Chapter 14

Investigations on the Effect of the Stabilizer on the brain uptake of Olanzapine loaded Solid lipid nanoparticles

14.1 INTRODUCTION

The ability of non ionic surfactants to inhibit the P glycoprotein (Pgp) efflux pump has been studied by various research teams across the globe. Rege and co workers studied the effect of non ionic surfactants on Pgp on Caco 2 cell lines (Rege BD et al., 2002). Hu and co workers observed that the di-ethyl fraction of Labrasol® enhanced the absorption of gentamycin than Labrasol® itself (Hu Z et al., 2002). Bogman and coworkers during their study in humans observed that Vitamin E TPGS increased the bioavailability of Pgp substrate talinolol when compared to poloxamer 188 (Bogman K et al., 2005).

Hugger studied the Pgp inhibitory ability of some polyethoxylated surfactants (Tween 80, Cremophor EL and PEG 300) on MDR1- transfected Madin Darby Canine Kidney (MDR1-MDCK) cell lines and compared the results with Caco 2 cell lines. He reported that PEG-300 (20%, v/v) caused almost complete inhibition of P-gp activity in both Caco-2 and MDR1-MDCK cell monolayers, whereas Cremophor EL (0.1%, w/v) and Tween 80 (0.05%, w/v) partially inhibited P-gp activity in Caco-2 cells. Cremophor EL (0.1%, w/v) and Tween 80 (0.05%, w/v) were inactive as P-gp inhibitors in MDR1-MDCK cell monolayers. This inability of Tween 80 and Cremphor EL to inhibit P-gp activity in MDR1-MDCK cells may be related to differences in the interactions of the surfactants with these different cell membranes. PEG-induced changes in P-gp activity are probably related to changes in the fluidity of the polar head group regions of cell membranes (Hugger ED et al., 2002). Cornaire and coworkers studied the effect of some surfactants to increase the absorption of digoxin and celiprolol in vitro. The most effective excipients with digoxin were (at 0.5%, w/v): Labrasol > Imwitor 742 > Acconon E = Softigen 767 > Cremophor EL > Miglyol > Solutol HS 15 > Sucrose monolaurate > Polysorbate 20 > TPGS > Polysorbate 80. With celiprolol, Cremophor EL and Acconon E had no effect, but transport was enhanced by Softigen 767 > TPGS > Imwitor 742 (Cornaire G et al., 2004). Constantinides and Wasan reported that incorporation of a nonspecific lipid and/or polymer excipient in the formulation can potentially increase the bioavailability of many drugs (Constantinides PP and Wasan KM., 2006).

Batrokova and coworkers studied the optimal structure requirements for pluronic block copolymers in modifying Pgp mediated drug efflux transporter activity in bovine brain microvessel endothelial cells (BBMECs). They scanned the whole series of Pluronics varying

in lengths of propylene oxide and ethylene oxide and overall lipophilicity. Using a wide range of block copolymers, differing in hydrophilic-lipophilic balance (HLB), and this study shows that lipophilic Pluronics with intermediate length of propylene oxide block (from 30 to 60 units) and HLB < 20 are the most effective at inhibiting Pgp efflux in BBMECs while the hydrophilic copolymers (F38, F68, F88 and F108) of the Pluronic group of surfactants had poor Pgp inhibitory activity in the BBMEC monolayers (Batrokova EV et al., 2003). Wang and co workers determined P-gp inhibition of some commonly used excipients using an integrated high-throughput process (Wang SW et al., 2004). From the list of excipients reported by Wang SW and co workers, we have selected Gelucire® 44/14 and Transcutol® for preparation of OL SLN. It was hypothesized that the above excipients can inhibit the P-gp drug efflux pump present at the BBB and may enhance the brain delivery of olanzapine. Inspite of the encouraging Pgp inhibitory results obtained with these non ionic surfactants on the Caco -2 and other cell lines very limited reports are available on the potential use of these surfactants on the Pgp inhibitory activity at the blood brain barrier and hence increase the brain delivery of some centrally acting drugs.

Panchagnula and Ritschel used Transcutol® as a co solvent (upto 50%v/v) for intracutaneous depot injection of dexamethasone. Use of Transcutol® through the parenteral route is therefore been explored earlier (Panchagnula R and Ritschel WA., 1991). But the potential use of Transcutol® stabilized nanoparticles in increasing the brain uptake in still unexplored. Gelucire® 44/14 has been proved to inhibit the Pgp efflux pump present in the intestine and increase the oral bioavailability of some Pgp substrates (Thambo and Lee., 2006). But the potential use of Gelucire® stabilized nanoparticles in increasing the brain uptake in still unexplored.

Gelucire® 44/14, Transcutol® and poloxamer 188 stabilized OL loaded Solid lipid nanoparticles were prepared and characterized. The SLNs were also subjected to stability evaluation at different storage conditions (Chapter 13). In the present chapter, ^{99m}Tc Radiolabelled complexes of OL aqueous solutions in both Gelucire® 44/14 (2%w/v) and Transcutol® (5%w/v) were administered intravenously to study the effect of the non ionic surfactant alone in increasing the brain delivery of OL (Positive control). ^{99m}Tc radiolabelled complex of Poloxamer 188 (surfactant that does not inhibit Pgp) stabilized SLN were also

prepared to study the change in brain concentration after formulation of OL into SLNs (negative control). ^{99m}Tc radiolabelled complexes of Gelucire® 44/14 stabilized SLN and Transcutol® stabilized SLN were administered intravenously and % radioactivity remaining in the brain was determined.

Different routes of administration may result in different biodistribution patterns. Allen and coworkers studied the effect of different routes of administration. In his study, Allen compared the biodistribution patterns of liposomes administered through different routes of administration and reported wide differences in the distribution patterns. Thus, the route of administration appears to be a major parameter in deciding the biodistribution of the drug in vivo. Presently all antipsychotics are given by intramuscular route when administered through the parenterally. The present investigation was aimed to study the differences in the in vivo biodistribution patterns and the brain deposition obtained with the intravenous route and the intramuscular route. ^{99m}Tc radiolabelled complexes of Gelucire® 44/14 stabilized SLN and Transcutol® stabilized SLN were administered intramuscularly and % radioactivity remaining in the brain was determined.

Preparation of olanzapine loaded solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) were prepared by the slight modification of melt emulsification and high pressure homogenization method reported earlier (Reddy et al., 2006). Briefly, the lipid was melted and OL was dissolved to obtain drug-lipid mixture. Hydrogenated soya phosphatidyl choline (HSPC) was dissolved in about 0.3 ml chloroform and added to the above lipid- drug mixture. The mixture was warmed at about 60 to 65°C to evaporate the chloroform totally and the clear lipid melt containing HSPC was added to the hot aqueous surfactant solution (2% poloxamer 188 incase of POL188 SLN; 5% Transcutol® incase of TROL SLN; 2% Gelucire® 44/14 incase of GOL SLN) preheated to 10°C above the melting point of the lipid, under high shear homogenization at 9500 rpm for 1 minute using Ultra Turrax (Ultra Turrax T-25, Germany) to result in a crude emulsion. The crude emulsion was subsequently homogenized in a high pressure homogenizer (Emulsiflex C5, Avestin, Canada) in a water bath maintained at 10°C above the melting point of the lipid. The nanoemulsion obtained was then cooled at room temperature to recrystallize the lipid back to the solid state. This resulted in the formation of drug entrapped SLN dispersions in an aqueous media.

14.2 EXPERIMENTAL

Pharmacokinetic and biodistribution studies

Animals

Albino rats of either sex weighing about 200-250 gm were selected for the study. The rats were fasted overnight before study and were accessed to water *ad libitum*. All the animal experiments were approved by CPCSEA and local animal ethics committee.

Radiolabeling protocol, in vitro stability and challenge tests

The radiolabeling protocol utilized for radiolabeling of plain olanzapine solution, poloxamer 188 stabilized olanzapine loaded SLN (POL188 SLN), Gelucire 44/14® stabilized olanzapine loaded SLN (GOL SLN) and Transcutol® stabilized olanzapine loaded SLN (TROL SLN) are described in detail in chapter 5. The radiolabelled complexes were also subjected to in vitro serum stability and transchelation studies to study their potential use as biomarkers for blood clearance and biodistribution studies (Chapter 5).

Blood clearance of plain olanzapine and its nanoparticle formulations in rabbits

White New Zealand Rabbits of either sex weighing 2.8 to 3.0 kg were selected for the blood clearance studies.Through the ear vein of rabbit, 0.5 mL of the ^{99m}Tc-labeled complexes of OL / POL188 SLN / GOL SLN / TROL SLN containing 500 µCi of ^{99m}Tc was intravenously injected. The blood samples were collected at 5 minutes, 15 minutes, 0.5 hour, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 6 hours, and 24 hours from the ear vein of rabbit and analyzed for the radioactivity in gamma ray spectrometer.

Further, to study the blood kinetics after intramuscular administration, GOL SLN and TROL SLN containing 500 µCi of ^{99m}Tc was injected intramuscularly into the hind limb of rabbits and the blood samples were withdrawn at the above mentioned predetermined time intervals and analyzed for the radioactivity in gamma ray spectrometer. The blood was weighed, and radioactivity in whole blood was calculated by considering the volume of blood as 7.3% of the total body weight.

Biodistribution studies

^{99m}Tc-labeled complexes of OL, GOL solution, TROL solution, GOL SLN, TROL SLN and POL188 SLN were injected intravenously through the tail vein into healthy Balb/c mice weighing ~ 25 to 30 g. The biodistribution studies of OL, GOL solution, TROL solution, POL188 SLN, GOL SLN and TROL SLN were performed after 1 hour, 4 hours, and 8 hours postinjection. At these time intervals, the blood was collected by cardiac puncture, the animals were humanely killed, and the organs were isolated.

Formulation	Purpose
Plain OL	To study the inherent ability of the drug to cross the BBB
GOL Solution / TROL Solution	*Negative Control:* To study the effect of the Pgp inhibitor alone in helping the drug to cross the BBB (Gelucire® 44/14 and Transcutol® are reported to inhibit the Pgp efflux pump) (Wang SW et al., 2004)
Poloxamer 188 stabilized SLN (POL188 SLN)	*Positive Control:* To study the effect of nanoparticle alone in helping the drug to cross the BBB (Poloxamer 188 does not inhibit the Pgp efflux pump)
GOL SLN / TROL SLN	To study the combined effect of both the Pgp inhibit and nanoparticle in crossing the BBB

Further, to study the biodistribution after intramuscular administration, GOL SLN and TROL SLN containing 500 μCi of ^{99m}Tc was injected intramuscularly into the hind limb of Balb/c mice and organs were isolated and analyzed for the radioactivity in gamma ray spectrometer. The organs were then weighed and measured for radioactivity in gamma ray spectrometer. The radioactivity was interpreted as percentage of injected dose per gram of organ/tissue. All the

animal experiments were approved by the Social Justice and Empowerment Committee for the purpose of control and supervision of experiments on animals, New Delhi, India.

Gamma scintigraphy

^{99m}Tc-labeled complexes of OL/ GOL solution / TROL solution / POL188 SLN / GOL SLN / TROL SLN containing 500 μCi of ^{99m}Tc was intravenously injected into the ear vein of healthy white New Zealand rabbits. After 1 hour post injection, the mice were fixed an animal fixing tray board and imaging was performed with Single Photon Emission Computed Tomography (SPECT, LC 75-005, Diacam, Siemens, USA) gamma camera.

Statistical Analysis

Statistical comparisons of the experimental data were performed by one way analysis of variance (ANOVA) at α level of 0.05.

14.3 RESULTS AND DISCUSSION

Blood Clearance Studies

Blood clearance studies of ^{99m}Tc-labeled complexes of OL / POL188 SLN / GOL SLN / TROL SLN were investigated in healthy white New Zealand Rabbits. After i.v. administration, all the SLN formulations exhibited higher blood concentrations compared with OL solution (Figure 14.1). The residence time of all the SLN formulations in the blood was significantly higher when compared to the plain drug. However, there was insignificant difference in the residence time in blood between the different SLN formulations studied NS. These data indicate the long circulation capability of the formulated SLN formulations.

Increase in the residence time of the POL 188 SLN formulation in the blood can be attributed to the adsorption of poloxamer 188 on the surface of the lipid nanoparticles which imparts a stealth property to the drug particle, resulting in reduced protein opsonization and subsequent RES uptake. The hydrophilic polyethylene oxide (PEO) segment of the poloxamer provides a protective barrier for nanoparticles against RES uptake. Many reports (Leroux JC et al., 1996; Redhead HM et al., 2001; Peracchia MT et al., 1999) are available that protein adsorption onto nanoparticles with hydrophilic shells is decreased and the body circulation time is increased in vivo studies. Lenaerts during his tumour targeting studies reported that poloxamer 407

copolymers would be a better choice as they are less easily desorbed from the particle surface (Lenaerts V., 1995).

Increase in the residence time of GOL SLN in the blood can be attributed to the adsorption of Gelucire® 44/14 on the nanoparticle surface. Gelucire® is chemically a mixture of glycerides of PEG1500 esters of long chain fatty acids. Colloidal drug carriers like nanoparticles and liposomes activate the complement system in the blood, which causes pseudoallergic reactions that damage heart and liver cells (Sebeni J., 1998). Pegylation can overcome this deficiency by attaching polyethylene glycol (PEG) chains to polypeptides or other candidate molecules. In fact, liposomes are now pegylated to improve the delivery of encapsulated drugs, such as the anticancer agent doxorubicin. Peracchia and coworkers studied the protein rejecting ability of pegylated stealth polycyanoacrylate nanoparticles. They also observed that the pegylated nanoparticles were associated with higher residence times when compared to the plain nanoparticles (Peracchia MT et al., 1999).

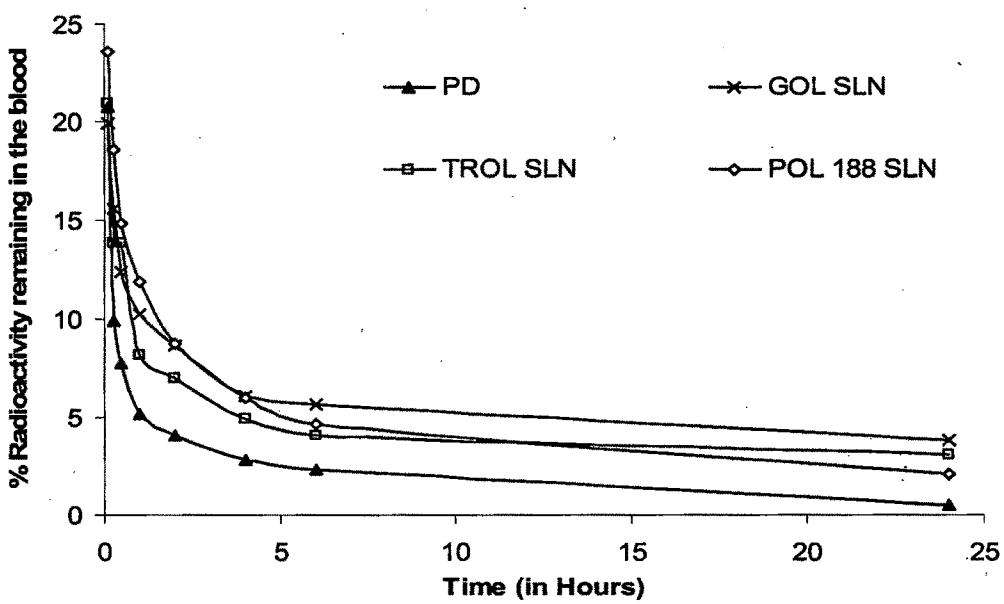

FIGURE 14.1: Pharmacokinetics of ^{99m}Tc-labeled olanzapine and olanzapine nanosuspensions after intravenous injection into ear vein of white New Zealand Rabbits.

The FDA has approved PEG for use as a vehicle or base in foods, cosmetics and pharmaceuticals, including injectable, topical, rectal and nasal formulations. PEG shows little toxicity, and is eliminated from the body intact by either the kidneys (for PEGs < 30 kDa) or in the faeces (for PEGs > 20 kDa) (Yamaoka T et al., 1994).

Pegylated nanoparticles have also been found to increase the brain concentrations of the drug incorporated in it. Calvo and coworkers revealed the potential use of polymer nanoparticles, such as n- hexadecylcyanoacrylate (PHDCA), to transport drugs across the BBB. Animal studies showed that PEG–PHDCA penetrated into the brain to a significantly greater extent than PHDCA alone. PEG–PHDCA distributed into deep areas of the brain, including the striatum, hippocampus, and hypothalamus. Furthermore, this movement occurred without damage to the BBB or other brain structures. The method seemed promising for the development of drug carriers for brain delivery (Calvo P et al., 2001).

In the present study, the peak plasma radioactivity was around 30 minutes for the OL solution and the different OL loaded SLN dispersions when administered through the intramuscular route. This maybe attributed to the fact that there exists a lag period for the drug / nanoparticle to enter the systemic circulation from its site of injection and this depends on the rate of blood blow to the particular muscle. The intravenous route of administration revealed significantly higher radioactivity in the blood when compared to the intramuscular route ($p < 0.005$). The percentage radioactivity remaining in the blood versus time plot after intramuscular administration is shown in figure 14.2.

Drug absorption from the i.m. sites into the venous blood will return to the heart and pass through the lungs before being distributed to the rest of the body. However, there will be an initial lag between the time of i.m. injection and when it enters the systemic circulation. Thus, the kinetics of drug administered through i.m. route is expected to show a decreased rate and extent of availability. The rate at which the drug leaves the muscular compartment depends on the blood flow in relationship to the size of the organ.

Of the different routes of administration available for the drug input to the systemic circulation, the intravenous (i.v.) administration yields one of the fastest and the most complete drug

availabilities. Further, intra-arterial injections might be employed when an even more complete and faster input of drug is desired. By administering the drug though an artery, the total drug enters the tissue to which the artery flows. But when a drug is administered through the i.v. route, the whole drug must be diluted into the venous system as the venous blood is pooled in the superior and inferior vena cava. It then enters the heart and is subsequently pumped to the lung before it can enter the arterial system and reach the target organ (s). In addition the fraction of drug reaching a desired site is dependent on the fraction of the arterial blood reaching that site. Enough through intra-arterial administration yields faster and complete input of drug to the body, they are considered much more dangerous than i.v. administration as they are associated with patient discomfort, bleeding and thrombosis.

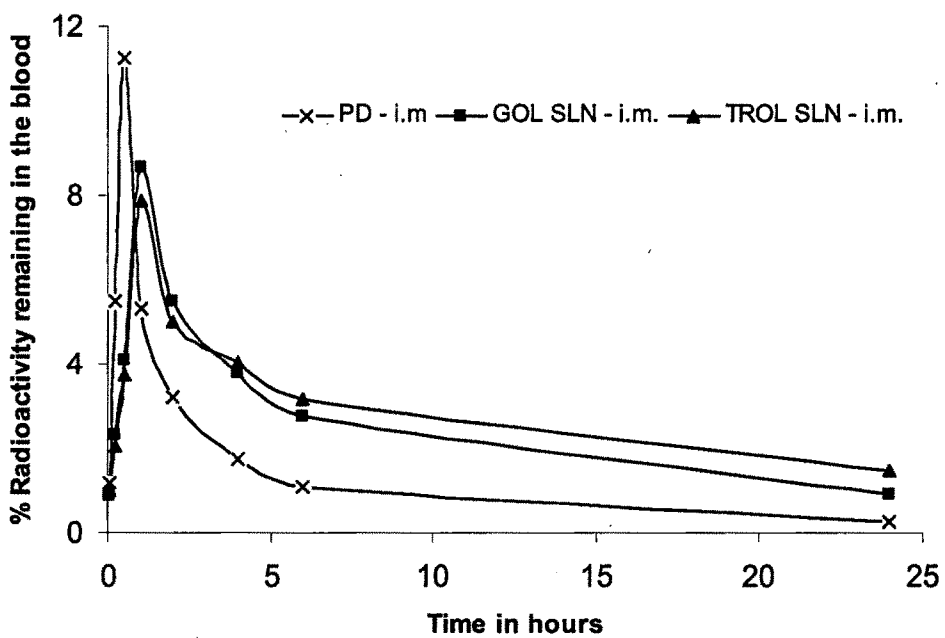

FIGURE 14.2: Pharmacokinetics of ^{99m}Tc-labeled olanzapine and olanzapine loaded solid lipid nanoparticles after intramuscular injection into hind limb of white New Zealand Rabbits.

Temporary or permanent drug loss can also occur when the drug is administered through the i.v. route during its passage through the lung. Apart from its cleansing activity, the lungs also

have some degree of metabolizing action (Gram TE., 1980) also and act as a metabolic site for some drugs (Vane JR., 1974). The lungs act as a good temporary storage site for some basic drugs, by partitioning of the drugs into the lipid tissues, as well as serving as a filtering function for the particulate matter that may be given through the i.v. route. Accumulation of lipophilic compounds and filtering of any compounds in the solid form can be considered as a temporary clearing or dilution of the drug, as it will ultimately leach back into the systemic circulation. Thus the lung acts as a clearing device, which is not present following intra-arterial injection. Drugs given through the i.v. route may, therefore, not necessarily be completely available to the site of action, since a certain fraction of the drug could be eliminated by the lung entering into the general circulation. This phenomenon is also considered as lung first pass metabolism (Chiou WL., 1979).

Evans and coworkers (Evans EF et al., 1975) studied the resting muscle blood flow through the gluteus maximus, vastus lateralis and deltoid muscles. Deltoid muscle blood flow was significantly greater than the gluteus muscle blood flow, with the vastus lateralis being intermediate. Since these two sites are commonly used for the adminsteration of i.m. injections, differences in rate of drug absorption may be expected. Lidocaine is one drug which has been investigated for its effect in response to the site of injection (Cohen LS et al., 1972; Schwartz ML et al., 1974). Deltoid injection gave higher peak plasma concentrations when compared with the gluteal injection. Doluisio and coworkers (Doluisio JT et al., 1971) observed that only 77-78% of the injected dose was absorbed from the i.m. dose. The authors explained by saying that there may be possible chemical or enzymatic decomposition of the drug at the site of injection. Wilensky and Lowden during their studies on phenytoin i.m. injection observed that there was a marked decrease in the rate and extent of absorption in comparison with the i.v. or oral doses. The authors demonstrated that this maybe due to the precipitation of drug as crystals in the muscle. Although these crystals eventually dissolve, the drug is essentially lost during a normal dosing interval (Wilensky AJ and Lowden JA., 1973).

Biodistribution Study

Plain OL solution exhibited higher concentrations in the organs of the reticuloendothelial system (RES), such as liver, spleen, and lung when compared to the SLN formulations. The overall uptake of GOL solution / TROL solution / GOL SLN / TROL SLN / POL188 SLN by liver and spleen was significantly lower than the free OL ($p < 0.005$) (Figures 14.5 and 14.6). The uptake of both plain OL solution and the SLN formulations was initially high but decreased with time. The liver uptake of nanoparticles was in the order of TROL SLN > GOL SLN > POL188 SLN. The results are shown in figure 14.3. These results were correlating with the surface hydrophilicity values obtained for these nanoparticles by the Rose Bengal adsorption method reported by Gessner and coworkers (Gessner A et al., 2000) (Chapter 13). TROL SLN gave the highest surface hydrophilicity value and the least liver uptake in vivo.

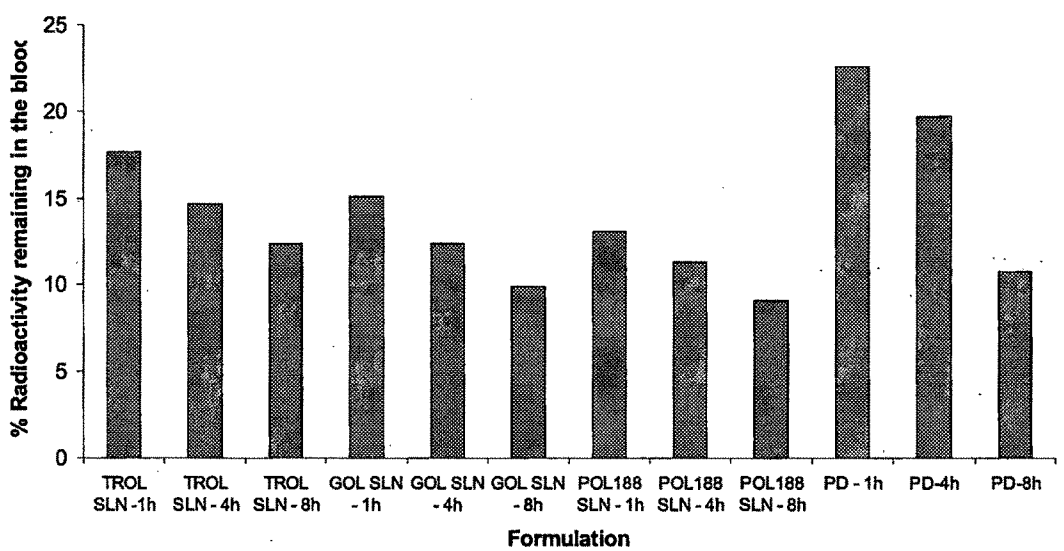

FIGURE 14.3: Liver Concentrations of ^{99m}Tc-labelled olanzapine and ^{99m}Tc-labelled olanzapine SLN after intravenous injection into Balb/c mice. The values plotted are the mean ± S.D of 3 experiments

The concentration of GOL SLN was relatively higher in brain (7.75 fold, 6.71 fold and 3.20 fold higher than plain OL solution at 1 hour, 4 hour and 8 hour respectively) (Figure 14.4). The concentration of TROL SLN was relatively higher in brain (5.50 fold, 4.85 fold and 2.50 fold higher than plain OL solution at 1 hour, 4 hour and 8 hour respectively). When the brain concentrations of GOL SLN and GOL solution were compared, 2.38 fold increase in the brain was achieved with the GOL SLN formulation. When the brain concentrations of GOL SLN and POL188 SLN were compared, 3.26 fold increase in the brain was achieved with the GOL SLN formulation. These results denote that nanoparticles stabilized with Pgp inhibitors are a better option for targeting the drug to the brain when compared to the plain drug - surfactant solution. Similar results were achieved TROL SLNs. The SLN stabilized with Transcutol® gave higher brain concentrations when compared to TROL solution. However, there was no significant difference in the brain uptake of OL between the GOL solution, TROL solution and POL188 SLN. Hydrolyzed technicium tends to accumulate in the stomach and intestine. A very low radioactivity was recovered from stomach and intestine and showed constancy with time indicating the in vivo stability of the radiolabeled complexes (Mishra P et al., 1999).

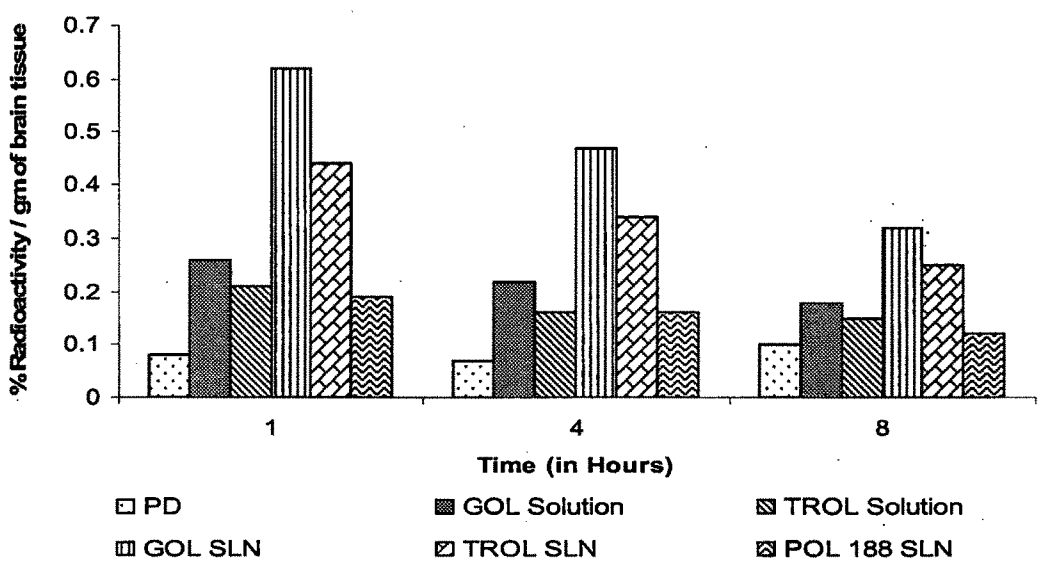

FIGURE 14.4: Brain Concentrations of ^{99m}Tc-labelled olanzapine and ^{99m}Tc-labelled olanzapine nanosuspension after intravenous injection into Balb/c mice. The values plotted are the mean ± S.D of 3 experiments

Figure 14.5 represents the organ/tissue concentrations after intravenous injection of ^{99m}Tc- OL and ^{99m}Tc radiolabelled OL / GOL solution / TROL solution / POL188 SLN. There was a significant uptake ($p < 0.01$) of plain OL solution in the kidney when compared to the SLN formulations at all the three time points studied. However there was no significant difference in the kidney uptake between the different nanoparticles. The kidney uptake was higher in case of GOL solution and TROL solution when compared with the GOL SLN and TROL SLN respectively. Further, the kidney uptake of OL solution and all the nanoparticle formulations decreased with time (Figures 14.5 and 14.6).

The value of blood flow per unit volume of tissue denotes how fast a drug can be delivered to a body region per unit volume of tissue and reflects the relatives rates at which tissues maybe expected to come in equilibrium with the blood. The amount of drug that can be stored or distributed into a tissue will depend on the size of the tissue (volume) and the ability of the drug to concentrate in the tissue (i.e partition coefficient between the organ and the blood). However presence of efflux protein in the blood – tissue barrier creates another hurdle for the drug delivery to the tissue.

The gamma scintigraphic images taken after 1 hour post intravenous injection of plain OL solution – ^{99m}Tc radiolabelled complex into New Zealand rabbits are shown in figure 14.7a. The images clearly showed the difference in the concentration of the radiolabelled complex in the brain after intravenous injection of GOL SLN (Figure 14.7e) and TROL SLN (14.7f).

Non ionic surfactants are commonly used as excipients in drug formulations. Many surfactants insert into lipid membranes, although to different extents, and change the lateral packing density of membranes at high concentrations. Moreover, they bind to the efflux transporter Pgp and most likely to related transporters and metabolizing enzymes with overlapping substrate specificities. If their affinity to Pgp is higher than that of the coadministered drug they act as modulators or inhibitors of Pgp and enhance drug absorption (Seelig A et al., 2006).

Demina and coworkers suggested that the inhibition of Pgp might be due to changes in its lipid surrounding. Further, high dependence of P-gp activity on the membrane microviscosity was also demonstrated, suggesting that the ability of non ionic surfactants to affect the P-gp activity is mediated by their effect on the membrane structure. They observed that adsorption of some

non ionic surfactants on lipid bilayers induced considerable disturbance of the lipid packing. It was found that not only bulk hydrophobicity but also the chemical microstructure of the copolymer determines its membrane disturbing ability. Demina observed that Pluronic copolymers containing polypropylene oxide caused higher acceleration of flip-flop and drug permeation than polysurfactants containing aliphatic chains. The effects of copolymers containing hyperbranched polyglycerol "corona" were more pronounced, as compared to the copolymers with linear poly (ethylene oxide) chains, indicating that a bulky hydrophilic block induces additional disturbances in the lipid bilayer. A good correlation between the copolymer flippase activity and a linear combination of copolymer bulk hydrophobicity and the van der Waals volume of its hydrophobic block was found. The relationship between the structure of a copolymer and its ability to disturb lipid membranes presented in this paper may be useful for the design of novel amphiphilic copolymers capable of affecting the activity of membrane transporters in living cells (Demina T et al., 2005).

Buckingham and coworkers studied the ability of fatty acid ester surfactants (molecular heterogeneity of these and other commercial surfactants) to modulate the multi-drug resistance (MDR) proteins. However the authors were not able to estimate the underlying mechanism(s) of MDR protein inhibition by these surfactants because of the molecular heterogeneity of these and other commercial surfactants. Surfactants comprising of 99% diesters were significantly more potent than monoester preparations for MDR modification activity in vitro. The nature of the fatty acid domains also proved to be important for activity, as was the relative length of the polyethylene glycol domain (which determines the hydrophile-lipophile balance). The ester linkage appeared unimportant since homologous diethers and diamides had activity similar to that of diesters (Buckingham LE et al., 1996). An interesting study indicated that the effect of surfactant strongly depends on its concentration: Triton X- 100 stimulated the activity of the ATPase active P-glycoprotein at low concentrations and inhibited it at higher concentrations (Cserhati T., 1994). Yu- li Lo reviewed the relationships between the hydrophilic–lipophilic balance values of pharmaceutical excipients and their multidrug resistance modulating effect in Caco-2 cells and rat intestines (Yu-li Lo., 2003). Using epirubicin as the Pgp substrate, the optimal effect on the Pgp substrate uptake was characteristic of excipients with intermediate HLB values ranging from 10 to 17. Moreover, the optimal net efficacy was observed for excipients with polyoxyethylene chains and intermediate chain length of fatty acid and fatty

alcohol (monolaurate for Tween 20, monooleate for Tween 80, monostearate for Myrj 52, and lauryl alcohol for Brij 30). Lo concluded that the optimal HLB values of surfactant systems with suitable hydrocarbon chains and polar groups are an important factor in designing promising epirubicin formulations for reversing MDR (Yu-li Lo., 2003).

Gelucire® 44/14 (Lauroyl Macrogol-32 Glycerides) is a saturated polyglycolized glyceride consisting of mono-, di- and triglycerides and of mono- and di-fatty acids of polyethylene glycol (PEG). Gelucire® 44/14 is obtained from the reaction of hydrogenated palm kernel oil with PEG 1500. Transcutol® is chemically diethylene glycol monoethyl ether. The exact mechanism of Pgp inhibition by these non ionic surfactants is still unclear. But they are believed to change the membrane fluidity or the membrane lipid packing. Pgp requires tightly bound annular lipids to express its transporter activity (Jones MN., 1999). Doige and coworkers have reported that phosphatidyl ethanolamines and phosphatidyl serines restore the Pgp activity because phospholipids are required for the catalytic activity of the ATPase in the Pgp domains. Hence the surfactants which affect the dynamic state of the membrane lipid bilayers will definitely have an impact on the Pgp efflux system (Doige CA et al., 1993). Changes in the membrane fluidity by the surfactant may also lead to conformational changes not only in the Pgp but also affect the other membrane bound transporters and enzymes systems. Rage and coworkers observed that Tween 80 and Cremophor® EL increase the cell fluidity and also inhibited Pgp. But other membrane transporters like the peptide transporter (PEPT1) were inhibited by Tween 80 only and the momocarboxylic acid transporter (MCT) was inhibited by Cremophor® EL only suggesting that membrane fluidization is not the generalized mechanism for Pgp inhibition. Hence it would be essential to study the molecular mechanisms for cell membrane perturbation by the surfactants followed by subsequent effect on the membrane transporters (Takaishi N et al., 2006).

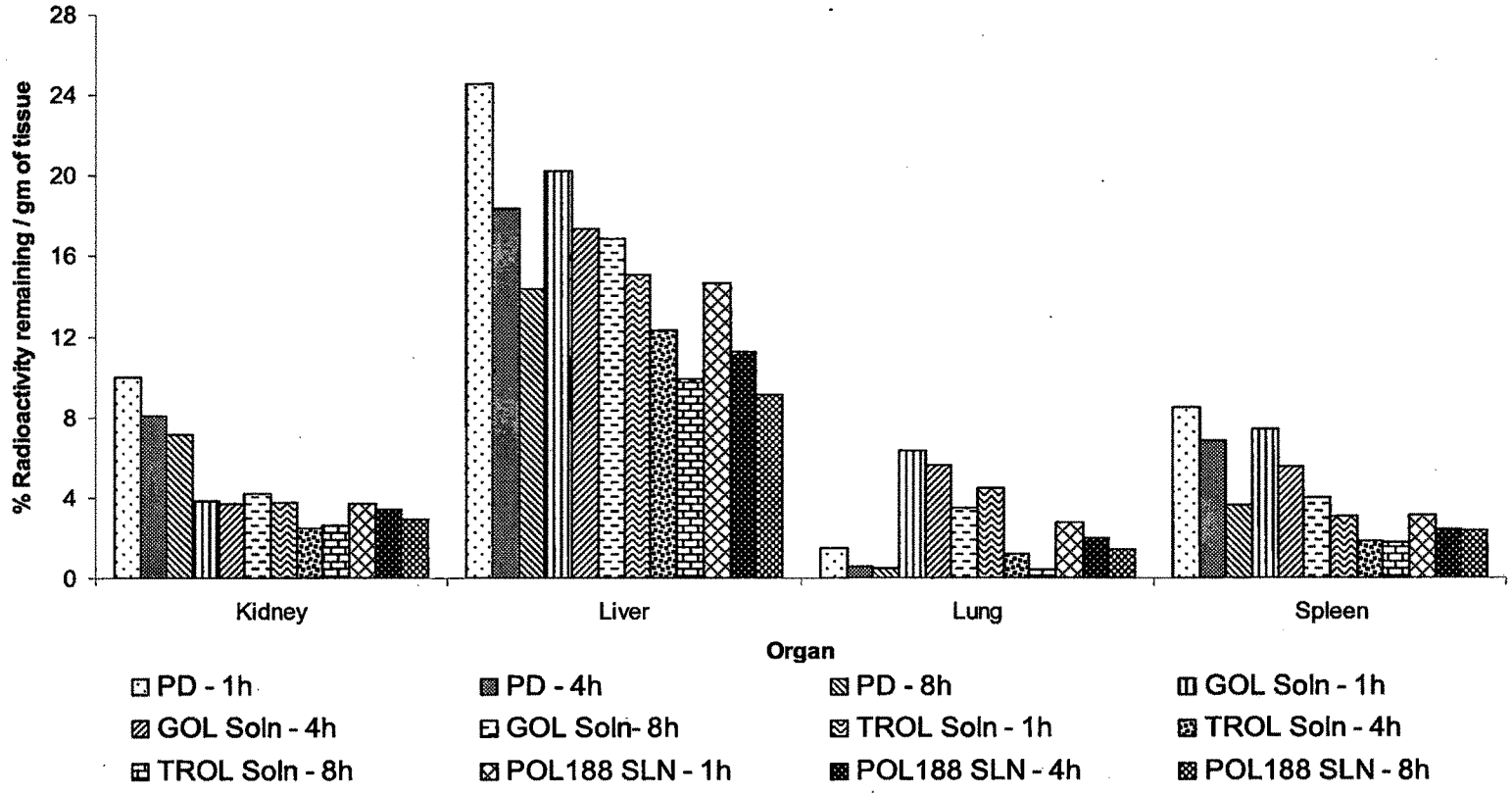

Figure 14.5: Tissue distribution of olanzapine after intravenous injection as plain solution (PD), Gelucire® 44/14 OL Solution (GOL Soln), Transcutol® OL Solution (TROL Soln) and poloxamer 188 stabilized SLN (POL188 SLN). The values plotted are the mean ± S.D of 3 experiments

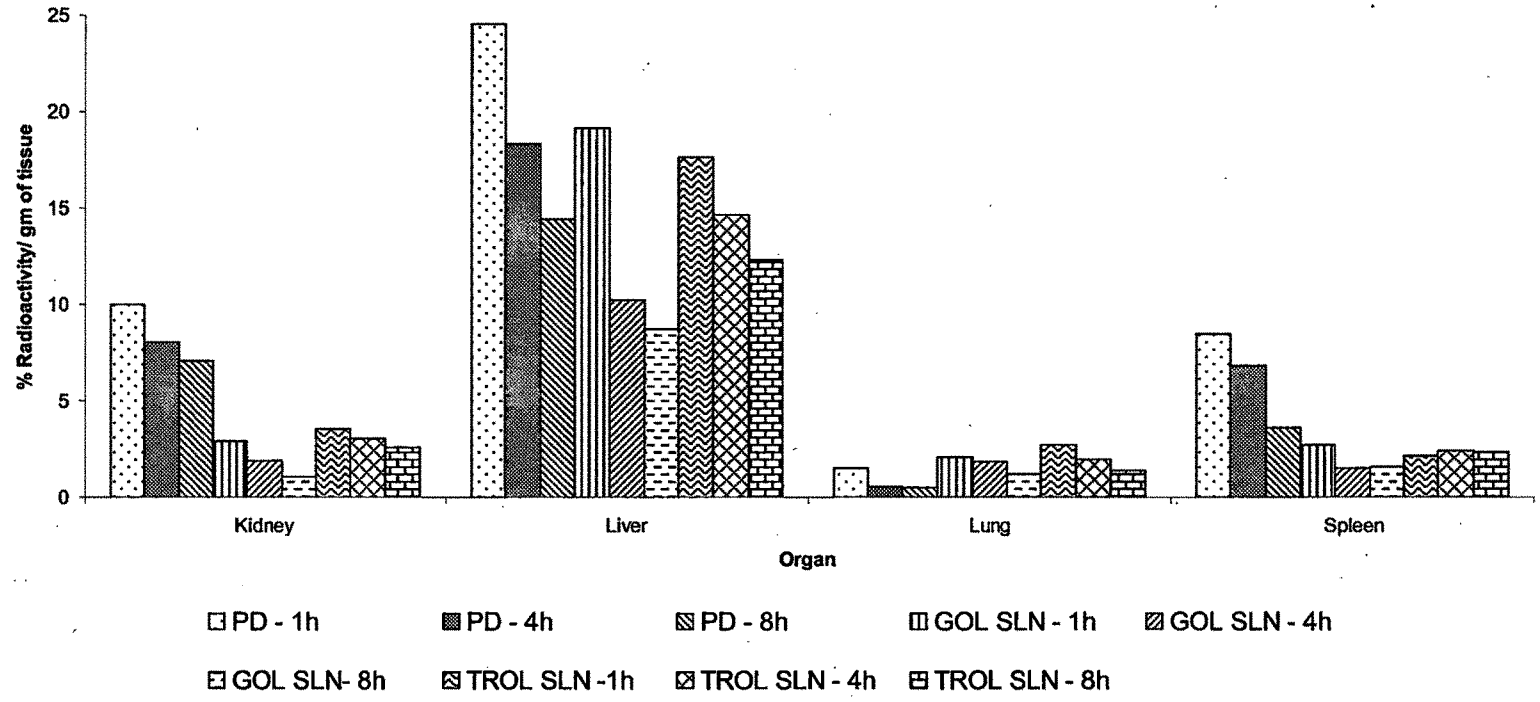

Figure 14.6: Tissue distribution of olanzapine after intravenous injection as plain solution (PD), Gelucire 44/14® stabilized SLN (GOL SLN) and Transcutol® stabilized SLN (TROL SLN). The values plotted are the mean ± S.D of 3 experiments.

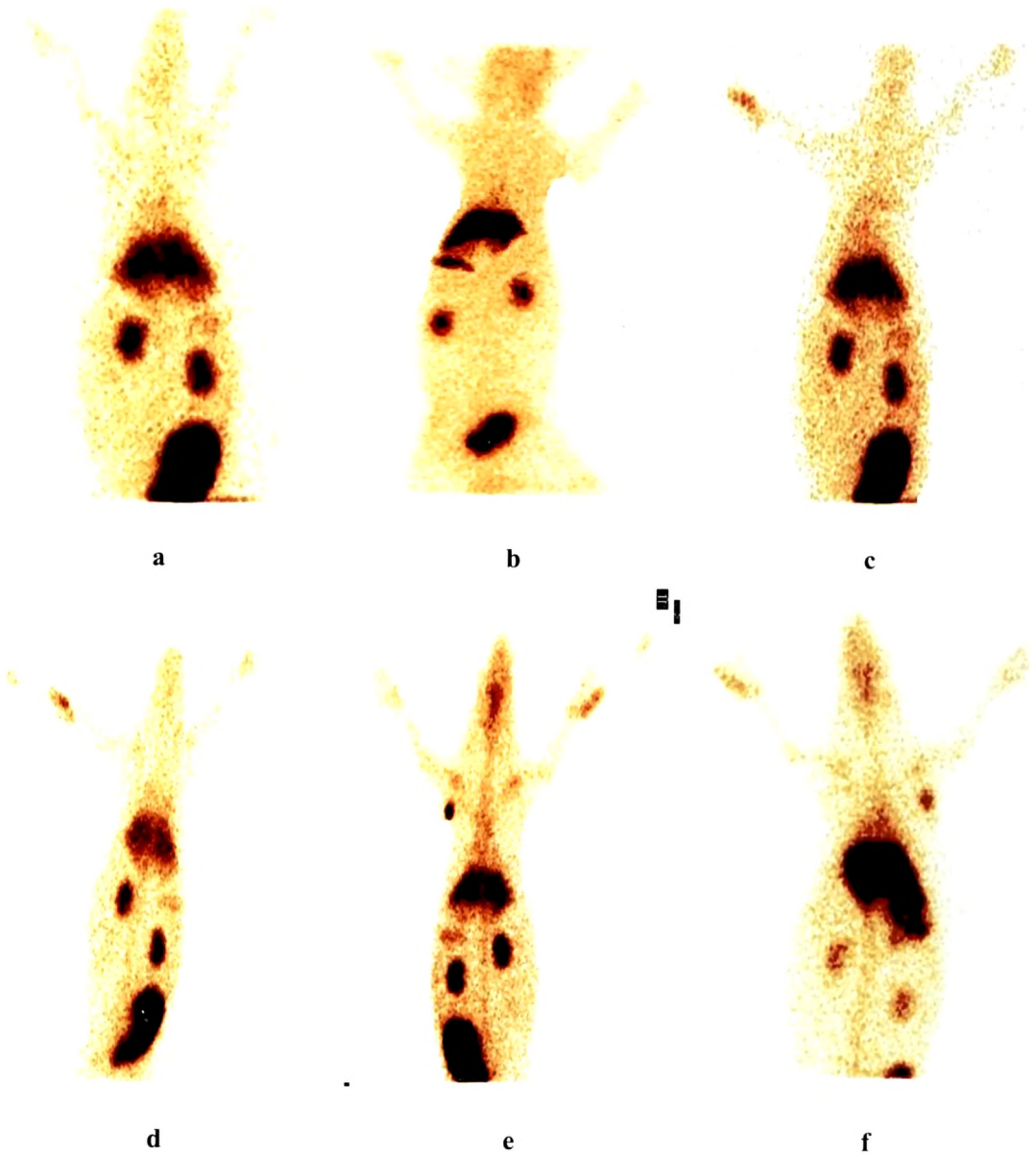

FIGURE 14.7: Gamma Scintigraphic image of plain olanzapine (a), GOL solution (b), TROL solution (c), POL188 SLN (d), GOL SLN (e), TROL SLN (f) after 1 h of intravenous injection in white New Zealand Rabbits. The black portion in the figure represents radiolabelled complex.

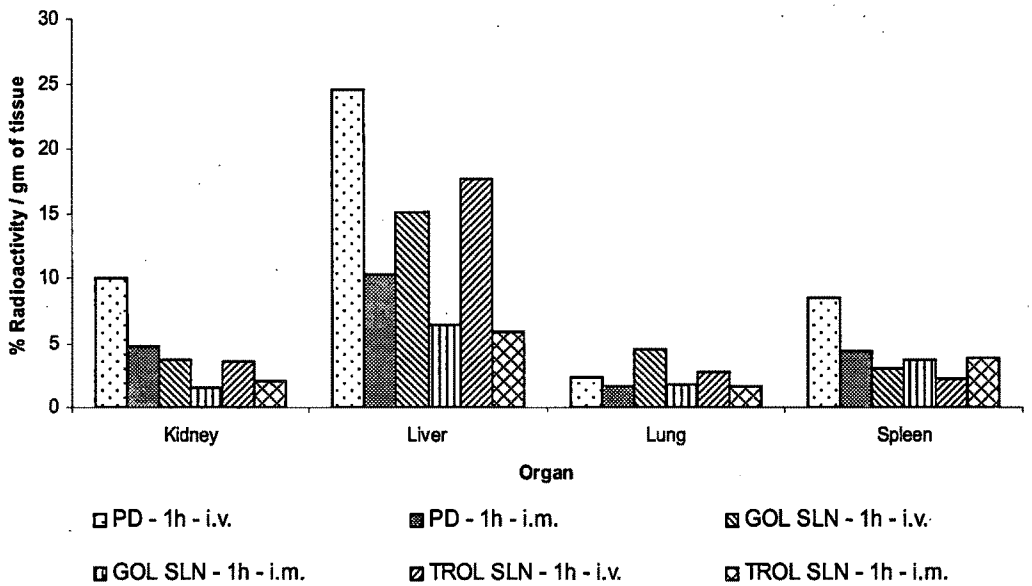

Figure 14.8: Tissue distribution after 8 hour of ^{99m}Tc-labelled olanzapine solution (PD), ^{99m}Tc-labelled Gelucire® 44/14 stabilized SLN (GOL SLN) and ^{99m}Tc-labelled Transcutol® stabilized SLN (TROL SLN) after intravenous injection (i.v.) and intramuscular injection (i.m.) into Balb/c mice. The values plotted are the mean ± S.D of 3 experiments.

There was a marked reduction in the liver uptake of the radiolabelled complexes by the liver after i.m. administration when compared to the i.v. route. Uptake by the other organs did not differ significantly after i.m. administration. The relative importance of the various organs as storage or elimination sites depends on how fast the drug gets to each organ and how much space or volume is available to hold the drug. In the present study, the intravenous route of administration yielded higher brain concentrations when compared to the intramuscular route. These results were in accordance with earlier reports of Doluisio and coworkers (Doluisio JT et al., 1971). This may be due to the fact that the drug that leaves the site of intramuscular administration will also be subject to the additional distribution and elimination similar to the intravenous administration. This further delays the distribution of the drug to the target organ and shows a decreased extent of distribution to the organ in comparison to the intravenous

route. The biodistribution to the different organs after 1hour, 4 hour and 8 hour post injection are shown in figure 14.8, 14.9, 14.10 respectivly.

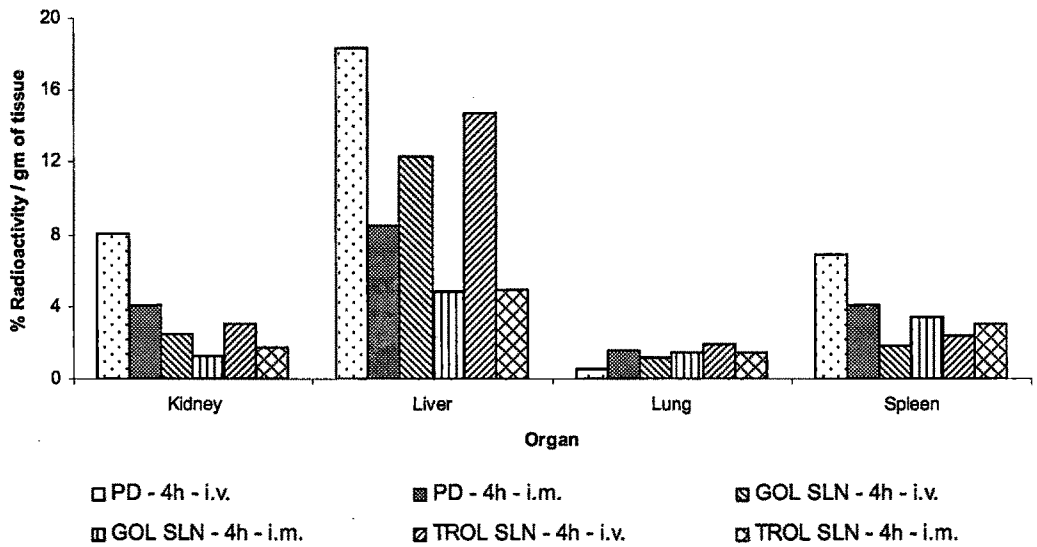

Figure 14.9: Tissue distribution after 4 hours of ^{99m}Tc-labelled olanzapine solution (PD), ^{99m}Tc-labelled Gelucire® 44/14 stabilized SLN (GOL SLN) and ^{99m}Tc-labelled Transcutol® stabilized SLN (TROL SLN) after intravenous injection (i.v.) and intramuscular injection (i.m.) into Balb/c mice. The values plotted are the mean ± S.D of 3 experiments

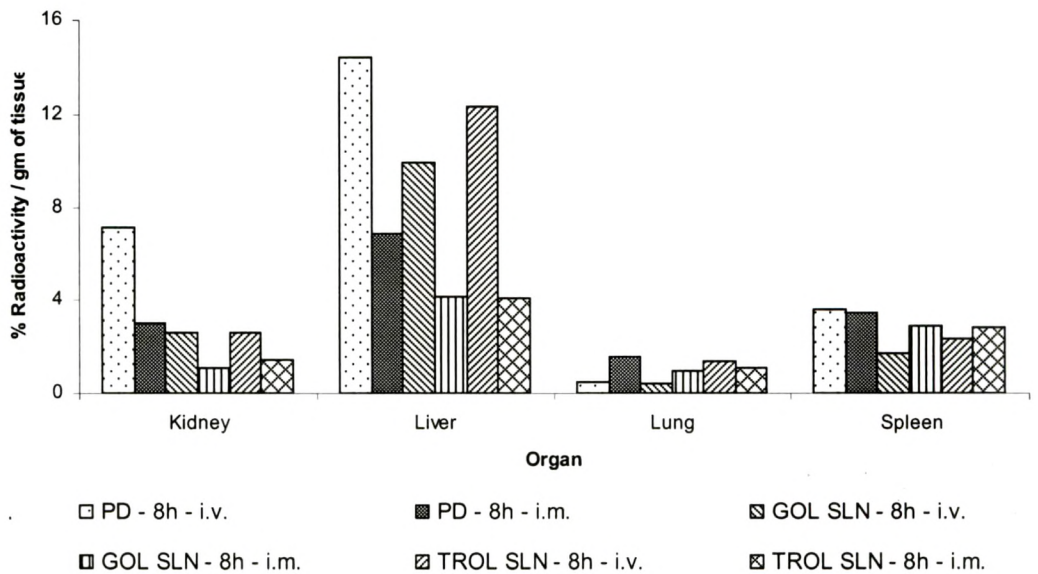

Figure 14.10: Tissue distribution after 8 hours of ^{99m}Tc-labelled olanzapine solution (PD), ^{99m}Tc-labelled Gelucire® 44/14 stabilized SLN (GOL SLN) and ^{99m}Tc-labelled Transcutol® stabilized SLN (TROL SLN) after intravenous injection (i.v.) and intramuscular injection (i.m.) into Balb/c mice. The values plotted are the mean ± S.D of 3 experiments

The radioactivity concentrations achieved in the brain after intravenous administration was also significantly higher ($p < 0.005$) when compared to those obtained after intramuscular administration at all the three time points studied for plain OL solution and the nanoparticle dispersions. The value of blood flow per unit volume of tissue denotes how fast a drug can be delivered to a body region per unit volume of tissue and reflects the relatives rates at which tissues maybe expected to come in equilibrium with the blood. The amount of drug that can be stored or distributed into a tissue will depend on the size of the tissue (volume) and the ability of the drug to concentrate in the tissue (i.e. partition coefficient between the organ and the blood). However presence of efflux protein in the blood – tissue barrier creates another hurdle for the drug delivery to the tissue

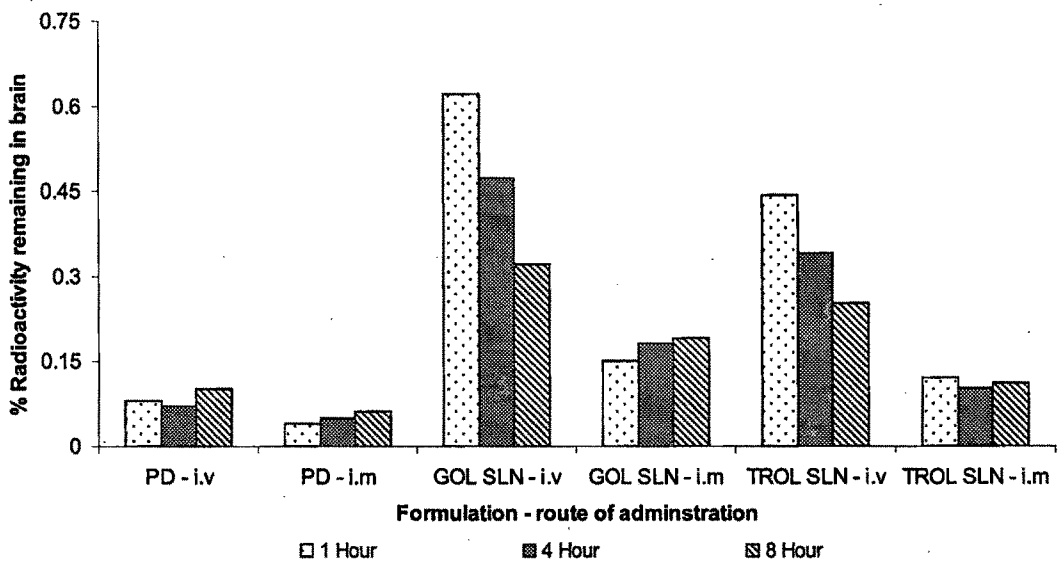

FIGURE 14.11: Brain Concentrations of ^{99m}Tc-labelled olanzapine solution (PD), ^{99m}Tc-labelled Gelucire® 44/14 stabilized SLN (GOL SLN) and ^{99m}Tc-labelled Transcutol® stabilized SLN (TROL SLN) after intramuscular injection (i.m.) into Balb/c mice. The values plotted are the mean ± S.D of 3 experiments

14.4 CONCLUSION

Radiolabeling of plain OL solution and the OL loaded SLNs was performed with ^{99m}Tc, with high labeling efficiency and in vitro and in vivo stability. In vivo, the GOL SLN and TROL SLN showed higher blood residence time and brain concentrations compared to the OL solution, indicating the potentiality of these SLNs in brain delivery of olanzapine. There was also a significant reduction in the uptake by the RES organs of OL after administration in nanoparticles. These data substantiate the potential of the above formulated Gelucire® 44/14 or Transcutol® stabilized olanzapine nanoparticles as a long circulating system in blood and higher brain deposition. The intravenous route of administration yielded higher brain deposition when compared to the intramuscular route of administration.

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