Chapter 9

Investigations on the in vivo performance of Ziprasidone nanosuspension

9.1 INTRODUCTION

Novel drug delivery systems which can effectively alter the in vivo biodistribution pattern of drugs incorporated in them. Rapid clearance from the blood by the reticuloendothelial system (RES) is a major barrier that has to be overcome by the drug delivery system. Various approaches have been tried to overcome this common setback. Simultaneous administration of drugs that suppress the RES (Profitt RT et al, 1983) and surface modification of the drug carrier (Illum L and Davis SS, 1984) are few attempts to reduce RES uptake. Out of the above two approaches, the surface modification method also lead to altered biodistribution patterns (Lue D et al, 1984).

In the present chapter, ZB nanosuspensions stabilized by poloxamer 407 were prepared by pearl milling (Chapter 9) and their pharmacokinetics and in vivo biodistribution after intramuscular administration to rats were studied.

9.2 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.

Blood kinetic profile and biodistribution studies

For the study of blood kinetic profiling, the rats were divided into three groups of the 3 each. The samples were injected intramuscularly into the hind leg of the rat using a $26_{1/2}$G needle. The first group was treated with 0.2 ml ZB solution (ZB in propylene glycol used as solubilizer equivalent to 1.14 mg/kg ZB). The second and third groups were injected with 0.2ml ZBLNS and ZBSNS (equivalent to 1.14 mg/kg ZB) which were reconstituted previously with pH 7.4 PB. Subsequently, the blood samples were collected from the retro-orbital plexus of rat eye, periodically at 5, 15, 30, 60, 120, 240 and 480 min into anticoagulant (3%w/v sodium citrate solution) treated vials. The collected blood was centrifuged at 3000 rpm for 10 minutes at 4°C in a cooling centrifuge (Sigma, Osterode, Germany) to isolate the plasma. The plasma was

extracted and analyzed spectrofluorimetrically using spectrofluorophotometer (Shimadzu RF-540, Shimadzu Coporation, Japan) as explained before in Chapter 4.

For biodistribution studies, the rats were divided into three groups of the three each. ZB, ZBLNS and ZBSNS were injected intramuscularly into the hind leg of the rat using a $26_{1/2}$G needle. At predetermined time intervals (1 h, 4 h and 8 h), the rats were sacrificed and the organs such as liver, lung, spleen, heart, kidney and brain were isolated. The tissues were blotted free of blood using a tissue paper, weighed separately and homogenized to a concentration of 10% w/v in pH 7.4 PB. The tissue homogenate was centrifuged at 6000 rpm for 10 min at 4°C in a cooling centrifuge to obtain the clear tissue homogenate. Further extraction and analysis the samples were carried out as explained before in Chapter 6.

Statistical analysis

All the data obtained are reported as the mean ± SD of three experiments. Statistical evaluation of the data was done by applying ANOVA at a significance level of $p < 0.005$.

9.3 RESULTS AND DISCUSSION

Blood kinetic profile and biodistribution studies

The blood clearance profiles of ZB solution, ZBLNS and ZBSNS are depicted in Figure 9.1. The pharmacokinetic parameters after intramuscular injection were calculated using Wagner Nelson method (Reddy LH and Murthy RSR., 2004) and are depicted in table 9.1.

The t_{max} of ZB solution, ZBLNS and ZBSNS was similar and was calculated as 30min. However, the C_{max} in case of the ZBLNS and ZBSNS was significantly higher than the ZB solution. The C_{max} value obtained for ZB solution, ZBLNS and ZBSNS were 198.03/mL ng, 251.43ng/mL and 273.62ng/mL respectively ($p < 0.005$). Both the ZBLNS and ZBSNS exhibited higher plasma concentrations all throughout the study compared to ZB solution.

On the other hand, the pharmacokinetic parameters of ZB solution and ZBLNS and ZBSNS differed significantly. The ZBLNS and ZBSNS exhibited about 2.51 fold and 2.22 fold higher plasma half life ($t_{1/2}$) ($p < 0.005$) compared to the ZB solution. The AUC $^{0-8}$ and AUC $^{0-\infty}$

were also significantly higher ($p<0.005$) for both ZBLNS and ZBSNS evidently revealing the long circulation property of these nanosuspension formulations in blood. Further, the plasma clearance and elimination rate constant were lower for the both ZBLNS and ZBSNS in comparison to ZB solution. However, the pharmacokinetic parameters of ZBLNS and ZBSNS did not differ significantly.

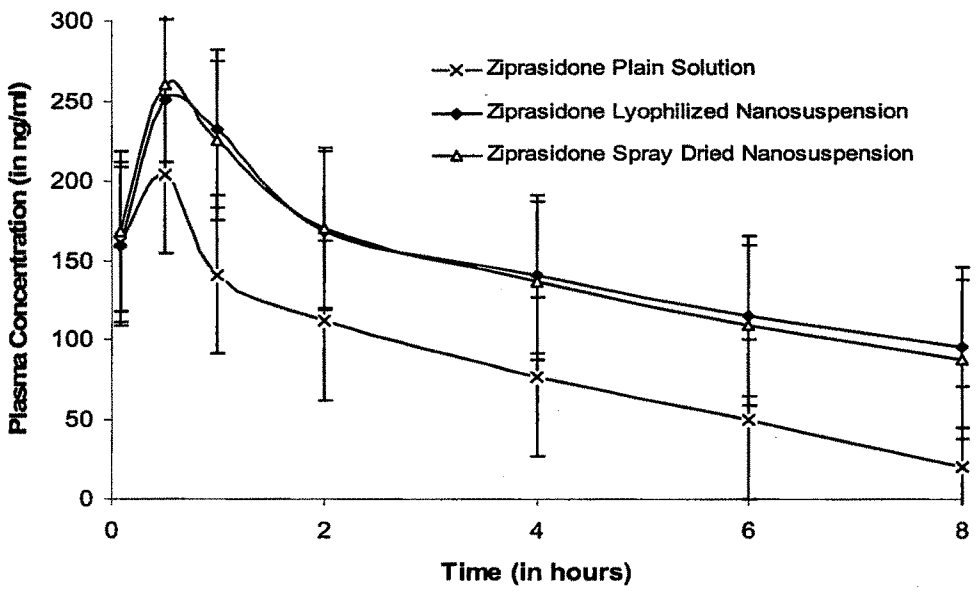


FIGURE 9.1: Blood levels of intramuscularly injected ziprasidone delivered as plain solution, lyophilized nanosuspension and spray dried nanosuspension. The values plotted are the mean ± S.D of 3 experiments.

This increase in the in vivo circulation time may be attributed to the adsorption of poloxamer 407 on the surface of the drug nanocrystals which imparts a stealth property to the drug particle, resulting in reduced protein opsonization and subsequent RES uptake. Earlier reports revealing the stealth property of poloxamer coated systems (Reszka R et al., 1997) also support our observations. Geyer demonstrated that intravenously injected lipid emulsions prepared with high molecular weight members of POE/POP copolymer nonionic surfactants such as poloxamers and poloxamines as emulsifiers remained in the blood for relatively long periods (Geyer RP., 1967). Later, Jeppsson and Rossner after their series of experiments suggested that

high molecular weight POP/POE surfactants prevent lipid particles from sticking to the blood vessel endothelium as well as inhibiting recognition by macrophages (Jeppsson R and Rossner S., 1975).

Parameters	ZB Solution	ZBLNS	ZBSNS
K_{el}, elimination constant, h^{-1}	0.2562	0.1021	0.1154
$T_{1/2}$, h	2.7037	6.7926	6.0062
V_d, ml/gm	0.0051	0.0046	0.0044
Cl, clearance , ml/min	0.0013	0.0004	0.0005
MRT, h	2.7517	3.3753	3.3189
AUC $^{0-8}$, h.ng/ml	683.5133	1190.6610	1194.44
AUC $^{0-\infty}$, h.ng/ml	765.6359	2127.2159	1956.02

TABLE 9.1: Comparative pharmacokinetic parameters of ziprasidone solution (ZB Solution) and ziprasidone nanosuspensions lyophilized (ZBLNS) and spray dried (ZBSNS) after intramuscular injection in rats (n = 3).

The biodistribution results of the ZB solution, ZBLNS and ZBSNS are presented in figure 9.3. The results indicate that the nanosuspension forms modify the biodistribution of ZB compared to the free solution form. ZB is a centrally acting D_2 and 5-HT_{2A} antagonist and hence the brain concentrations are important to be measured. The brain concentrations were increased from 0.4102μg/g at the 8 hours for the plain drug solution to 0.7633μg/g and 0.8559μg/g for ZBLNS and ZBSNS respectively (Figure 9.2). Pluronic block copolymers are reported to inhibit the P-glycoprotein (Pgp) drug efflux system at the blood-brain barrier (BBB) and increase the permeability of a broad spectrum of drugs (Batrakova EV et al., 2002). The possible mechanisms involved in efflux inhibition are simultaneous alterations in intracellular ATP levels and membrane fluidization in the BBB by poloxamer co polymers as potential reasons for inhibition of the drug efflux system (Batrakova EV et al., 2001).

ZB is prone to first pass metabolism and hence deposited in the higher concentrations (on comparison with ZBLNS and ZBSNS) in the liver. The particle uptake by RES is an obstacle

for drug delivery to the brain. Following parenteral adminstration, non-stealth nanoparticles are cleared rapidly from the blood (within minutes) by elements of the RES, particularly the Kupffer cells of the liver. This RES clearance results in reduced amount of drug in circulation, and hence the amount available for brain delivery is also significantly reduced. The reduction in the RES uptake of the nanosuspension formulations may be credited to poloxamer-407 adsorption on the surface of the drug nanoparticle, which leads to increase in surface hydrophilicity of the particle, hence leading to reduced identification by circulating macrophages. The tissue distribution pattern of ZBLNS and ZBSNS was not significantly different.

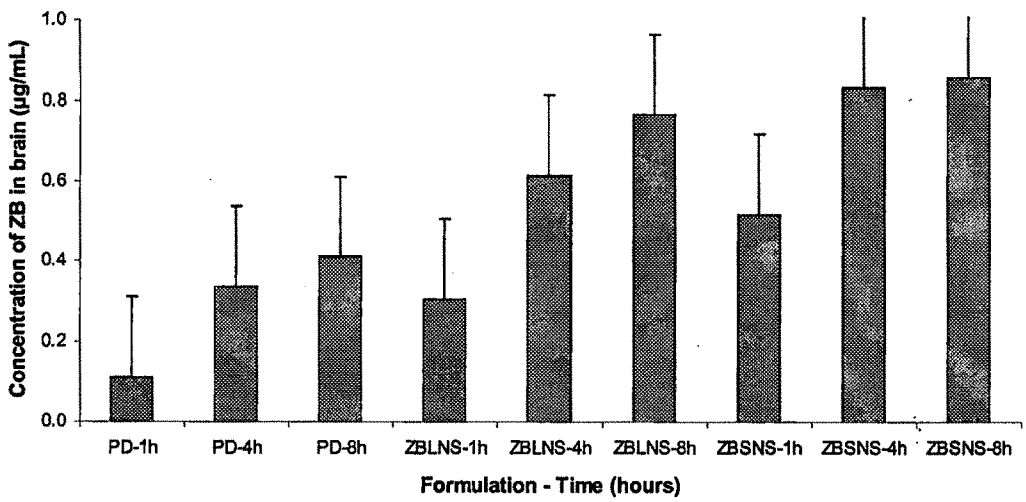
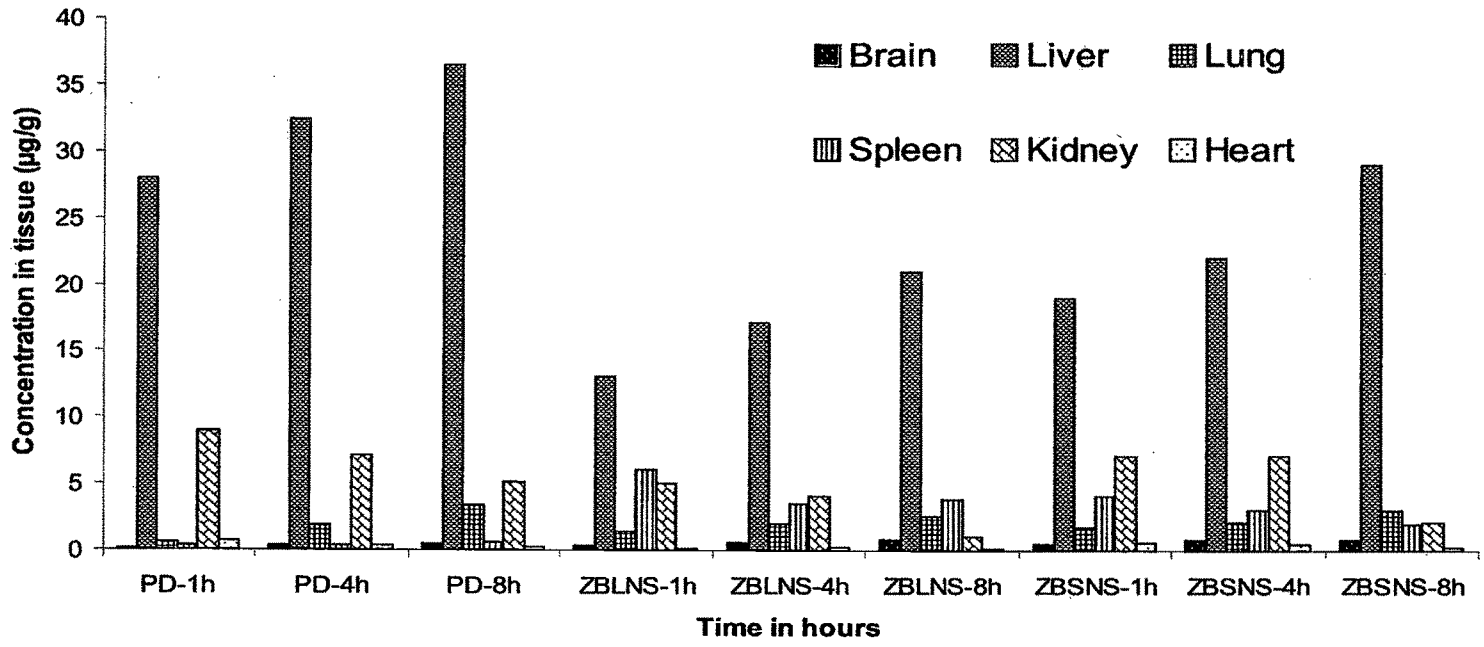


FIGURE 9.2 Brain Concentrations of ziprasidone after intramuscular injection as plain solution, lyophilized nanosuspension (ZBLNS) and spray dried nanosuspension (ZBSNS). The values plotted are the mean ± S.D of 3 experiments.



PD - Ziprasidone Solution
ZBLNS - Ziprasidone Lyophilized Solution
ZBSNS - Ziprasidone Spray Dried Solution

FIGURE 9.3 Tissue distribution of ziprasidone after intramuscular injection as plain solution, lyophilized nanosuspension (ZBLNS) and spray dried nanosuspension (ZBSNS). The values plotted are the mean ± S.D of 3 experiments

9.4 CONCLUSION

In vivo, the nanosuspensions exhibited higher blood circulation time and brain concentration compared to the ziprasidone solution, indicating the potentiality of nanosuspension forms. There was a significant reduction in the uptake by the RES organs of ZB after administration in nanosuspension form. These data substantiate the potential of the above formulated poloxamer coated ZB nanosuspension as a long circulating system in blood and higher brain deposition. Looking at the experimental results, it appears that the proposed nanosuspension forms can be an alternative to the commercially available ziprasidone parenteral formulation for intramuscular injection.

9.5 REFERENCES

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