Chapter 7

Investigations on the in vivo performance of Ziprasidone loaded poly (lactide co -glycolide) microparticles

7.1 INTRODUCTION

Exploitation of polymeric drug delivery systems for parenteral controlled release of proteins, peptides, and poorly soluble compounds provides a number of advantages over daily injections with patient compliance being an important benefit. Microsphere based new drug delivery systems are injected or implanted into the subcutaneous tissue and release the incorporated drug in a controlled manner, allowing the adjustment of release rates over extended periods of time ranging from several days to up to one year (Danckwerts and Fassihi, 1991). Interest in the field of polymeric drug delivery has increased considerably especially after the commercial success of products such as Lupron Depot®, Zoladex®, Norplant®, and Gliadel®, all which use the principles of sustained and localized drug delivery. In case of localized drug delivery, one attempts to achieve high drug concentrations at the site of implantation without exposing the non-affected tissue. This aspect can be utilized for the effective delivery of antineoplastic agents, which have a considerable level of tissue toxicity in addition to a narrow therapeutic window.

In contrast to conventional parenteral formulations, novel injectable depot systems using biodegradable polymers allow the control and modulation of drug release. Parenteral depot systems can be classified into implants or microparticles. Implants are cylindrical devices injected through a large bore needle (trocar) into the subcutaneous (s.c.) tissue. Goserelin (Zoladex, AstraZeneca), leuprolide (Viadur, Alza) and buserelin (Biogonist, Laboratories Cassenne), three synthetic LHRH analogues have received regulatory approval in implant form. In contrast to implants, microparticles can be injected intramuscularly or subcutaneously through a hypodermic needle. There are a few therapeutic peptides commercially available as microsphere-based depot systems like Lupron depot (Leuprolide, Takeda), Sandostatin LAR (Octreotide, Novartis), Suprefact depot (Buserelin, Aventis Pharma), Trelstardepot (Triptorelin, Debio Pharma). A few more peptides like Orntide, Deslorelin and a recombinant growth hormone (Nutropin depot) are undergoing clinical evaluation as microsphere-based implantable therapeutic systems.

Medication non-compliance is a major barrier to better health outcomes for people with schizophrenia. Atleast 50% of out patients with schizophrenia stop their medication within a year of hospital discharge and this non-compliance is a major risk factor for relapse which may

turn out to be more severe and dangerous (Babiker IE, 1986).The factors contributing to non-adherence behavior are lack of motivation, depressive states, lack of insight and unawareness of illness and need of its treatment, sociodemographic characteristics such as young age, being single and lack of family involvement, complex dosing regimens and lack of reasonable access to medication. Most physicians feel that high rate of relapse; hospitalization and suicidal behavior associated with untreated schizophrenia indicate that continuous antipsychotic treatment is the most likely solution to successful therapy. Generally schizophrenic patients have difficulty to comply with their medication regimen because of the nature of the illness. Therefore, a long acting injectable medication that does not require the patient to take the medication daily might increase compliance and substantially improve patient symptoms.

In the present chapter, Ziprasidone (ZB) loaded PLGA 50:50 microspheres (PLGA 50:50 MS) prepared by o/w emulsification solvent evaporation method (Chapter 6) was administered intramuscularly into rats and the pharmacokinetics was studied.

7.2 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 3 each. The samples were injected intramuscularly into the hind leg of the rat using a 24G 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 group was injected with 0.5ml of ZB loaded PLGA 50:50 microspheres (equivalent to 4.0mg of ZB) which was reconstituted previously with pH 7.4 PB. The third group was injected with 0.5ml of ZB loaded PLGA 50:50 microspheres (equivalent to 2.0mg of ZB) which was 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.

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 $p < 0.005$.

7.3 RESULTS

Blood kinetic profile studies

The blood clearance profiles of ZB solution in propylene glycol, ZB loaded PLGA 50:50 MS are depicted in Figure 7.1 and figure 7.2. The pharmacokinetic parameters after intramuscular injection were calculated using Wagner Nelson method (Reddy LH and Murthy RSR., 2004) and are depicted in table 7.1.

The pharmacokinetics of ZB loaded biodegradable microspheres (upon injection of single dose) was carried out in white albino rats. ZB in the plasma was estimated by the spectrofluorimetric method reported earlier. The objective of the experiment was to design a controlled release drug delivery system for attaining a steady plasma drug concentration over long periods of time so as to minimize the frequency of injections and fluctuations in plasma drug concentration. The plasma concentrations of free BLM were found to be between 67.62 ng/mL and 54.47 ng/mL after 2 days at 4mg and 2mg dose levels respectively. The plasma ZB levels after i.m. administration of plain ZB solution in PG went below detection limits after 8 hours post injection. No further blood sampling was done for plain ZB treated groups, as they were injected with single injections only and the plasma drug levels were compared at similar sampling intervals.

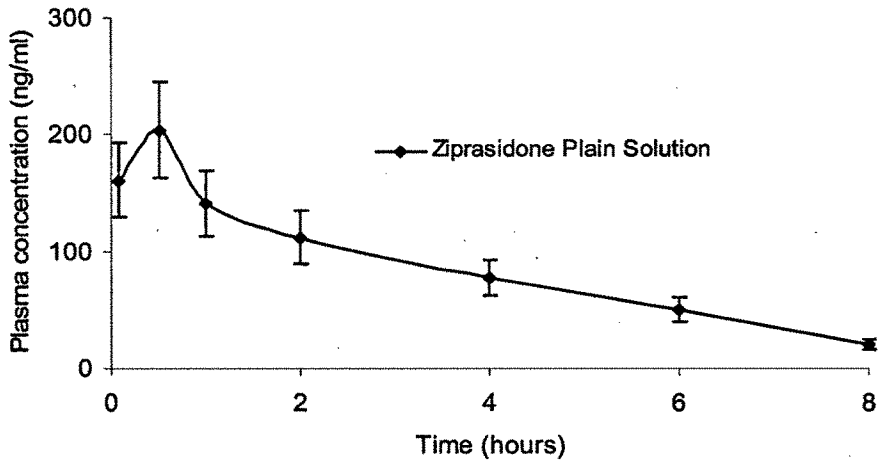

FIGURE 7.1: Blood levels of intramuscularly injected ziprasidone delivered as plain solution. The values plotted are the mean ± S.D of 3 experiments

On the 8th day post-injection, plasma concentrations of 57.26 and 35.84 ng/mL were obtained at 4mg and 2mg dose levels respectively. A steady state plasma level concentration of ZB was obtained between the time period of 2-8 days (67.62 ng/mL to 57.26 ng/mL for 4mg dose and 54.47 ng/mL to 35.84 ng/mL for 2mg dose). The drug-release rate was just picking up upto the 10th day post-injection, the elimination phase just commenced and the plasma ZB values went below detection limits after 17 days for 4 mg dose and 10 days for 2mg dose respectively. The rate of drug release in-vivo increased with the dose injected for both the copolymers.

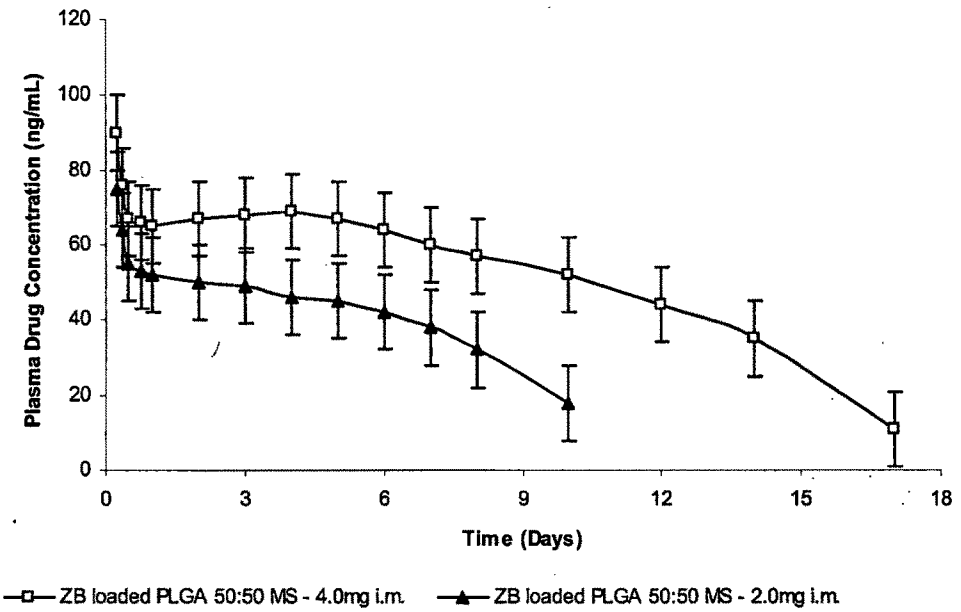

FIGURE 7.2: Blood levels of intramuscularly injected ziprasidone delivered as ZB loaded PLGA 50:50 MS dispersion. The values plotted are the mean ± S.D of 3 experiments.

Development of intramuscular depots for prolonging the drug release in vivo is catching attention of researchers across the globe. Shah and researchers from Atrix Laboratories (Shah NH et al., 1993), Dunn and coworkers (Dunn RL et al., 1991, 1992, 1994, 1995), Tipton and coworkers (Tipton AJ., 1992; Lowe BK et al., 1993; Frank KR et al., 1994) have described a novel implant system which is parenterally administered as a liquid and subsequently solidifies into a gel matrix (implant) *in situ*, from which the drug is released in a controlled manner. Although this implant system precludes the need for any surgery for its administration, it has a number of disadvantages:

(1) The safety of solvents like N-methyl-2-pyrrolidone (NMP) used to formulate these systems is questionable and not well documented,

(2) The injection of these liquid implant systems and their subsequent solidification produce non-uniform matrix implants having variable consistency and geometry, and

(3) Due to the formation of matrix implants having inconsistent texture, shape and size, the drug release from them is likely be variable and unpredictable.

Similarly, Shenoy and coworkers (Shenoy DB et al., 2002) studied the tumor regression efficiency of Bleomycin sulphate (antineoplastic agent) loaded PLGA 75:25 and PLGA 85:15 microsphere based depot. The authors concluded that the extent of biodegradation of the polymer matrix plays a major role in deciding the drug fraction available for anti-tumor action of the encapsulated antineoplastic agent.

Presently, Risperidone loaded PLGA microsphere based intramuscular depot is available in the market. After a single intramuscular (gluteal) injection of the depot, lead to a small initial release of the drug (< 1% of the dose), followed by a lag period of 3 weeks. The main release of the drug started from week 3 onwards and was maintained from week 4 to 6 and subsided by week 7. The manufacturer recommends oral antipsychotic supplementation to be given during the first 3 weeks of treatment with Risperidone Depot to maintain therapeutic levels until the main release of Risperidone from the injection site has began. However, in the present study, we did not observe any lag period for the drug release in vivo. Fluctuations in the plasma concentrations were lesser with the depot formulation on comparison with oral Risperidone. The efficicay of the Risperidone depot was evaluated by assessing the Positive and Negative Syndrome Scale (PANSS). Total PANSS scores showed significant improvement in the change from the baseline to endpoint in Schizophrenia patients treated with the Risperidone depot. However, there was no statistically significant difference between the treatment effects for the three dose groups.

Parameters	ZB Solution	ZB loaded PLGA 50:50 MS – 4.0mg	ZB loaded PLGA 50:50 MS – 2.0mg
K_{el}, elimination constant, h^{-1}	0.2562	-	-
K_{el}, elimination constant, day^{-1}	-	0.0502	0.0831
$T_{1/2}$, h	2.7037	-	-
$T_{1/2}$, day	-	13.8033	8.336
V_d, ml/gm	0.0051	0.0131	0.0159
Cl, clearance , ml/min	0.0013	-	-
Cl, clearance , ml/day	-	0.0007	0.0013
MRT, h	2.7517	-	-
MRT, day	-	6.6667	4.6476
AUC $^{0-8}$, h.ng/ml	683.5133	-	-
AUC $^{0-8}$, day.ng/ml	-	831.6258	476.1259
AUC $^{0-\infty}$, h.ng/ml	765.6359	-	-
AUC $^{0-\infty}$, day.ng/ml	-	1389.3346	704.6599

TABLE 7.1: Comparative pharmacokinetic parameters of ziprasidone solution (ZB Solution), ZB loaded PLGA 50:50 MS – 2.8mg and ZB loaded PLGA 50:50 MS – 1.4mg after intramuscular injection in rats (n = 3).

7.4 CONCLUSION

The ZB loaded PLGA 50:50 microspheres exhibited sustained plasma ZB levels in vivo compared to the ziprasidone solution, indicating the potentiality of the formulated PLGA microspheres as long acting injectable forms. Out of the two doses studied, the 4mg dose gave higher plasma levels throughout the time period studied. The formulated microspheres were able to sustain the release upto a period of 17 days when 4mg depot and 10 days when 2mg depot was administered. Steady state plasma concentrations between 57ng/mL to 67ng/mL and 38ng/mL to 53 ng/mL were observed between day 2 to day 10 post injection of the 4mg depot and 2mg depot respectively. Interpreting the experimental results, it appears that the above formulated microsphere formulation can be a potential long acting formulation for combating non-compliance associated with Schizophrenia.

REFERENCES

Babiker IE, Non-compliance in schizophrenia, *Psychiatr Dev.* 1986, 4,329-337.

Danckwerts M and Fassihi A, Implantable controlled release drug delivery system: a review. *Drug Dev Ind Pharm*, 1991, 17, 1465–1502.

Dunn RL, Tipton AJ, Menardi EM, A biodegradable *in- situ* forming drug delivery system, *Proceedings of the International Symposium on Controlled Release of Bioactive Materials*, 1991, 18, 465.

Dunn RL, Tipton AJ, Yewey GL, Reinhart PC, Menardi EM, Rogers JA, Southard GL, An injectable implant delivery system for antimicrobial agents, *American Association of Pharmaceutical S cientists*, Western Regional Meeting (Reno, NV), 1992, pp. 189- 191.

Dunn RL, Yewey GL, Duysen EG, Frank KR, Huffer WE, Pieters R, Bone regeneration with the Atrigel® polymer system. *Portland BoneS ymposium*, Portland, Oregon, 1995.

Frank KR, Duysen EG, Yewey GL, Dunn RL, Huer WE, Pieters R, , Controlled release of bioactive growth factors from a biodegradable delivery system. *Pharmaceutical Research*, 1994, 1, S-88.

Lowe BK, Norton RL, Keeler EL, Frank KR, Tipton AJ, The effects of gamma irradiation on poly(DL-lactide) as a solid and in *N*-methyl-2- pyrrolidone solutions. *19th Annual Meeting of the Society for Biomaterials*, Birmingham, AL. 1993.

Reddy LH, Murthy RSR, Pharmacokinetics and Biodistribution Studies of Doxorubicin loaded Poly (Butyl Cyanoacrylate) Nanoparticles synthesized by two different techniques, *Biomed. Papers*, 148, 161–166.

Shah NH, Railkar AS, Chen FC, Tarantino R, Kumar S, Murjani M, Palmer D, Infeld MH, Malick AW, A biodegradable injectable implant for delivering micro and macromolecules using poly(lactic-co-glycolic) acid (PLGA) copolymers. *Journal of Controlled Release*, 1993, 27, 138- 147.

Shenoy DB, D'Souza RJ, Udupa N, Poly (DL-lactide-co-glycolide) microporous microspherebased depot formulation of a peptide-like antineoplastic Agent, *J. Microencapsulation*, 2002, 19, 523-535

Tipton AJ, Fujita SM, Frank KR, Dunn RL, Release of naproxen from a biodegradable, injectable delivery system, *Proceedings of the International Symposium on Controlled Release of Bioactive Materials*, 1992, 19, 314.