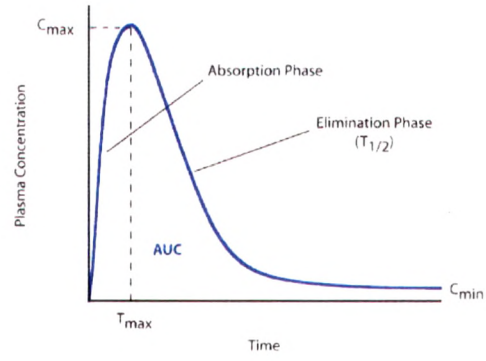
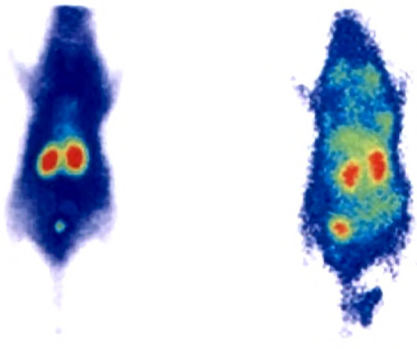


Chapter 6: Pharmacokinetic studies



6. PHARMACOKINETIC STUDIES

Previous literature has indicated the usefulness of radiolabeling techniques for pharmacokinetic and biodistribution studies. The radiolabeled formulations were better traced out in the biological system for their fate rather than conventional tissue extraction followed by instrumental analytical studies. Particularly, the radiolabeling with short lived radionuclides has been preferred due to their rapid decay and low toxicity. Drugs or colloidal drug carriers are linked to the radionuclides that are tailored for preferable concentration by a particular organ or physiologic process. In practice, the majority of radiopharmaceuticals are used for diagnosis (Mishra et al, 1999), but there a number of radionuclides available for the treatment of some disorders, especially cancer (Babbar et al, 2003). In the typical radiopharmaceutical formulation, the quantities of radionuclides and pharmaceutical agent used are normally quite less. The radiopharmaceutical differs from the conventional pharmaceutical in that it is not intended to elicit a pharmacological response due to the sub therapeutic doses administered. Hence, the radiopharmaceutical does not disturb the normal physiological process being measured, function as a true tracer, and they are generally free from hypersensitivity reactions. Since the dose administered is very low, the control of parameters such as tonicity and pyrogenicity is also not so important. The natural decay process may result in change in the final radionuclide composition and in the degradation of the stable materials. Variation in quality of radiopharmaceutical can greatly affect the biodistribution pattern and thereby the ultimate scan quality, causing problems in interpretation.

Quality control is an important aspect in the formulation and use of radiopharmaceuticals as it decides the efficacy for the purpose used. Before using the radionuclide for linking to the compound, the quality control testing is necessary to assure the efficacy of radionuclide. They include – radioactivity, radionuclide concentration, radionuclide purity and identity, radiochemical purity, chemical purity, sterility, apyrogenicity, absence of foreign particulate matter, particle Size (Babbar AK and Sharma RK., 2003).

The emergence of scintigraphy or imaging techniques for studying the biodistribution patterns in the sixties and seventies has lead to the increase in the popularity of the application of nuclear medicine. These techniques allow non invasive biodistribution

study by tracing using an external detection system viz. gamma camera (Single Photon Emission Computed Tomography - SPECT). SPECT imaging represents methods for acquiring and processing the scintigraphic data to reconstruct a three dimensional tomographic image displaying the distribution of radioactivity within certain organ system using emitted gamma rays upon administration of a radio tracer (Sorensen JA and Phelps ME., 1980; Budinger TF., 1980). Gamma imaging has lead to an increase in the demand for short lived radio tracers which can be safely administered in larger doses with minimal radiation dose. For biological experiments, the radionuclides are linked to the compounds of interest by various techniques. The effective binding of radiolabelled to the compound is determined by the quality control tests such as labeling efficiency, stability of radiolabeled complexes, challenge tests using substances having high affinity to the radiolabel and serum stability.

In practice, the radiopharmaceutical preparation is administered to the species of interest, by the parenteral route. At specified time intervals, the organs or tissues of interest are removed and measured for radioactivity using a gamma counter. The images of organs/tissues can also be taken without sacrificing the host using the SPECT camera. Various radionuclides are used for the above mentioned purposes include ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{99}Mo, ^{131}I,^{123}I, ^{133}Xe, ^{201}Tl, ^{99m}Tc, ^{67}Ga, ^{111}In (Ramamoorthy N and Desai CN., 1997).

Various reports are available where ^{99m}Tc has been widely used for the pharmacokinetic and biodistribution studies of many drugs and their delivery systems. Technetium is prepared by the following reaction from Uranium (^{235}U)

$$^{235}U \xrightarrow{\text{Irradiated with neutron flux}} {}^{99}Mo \longrightarrow {}^{99m}Tc$$

Common methods of separation of ^{99m} Technicium and ^{99} Molybdenum

1. Column Chromatography over acidic alumina
2. Solvent extraction of ^{99m}Tc with methyl ethyl ketone
3. Sublimination of Tc oxides from Mo compounds

The principle involved in the measurement of radioactivity is as follows: the gamma rays emitted by the isotopes enter a stainless steel casing and generate electrons,

which are absorbed by the sodium iodide (NaI) crystal. The NaI crystal undergoes excitation and further de-excitation to produce a flash of light. This flash of light passes through an optically coupled photomultiplier tube. In the photomultiplier tube, the intensity of light is enhanced and passes through a pre-amplifier and linear amplifier and consequently to the pulse height analyzer. The signals are then tuned in a tuner and recorded in the recorder in case of gamma camera. The gamma camera is equipped with a scaler instead of recorder. In scaler, the signals are converted into digits in terms of counts.

Physical Properties of ^{99m}Technicium

^{99m}Tc decays by isomeric transition with the physical half life of 6.02h. The principle photon useful for the detection and imaging studies is gamma-2 with the mean energy of 140.5keV. The specific gamma ray constant for ^{99m}Tc is 0.8R/mCi-hr at 1cm (5.58μCi/kg/hr/MBq at 1cm). The use of 2.5mm thickness of lead can effectively attenuate the radiation emitted by a factor of 1000.

Principles of radiolabeling of compounds with ^{99m}Tc

The majority of ^{99m}Tc compounds employ the stannous chloride reduction method, which makes use of the fact that stannous chloride is one of the most powerful reducing agent. ^{99m}Tc obtained from the Mo / Tc generator is in chemical form of TcO_4^-, or pertechnetate. While the anion has an overall negative charge of -1, the oxidation number of technetium is +7. The chelating agents commonly used to prepare ^{99m}Tc products are also anions with an overall negative charge due to the presence of N, O and P atoms, each of which has 1 or more extra pairs of electrons. These negative charges repel each other so pertechnetate will not form chelates. A reducing agent is therefore required to convert the ^{99m}Tc into an electropositive cationic form capable of binding to chelating agents. ^{99m}Tc sulfur colloid and ^{99m}Tc DMSA are the only two commercially available compounds that do not use the stannous reduction method. In the reaction, the stannous ion is the reducing agent, and therefore the substance oxidized, while pertechnetate is the oxidizing agent and therefore the substance reduced. Most soluble ^{99m}Tc compounds, excluding those containing a protein have octahedral structures and are said to be hexa coordinated since there are typical 6 binding sites available consisting of N, O, or P atoms. Technetium-99m is a radionuclide of choice because of its unique properties like

visualization of coupled complex in organs, sensitivity in detection even in extreme low levels in the organs.

6.1 RADIOLABELLING OF FORMULATIONS

6.1.1 Materials

Stannous chloride dihydrate ($SnCl_2.2H_2O$) was purchased from Sigma Chemical Co.(St. Louis, MO), sodium pertechnetate, separated from molybdenom-99 (99m) by solvent extraction method, was provided by Regional Center for Radiopharmaceutical Division (Northern Region) Board of Radiation and Isotope Technology (BRIT, Delhi, India)

6.1.2 Methods

The radiolabelling of NG, SB and HG formulations, viz drug solutions NS, SS and HS, drug nanoparticles NNp, SNp and HNp, drug antibody conjugated nanoparticles PE-mAb-Tfr-NNp, PE-mAb-Tfr-SNp and PE-mAb-Tfr-HNp, drug microemulsions NME, SME and HME and drug mucoadhesive microemulsions NMME, SMME and HMME, was performed with ^{99m}Tc by direct labelling method [Richardson et al 1977, Babbar et al 1991]. Briefly, 1ml of formulation (in case of microemulsions) or formulation dispersion (in case of nanoparticles) was mixed with 50µl of stannous chloride solution (5mg/ml). The pH was adjusted to 6.8±0.5 with 0.5M sodium bicarbonate solution and the preparation was incubated for 10mins at room temperature with required volume of freshly eluted ^{99m}Tc-pertechnetate solution (15 to 30 mCi/ml)) such that the resultant preparation has a radioactivity of 50 to 200 µCi/ml. The quality control (percentage labeling efficiency and stability of the labelled complexes) was performed as described earlier. [Theobald, 1990].

The labelling efficiency of ^{99m}Tc-labelled formulations was determined using ascending instant thin layer chromatography (ITLC) using silica gel (SG)-coated fibre glass sheets (Gelman Sciences Inc, Ann Arbor, MI). The ITLC was performed using acetone as the mobile phase. Approximately 2 to 3µL of the radiolabelled complex was applied at a point 1cm from one end of an ITLC-SG strip. The strip was eluted in acetone. The solvent front was allowed to reach 7-8cm from the point of application. The strip was cut horizontally into 2 halves, and the radioactivity in each half was

determined in a gamma ray counter (Gamma ray spectrometer, Captec-R, Capintec, USA). The free ^{99m}Tc-pertechnetate that moved with the solvent (R_f = 0.9) was determined.

The radiocolloid (reduced/hydrolyzed) technetium along with the labelled complex remained at the point of application. The amount of radiocolloids was determined using ITLC with pyridine: acetic acid: water (3:5:1.5 v/v) as mobile phase. The radiocolloids remained at the point of application, while both the free pertechnetate and the labelled complex moved away with the solvent front. The activity migrate using pyridine: acetic acid: water as a mixture was subtracted from that with the solvent front using acetone, the net amount of ^{99m}Tc labelled complex was calculated.

The radiolabelling was optimized for incubation time and the concentration of $SnCl_2.2H_2O$ using NG formulations and the results tabulated in tables 6.1. The pH of the radiolabelled formulations was maintained at 6.8±0.5.

In vitro stability study of radiolabeled complex

The stability study of ^{99m}Tc labelled formulations was determined in vitro using 0.9%w/v sodium chloride and rat serum by ascending thin layer chromatography for NG formulations. The complex (0.1 mL) was mixed with 1.9 mL of rat serum and incubated at 37°C. The samples at definite time point upto 24hrs were subjected to ITLC using acetone solvent system. The % labelling efficiency for formulations was determined. The results for stability in sodium chloride and rat serum are tabulated in table 6.2.

Transchelation challenge test using diethylene triamine penta acetic acid

To evaluate stability and bonding strength of the radiolabelled drug formulations transchelation challenge test was preformed for NG formulations with 1mL of the radiolabelled formulation being challenged against various concentrations (10, 30 and 50 mM) of diethylene triamine penta acetic acid (Babbar A K et al., 2000). The mixtures were incubated for 4 hours at 37°C and the labeling efficiency was measured using ITLC-SG; acetone and PAW system as mobile phase. Approximately 2 to 3 µL

complex was applied at 1cm distance on the ITLC-SG and mobile phase was allowed to run up to 8 cm from the point of application. The separated pertechnetate and DTPA complex was separated at migration value 0.90 (R_f = 0.90) while ^{99m}Tc-labelled complex remained at the point of application (R_f = 0). Effect of different molar concentration and percent transchelation is illustrated in Figure 6.1.

6.2 PHARMACOKINETIC STUDIES

6.2.1 Methods

6.2.1.1 Animals

All animal experiments conducted were approved by the Social Justice and Empowerment Committee for the purpose of control and supervision on animals and experiments, Ministry of Government of India. Balb/C mice weighing between 25-30gm were selected for pharmacokinetic studies.

6.2.1.2 Pharmacokinetics of radiolabelled formulations

Three mice were used at each time point for each formulation. The mice were divided into various groups as tabulated in tables 6.3, 6.4 and 6.5 for NG, HG and SB formulations respectively. For intravenous administrations, formulation (0.1mL equivalent to 50μCi) was injected via the tail vein of the animals. During intranasal administrations, animals were held from back in slanted position and formulation (7μL per nostril) was instilled into each nostril with the help of micropipette (1μL to10μL) attached with low-density polyethylene tubing, having 0.1mm internal diameter at the delivery site. For oral administrations, formulation (0.1 mL equivalent to 50μCi) was administered using oral feeding cannula attached to 1mL syringe. The animals were sacrificed humanely at different time intervals of 0.5hrs, 1hr, 2 hr, 4hr, 24hrs and blood was collected via cardiac puncture. 10min, 30 min, 90 min, 240min and 1440min, and blood collected via cardiac puncture. Subsequently, brain removed, washed twice with normal saline, made free from any adhering tissues and weighed. The radioactivity present in blood and brain was determined using shielded well-type

gamma scintillation counter along with 3 samples of standard solution representing 100% of the administered dose. Radiopharmaceutical uptake per gram in each tissue/organ was calculated as a fraction of administered dose (Babbar AK et al, Vyas et al) using equation:

$$\%ID/Gram\ of\ tissue = \frac{\left(\frac{Sample\ count}{Sample\ weight} \times 100\right)}{Standard\ counts}$$

The results of radioactivity measured at various time points in brain and blood are recorded in Tables 6.3, 6.4 and 6.5 for NG, HG and SB formulations respectively. The brain concentrations versus time plot for the formulations were constructed and illustrated in figures 6.2, 6.3 and 6.4 for NG, HG and SB formulations respectively. The blood concentrations versus time profile are plotted in Figures 6.5, 6.6 and 6.7 for NG, SB and HG formulations respectively.

Pharmacokinetic parameters were calculated using kinetica software (version 5, Therma, Philadelphia, PA, USA) and recorded in tables 6.6, 6.7 and 6.8 for NG, HG and SB formulations respectively. For intranasally administered formulations brain targeting efficiency was assessed based on the values of drug targeting index (DTI) which represents time average partitioning of drug between brain and blood, and brain drug targeting percentage (DTP%), which represents the percentage of drug directly transported to the brain via the neural pathway, and were calculated using following equations and tabulated in table 6.9 for NG, HG and SB formulations (Babbar AK et al, Vyas et al, Wang et al).

$$\mathbf{DTI} = \frac{(AUC_{brain}/AUC_{blood})_{i.n.}}{(AUC_{brain}/AUC_{blood})_{i.v.}}$$

Where, AUC = Area under the curve.

$$DTP\% = \frac{B_{i.n} - B_x}{B_{i.n}} \times 100$$

Where,

$$B_x = \frac{B_{i.v.}}{P_{i.v.}} \times P_{i.n.}$$

$B_{i.n.}$ = $AUC_{0\rightarrow24}$ brain following intranasal administration.

B_x = Brain AUC fraction contributed by systemic circulation through the blood brain barrier following intranasal administration.

$B_{i.v.}$ = $AUC_{0\rightarrow24}$ brain following intravenous administration.

$P_{i.v.}$ = $AUC_{0\rightarrow24}$blood following intravenous administration.

$P_{i.n.}$ = $AUC_{0\rightarrow24}$blood following intranasal administration.

6.2.1.3 Statistical Analysis

All data are reported as mean ± SEM, and the difference between the groups were tested using Student's t test at the level of $p<0.05$, and differences greater at $p<0.05$ were considered insignificant.

6.2.1.4 Gamma Scintigraphy Studies

Gamma Scintigraphy was done in mice for NG formulations to ascertain localization of drug in brain. Balb/C mice weighing between 25-30gm were selected for the imaging. Radiolabelled NG formulations (50μCi/7μL) were administered intranasally (7μL in each nostril), intravenously (50μCi/100μL) and orally (50μCi/100μL) respectively as described under section 6.3.1.2. The animals were distantly positioned on a board using surgical tape and images captured after 15min following formulation administrations using single positron emission computerized tomography (SPECT, LC 75-005, Diacam, Siemens AG, Erlanger, Germany) gamma camera (Babbar et al 2000, Vyas et al 2006) and demonstrated in figures 6.8.

6.3 RESULTS

Table 6.1: Influence of incubation time and amount of stannous chloride on the radiolabeling efficiency of nicergoline formulations

Formulation	% Radiolabeling					
	Incubation time (min)			Stannous chloride (µg)		
	5	10	20	200	250	300
^{99m}Tc-NS	95.1 ± 1.5	98.6 ± 0.6	98.4 ± 1.2	93.5 ± 1.1	99.1 ± 0.5	98.1 ± 1.1
^{99m}Tc-NNp	94.5 ± 1.8	99.1 ± 0.8	97.5 ± 1.9	94.3 ± 1.9	98.7 ± 1.1	97.6 ± 1.6
^{99m}Tc-PE-mAb-Tfr-NNp	94.8 ± 1.2	98.9 ± 0.5	99.1 ± 0.9	94.2 ± 1.4	98.9 ± 0.9	98.8 ± 0.9
^{99m}Tc-NME	94.2 ± 1.1	98.7 ± 1.0	99.0 ± 0.7	94.1 ± 1.2	98.8 ± 0.8	99.2 ± 0.7
^{99m}Tc-NMME	94.4 ± 0.9	98.8 ± 0.7	99.2 ± 1.0	94.6 ± 1.1	98.6 ± 0.7	99.1 ± 1.1

Table 6.2: In vitro stability of ^{99m}Tc labeled nicergoline formulations in 0.9%w/v sodium chloride and rat serum at 37°C

Time (hrs)	% Radiolabeling									
	In 0.90% Sodium Chloride					In Serum				
	^{99m}Tc-NS	^{99m}Tc-NNp	^{99m}Tc-PE-mAb-Tfr-NNp	^{99m}Tc-NME	^{99m}Tc-NMME	^{99m}Tc-NS	^{99m}Tc-NNp	^{99m}Tc-PE-mAb-Tfr-NNp	^{99m}Tc-NME	^{99m}Tc-NMME
0.5	99.0 ± 0.8	98.6 ± 0.6	98.5 ± 1.2	99.1 ± 1.0	98.9 ± 0.7	98.6 ± 0.9	97.7 ± 0.5	97.4 ± 1.9	99.3 ± 1.0	98.9 ± 0.7
1	98.7 ± 0.9	97.2 ± 1.2	98.1 ± 1.3	98.8 ± 0.9	98.4 ± 1.0	98.1 ± 0.8	97.2 ± 1.8	97.1 ± 1.6	98.9 ± 0.8	98.6 ± 0.9
2	98.4 ± 0.6	96.6 ± 1.1	97.7 ± 0.9	98.4 ± 1.1	98.0 ± 0.9	97.7 ± 0.9	95.5 ± 2.5	96.8 ± 1.4	98.5 ± 0.9	98.2 ± 0.8
4	98.2 ± 1.1	96.1 ± 2.6	97.3 ± 1.2	98.1 ± 0.8	97.7 ± 1.1	96.8 ± 1.1	94.9 ± 0.9	95.9 ± 1.8	98.1 ± 1.1	97.7 ± 1.1
6	97.1 ± 1.3	95.5 ± 2.1	96.1 ± 2.3	97.7 ± 0.6	97.3 ± 1.2	96.2 ± 0.7	94.1 ± 1.3	94.8 ± 1.2	97.5 ± 1.2	97.3 ± 0.9
24	96.3 ± 2.1	95.1 ± 1.4	94.4 ± 1.8	97.2 ± 1.1	96.4 ± 1.3	95.8 ± 1.2	93.6 ± 0.6	92.2 ± 1.6	96.8 ± 0.7	96.6 ± 1.0

Figure 6.1: Effect of variable molar concentration of DTPA on radiolabeled ^{99m}Tc complex of nicergoline formulations

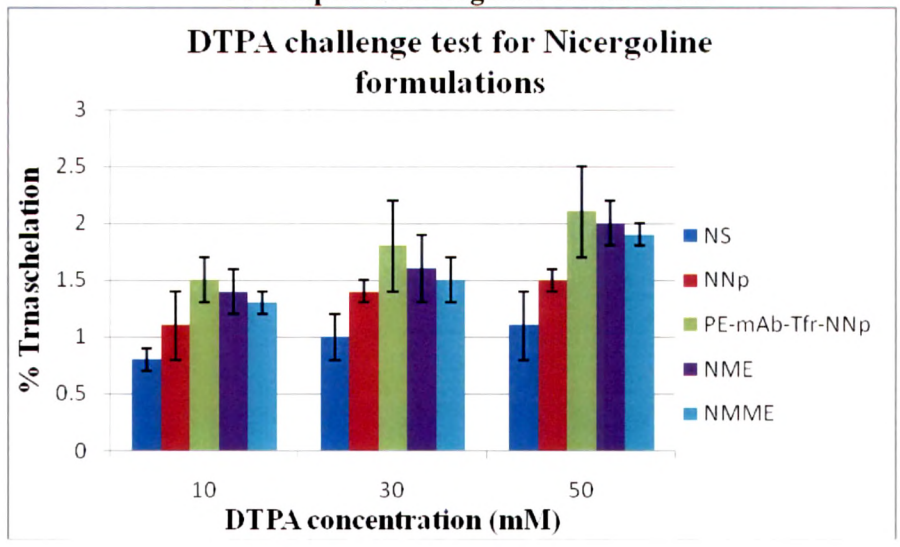


Table 6.3: Compartmental distribution of ^{99m}Tc-labeled nicergoline formulations at predetermined time intervals in balb/C mice*

Formulation (route of administration)	Distribution of NG in blood and brain at predetermined time intervals (%ID/g)					
	Organ	10 min	30 min	90 min	240 min	1440 min
NS (i.n.)	Blood	0.099 ± 0.04	0.102 ± 0.03	0.083 ± 0.02	0.068 ± 0.004	0.031 ± 0.003
	Brain	0.063 ± 0.02	0.047 ± 0.004	0.028 ± 0.005	0.0086 ± 0.003	0.0029 ± 0.0005
NNp (i.n.)	Blood	0.048 ± 0.03	0.08 ± 0.01	0.102 ± 0.03	0.093 ± 0.01	0.02 ± 0.004
	Brain	0.091 ± 0.03	0.089 ± 0.02	0.075 ± 0.01	0.048 ± 0.002	0.009 ± 0.003
PE-mAb-Tfr-NNp (i.n.)	Blood	0.035 ± 0.02	0.114 ± 0.03	0.162 ± 0.04	0.19 ± 0.03	0.067 ± 0.01
	Brain	0.095 ± 0.04	0.285 ± 0.04	0.224 ± 0.05	0.132 ± 0.02	0.028 ± 0.002
NME (i.n.)	Blood	0.017 ± 0.007	0.021 ± 0.003	0.032 ± 0.005	0.029 ± 0.006	0.019 ± 0.001
	Brain	0.043 ± 0.02	0.0374 ± 0.005	0.029 ± 0.003	0.018 ± 0.004	0.007 ± 0.002
NMME (i.n.)	Blood	0.034 ± 0.01	0.046 ± 0.008	0.071 ± 0.01	0.104 ± 0.03	0.032 ± 0.006
	Brain	0.11 ± 0.05	0.108 ± 0.03	0.087 ± 0.02	0.079 ± 0.01	0.013 ± 0.003
NNp (p.o.)	Blood	0.0097 ± 0.004	0.0119 ± 0.006	0.0132 ± 0.003	0.0111 ± 0.004	0.006 ± 0.002
	Brain	0.0052 ± 0.003	0.00758 ± 0.001	0.00612 ± 0.002	0.00498 ± 0.001	0.0003 ± 0.0001
NME (p.o.)	Blood	0.00087 ± 0.0003	0.00123 ± 0.0004	0.002 ± 0.0004	0.01 ± 0.003	0.003 ± 0.0006
	Brain	0.00055 ± 0.0001	0.00086 ± 0.0002	0.001 ± 0.0003	0.0024 ± 0.0005	0.0002 ± 0.0001
NME (i.v.)	Blood	0.095 ± 0.03	0.102 ± 0.02	0.083 ± 0.02	0.068 ± 0.01	0.019 ± 0.005
	Brain	0.0049 ± 0.0006	0.0037 ± 0.0007	0.0029 ± 0.0005	0.0015 ± 0.0003	0.0002 ± 0.0001
NNp (i.v.)	Blood	0.253 ± 0.05	0.261 ± 0.03	0.242 ± 0.05	0.213 ± 0.02	0.081 ± 0.03

	Brain	0.0142 ± 0.006	0.0134 ± 0.004	0.0119 ± 0.006	0.0078 ± 0.002	0.002 ± 0.0004
PE-mAb-Tfr-NNp (i.v.)	Blood	0.387 ± 0.04	0.413 ± 0.02	0.369 ± 0.04	0.323 ± 0.05	0.138 ± 0.02
	Brain	0.0109 ± 0.007	0.0152 ± 0.006	0.0131 ± 0.006	0.0104 ± 0.004	0.004 ± 0.0006
Brain to blood ratio						
NS (i.n.)	Brain/ Blood	0.64 ± 0.04	0.46 ± 0.03	0.34 ± 0.05	0.127 ± 0.04	0.094 ± 0.02
NNp (i.n.)	Brain/ Blood	1.9 ± 0.02	1.11 ± 0.04	0.74 ± 0.02	0.52 ± 0.02	0.45 ± 0.04
PE-mAb-Tfr-NNp (i.n.)	Brain/ Blood	2.71 ± 0.05	2.5 ± 0.05	1.38 ± 0.03	0.695 ± 0.05	0.42 ± 0.05
NME (i.n.)	Brain/ Blood	2.53 ± 0.03	1.78 ± 0.02	0.91 ± 0.05	0.62 ± 0.04	0.37 ± 0.02
NMME (i.n.)	Brain/ Blood	3.24 ± 0.06	2.35 ± 0.04	1.23 ± 0.03	0.76 ± 0.06	0.41 ± 0.03
NNp (p.o.)	Brain/ Blood	0.54 ± 0.04	0.637 ± 0.02	0.464 ± 0.03	0.359 ± 0.03	0.05 ± 0.003
NME (p.o.)	Brain/ Blood	0.63 ± 0.05	0.7 ± 0.06	0.5 ± 0.04	0.24 ± 0.02	0.067 ± 0.01
NME (i.v.)	Brain/ Blood	0.052 ± 0.02	0.036 ± 0.01	0.035 ± 0.01	0.022 ± 0.01	0.011 ± 0.004
NNp (i.v.)	Brain/ Blood	0.056 ± 0.03	0.051 ± 0.02	0.049 ± 0.007	0.037 ± 0.004	0.025 ± 0.002
PE-mAb-Tfr-NNp (i.v.)	Brain/ Blood	0.028 ± 0.02	0.037 ± 0.014	0.036 ± 0.004	0.032 ± 0.003	0.029 ± 0.01

The rats were administered with 50μCi ^{99m}Tc-NG and the radioactivity was measured in percent per g of tissue of the administered dose. Values are expressed as mean ± SEM of three estimations. '' Indicates that the variation in values between NS (i.n.), NNp (i.n.), PE-mAb-Tfr-NNp (i.n.) or NNp (i.v.) when compared to PE-mAb-Tfr-NNp (i.v.) are significant ($p < 0.05$)

Table 6.4: Compartmental distribution of ^{99m}Tc-labeled hydergine formulations at predetermined time intervals in balb/C mice*

Formulation (route of administration)	**Distribution of HG in blood and brain at predetermined time intervals (%ID/g)**					
	Organ	**10 min**	**30 min**	**90 min**	**240 min**	**1440 min**
HS (i.n.)	Blood	0.106 ± 0.01*	0.135 ± 0.009*	0.112 ± 0.02*	0.097 ± 0.008*	0.023 ± 0.03*
	Brain	0.087 ± 0.04*	0.072 ± 0.008*	0.052 ± 0.007*	0.026 ± 0.01*	0.009 ± 0.02*
HNp (i.n.)	Blood	0.079 ± 0.01*	0.09 ± 0.009*	0.12 ± 0.007*	0.086 ± 0.01*	0.032 ± 0.009*
	Brain	0.1 ± 0.02*	0.088 ± 0.01*	0.073 ± 0.009*	0.046 ± 0.01*	0.01 ± 0.008*
PE-mAb-Tfr-HNp (i.n.)	Blood	0.083 ± 0.007*	0.177 ± 0.009*	0.143 ± 0.04*	0.071 ± 0.03*	0.025 ± 0.01*

	Brain	0.165 ± 0.03*	0.291 ± 0.04*	0.208 ± 0.02*	0.093 ± 0.04*	0.026 ± 0.02*
HME (i.n.)	Blood	0.054 ± 0.006	0.061 ± 0.011	0.068 ± 0.015	0.049 ± 0.007	0.018 ± 0.004
	Brain	0.077 ± 0.01	0.072 ± 0.014	0.059 ± 0.012	0.028 ± 0.004	0.006 ± 0.0005
HMME (i.n.)	Blood	0.047 ± 0.004	0.059 ± 0.01	0.064 ± 0.011	0.041 ± 0.006	0.02 ± 0.004
	Brain	0.099 ± 0.011	0.091 ± 0.02	0.077 ± 0.014	0.044 ± 0.005	0.014 ± 0.006
HNp (p.o.)	Blood	0.0123 ± 0.004	0.0131 ± 0.005	0.0137 ± 0.007	0.0126 ± 0.004	0.008 ± 0.002
	Brain	0.0054 ± 0.006	0.0061 ± 0.0013	0.0069 ± 0.001	0.0057 ± 0.0008	0.0001 ± 0.0001
HME (p.o.)	Blood	0.00106 ± 0.001	0.00125 ± 0.0008	0.0113 ± 0.004	0.00898 ± 0.001	0.0038 ± 0.0006
	Brain	0.00072 ± 0.0002	0.00087 ± 0.0003	0.0031 ± 0.0006	0.0023 ± 0.0009	0.00029 ± 0.0001
HME (i.v.)	Blood	0.112 ± 0.03	0.128 ± 0.02	0.119 ± 0.03	0.087 ± 0.02	0.019 ± 0.008
	Brain	0.0058 ± 0.01	0.0065 ± 0.009	0.0059 ± 0.0013	0.0038 ± 0.0009	0.0004 ± 0.0001
HNp (i.v.)	Blood	0.64 ± 0.05	0.57 ± 0.03	0.46 ± 0.03	0.35 ± 0.03	0.12 ± 0.03
	Brain	0.026 ± 0.009	0.02 ± 0.007	0.013 ± 0.005	0.009 ± 0.002	0.002 ± 0.0007
PE-mAb-Tfr-HNp (i.v.)	Blood	0.51 ± 0.05	0.586 ± 0.06	0.523 ± 0.06	0.26 ± 0.02	0.118 ± 0.04
	Brain	0.025 ± 0.009	0.031 ± 0.009	0.024 ± 0.008	0.011 ± 0.004	0.004 ± 0.0009
Brain to blood ratio						
HS (i.n.)	Brain/ Blood	0.821 ± 0.02*	0.533 ± 0.04*	0.464 ± 0.02*	0.268 ± 0.02*	0.391 ± 0.03*
HNp (i.n.)	Brain/ Blood	1.266 ± 0.05*	0.978 ± 0.02*	0.608 ± 0.03*	0.535 ± 0.04*	0.313 ± 0.02*
PE-mAb-Tfr-HNp (i.n.)	Brain/ Blood	1.988 ± 0.04*	1.644 ± 0.05*	1.455 ± 0.04*	1.31 ± 0. 0.31*	1.04 ± 0.05*
HME (i.n.)	Brain/ Blood	1.43 ± 0.03	1.18 ± 0.04	0.87 ± 0.02	0.57 ± 0.02	0.33 ± 0.05
HMME (i.n.)	Brain/ Blood	2.11 ± 0.05	1.54 ± 0.05	1.2 ± 0.03	1.07 ± 0.04	0.7 ± 0.04
HNp (p.o.)	Brain/ Blood	0.44 ± 0.02	0.47 ± 0.03	0.5 ± 0.01	0.45 ± 0.03	0.013 ± 0.008
HME (p.o.)	Brain/ Blood	0.68 ± 0.04	0.7 ± 0.05	0.27 ± 0.03	0.25 ± 0.01	0.076 ± 0.02
HME (i.v.)	Brain/ Blood	0.052 ± 0.03	0.051 ± 0.04	0.05 ± 0.02	0.044 ± 0.02	0.021 ± 0.01
HNp (i.v.)	Brain/ Blood	0.041 ± 0.02	0.035 ± 0.02	0.028 ± 0.01	0.026 ± 0.01	0.017 ± 0.007
PE-mAb-Tfr-HNp (i.v.)	Brain/ Blood	0.049 ± 0.03	0.053 ± 0.03	0.046 ± 0.03	0.042 ± 0.03	0.034 ± 0.04

The rats were administered with 50μCi ^{99m}Tc-HG and the radioactivity was measured in percent per g of tissue of the administered dose. Values are expressed as mean ± SEM of three estimations. '' Indicates that the variation in values between HS (i.n.), HNp (i.n.), PE-mAb-Tfr-HNp (i.n.) or HNp (i.v.) when compared to PE-mAb-Tfr-HNp (i.v.) are significant ($p < 0.05$)

Table 6.5: Compartmental distribution of ^{99m}Tc-labeled sibutramine formulations at predetermined time intervals in balb/C mice*

Formulation (route of administration)	Distribution of SB in blood and brain at predetermined time intervals (%ID/g)					
	Organ	10 min	30 min	90 min	240 min	1440 min
SS (i.n.)	Blood	0.079 ± 0.006	0.086 ± 0.005	0.064 ± 0.011	0.047 ± 0.004	0.009 ± 0.002
	Brain	0.053 ± 0.004	0.045 ± 0.003	0.033 ± 0.006	0.02 ± 0.003	0.004 ± 0.0006
SNp (i.n.)	Blood	0.063 ± 0.01	0.075 ± 0.004	0.059 ± 0.005	0.042 ± 0.006	0.015 ± 0.009
	Brain	0.07 ± 0.005	0.061 ± 0.006	0.045 ± 0.003	0.031 ± 0.009	0.007 ± 0.001
PE-mAb-Tfr-SNp (i.n.)	Blood	0.059 ± 0.003	0.077 ± 0.011	0.096 ± 0.009	0.082 ± 0.007	0.029 ± 0.005
	Brain	0.113 ± 0.02	0.125 ± 0.013	0.117 ± 0.014	0.078 ± 0.005	0.018 ± 0.004
SME (i.n.)	Blood	0.061 ± 0.005	0.067 ± 0.009	0.074 ± 0.005	0.059 ± 0.004	0.015 ± 0.007
	Brain	0.086 ± 0.003	0.081 ± 0.01	0.069 ± 0.004	0.046 ± 0.003	0.008 ± 0.001
SMME (i.n.)	Blood	0.066 ± 0.002	0.078 ± 0.006	0.094 ± 0.007	0.089 ± 0.009	0.015 ± 0.003
	Brain	0.151 ± 0.009	0.144 ± 0.05	0.131 ± 0.013	0.104 ± 0.011	0.009 ± 0.002
SNp (p.o.)	Blood	0.0093 ± 0.0011	0.0126 ± 0.004	0.0137 ± 0.004	0.0104 ± 0.004	0.002 ± 0.0005
	Brain	0.0061 ± 0.0005	0.0088 ± 0.002	0.0075 ± 0.001	0.0056 ± 0.001	0.001 ± 0.0004
SME (p.o.)	Blood	0.002 ± 0.0004	0.0035 ± 0.0005	0.005 ± 0.0006	0.003 ± 0.0011	0.001 ± 0.0003
	Brain	0.0007 ± 0.0001	0.0013 ± 0.0003	0.0019 ±0.0003	0.0011 ± 0.0004	0.0002 ± 0.0001
SME (i.v.)	Blood	0.103 ± 0.02	0.115 ± 0.022	0.094 ± 0.012	0.072 ± 0.012	0.021 ± 0.006
	Brain	0.0058 ± 0.0003	0.0046 ± 0.001	0.0033 ± 0.0007	0.0021 ± 0.0005	0.0004 ± 0.0001
SNp (i.v.)	Blood	0.261 ± 0.013	0.275 ± 0.013	0.242 ± 0.02	0.213 ± 0.023	0.031 ± 0.004
	Brain	0.0149 ± 0.005	0.0137 ± 0.002	0.0119 ± 0.004	0.0078 ± 0.0011	0.001 ± 0.0005
PE-mAb-Tfr-SNp (i.v.)	Blood	0.317 ± 0.012	0.331 ± 0.021	0.322 ± 0.03	0.294 ± 0.012	0.058 ± 0.007
	Brain	0.0129 ± 0.005	0.0154 ± 0.004	0.0116 ± 0.005	0.0091 ± 0.001	0.0036 ± 0.0004
Brain to blood ratio						
SS (i.n.)	Brain/Blood	0.67 ± 0.05	0.523 ± 0.04	0.516 ± 0.03	0.426 ± 0.04	0.364 ± 0.02
SNp (i.n.)	Brain/Blood	1.11 ± 0.03	0.81 ± 0.05	0.763 ± 0.04	0.738 ± 0.05	0.47 ± 0.04
PE-mAb-Tfr-SNp (i.n.)	Brain/Blood	1.92 ± 0.06	1.62 ± 0.03	1.22 ± 0.06	0.95 ± 0.05	0.62 ± 0.03

SME (i.n.)	Brain/Blood	1.41 ± 0.07	1.21 ± 0.06	0.93 ± 0.04	0.78 ± 0.04	0.53 ± 0.04
SMME (i.n.)	Brain/Blood	2.29 ± 0.04	1.85 ± 0.05	1.39 ± 0.07	1.17 ± 0.06	0.6 ± 0.03
SNp (p.o.)	Brain/Blood	0.66 ± 0.05	0.698 ± 0.03	0.548 ± 0.02	0.539 ± 0.05	0.5 ± 0.02
SME (p.o.)	Brain/Blood	0.35 ± 0.06	0.372 ± 0.04	0.38 ± 0.03	0.37 ± 0.04	0.2 ± 0.05
SME (i.v.)	Brain/Blood	0.056 ± 0.02	0.04 ± 0.02	0.035 ± 0.01	0.029 ± 0.01	0.019 ± 0.01
SNp (i.v.)	Brain/Blood	0.057 ± 0.03	0.05 ± 0.01	0.049 ± 0.02	0.037 ± 0.02	0.032 ± 0.02
PE-mAb-Tfr-SNp (i.v.)	Brain/Blood	0.076 ± 0.01	0.08 ± 0.04	0.073 ± 0.01	0.065 ± 0.02	0.062 ± 0.01

The rats were administered with 50μCi ^{99m}Tc-SB and the radioactivity was measured in percent per g of tissue of the administered dose. Values are expressed as mean ± SEM of three estimations. '' Indicates that the variation in values between SS (i.n.), SNp (i.n.), PE-mAb-Tfr-SNp (i.n.) or SNp (i.v.) when compared to PE-mAb-Tfr-SNp (i.v.) are significant ($p < 0.05$)

Figure 6.2 Brain concentrations versus time (hr) plot for NG formulations

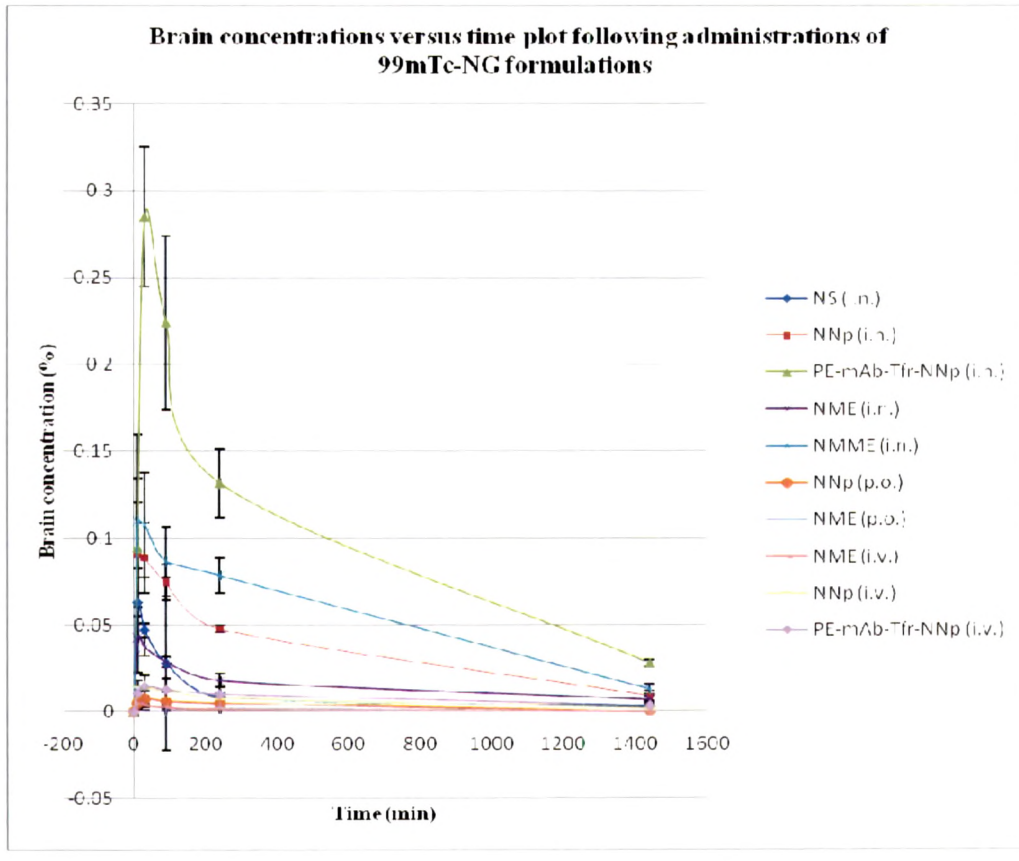


Figure 6.3 Brain concentrations versus time (hr) plot for HG formulations

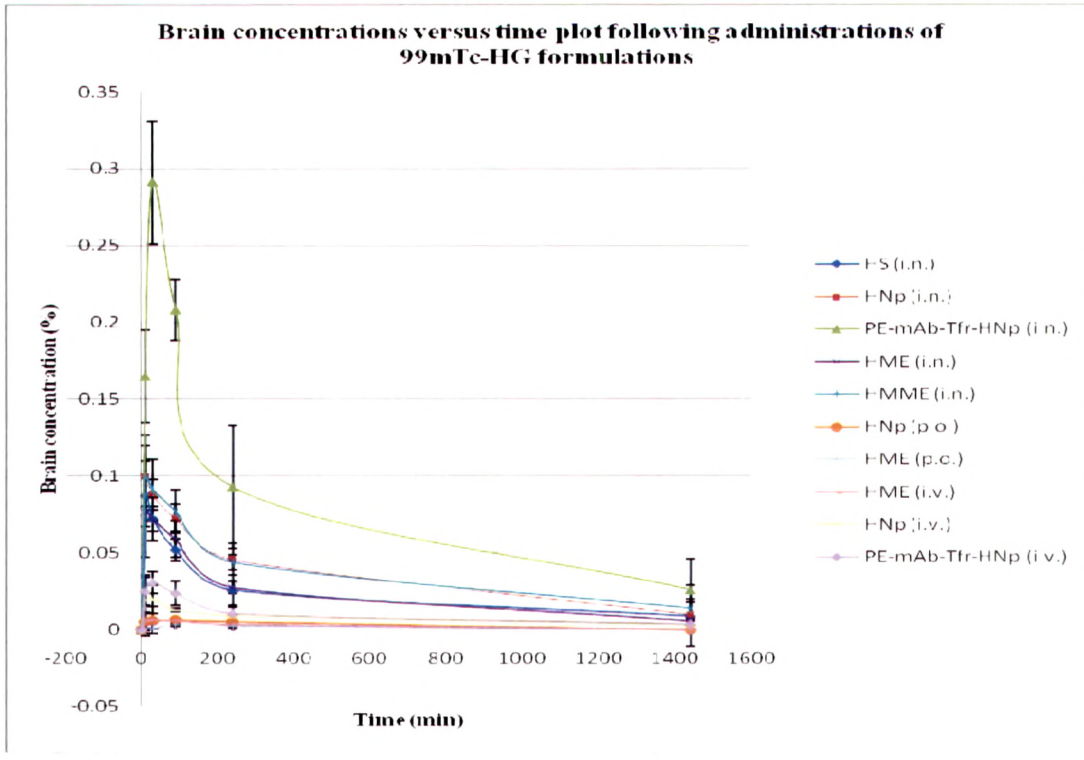


Figure 6.4 Brain concentrations versus time (hr) plot for SB formulations

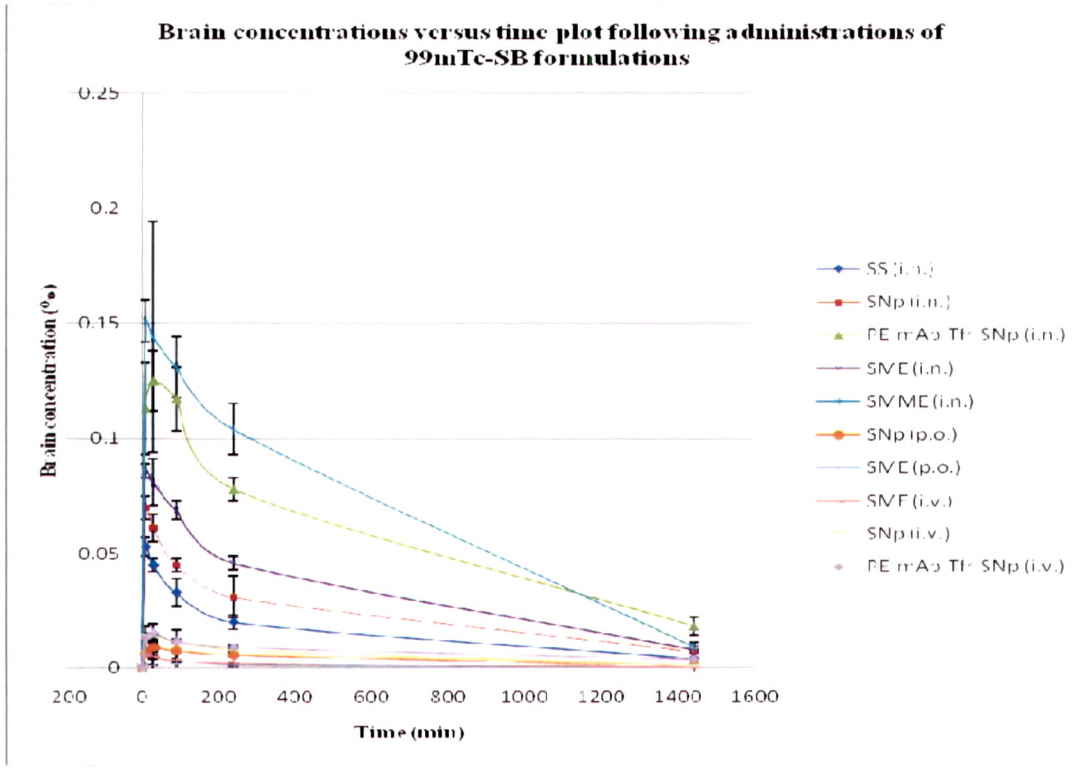


Figure 6.5 Blood concentrations versus time (hr) plot for NG formulations

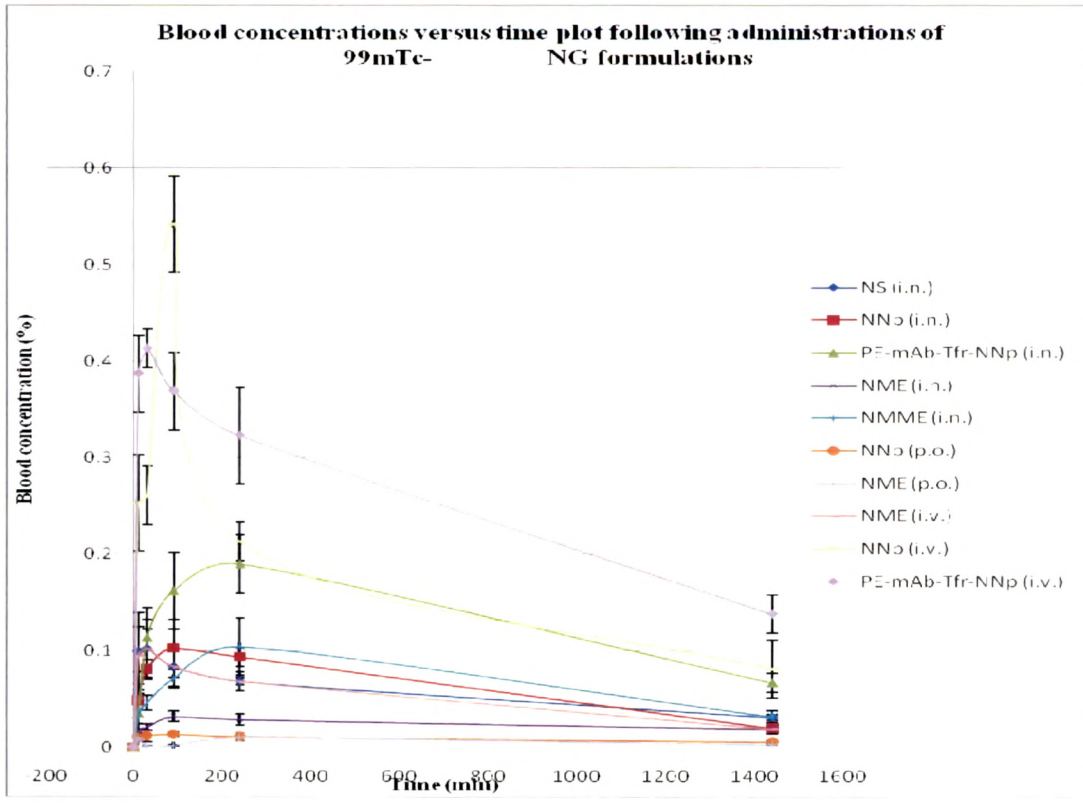


Figure 6.6 Blood concentrations versus time (hr) plot for HG formulations

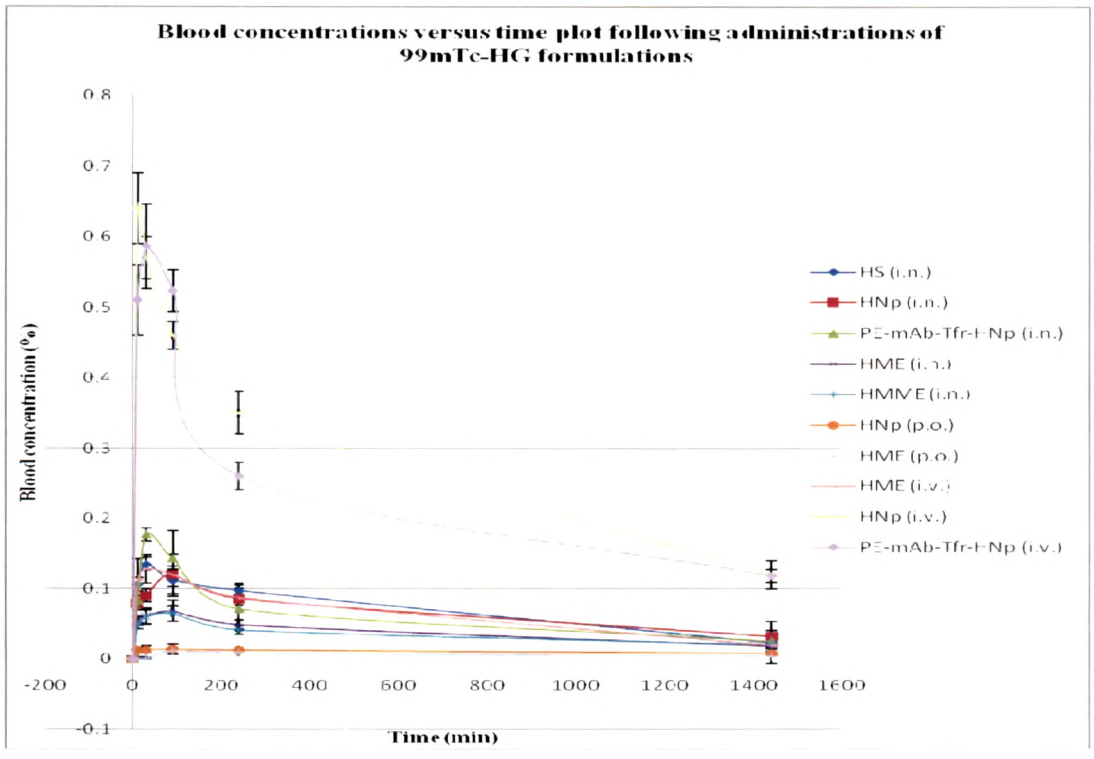


Figure 6.7 Blood concentrations versus time (hr) plot for SB formulations

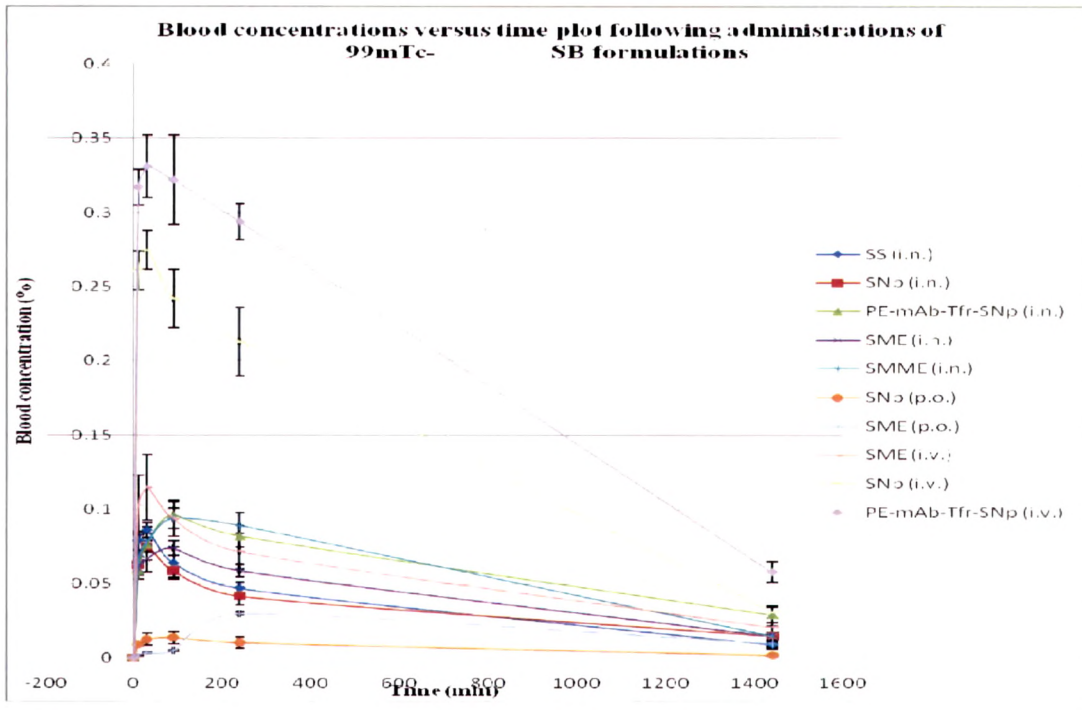


Table 6.6: Pharmacokinetics of ^{99m}Tc labeled NG formulations at predetermined time intervals in balb/C mice*

Formulation (route of administration)	Organ/ Tissue	C_{max} (%/g)	T_{max} (min)	$AUC_{0\rightarrow1440}$ (min*%/g)	$AUC_{0\rightarrow\infty}$ (min*%/g)	MRT (min)	$T_{1/2}$ (min)
NS (i.n.)	Blood	0.102 ± 0.017	30	75.85 ± 5.7	120.08 ± 11.8	1433.3 ± 14	989.1 ± 10
	Brain	0.063 ± 0.005	10	12.37 ± 1.6	13.93 ± 2.8	590.67 ± 11	373.27 ± 7
NNp (i.n.)	Blood	0.102 ± 0.01	90	78.6 ± 6.2	94.79 ± 7.1	829.71 ± 12	561.17 ± 9
	Brain	0.091 ± 0.004	10	44.2 ± 5.3	49.9 ± 3.6	652.35 ± 10	436.9 ± 6
PE-mAb-Tfr-NNp (i.n.)	Blood	0.142 ± 0.013	90	92.96 ± 8.1	103.59 ± 9.7	433.44 ± 9	652.11 ± 12
	Brain	0.285 ± 0.015	30	126.05 ± 11.5	145.43 ± 12.3	645.11 ± 14	424.91 ± 8
NME (i.n.)	Blood	0.032 ± 0.007	90	35 ± 3.2	85.92 ± 6.9	2714.71 ± 13	1857.33 ± 16
	Brain	0.043 ± 0.009	10	20.44 ± 2.3	27.8 ± 2.4	1060.3 ± 10	728.93 ± 13
NMME (i.n.)	Blood	0.104 ± 0.018	90	77.16 ± 6.7	117.52 ± 9.8	1328.51 ± 14	874.16 ± 11
	Brain	0.11 ± 0.011	10	65.32 ± 6.4	74.18 ± 5.6	684.28 ± 11	469.65 ± 8
NNp (p.o.)	Blood	0.0132 ± 0.006	90	12.78 ± 2.2	23.56 ± 3.3	1824.09 ± 12	1245.19 ± 13
	Brain	0.0052 ± 0.001	10	3.02 ±1.1	3.15 ± 0.7	456.58 ± 8	314.6 ± 7
NME (p.o.)	Blood	0.01 ± 0.004	90	3.94 ± 0.9	4.61 ± 1.1	742.49 ± 10	463.8 ± 6
	Brain	0.0024 ± 0.002	90	0.95 ± 0.4	1.07 ± 0.3	655.36 ± 11	420.32 ± 8
NME (i.v.)	Blood	0.102 ± 0.009	30	74.18 ± 7.2	112.72 ± 11.6	1334.41 ± 13	921.06 ± 10
	Brain	0.0049 ± 0.001	10	1.4 ± 0.8	1.51 ± 0.4	517.16 ± 14	371.64 ± 8

NNp (i.v.)	Blood	0.261 ± 0.011	30	219.4 ± 14.2	319.55 ± 13.2	1241.26 ± 15	857 ± 11
	Brain	0.0142 ± 0.009	10	7.68 ± 2.4	9.18 ± 1.5	784.91 ± 9	521.85 ± 8
PE-mAb-Tfr-NNp (i.v.)	Blood	0.413 ± 0.012	30	346.25 ± 16.7	537.67 ± 16.2	1392.23 ± 16	961.47 ± 10
	Brain	0.0152 ± 0.007	30	10.96 ± 2.7	15.68 ± 3.1	1193.43 ± 14	818.25 ± 9

*The rats were administered with 50μCi ^{99m}Tc-NG and the radioactivity was measured in percent per g of tissue of the administered dose. Values are expressed as mean ± SEM of three estimations.

Table 6.7: Pharmacokinetics of ^{99m}Tc labeled HG formulations at predetermined time intervals in balb/C mice*

Formulation (route of administration)	Organ/ Tissue	C_{max} (%/g)	T_{max} (min)	$AUC_{0\rightarrow1440}$ (min*%/g)	$AUC_{0\rightarrow\infty}$ (min*%/g)	MRT (min)	$T_{1/2}$ (min)
HS (i.n.)	Blood	0.135 ± 0.009	30	87.68 ± 15.4	107.12 ± 21.3	846.94 ± 10	585.99 ± 11
	Brain	0.087 ± 0.04	10	30.56 ± 3.9	37.49 ± 3.9	816 ± 11	533.7 ± 10
HNp (i.n.)	Blood	0.12 ± 0.007	90	89.24 ± 7.3	124.1 ± 10.3	1125 ± 16	753.8 ± 12
	Brain	0.1 ± 0.02	10	44.27 ± 3.1	51.44 ± 3.7	715.34 ± 10	496.7 ± 5
PE-mAb-Tfr-HNp (i.n.)	Blood	0.177 ± 0.009	30	80.89 ± 6.9	100.81 ± 9.8	859.13 ± 12	552.4 ± 9
	Brain	0.291 ± 0.07	30	104.73 ± 9.6	121.76 ± 11.5	699.25 ± 9	454.1 ± 6
HME (i.n.)	Blood	0.068 ± 0.02	90	51.13 ± 6.6	70.55 ± 5.9	1107.22 ± 14	747.72 ± 6
	Brain	0.077 ± 0.006	10	29.17 ± 2.2	32.69 ± 3.2	618.59 ± 11	406.39 ± 6
HMME (i.n.)	Blood	0.064 ± 0.01	90	47.84 ± 3.7	74.13 ± 6.8	1361.28 ± 16	911.36 ± 6
	Brain	0.099 ± 0.02	10	47.71 ± 4.1	58.57 ± 4.4	831.52 ± 12	537.99 ± 6
HNp (p.o.)	Blood	0.0137 ± 0.004	90	15.25 ± 2.6	35.71 ± 4.6	2572.9 ± 17	1773.49 ± 6
	Brain	0.0069 ± 0.001	90	3.14 ± 0.5	3.17 ± 0.4	344.41 ± 9	214.88 ± 6
HME (p.o.)	Blood	0.0113 ± 0.005	90	4.68 ± 0.6	17.82 ± 2.7	3819.51 ± 13	2396.32 ± 6
	Brain	0.0031 ± 0.001	90	1.21 ± 0.2	1.4 ± 0.3	707.98 ± 11	450.55 ± 6
HME (i.v.)	Blood	0.128 ± 0.03	30	79.32 ± 7.1	93.51 ± 8.2	761.22 ± 12	517.65 ± 6
	Brain	0.0065 ± 0.001	30	3.05 ± 0.9	3.26 ± 0.6	516.4 ± 10	352.91 ± 6
HNp (i.v.)	Blood	0.64 ± 0.05	10	364.28 ± 25.3	489.84 ± 24.8	1048.34 ± 14	725.26 ± 11
	Brain	0.026 ± 0.009	10	8.78 ± 1.3	10.28 ± 1.4	728.63 ± 11	519.03 ± 9
PE-mAb-Tfr-HNp (i.v.)	Blood	0.586 ± 0.06	30	318.89 ± 16.4	433.04 ± 15.7	1039.6 ± 16	670.56 ± 10
	Brain	0.031 ± 0.007	30	13.13 ± 2.2	16.23 ± 3.1	832.38 ± 12	536.84 ± 8

*The rats were administered with 50μCi ^{99m}Tc-HG and the radioactivity was measured in percent per g of tissue of the administered dose. Values are expressed as mean ± SEM of three estimations.

Table 6.8: Pharmacokinetics of ^{99m}Tc labeled SB formulations at predetermined time intervals in balb/C mice*

Formulation (route of administration)	Organ/ Tissue	C_{max} (%/g)	T_{max} (min)	$AUC_{0\rightarrow1440}$ (min*%/g)	$AUC_{0\rightarrow\infty}$ (min*%/g)	MRT (min)	$T_{1/2}$ (min)
SS (i.n.)	Blood	0.086 ± 0.011	30	42.2 ± 4.3	48.52 ± 4.8	700.06 ± 13	486.74 ± 6
	Brain	0.053 ± 0.005	10	19.39 ± 2.9	22.09 ± 3.4	664.57 ± 11	468.98 ± 5
SNp (i.n.)	Blood	0.075 ± 0.009	30	44.67 ± 4.1	60.38 ± 4.7	1056.92 ± 15	726.24 ± 10
	Brain	0.07 ± 0.011	10	29.8 ± 2.2	35.08 ± 3.2	746.4 ± 10	523.01 ± 7
PE-mAb-Tfr-SNp (i.n.)	Blood	0.096 ± 0.013	90	81.36 ± 3.6	114.35 ± 9.3	1160.85 ± 14	788.71 ± 12
	Brain	0.125 ± 0.012	30	73.73 ± 5.2	87.07 ± 7.6	759.93 ± 12	513.46 ± 8
SME (i.n.)	Blood	0.074 ± 0.011	90	54.3 ± 4.2	67.16 ± 5.2	874.08 ± 10	594.21 ± 7
	Brain	0.086 ± 0.009	10	41.17 ± 3.3	46.35 ± 4.7	648.2 ± 10	449.23 ± 8
SMME (i.n.)	Blood	0.094 ± 0.013	90	90.08 ± 6.9	136.79 ± 9.6	726.58 ± 13	492.59 ± 6
	Brain	0.151 ± 0.016	10	76.08 ± 6.2	80.57 ± 6.6	506.8 ± 8	345.71 ± 4
SNp (p.o.)	Blood	0.0137 ± 0.006	90	8.97 ± 1.7	10.39 ± 2.1	729.29 ± 11	493.13 ± 7
	Brain	0.0088 ± 0.002	30	4.85 ± 0.9	5.53 ± 1.1	686.85 ± 9	471.3 ± 5
SME (p.o.)	Blood	0.005 ± 0.0011	90	3.09 ± 1.1	4.03 ± 0.9	968.1 ± 12	638.22 ± 8
	Brain	0.0019 ± 0.0007	90	0.97 ± 0.3	1.1 ± 0.3	667.91 ± 11	440.69 ± 5
SME (i.v.)	Blood	0.115 ± 0.019	30	70.99 ± 6.4	90.47 ± 7.4	931.63 ± 13	642.87 ± 9
	Brain	0.0058 ± 0.0013	10	2.0 ± 0.3	2.26 ± 0.5	654.96 ± 10	464.08 ± 7
SNp (i.v.)	Blood	0.275 ± 0.021	30	169.55 ± 11.3	189.55 ± 11.6	648.45 ± 11	447.31 ± 6
	Brain	0.0149 ± 0.009	10	6.56 ± 1.5	7.1 ± 1.8	556 ± 8	380.65 ± 5
PE-mAb-Tfr-SNp (i.v.)	Blood	0.331 ± 0.016	30	248.29 ± 12.3	292.86 ± 12.1	774.11 ± 11	532.57 ± 8
	Brain	0.0154 ± 0.005	30	9.81 ± 1.7	14.15 ± 1.8	834.42 ± 12	1207.9 ± 16

*The rats were administered with 50μCi ^{99m}Tc-SB and the radioactivity was measured in percent per g of tissue of the administered dose. Values are expressed as mean ± SEM of three estimations.

Figure 6.8: Gamma scintigraphy images of Balb/C mice (A/P view) showing the presence of radioactivity in brain following administrations of ^{99m}Tc labeled NG formulations

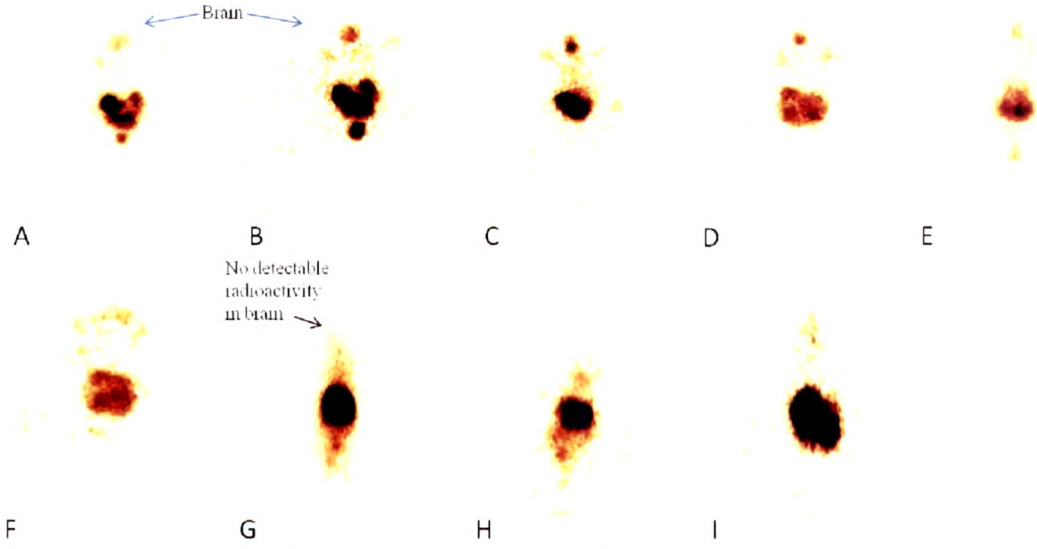


Figure 6.8 Gamma scintigraphy images of Balb/C mice (A/P view) showing the presence of radioactivity in brain following administrations of ^{99m}Tc-NG formulations A NS (i.n.), B NNp (i.n.), C PE-mAb-Tfr-NNp (i.n.), D NME (i.n.), E NMME (i.n.), F NNp (i.v.), G PE-mAb-Tfr-NNp (i.v.), H NME (i.v.), I NNp (p.o.)

Table 6.9: Drug targeting index and direct brain drug targeting percentage following intranasal administration of NG, HG and SB formulations

Formulation and route of administration	Drug Targeting Index (DTI)	Direct Brain Drug Targeting Percentage (%DTP)
NS (i.n.)	3.98 ± 0.4	74.86 ± 3.2
NNp (i.n.)	18.05 ± 2.3	94.46 ± 5.4
PE-mAb-Tfr-NNp (i.n.)	48.14 ± 2.6	97.92 ± 4.7
NME (i.n.)	11.1 ± 0.9	90.99 ± 3.3
NMME (i.n.)	21.64 ± 1.3	95.38 ± 4.1
HS (i.n.)	9.34 ± 0.5	89.29 ± 2.9
HNp (i.n.)	11.06 ± 1.2	90.96 ± 4.5
PE-mAb-Tfr-HNp (i.n.)	32.23 ± 3.5	96.9 ± 3.6
HME (i.n.)	12.36 ± 1.1	91.91 ± 2.2
HMME (i.n.)	21.08 ± 1.6	95.26 ± 3.1
SS (i.n.)	9.42 ± 0.6	89.39 ± 2.4
SNp (i.n.)	12.03 ± 1.5	91.68 ± 3.5
PE-mAb-Tfr-SNp (i.n.)	15.76 ± 1.2	93.66 ± 2.7
SME (i.n.)	14.28 ± 1.1	93 ± 4.1
SMME (i.n.)	12.19 ± 1.7	91.8 ± 2.3

Values are expressed as mean ± SEM of three estimations.

6.4 DISCUSSION

NG, HG and SB formulations were labelled with ^{99m}Tc with high labeling efficiency using direct labelling method. The quantity of stannous chloride to reduce ^{99m}Tc plays an important role in the labelling efficiency. Lower quantity of stannous chloride leads to low labelling efficiency where as higher amount of stannous chloride leads to formation of undesirable radiocolloids. The optimum quantity of stannous chloride for high labeling efficiency and low free and reduced/hydrolyzed ^{99m}Tc, was found to be 250µg for all preparations. The incubation time was optimized as 10min. The pH for all the formulations was kept around 6.8±0.5. The labeling efficiency and the stability of labeled complex were ascertained by ascending TLC using ITLC strips. The labelling efficiency for ^{99m}Tc labelled drug formulations was found to be more than 98%. The invitro stability of radiolabelled preparations was checked in presence of rat serum and 0.9%w/v sodium chloride. Rat serum was selected to mimic the experiment in-vivo conditions related to serum proteins and physiological pH. The labelling efficiency of ^{99m}Tc labelled formulation at all the time points is found to be greater than 90%. Bonding strength of ^{99m}Tc labelled drug formulations was also investigated by the DTPA challenging test, and the percent transchelation of the labeled complex was below 4%w/w at highest concentration tested (50mM). The results suggested high bonding strength and stability of ^{99m}Tc labelled drug formulations. Thus these formulations were found suitable for biodistribution studies.

Drug concentrations in brain following intranasal administration of ^{99m}Tc labelled drug formulations were found to be significantly higher at all time points compared to intravenous and oral administrations. Also, at all time points the brain/blood ratios were calculated for the formulations and were found to be significantly higher for intranasally administered formulations than formulations administered intravenously or oral, demonstrating intranasal delivery a promising strategy to deliver drugs quickly and effectively to the brain. Also, the brain/blood ratios for ^{99m}Tc-PE-mAb-Tfr-HNp (i.n.) at all time points were found to be more than 10 folds compared to ^{99m}Tc-PE-mAb-Tfr-HNp (i.v.). The significantly high brain/blood ratios for ^{99m}Tc-PE-mAb-Tfr-HNp (i.n.) compared to ^{99m}Tc-HS (i.n.) and ^{99m}Tc-HNp (i.n.) demonstrates selective uptake of antibody conjugated nanoparticles into the brain compared to unconjugated nanoparticles and drug solution across transferrin receptors at the olfactory bulb of animals. These receptors are expressed abundantly on the olfactory

bulb of rodents resulting in selective and effective distribution of antibody (specific to transferrin receptors) conjugated nanoparticles into the brain than other formulations.

Intranasal antibody conjugated nanoparticles showed highest C_{max} (brain) compared to other intranasally administered formulations. Also, the C_{max} (brain) for intranasal antibody conjugated nanoparticles was found to be approximately 10-folds and more than 50-folds higher when compared to intravenous and oral administrations respectively. C_{max} (brain) and $AUC_{0\rightarrow\infty}$ (brain) of intranasal antibody conjugated nanoparticles is approximately 2.5 folds than other intranasal formulations demonstrating antibody conjugated nanoparticles a suitable formulation to deliver drugs effectively to the brain intranasally.

The antibody conjugated nanoparticles showed the highest DTI and DTP% values among all intranasally administered formulations. Higher DTI and DTP% for antibody conjugated nanoparticles may be attributed to the higher brain concentrations of drug following its intranasal administration resulting from selective uptake of antibody conjugated nanoparticles across the transferrin receptors on the murine olfactory bulb.

Gamma scintigraphy imaging demonstrate significantly high radioactivity in the animal brain for intranasal antibody conjugated nanoparticles compared to other intranasal formulations and formulations administered intravenously and orally and supports selective and effective transport of drug to the brain following intranasal administration of antibody conjugated nanoparticles. Thus, gamma scintigraphy images are consistent with the biodistribution data shown in tables.

6.5 CONCLUSION

The results of the study demonstrate intranasal delivery a practical strategy to deliver drugs effectively to the brain. Also, the results demonstrate antibody conjugated nanoparticles a suitable delivery system to deliver drugs selectively to the brain as supported by high AUC, DTI and DTP% values for antibody conjugated nanoparticles compared to other formulations. The increased brain AUC and DTI and DTP% illustrate selective nose to brain transport of drugs following intranasal delivery than intravenous administration. Thus, the developed intranasal antibody conjugated

nanoparticles would demonstrate advantage over conventional nasal formulations in treating brain disorders by being more brain selective with a possibility of reducing drug dose and/or frequency of dosing and, possibly the cost of therapy.

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