ORIGINAL ARTICLE

Development of novel risperidone implants using blends of polycaprolactones and *in vitro in vivo* correlation studies

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ABSTRACT

The objective of this study was to develop a novel implant containing risperidone intended for long-term treatment in Schizophrenia utilizing *in vitro in vivo* correlation (IVIVC) studies. Different implants (F1-F8) containing an antipsychotic drug, risperidone, were prepared using a hot melt extrusion technique by taking polycaprolactones of different molecular weights (Mwt. 15000, 45000, 80000) either alone or as their blends, and PLGA (75:25). The implants contained 40% of the drug. After fabrication, the implants were characterized for various *in vitro* properties such as drug release and physical strength. Prior to conducting drug release studies, optimum drug release method was developed based on IVIVC studies. An optimized formulation based on drug release and physical strength at the end of fabrication was selected from the various implants fabricated. The bioactivity, reversibility, and IVIVC of optimized formulation were determined using pharmacokinetic studies in rats. Short-term stability studies were conducted with optimized formulation. Drug release depended on polymer molecular weight. Implant fabricated using 50:50 polycaprolactone 45,000 and polycaprolactone 80,000 was considered optimized implant. Optimized formulation selected released the drug for 3-months *in vitro* and was physically rigid. The optimized implant was able to release the drug *in vivo* for a period of 3 months, the implants are reversible throughout the delivery interval and, a 100% IVIVC was achieved with optimized implant, suggesting the development of 3-month drug-releasing implant for risperidone. The optimized implant was stable for 6 months at room temperature (25°C) and 45°C. A novel implant for risperidone was successfully prepared and evaluated.

Key words: Implant, *in vitro in vivo* correlation, removability, risperidone, schizophrenia, stability

INTRODUCTION

Schizophrenia and schizoaffective disorders are severe mental disorders characterized by breakdown of thought process and deficiency of emotional responses.[1,2] Common symptoms include delusions, disorganized thinking, auditory hallucinations, disorganized speech, etc. There is a significant social and occupational dysfunction in this disease. The drugs used to treat this disease are called as antipsychotics. Non-adherence with antipsychotic medication remains a principal obstacle undermining better treatment outcomes in schizophrenia and schizoaffective disorder. The reduced quality of life in these patients because of non-adherence was investigated early-on.[2] Long-term depots were the first treatment options developed.[3] Later, parenteral microspheres were developed. Both these formulations are found to be advantageous than daily oral administrations. However, there is a need for improvement in such delivery methods. Many antipsychotics are unable to make the required ester linkages to form depot suspensions. Presently marketed microsphere formulation requires oral antipsychotic supplementation.[2] Both these strategies are irreversible.

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Depot treatment also causes pain at the site of injection resulting in patient discontinuing the therapy.[4] Thus, several groups addressed implantable delivery systems, which are reversible, do not require oral supplementation, do not cause pain, and can be administered via simple techniques for the treatment of schizophrenia.[4,5] All the implants that are reported so far are based on PLGAs and are able to release the drug for one or two months. On the other hand, implants and microspheres with different and more prolonged drug-releasing behavior can be prepared using other biodegradable polymers such as polycaprolactones. For several drugs, the drug-releasing behavior was found to be variable in PLGA and polycaprolactone.[6] Either quick or prolonged drug release can be achieved in polycaprolactone-based delivery systems when compared to PLGA-based delivery systems. For paclitaxel, quicker release was achieved with polycaprolactone films compared to PLGA films.[7] On the other hands, ketorolac tromethamine from polycaprolactone-based microspheres had more prolonged release compared to the microspheres prepared using PLGA.[8] The hypothesis of this study is that a 3-month drug-releasing clinically useful implant for risperidone can be prepared with polycaprolactone and its blends. As per our knowledge, for risperidone, 3-month drug-releasing implants were not previously reported. Further, formulation development based on IVIVC leads to strong formulation development programs. Such a novel formulation development methodology utilizing IVIVC for development of parenteral drug delivery systems is currently gaining prominence.[6,9,10] Thus, the objective of this study was to develop inexpensive and novel implants for antipsychotic drug that can release the drug for 3 months using polycaprolactones and its blends, conduct biostudies such as pharmacokinetics, IVIVC and reversibility with this delivery system.

MATERIALS AND METHODS

Materials

Drug and chemicals used in the study were of analytical grade and procured either gift samples or purchased. Paclitaxel (PTX) and PLGA (75:25) were gift samples from Relisys Medical Devices Pvt. Ltd., Hyderabad, India. Polycaprolactones (M.Wts 14000, 45000 and 80000) were purchased from Sigma-Aldrich Ltd., Germany. Dichloromethane and polyvinyl alcohol were purchased from SD Fine Chemicals Ltd. A UV-Vis Spectrophotometer from Fisher Scientific and a HPLC with UV detection from Waters Corporation was used in the analysis of the samples. Differential scanning calorimetry (DSC) used to characterize the solid state of the drug in the formulation was from Shimadzu. A JSM-5200 Scanning Electron Microscope (SEM), Japan, was used to study the surface morphology of the microspheres. All the other equipments used were all from standard sources.

Methods

Preparation of the implant

Risperidone implants (F1-F8) were prepared by hot melt extrusion method as reported previously.[9] The formulae used to prepare the implant are shown in Table 1. Briefly, specific amount of polymer was dissolved in 5 ml of dichloromethane organic solvent. Risperidone drug was weighed and dissolved in 5 ml dichloromethane. The drug solution was poured into the polymer solution, stirred well, and then dichloromethane was allowed to evaporate to obtain a powder. This powder was melt extruded at 150°C in a Teflon tube to form larger implants, which were then cut to desired sizes and used. The methodology of fabrication of implants is depicted in Figure 1. Upon fabrication, the hardness of the implants was tested using a Monsanto hardness tester.

In vitro characterization of the implant

The implant surface morphology was investigated using SEM. Risperidone implant was sliced kept over a slab. Photographs were taken using a JSM-5200 Scanning Electron Microscope (Tokyo, Japan) operated at 20 kV. Pictures of risperidone implant were taken by random scanning of slab. Solid state of the drug in the implant was determined using a DSC. Approximately, 4 mg of sample was taken in an open aluminum pan and heated at scanning rate of 10°C/min between 0°C and 300°C, and the corresponding thermograms were obtained. Aluminum was used as the standard reference material. The thermograms

Table 1: Risperidone implants formulations

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Respiridone (mg)	80	80	80	80	80	80	80	80
PCL 14000 (mg)	120	-	-	60	60	-	40	-
PCL 45000 (mg)	-	120	-	60	-	60	40	-
PCL 80000 (mg)	-	-	120	-	60	60	40	-
PLGA 75:25 (mg)	-	-	-	-	-	-	-	120
DCM (ml)	10	10	10	10	10	10	10	10

PCL: Polycaprolactone, DCM: Dichloromethane

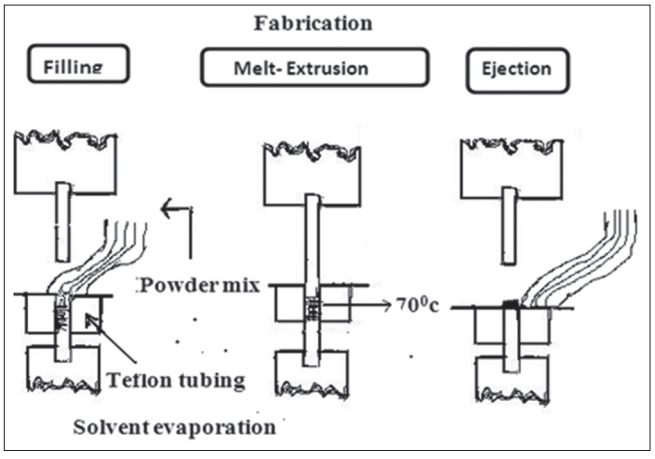

Figure 1: Procedure for fabrication of the implant

of risperidone, mixture of optimized polymers (50:50 ratio of PCL 14000 and PCL 45000, PCL 80000), placebo implant, pure respiridone with mixture of polymers, and risperidone implant were obtained.

Establishing suitable drug release study methodology

To determine *in vitro* release, the drug release method was first optimized. For this purpose, F3 implant was used. Three different drug release study methodologies were investigated. In the first method, one implant (F3) was taken in a 1 L phosphate buffer in USP apparatus 1. The release samples of 1 ml were taken periodically and replaced with 1 ml of the buffer. The drug was analyzed in the samples, and the cumulative drug release plot was developed. This method was used so as to provide best sink condition for drug release. The aqueous solubility of respiridone is 2.8 mg/L. In the second method, the release studies were performed in a USP type-I apparatus containing 200 ml of phosphate buffer. An aliquot of 5 ml sample was periodically withdrawn and was replaced with same amount of the buffer. The drug was estimated in the samples, and the cumulative amount of drug release vs. time was plotted. This method was based on the volume of distribution as the volume of the drug-releasing medium. The volume of distribution of respiridone was 1 L/Kg. In the third method, each implant was taken in microtubes and suspended in 2.0 ml phosphate buffer solution. These microtubes were placed in horizontal water bath shaker. At the end of 24 h, 0.5 ml of supernatant solution was withdrawn, filtered, and it was further diluted with PBS (7.4 pH). Meanwhile, the volume withdrawn was replenished with an equal volume of fresh PBS (7.4 pH) in the microtubes and placed in the water bath. This technique is based on the volume of intraperitoneal (IP) space in rats. The volume of IP space is around 2 mL. In all the three methods of release studies, the temperature was maintained at 37°C. The amount of risperidone released was analyzed by UV spectrophotometer at the λ max value of 278 nm using PBS as the blank. Release studies were performed till no more drug was released. The method for drug release studies was optimized based on the *in vivo* drug release studies in rats with one implant prepared using polycaprolactone of molecular weight 80,000 after determining IVIVC as described in the next sections. This implant was selected to establish the release medium, because it has been previously reported that robust implants can be prepared with this polycaprolactone with drugs.

Drug release studies with the implants were performed in the optimized drug release method. The third method in the above three methods was considered optimized from IVIVC data. This method was used to study drug release for further development of desired implants.

Determination of content uniformity and drug release

Content uniformity was determined using a UV-Vis assay method at a λ_{max} of 278 nm. For this purpose, each implant was dissolved in dichloromethane, and the amount of the drug was determined using UV-Vis spectrophotometer. Placebo implant was taken to prepare blank used in the assay. Implants of different batches containing 54 mg of drug were taken in microtubes and suspended in 2.0 ml phosphate buffer solution. These microtubes were placed in horizontal water bath shaker. At the end of specific time points, 0.5 ml of supernatant solution was withdrawn, filtered, and it was further diluted with PBS (7.4 pH). Meanwhile, the volume withdrawn was replenished with an equal volume of fresh PBS (7.4 pH) in the microtubes and placed in the water bath. The cumulative percentage drug release versus time was then plotted and used. To analyze the mechanism of drug release rate kinetics of the optimized dosage form, the obtained data was fitted into zero-order, first-order, Higuchi, and Korsmeyer-Peppas release models.

In vivo studies

The Institutional Animal Ethical Committee of College of Pharmacy approved (024/IAEC/St.JCOP/2013) all protocols, and the study was conducted after following the CPSCEA guidelines. A total of 30 rats were used in the studies. These rats were divided into five groups; one group given with intravenous dose of respiridone, the second group was administered one time with F-3 (for developing IVIVC for optimization of release medium), and the third group with the optimized implant (F-6) prepared in the study and the fourth group with the optimized implant (F-6) to study the reversal of the implant. The study in the second group was performed prior to the studies in first, third, and fourth groups. Such a methodology was followed for optimizing drug release methodology conducted earlier. Groups of rats were administered the implant on day 1 via IP route. The IP route was selected to deliver the drug systemically because of the convenience this route offers compared to other parenteral routes in animal models. The implants were sterilized under UV light for 24 hours prior to administration. Over a period of 90 days, blood was collected and analyzed for plasma respiridone level using a solid phase extraction protocol followed by HPLC. A previously published method was used for this purpose.[4] To study reversibility in fourth group, the implant was allowed in the rat for two months, at the end of which the implant was removed from the IP space and the experiment was continued. IVIVC studies were also conducted with the optimized implant. For these studies, another set of rats were used. IVIVC studies were performed as described in the next paragraph.

IVIVC was established according to Aukunuru *et al*.[9,10] This method is used to establish IVIVC with long-term parenteral depot.[11] The parameters compared were cumulative absorption profile to that of *in vitro* release profile i.e., correlation of the amount of drug released to that of respective fraction of dose absorbed. *In Vitro* release profile was then obtained for F3 implants using

various drug release methodologies as mentioned earlier. Cumulative amount of the drug absorbed was calculated using Wagner-Nelson method approximating the kinetics of the drug to one compartment open model. According to Wagner-Nelson method, the cumulative amount of drug released from the microspheres into the systemic circulation in a rat was calculated using the equation given below:

$$A_b/Ab^{\infty} = (C_p + K[AUC]_0^t]/K[AUC]_0^{\infty}$$

Where A_b is the cumulative amount released at any time, Ab^{∞} is the dose administered, C_p is the plasma concentration at any time t, K is the elimination rate constant, and AUC is the area under the curve.

The effects of continual respiridone treatment in the rats administered optimized implant; F6 was also assessed using Locomotor Testing as a pharmacodynamic measure. Such a methodology was previously used for respiridone implants.[4] The locomotor activity was determined using an actophotometer.

Stability studies

Stability studies were performed according to previously reported method. The formulation was stored in amber-colored glass bottles at 4 ± 1°C, room temperature 25 ± 1°C, and in hot air oven at 40 ± 1°C for a period of six months. The samples were analyzed every 10 days by HPLC as indicated in the pharmacokinetic studies. The amount of the drug that remained in the implant was considered to be the stable portion for every time point of analysis.

Statistical analysis

All experiments were done six times, and the data were expressed as mean ± STDEV, and Tukey's post-hoc test was done to analyze significance of difference between different groups using the statistical analysis software package SPSS (Version 16.0, IBM, USA).

RESULTS AND DISCUSSION

Although new drug discovery for schizophrenia is very actively pursued by major pharmaceutical companies, non-adherence is still the major problems with antipsychotics.[1-5] Thus, new methods apart from discovery of new drugs are actively being pursued by various groups. In this regard, depot implants, depot suspensions, and microspheres are the methods that can be actively investigated. For instance, depot forms for antipsychotic are available in the market to treat Schizophrenia patients. This study is another attempt on those lines. We particularly focused on implants rather than depot suspensions or microspheres. Further, IVIVC studies which help in rigorous formulation development of parenteral sustained release dosage forms as described previously[12] were conducted. Implants (F1-F8) were successfully prepared with the technique used in this study with all the formulations. However, the hardness of the implants varied with the polymer type and molecular weight. The implants prepared using PLGA (75/25), PCL 45000 and PCL 80000 were hard while the implants prepared with PCL 14000 had very low hardness. Upon fabrication, surface morphology of the implant was examined by SEM. All the implants prepared using polycaprolactones (Mwt. 14,000; 40,000; 80,000) and PLGA (75:25) had smooth surface**.** A representative SEM picture of one implant is shown in Figure 2. *In vitro* drug release study methodology was optimized using formulation F3. The cumulative amount of the drug released from the implants *in vitro* was studied in three different methodologies. The results are shown in Figure 3. The calibration curve of the drug was constructed to determine the concentration of the drug from the absorbance values. From this, the cumulative % drug release was determined**.** Since a 100% correlation was obtained between *in vitro* drug release and *in vivo* drug absorbed with the third methodology [Figure 4], we considered it to be optimized drug release study methodology and used it for further formulation development. The plots of cumulative percentage drug release vs. time for all the eight formulations were developed. The drug release from implant prepared using pure polymers is shown in Figure 5. The drug release from implants prepared using blends of polycaprolactones is shown in Figure 6. PLGA-based implant released the drug to an extent of 100% in 50 days while polycaprolactone-based

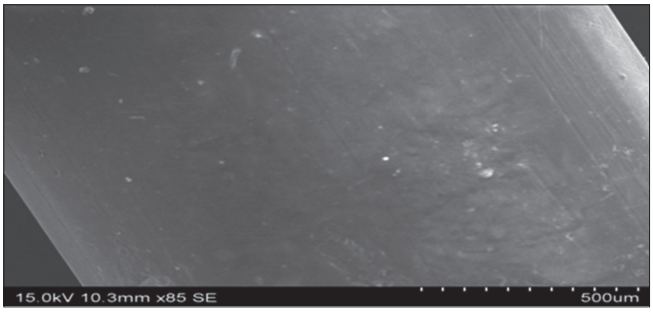

Figure 2: Scanning electron microscope picture of the surface of the implants

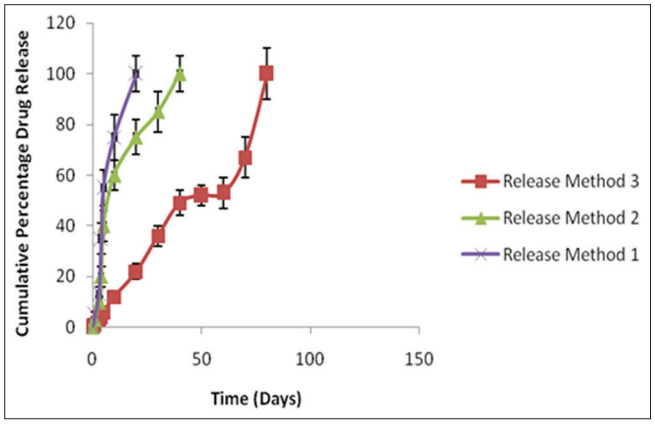

Figure 3: Drug release in various release methods with implant f3

implants demonstrated more prolonged drug release. As the molecular weight of polycaprolactones was increased, there was more prolonged drug release. A 100% of drug release was achieved with PCL 14000, PCL 45000, and PCL 80000 implants in 70 days, 80 days, and 110 days, respectively. The hardness of the implants was in the order PCL 80000 > PCL 45000 > PCL 14000 with PCL 80000 giving the best results. With the implants prepared using blends of PCLs, there was altered drug release when compared to that of the implants prepared using pure polymers. Both the implants F6 and F7 achieved the target release of 100% in 90 days. However, the hardness of F6 was more when compared to that of F7, and thus F6 was considered the optimized implant. F6 was prepared using a blend of PCL 45,000 and PCL 80,000 at 1:1 ratio. The optimized implant, F6, released the drug for 3-months *in vitro*. Thus, the objective of this study in terms of drug release was met from *in vitro* release data. Drug release mechanisms were determined by fitting *in vitro* drug release data to various kinetic models. It can be concluded that the optimized formulation gave a good fit to the Korsemeyer-Peppas model. The diffusion exponent (n) values were greater than 1, so the drug release follows super case II transport. Thermal behavior of drug in this formulation, *in vivo* drug release, and reversibility were studied with this formulation. *In vivo* drug release was assessed using plasma drug levels as well as locomotor testing.

DSC studies were performed to understand the nature of the entrapped drug in the polymer. The physical state of risperidone in the polymer would also influence its release characteristics. To probe this effect, DSC analysis was performed on different samples. In the DSC curves displayed in Figure 7, melting endotherm of pure risperidone was found to be 170°C. There was no peak detected in the temperature ranges of 150-200°C for blank implant and in the optimized formulation (F6); the peak appeared at the same temperature (170°C) but with reduced peak area. The reduction of drug peak may be due to conversion of risperidone from crystalline state to amorphous, partly or fully.

The optimized implant (F6) was injected into the rats, and the pharmacokinetics, IVIVC, and pharmacodynamics were tested to demonstrate the fulfillment of the target of the study. *In vivo* onset was rapid, and plasma concentration was in the range of 10-120 ng/ml for

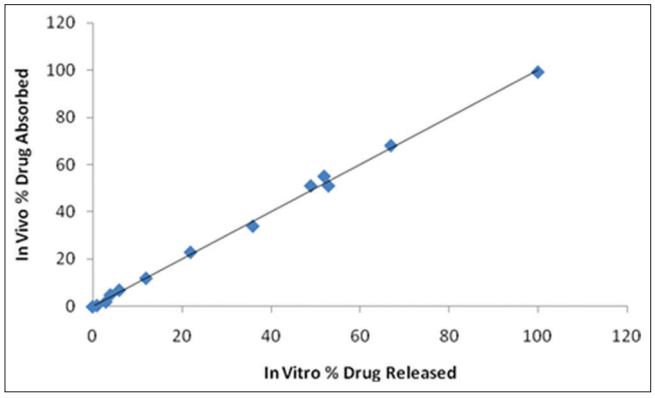

Figure 4: *In vitro in vivo* correlation of drug released and drug absorbed in the optimized drug release method

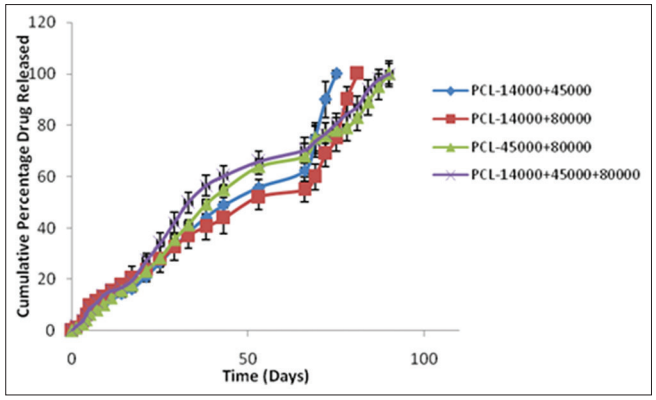

Figure 6: Cumulative % drug released from respiridone implants prepared using blends of polycaprolactones

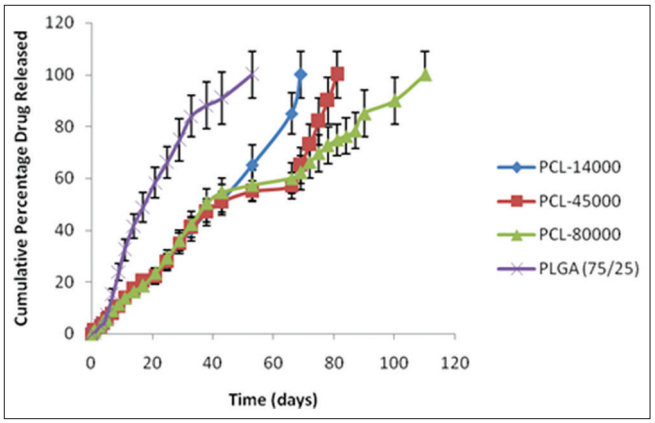

Figure 5: Cumulative % drug release from various respiridone implants fabricated using pure polymers

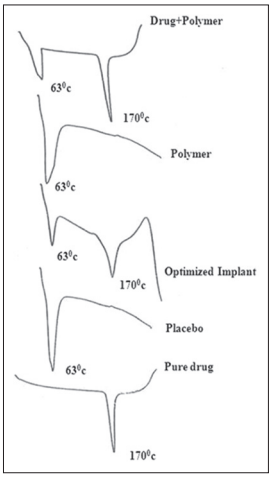

Figure 7: Differential scanning thermograms of various samples

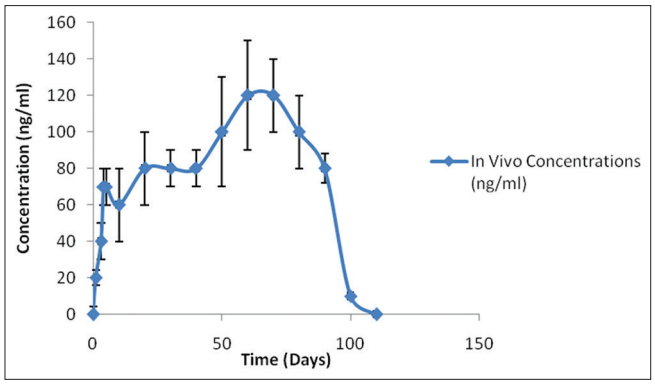

Figure 8: *In vivo* plasma profile of respiridone with the optimized implant

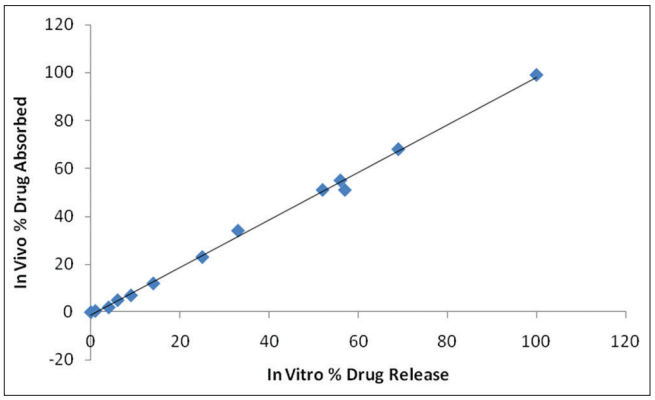

Figure 9: *In vitro in vivo* correlation with optimized implant

a substantial portion of release interval. Implants released the drug for 3-months *in vivo* [Figure 8]. *In vitro in vivo* studies with the optimized implant indicated a 100% correlation authenticating the development of a 3-month drug-releasing implant for respiridone with the implants [Figure 9]. In the set of rats where the implants were removed intermittently, the plasma levels almost reached zero within 2 days, suggesting the reversibility of the implant. In the pharmacodynamic assessment, the animals were observed for first one week to evaluate the signs of high initial drug release. From the observations, no burst release appeared to develop *in vivo*. For 90 days, antipsychotic effects were demonstrated in rats administered F6 implant. This was demonstrated based on locomotor activity. Thus, the optimized implant released the drug *in vivo* over a period of 3 months, and the drug release was reversible. The drug levels were found to be in the therapeutic window for all the 3 months suggesting the superior performance of the implant *in vivo* as well. This has been confirmed both by drug levels, IVIVC and pharmacodynamic end points. The stability studies were performed for 6 months, and the formulations were found to be stable at all temperature conditions.

CONCLUSIONS

The results of this study demonstrate a 3-month drug-releasing implant for risperidone intended for long-term therapy in Schizophrenia. The implants sustain the drug release and were reversible indicating that the implants are better than long-term depots and microspheres.

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REFERENCES

1. Amresh SI, Sangeeta RJ. Schizoaffective disorder: Consistency of diagnosis. Indian J Psychiatry 1999;41:329-32.
2. Schooler NR. Relapse and rehospitalization: Comparing oral and depot antipsychotics. J Clin Psychiatry 2003;64 Suppl 16:S14-7.
3. Barnes TR, Curson DA. Long-term depot antipsychotics. A risk-benefit assessment. Drug Saf 1994;10:464-79.
4. Rabin C, Liang Y, Ehrlichman RS, Budhian A, Metzger KL, Majewskitideken C, *et al*. *In vitro* and *in vivo* demonstration of risperidone implants in mice. Schizophr Res 2008;98:66-78.
5. Amann LC, Gandal MJ, Lin R, Liang Y, Siegel SJ. *In vitro in vivo* correlations of scalable PLGA-Risperidone implants for the treatment of schizophrenia. Pharm Res 2010;27:1730-7.
6. Shiny J, Ramchander T, Habibuddin M, Goverdhan P, Aukunuru JV. Development and evaluation of a novel biodegradable sustained release microsphere formulation of paclitaxel intended to treat breast cancer. Int J Pharm Investig 2013;3:119-25.
7. Lao LL, Venkatraman SS, Peppas NA. Modeling of drug release from biodegradable polymer blends. Eur J Pharm Biopharm 2008;70:796-803.
8. Sinha VR, Trehan A. Development, characterization, and evaluation of ketorlac tromethamine loaded biodegradable microspheres as a depot system for parenteral delivery. Drug Deliv 2008;15:365-72.
9. Aukunuru JV, Sunkara G, Ayalasomayajula SP, DeRuiter J, Clark RC, Kompella UB. A biodegradable injectable implant sustains systemic and ocular delivery of an aldose reductase inhibitor and ameliorates biochemical changes in a galactose-fed rat model for diabetic complications. Pharm Res 2002;19:278-85.
10. Aukunuru J, Sankavarapu V. Pharmacokinetics and *in vitro* and *in vivo* correlation of NN-dimethiaminocurcumin (NNDMAC) loaded polycaprolactone microspheres in rats. Curr Trends Biotechnol Pharm 2010;4:578-88.
11. Amitava M, Yunhui W. Use of *in vito-in vivo* correlation (IVIVC) to facilitate the development of polymer-based controlled release injectable formulations. Recent Pat Drug Deliv Formul 2010;4:94-104.
12. Burgess DJ, Hussain AS, Ingallinera TS, Chen ML. Assuring quality and performance of sustained and controlled release parenterala: Workshop report. AAPS PharmaSci 2002;4:1-11.

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