalent to 0.47 mg/kg B.W.) was administered i.n. (50 μL in each nostril). The rabbits were held from the back in slanted position during nasal administration of formulations. The rabbits were anaesthetized using 1 mL ketamine hydrochloride intramuscular injection (50 mg/mL) 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[35, 44]. The scintigraphy images following i.v. and i.n. administrations of different ^{99m}Tc-tacrine formulations are shown in Figure 2.

Morris Water Maze Test

To evaluate the influence of developed formulation on learning and memory capacities, Morris water maze test was performed in scopolamine induced amnesia model in mice[45, 46]. Morris water maze is a circular pool (90 cm in diameter and 45 cm in height) with a featureless inner surface. The pool was filled to a height of 25 cm with water. The pool was

divided into four quadrants of equal area and a clear plexiglass column platform (6 cm diameter) was centered in one of the four quadrants of the pool and submerged just below the water surface. The mice could escape from water onto the platform and the time (in seconds) taken by the mouce was measured as escape latency. Mice were evaluated once daily for 2 water maze test for 4 consecutive days. In the 1st test mice were placed on the platform for 15 seconds and then placed in the water. Escape latency (indicative of Learning and Intact Reference Memory) was measured (Figure 3). After 15 seconds on the platform the animals were placed back in the water (in previous position) and allowed to search for platform (retained in previous position). Escape latency (indicative of Short-term Working Memory i.e. 2nd test) was recorded (Figure 3). Amnesia was induced by intraperitoneal (i.p.) injection of scopolamine hydrochloride (0.4mg/kg of body weight) in 0.9% saline 30 minutes prior to testing. Mice were divided in 2 groups: Saline treated and Scopolamine treated. In both groups, animals (n=4) were treated with different i.v. and i.n. formulations of tacrine (i.e. $TS_{i.n.}$, $TME_{i.n.}$, $TMME_{i.n.}$, and $TS_{i.v.}$- 1.3 mg/kg as described in biodistribution studies) 1 hr prior to testing (i.e. 30 minutes prior to Scopolamine treatment) and evaluated once daily for 2 water maze test for 4 consecutive days as described above. Scopolamine-treated mice were also administered i.v. and i.n., the placebo formulations to check the influence of formulation components on scopolamine induced amnesia.

Statistical Analysis

All data are reported as mean ± SEM and the difference between the groups were tested using Student's t-test and value of $p<0.05$ was considered statistically significant.

Results

The tacrine content was found to be 99.43%, 98.95%, and 98.78% for TS, TME, and TMME, respectively. The mean globule size and zeta potential of TME and TMME were found to be 23.50 ± 0.40, 26.23 ± 0.40 nm and -20.50 ± 0.51, -34.30 ± 0.57 mV respectively (Table 1). Radiochemical purity achieved for ^{99m}Tc-TS/TME/TMME was 96.35%, 97.66% and 98.31% respectively when evaluated for reduced/hydrolyzed ^{99m}Tc and free ^{99m}Tc. The optimal $SnCl_2.2H_2O$ concentration was found to be 200 µg/mL at pH 6.50 with an incubation time of 30min. ^{99m}Tc-TS/TME/TMME showed <5% w/w degradation in normal saline solution and in mice serum up to 24h. Bonding strength of ^{99m}Tc-TS/TME/TMME was also investigated by the DTPA challenge test, and the percent transchelation of the labeled complexes were <1.60 % w/w at 25mM DTPA concentration, while at 100mM, it increased up to only around 5.00 % w/w.

Biodistribution studies of ^{99m}Tc-TS following i.v. and ^{99m}Tc-TS/TME/TMME following i.n. administration in BALB/c mice were performed and the radioactivity was estimated at predetermined time intervals up to 480min (Table 2). The brain/blood ratio and pharmacokinetic parameters of the drug were also calculated and recorded in Table 2 and 3. After nasal administration, tacrine was delivered to the brain quickly compared to i.v. administration (T_{max} – 60min versus 120min). Similarly, lower T_{max} values for brain (60 min) compared to blood (120 min) were observed for all the three nasally administered formulations. The brain/blood ratios of the drug were found to be higher at all time points ($P<0.05$) following i.n. administration of formulations (Table 2). The concentrations of drug in the brain following i.n. administration were found to be significantly higher ($P<0.05$) at all sampling time points compared to i.v. administration up to 480 min. The $T_{1/2}$ and K_{el} of drug in blood was found to be significantly different ($P<0.05$) for i.v. and i.n. administration of different tacrine formulations, but insignificant differences in these values were observed in

brain irrespective of the routes of administration and the type of the formulations (Table 2). Significantly higher ($P<0.05$) C_{max} (brain) and AUC (brain) were observed when $TS_{i.n.}$, $TME_{i.n.}$ and $TMME_{i.n.}$ were compared to $TS_{i.v.}$. The bioavailability of tacrine in brain after i.n. compared to i.v. administrations was 131.99%, 218.67% and 287.09% for TS, TME and TMME respectively (Table 3). When $TME_{i.n.}$ was compared to $TS_{i.n.}$, significantly higher ($P<0.05$) C_{max} and AUC were observed. Incorporation of mucoadhesive agent in the formulation (TMME) resulted in further increase in C_{max} and AUC. To evaluate the brain targeting efficiency, DTE (%) and DTP (%) were also calculated (Table 4) from the pharmacokinetics data (Table 3). Amongst all the three nasally administered formulations, TMME showed highest DTE (295.87 %) and DTP (66.20 %) values followed by TME (242. 82 % and 58.82 %) and then TS (207.23 % and 51.75 %).

Gamma scintigraphy images (Figure 2) shown accumulation of significantly higher radioactivity in the rabbit brain following i.n. administration of tacrine compared with i.v. administration. Amongst i.n. formulations, TMME shows higher radioactivity compared to TS and TME.

The effects of tacrine formulations after i.v. and i.n. administration on the escape latency achieved in the Morris water maze test in saline- and scopolamine-treated mice are shown in Figure 3. Saline-treated mice rapidly learned the location of the platform as indicated by a gradual decrease in escape latency. Minimum escape latency was achieved on day 3 and thereafter there was no significant decrease in escape latency observed. While in scopolamine-treated mice, a characteristic swimming behavior consisting of circling around the pool was observed and the latency period in learning and intact reference memory (test 1) and short-term working memory (test 2) remained unchanged throughout 4 days of testing period. Intravenous and i.n. administration of different tacrine formulations in saline-treated mice and i.v./ i.n. administration of placebo formulations in scopolamine-treated animals did

not resulted into any noticeable change in escape latency (Data not shown). Following i.v. and i.n. administration of tacrine formulations in scopolamine-treated mice significant decrease ($P<0.05$) in escape latency in both of the tests was observed. Following i.n. administration of TMME mice learned to reach the platform within 3 days and exhibited behavioral pattern identical to saline-treated control mice. While, following $TS_{i.n.}$ and $TME_{i.n.}$, similar behavior was observed at the end of 4 days. In case of $TS_{i.v.}$, a noticeable decrease in the escape latency was observed, but, it was slow compared to i.n. administrations and mice did not learn to reach the platform by end of 4 days.

Discussion

TME and TMME were successfully prepared and showed the mean globule size <27 nm and zeta potential < -20 mV. The TME showed net negative charge and addition of mucoadhesive agent further contributed negatively to the system. This may be attributed to the fact that increase in surfactant level results in to decrease in surface tension and surface free energy of the formed micelles and therefore, net negative charge (anionic) of the microemulsion increased[47]. A zeta potential less than -25 mV for a microemulsion is reported[33, 48, 49] to discourage phase separation and/or flocculation. Hence, developed microemulsions are expected to be physically stable and addition of mucoadhesive agent (Carbopol 934 P) stabilizes it further.

TS, TME and TMME were effectively radiolabeled with ^{99m}Tc and optimized for maximum labeling efficiency and stability. The results of *in vitro* stability (in normal saline and mice serum) and DTPA challenge test suggested high bonding strength and stability of ^{99m}Tc-labelled tacrine formulations. Thus, these complexes were found suitable for *in vivo* studies.

After nasal administration of different tacrine formulations, rapid delivery of tacrine to the brain compared to i.v. administration may be because of preferential nose-to-brain transport

following i.n. administration. This was further supported by the lower T_{max} values for brain compared to blood for all the three nasally administered formulations. Higher brain/blood ratios of the drug further confirms direct nose-to-brain transport[50]. The substantially higher uptake in the brain with i.n. administration (as evidenced by significantly higher ($P<0.05$) concentrations at all sampling time points, C_{max}, AUC and bioavailability) compared to i.v. administration suggests a larger extent of selective transport of tacrine from nose-to-brain[51]. This is in agreement with many scientists who believe in this unique connection between the nose and the brain, and drug transport to brain circumventing the BBB after i.n. administration[17, 22-24, 43, 52, 53]. Higher C_{max} and AUC values for $TMME_{i.n.}$ followed by $TME_{i.n.}$ compared to $TS_{i.n.}$ may be explained on the basis of following facts: The microemulsion by virtue of their low globule size and lipophilicity and mucoadhesive agent by increasing the retention of formulation at the absorption site enhances transport of drug across nasal mucosa. These findings are in congruence with the observations reported by Zhang et al (2004)[42] that microemulsion enhances transport of drug across nasal mucosa resulting in direct nose-to-brain transport of the drugs[42, 54]. Under normal circumstances, nasally administered formulations get cleared quickly from the nasal cavity due to mucociliary clearance. Mucoadhesive agents are well reported to prolong the contact time of the formulation with the nasal mucosa and thereby enhance rate and extent of absorption of the drug[27]. Higher DTE (%) and DTP (%) values for $TMME_{i.n.}$ compared to $TME_{i.n.}$ and $TS_{i.n.}$ demonstrated the significance of the mucoadhesive microemulsion formulation in targeting the drug efficiently to the brain following i.n. administration preferentially due to selective nose-to-brain transport.

The gamma scintigraphy images clearly demonstrate the accumulation of formulations in brain at 15 min post dosing when administered via i.n. routes. However, following i.v. administration very little or no accumulation of radioactive formulation was observed. This

suggested selective nose-to-brain direct transport of drug. The accumulation of higher radioactivity in the brain following intranasal administration of TMME compared to TS and TME demonstrates the role of mucoadhesive microemulsion in brain targeting. Thus, scintigraphy images are consistent with the observations of biodistribution studies.

Scopolamine, a nonselective muscarinic receptor antagonist, has been widely used as a pharmacological tool to evaluate the effects nootropic drugs on memory deficits in experimental animals[55]. Numerous studies show positive effects of acetylcholinesterase (AChE) inhibitors on behavioral deficits in animals induced by the scopolamine[56-58]. Intravenous and i.n. administration of placebo formulations in scopolamine-treated animals did not resulted into any noticeable improvement in learning and memory capacities. Whereas, i.v. and i.n. administration of different tacrine formulations in scopolamine-treated mice antagonize scopolamine induced amnesia as evidenced by significant decrease ($P<0.05$) in escape latency in both of the tests. These results indicated an increase in learning and memory capacities with tacrine but not with placebo. Compared to i.v. administration, i.n. administration resulted in faster memory regain in scopolamine induced amnesic mice with $TMME_{i.n.}$ being the superior. These results further support the findings of biodistribution studies.

Intranasal delivery of therapeutics has already been successfully used to improve memory in both normal human adults and in those with AD. Benedict et al (2004)[59] have investigated the effects of prolonged intranasal administration of human regular insulin (RH-I; 4 x 40 IU/d over 8 weeks) on declarative memory (immediate and delayed recall of word lists), attention (Stroop test), and mood in 38 healthy human subjects. Intranasal insulin did not alter blood glucose and plasma insulin levels indicating direct nose to brain transport of insulin. Delayed recall of words significantly improved after 8 weeks of intranasal insulin administration. Moreover, subjects after intranasal insulin reported signs of enhanced mood as indicated by

reduced anger and enhanced self-confidence. In a later study[60], authors compared the acute (40 IU) and longterm (4 x 40 IU/day over 8 weeks) effects of intranasally administered RH-I and the rapid-acting insulin analog insulin aspart (ASP-I) on declarative memory in healthy human subjects. Authors reported improvement in declarative memory as indicated by improved word list recall after longterm intranasal insulin treatment compared to placebo while no improvement with acute treatment. Out of two insulins, ASP-I performed better than RH-I. These studies demonstrate the importance of intranasal delivery of insulin for the treatment of patients with memory disorders like in AD. Reger et al (2006)[61] have reported that acute intranasal insulin administration improved verbal memory in AD and amnestic mild cognitive impaired subjects without apolipoprotein E-ε4 allele without changing plasma insulin or glucose levels. To conclude, mucoadhesive microemulsion of tacrine was successfully prepared, demonstrated to deliver tacrine to the brain quickly and in larger quantities following i.n. administration in mice. Thus, studies of this investigation amply demonstrate direct nose-to-brain tacrine delivery and regain of memory loss in scopolamine induced amnesic mice following i.n. administration and hence, suggest possible role of i.n. tacrine delivery in treating Alzheimer's patients possibly by reducing / eliminating dose dependent hepatotoxicity, a reason of restricting its use in clinical practice. However, clinical studies with special focus on hepatic toxicity evaluation on chronic use of the developed formulations is necessary for establishing suitability in clinical practice in the treatment of AD.

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Table 1. Composition and characterization* of Tacrine formulations.

Formulation[a]	O (%)	S (%)	CoS (%)	AQ (%)	Drug Content (%)	Globule size (nm)	Zeta potential (mV)	Radiochemical Purity (%)
TS	-	-	-	100	99.43	-	-	96.35%
TME	15	41.25	13.75	30	98.78	23.50 ± 0.40	-20.50 ± 0.51	97.66%
TMME[b]	15	41.25	13.75	30	98.95	26.23 ± 0.40	-34.30 ± 0.57	98.31%

[a] TS – Tacrine Solution, TME – Tacrine Microemulsion, TMME – Tacrine Mucoadhesive Microemulsion

[b] TMME additionally contains 0.5%w/w of Carbopol 934 P as mucoadhesive agent

* The results are mean values ± SEM derived from six different experimental batches. O is denoted for oil Phase (Labrafil M 19944 CS®), S for surfactant (Cremophor RH 40®), CoS for co-surfactant (Transcutol P®) and AQ is denoted for aqueous phase (Propylene glycol for TS and Purified water for TME and TMME). The formulations (TS, TME and TMME) contain tacrine 33 mg/ml.

Table 2. Distribution of ^{99m}Tc-TS$_{i.v.}$, ^{99m}Tc-TS$_{i.n.}$, ^{99m}Tc-TME$_{i.n.}$ and ^{99m}Tc-TMME$_{i.n.}$ in BALB/c mice$^{\$}$ at predetermined time intervals

Formulation$_{route\ of\ administration}$	Organ/Tissue	Sampling time points (minutes)					
		15	30	60	120	240	480
TS$_{i.v.}$	Blood	4.17 ± 0.47	3.58 ± 0.36	2.45 ± 0.32	1.75 ± 0.25	0.84 ± 0.14	0.37 ± 0.04
	Brain	0.28 ± 0.07	0.33 ± 0.10	0.36 ± 0.04	0.41 ± 0.09	0.29 ± 0.05	0.11 ± 0.02
TS$_{i.n.}$	Blood	0.71 ± 0.07	0.82 ± 0.08	1.13 ± 0.12	1.23 ± 0.18	0.80 ± 0.12	0.43 ± 0.06
	Brain*	0.46 ± 0.12	0.48 ± 0.09	0.53 ± 0.10	0.50 ± 0.06	0.38 ± 0.06	0.14 ± 0.03
TME$_{i.n.}$	Blood	0.56 ± 0.09	0.76 ± 0.11	1.45 ± 0.14	1.72 ± 0.12	1.43 ± 0.11	0.36 ± 0.06
	Brain*	0.54 ± 0.10	0.62 ± 0.09	0.88 ± 0.07	0.83 ± 0.07	0.67 ± 0.09	0.23 ± 0.06
TMME$_{i.n.}$	Blood	0.78 ± 0.11	0.98 ± 0.10	1.54 ± 0.14	1.86 ± 0.13	1.50 ± 0.12	0.40 ± 0.06
	Brain*	0.72 ± 0.10	0.87 ± 0.12	1.22 ± 0.08	1.07 ± 0.08	0.90 ± 0.10	0.25 ± 0.07
TS$_{i.v.}$	Brain/Blood	0.07 ± 0.01	0.09 ± 0.03	0.15 ± 0.04	0.24 ± 0.07	0.35 ± 0. 09	0.30 ± 0.06
TS$_{i.n.}$	Brain/Blood*	0.64 ± 0.10	0.59 ± 0.14	0.48 ± 0.14	0.42 ±0.12	0.48 ± 0.07	0.33 ± 0.09
TME$_{i.n.}$	Brain/Blood*	0.99 ± 0.27	0.82 ± 0.03	0.61 ± 0.03	0.48 ±0.03	0.47 ± 0.05	0.68 ± 0.32
TMME$_{i.n.}$	Brain/Blood*	0.93 ± 0.18	0.89 ± 0.04	0.79 ± 0.06	0.58 ±0.01	0.60 ± 0.02	0.62 ± 0.12

$^{\$}$ The mice were administered with 100 µCi ^{99m}Tc-Tacrine formulations and the radioactivity was measured in %/g of tissue of the administered dose. Radio activity was measured at 0min and all the measurements were performed using 0min sample of corresponding tissue/organ as blank sample. Each value is the mean ± SEM (n = 3).

* Significantly higher from corresponding values for TS$_{i.v.}$ at all time points

Table 3. Pharmacokinetics of ^{99m}Tc-TS$_{i.v.}$ ^{99m}Tc-TS$_{i.n.}$, ^{99m}Tc-TME$_{i.n.}$ and ^{99m}Tc-TMME$_{i.n.}$ in BALB/c mice[$]

Pharmacokinetic Parameter	^{99m}Tc-TS$_{i.v.}$		^{99m}Tc-TS$_{i.n.}$		^{99m}Tc-TME$_{i.n.}$		^{99m}Tc-TMME$_{i.n.}$	
	Blood	**Brain**	**Blood**	**Brain**	**Blood**	**Brain**	**Blood**	**Brain**
C_{max} (% radioactivity/g)[#]	4.17 ± 0.47	0.41 ± 0.09	1.23 ± 0.18	0.53 ± 0.10[*]	1.72 ± 0.12	0.88 ± 0.07[*,%]	1.86 ± 0.13	1.22 ± 0.08[*,@]
T_{max} (min)	15	120	120	60	120	60	120	60
$AUC_{0\rightarrow480}$ (min*% radioactivity/g)	606.45	130.13	386..25	171.75[*]	546.15	284.55[*,%]	588.45	373.58[*,@]
$AUC_{0\rightarrow\infty}$ (min*% radioactivity/g)	683.29	160.37	533.44	211.63[*]	631.29	349.77[*,%]	684.34	446.67[*,@]
K_{el} (L/min)	0.0045	0.0037	0.0029	0.0036	0.0045	0.0037	0.0044	0.0038
$T_{1/2}$ (min)	155.03	186.85	241.36	191.20	152.52	187.74	155.91	181.62
Bioavailability (%)	-	-	63.69	131.99	90.06	218.67	97.03	287.09

[$] The mice were administered with 100 µCi ^{99m}Tc-Tacrine and the radioactivity was measured in percent per gram of tissue of the administered dose.

[#] Each value is the mean ± SEM (n = 3)

[*] Significantly higher from corresponding values for TS$_{i.v.}$

[%] Significantly higher from corresponding values for TS$_{i.n.}$

[@] Significantly higher from corresponding values for TS$_{i.n.}$ and TME$_{i.n.}$

Table 4. Drug targeting efficiency (DTE (%)) and direct nose-to-brain transport (DTP (%)) following intranasal administration of ^{99m}Tc-TS/^{99m}Tc-TME/^{99m}Tc-TMME Formulation.

Formulation	DTE (%)	DTP (%)
$TS_{i.n.}$	207.23	51.75
$TME_{i.n.}$	242.82	58.82
$TMME_{i.n.}$	295.87	66.20

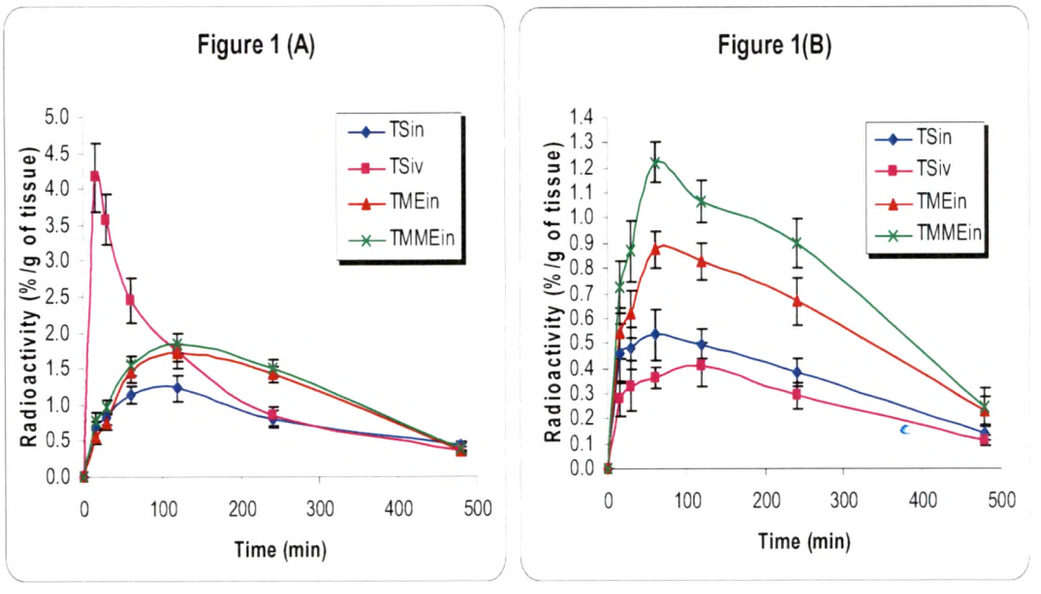

Figure 1. Tacrine concentration in mice (n = 3) (A) blood and (B) brain at different time intervals following ^{99m}Tc-TS$_{i.v.,}$ ^{99m}Tc-TS$_{i.n.,}$ ^{99m}Tc-TME$_{i.n.}$ and ^{99m}Tc-TMME$_{i.n.}$ administrations.

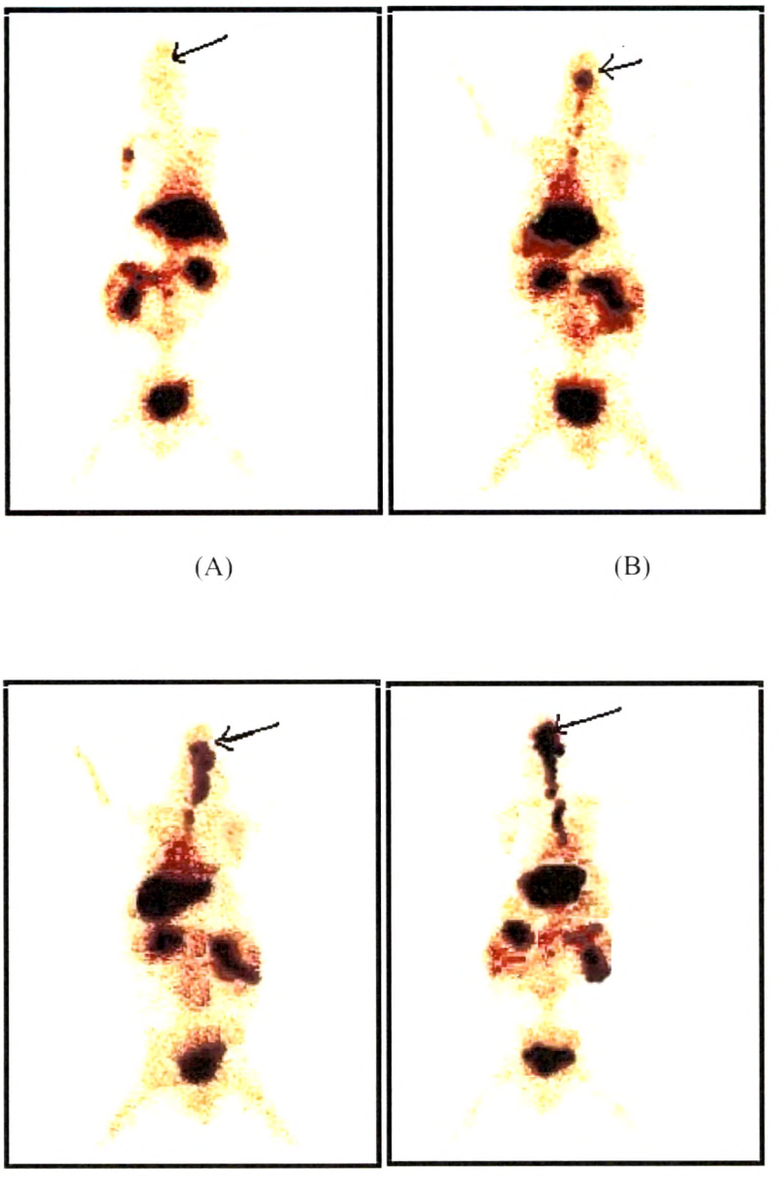

Figure 2. Gamma scintigraphy images of rabbit showing the presence of radioactivity into the brain (arrows). (A) ^{99m}Tc-TS$_{i.v.}$ (100 μCi); (B) ^{99m}Tc-TS$_{i.n.}$ (100 μCi); (C) ^{99m}Tc-TME$_{i.n.}$ (100 μCi); and (D) ^{99m}Tc-TMME$_{i.n.}$ (100 μCi)

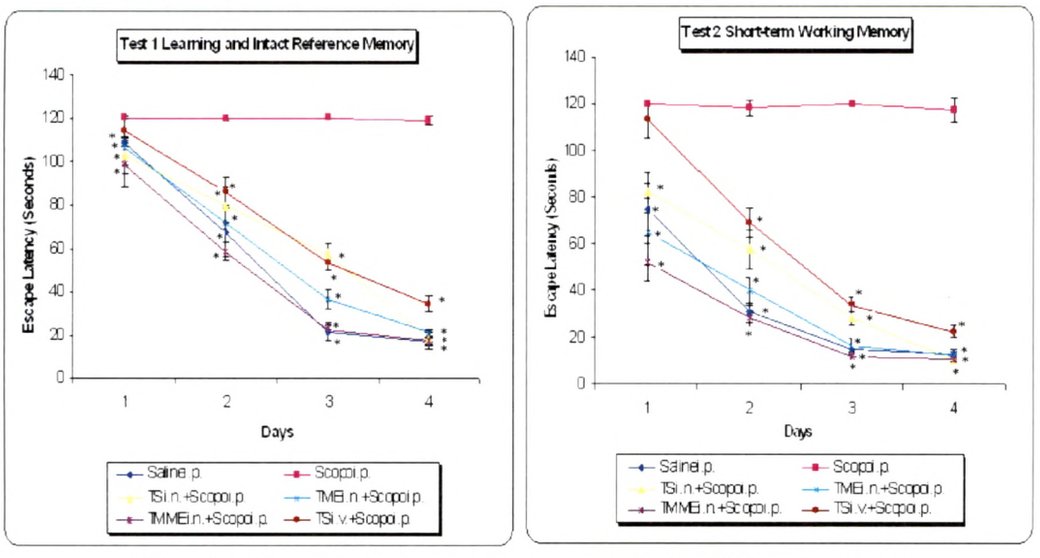

Figure 3. Effects of different tacrine formulations (TS – Tacrine Solution, TME – Tacrine Microemulsion, TMME – Tacrine Mucoadhesive Microemulsion) after i.v. and i.n. administration on the escape latency achieved in the Morris water maze test.

Figure Legends:

Figure 1. Tacrine concentration in mice (n = 3) (A) blood and (B) brain at different time intervals following ^{99m}Tc-TS$_{i.v.}$, ^{99m}Tc-TS$_{i.n.}$, ^{99m}Tc-TME$_{i.n.}$ and ^{99m}Tc-TMME$_{i.n.}$ administrations.

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