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Research Article

Development of Risperidone PLGA Microspheres

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The aim of this study was to design and evaluate biodegradable PLGA microspheres for sustained delivery of Risperidone, with an eventual goal of avoiding combination therapy for the treatment of schizophrenia. Two PLGA copolymers (50 : 50 and 75 : 25) were used to prepare four microsphere formulations of Risperidone. The microspheres were characterized by several *in vitro* techniques. *In vivo* studies in male Sprague-Dawley rats at 20 and 40 mg/kg doses revealed that all formulations exhibited an initial burst followed by sustained release of the active moiety. Additionally, formulations prepared with 50 : 50 PLGA had a shorter duration of action and lower cumulative AUC levels than the 75 : 25 PLGA microspheres. A simulation of multiple dosing at weekly or 15-day regimen revealed pulsatile behavior for all formulations with steady state being achieved by the second dose. Overall, the clinical use of *Formulations A, B, C, or D* will eliminate the need for combination oral therapy and reduce time to achieve steady state, with a smaller washout period upon cessation of therapy. Results of this study prove the suitability of using PLGA copolymers of varying composition and molecular weight to develop sustained release formulations that can tailor *in vivo* behavior and enhance pharmacological effectiveness of the drug.

1. Background

The treatment of schizophrenia using oral conventional antipsychotics dates back to the mid-1950s. Administration of antipsychotic drugs via the oral route offered several advantages in terms of ease of administration, noninvasiveness of therapy, and portability of medication. It is common knowledge that injectable depot formulations possess a number of advantages over oral dosage forms such as avoidance of first-pass metabolism and the certainty of delivery of the therapeutic agent [1–3]. Therefore, by the 1960s, the first injectable depot conventional antipsychotic was introduced [1]. The sustained release properties of the injectable depot led to significant strides in the treatment of schizophrenia as it reduced relapse rates in comparison to the oral dosage form. A reduction in the number of days of hospitalization for patients on injectable antipsychotics over those on oral medication was also documented by researchers [4]. Despite being an injectable, it was noted that patients preferred injectable depot antipsychotics over oral agents. Additionally, the use of injectable depot preparations for the treatment of schizophrenia was considered beneficial as it ensured adherence to treatment over an extended duration leading to improved health outcomes [4–7]. Compliance with treatment regimens sharply increased when patients were switched to depot agents, allowing physicians a better mechanism to detect noncompliance to therapy. Further, the injectable depot allowed better control over drug management and more predictable and consistent plasma drug concentrations when compared with oral formulations [8]. In general, injectable depots were well tolerated and more clinically efficacious than oral preparations [4, 9].

The second generation antipsychotics or atypical antipsychotics were introduced in the 1980s and led to significant improvements in the treatment of schizophrenia. Atypicals, effective for the positive symptoms of schizophrenia, demonstrated a lack of negative symptoms leading to greater efficacy and reduced side effects. Indeed, atypical antipsychotics have

a substantially better adverse effect profile than first generation antipsychotics with respect to movement disorders, akathisia, and tardive dyskinesia [10]. Notably, concerns with extrapyramidal symptoms (EPS) and the risk of tardive dyskinesia with older antipsychotics led to a reluctance in accepting injectable depots of first generation antipsychotics and a preference for oral atypical antipsychotics [11].

Risperidone, a novel benzisoxazole-type atypical antipsychotic, is effective in the treatment of positive as well as negative symptoms of schizophrenia and has a low incidence of extrapyramidal side effects [12–16]. *In vivo*, Risperidone is extensively metabolized by cytochrome P450 2D6 (subject to genetic polymorphism) to form its main metabolite, 9-hydroxyrisperidone, via hydroxylation and N-dealkylation pathways [17, 18]. 9-Hydroxyrisperidone displays similar pharmacological activity to the parent compound; thus, the active moiety *in vivo* is a summation of both species. Clinically, the efficacy of Risperidone has been well established and is effective against positive and negative symptoms of schizophrenia [19, 20]. Risperidone is an antagonist of the 5HT2A receptor compared with the D2 receptor which allows for a greater efficacy against negative symptoms and a lower rate of EPS which makes it a suitable candidate for treatment of schizophrenia [19].

Two decades of clinical usage have clearly established that atypical antipsychotics like Risperidone offer several benefits including reduced concerns with movement disorders and greater efficacy for negative and mood symptoms than first generation antipsychotics [21]. However, these benefits diminish greatly in patients who suffer from severe psychiatric ailments primarily due to non-adherence to oral therapy. Several reports have documented the reduced effectiveness of oral Risperidone therapy in young and old schizophrenic patients [22, 23]. Daily dosing of oral Risperidone is non-ideal due to patient resistance to treatment and often ineffective given that efficacy depends on constant adherence to therapy. Despite the fact that adherence to daily medication has been better in schizophrenic patients dosed with atypical antipsychotics than conventional antipsychotics, Dolder et al. recorded that poor compliance issues persisted in schizophrenic patients [24].

A critical factor in achieving beneficial long term outcomes is in establishing a mechanism wherein the schizophrenic patients demonstrate adherence to treatment cycles. Infrequent intake of medication or partial dosing is far more common than complete non-adherence to therapy posing a significant challenge to patients, caregivers, and society at large. Robinson et al. reported a five-fold increase in the risk of relapse with patients who partially adhered to treatment [25]. Incidence of relapse in schizophrenic patients carries a large economic and personal cost. Relapsed patients suffer from reversal of gains achieved during therapy, loss of function, demoralization, loss of confidence, danger to self or others, and loss of job leading to a loss in productivity and opportunity. Further, rehospitalization of relapsed patients places a huge economic burden on existing healthcare system in the US [26].

Continuous delivery of the atypical antipsychotic is an effective way to ensure adherence to therapy with minimal relapse. Analogous to the first generation antipsychotics, injectable depot formulations of Risperidone were developed and marketed. Studies on long acting Risperidone revealed its efficaciousness in the treatment of schizophrenia and schizoaffective disorder [8, 27]. Extended treatment with long acting Risperidone also reduced movement disorders relative to baseline in patients clinically stable on a variety of antipsychotic drugs [28].

However, a major drawback of the currently marketed long acting Risperidone, administered every 15 days, necessitates an additional supplementation with oral Risperidone for three weeks after administration of the injectable formulation. While challenges related to patient compliance continue to persist with oral therapy, oral supplementation is necessary due to the delayed response profile obtained with the injectable preparation where drug release occurs approximately 3 weeks after administration. Published literature cites that *in vivo* levels peak 4-5 weeks after dosing, for a 7-week duration of action [27, 29]. Co-administration of oral Risperidone, while necessary in an inpatient or outpatient setting, is inconvenient and poses major compliance issues in patients with psychotic disorders. Additionally, costs incurred with co-administration therapy of Risperidone are high [30, 31]. Thus, the latency in drug release is a major shortcoming of the long acting Risperidone depot preparation. Therefore, there is a strong need for a non-oral controlled delivery dosage form for this drug.

Over the years, several polymers have been evaluated for development of controlled release injectable formulations. Of these polymers, one class of polymers has achieved significant commercial success in the pharmaceutical market. The polylactide (PLA) and polylactide-co-glycolide (PLGA) class of polymers are biodegradable, biocompatible, and nontoxic and have a long history of use [32]. *In vivo*, they are hydrolyzed into metabolic products that are easily eliminated from the body. Initially approved for surgical use in humans by the US Food and Drug Administration, they have since been used to formulate a wide range of therapeutic agents [33, 34]. A few commercially available formulations using PLA or PLGA polymers include Lupron Depot, Somatuline LA, and Trelstar Depot [35]. These polymers have been shown to be efficacious in the delivery of biologically active agents and also improve patient compliance by eliminating the need for frequent administration [36].

PLGA polymers are well suited for controlled delivery of drugs via the parenteral route as they exhibit good mechanical properties and demonstrate predictable degradation kinetics. Notably, polymeric microspheres prepared using PLGA have been successful in ensuring sustained release of therapeutic agents for various drugs [37]. Several examples in literature discuss their effectiveness in providing targeted drug levels *in vivo*, for long periods of time [38–40]. For this reason, they are popular as delivery vehicles for drugs where sustained release is desired for extended intervals, ranging from a few weeks to several months [41, 42]. These polymers are also used in marketed injectable formulations as carriers to deliver antipsychotic drugs and are noted to provide benefits over conventional oral therapy [43]. A striking benefit of using PLGA polymers to deliver atypical

antipsychotics includes a reduction in dosing frequency leading to measurable increase in adherence to treatment regimens in a schizophrenic patient population [44, 45]. In general, the success of PLGA polymers as delivery systems is due to the fact that polymer properties are well understood and can be customized to afford sustained drug release. For instance, selection of copolymers of various lactide : glycolide with variable molecular weights is an effective way to control polymer degradation rate and drug release. By changing the composition of lactide or glycolide in the copolymer, a wide range of degradation rates can be obtained. An increase in the more hydrophobic lactide moiety ensures a slower degradation rate of the PLGA polymer leading to extended duration of drug release [46]. Similarly, utilization of a higher molecular weight copolymer increases degradation times leading to prolonged drug release. Additional properties that can be varied include polymer crystallinity and glass transition temperature. These physical and chemical properties have been well studied and characterized leading to predictable degradation kinetics of the PLGA polymer, *in vitro* and/or *in vivo*.

Upon *in vivo* administration of a PLGA based injectable depot, water interacts with the polymer and hydrolysis of the ester bonds commences. As the polymer degrades, its hydrophobicity decreases and the number of hydrophilic hydroxyl and carboxylic acid end groups in the matrix increases. An accumulation of hydrophilic acidic end groups has a twofold effect: (1) it increases the amount of water incursion into the polymer and (2) initiates autocatalysis of the polymer matrix [47]. Therefore, polymer degradation and, consequently, drug release from PLGA is a very complex and dynamic process. This is of particular significance as it provides the researcher a scientifically sound approach to select an appropriate polymer specific to a therapeutic need or treatment regimen.

When plotted as a function of time, drug release from a PLGA matrix occurs in three phases [32]. The first phase of release is known as "initial burst" and occurs as a result of detachment of surface associated drug or drug that is easily dissociated from accessible pores in the polymeric microspheres. Depending on the surface area and porosity, a high or low initial burst may be observed. The second phase of release, that is, diffusional release, is a consequence of initial polymer hydration and is followed by "erosional release" or the final phase of drug release. Once the polymer is hydrated, polymer autocatalysis ensues causing bulk hydrolysis, that is, complete polymer degradation and erosion (mass loss). Previous reports have also documented that properties of the formulation have an impact on drug release kinetics [48]. Therefore, depending on the properties of the polymer and the microsphere dosage form, the rate and extent of each of these phases can be altered to customize drug release profiles. Hence, in this study, two PLGA copolymers having varying molecular weights and lactide : glycolide ratios as well as drug loading were evaluated with an aim to obtain Risperidone PLGA microspheres having varying duration of action. Results and discussions related to the findings of the study demonstrate the suitability of this approach in developing sustained release formulations where *in vivo* behavior can be customized to meet patient needs.

2. Materials and Methods

2.1. Materials. Risperidone was purchased from Cipla Ltd., India, and PLGA 50 : 50 (45 and 74 kDa) and 75 : 25 (54 and 65 kDa) from Boehringer Ingelheim (Ingelheim, Germany) and Alkermes (Cambridge, MA). All other chemicals were obtained commercially as analytical grade reagents.

2.2. Preparation of Microspheres. The four formulations evaluated were

(a) 45 kDa PLGA, 50 : 50 lactide : glycolide (*Formulation A*),

(b) 74 kDa PLGA, 50 : 50 lactide : glycolide (*Formulation B*),

(c) 54 kDa PLGA, 75 : 25 lactide : glycolide (*Formulation C*),

(d) 65 kDa PLGA, 75 : 25 lactide : glycolide (*Formulation D*).

Briefly, four Risperidone PLGA (*Formulations A, B, C, and D*) microspheres formulations were prepared by a solvent extraction/evaporation method [41]. Briefly, a solution of drug and polymer (10–20% polymer concentration) in dichloromethane was injected into an aqueous continuous phase at a ratio between 250 and 350 parts of polymer phase : aqueous phase, under stirring with a Silverson L4R mixer (Silverson machines, MA, USA) at 5000 rpm. Subsequently, the solvents were removed by stirring after which the microspheres were recovered by filtration, suspended in a suitable vehicle, filled into vials, and freeze-dried. The microspheres were characterized as described in Section 2.3.

2.3. Characterization of Microspheres

2.3.1. Particle Size. Particle size distribution of the microspheres prior to vialing was determined using a laser diffraction technique (Malvern 2600c Particle Sizer, Malvern, UK). The particles were suspended in 0.05% Tween 80 and counted using a laser sensor [41]. The average particle size was expressed as volume mean diameter in microns (μm).

2.3.2. Surface Morphology. The surface morphology was examined by scanning electron microscopy (SEM) (Hitachi S800, Japan) at an appropriate magnification, after palladium/gold coating of the microsphere sample on an aluminum stub.

2.3.3. Bulk Density. Bulk density of the microspheres was determined by transferring a weighed amount of microspheres to a graduated cylinder. The cylinder was subsequently tapped 50 times from a vertical distance of approximately 0.5 inches and the occupied volume recorded. The tapping process was repeated until the volume occupied

Table 1: Properties of Risperidone PLGA microspheres.

Formulation	A	B	C	D
MW	45 kDa	74 kDa	54 kDa	65 kDa
PLGA type	50 : 50	50 : 50	75 : 25	75 : 25
Drug load (%)	25	34	34	33
Bulk density (g/cc)	0.76	0.67	0.65	0.68
Mean particle size (μm)	24.6	18.9	17.1	21.9
Dose of Risperidone (mg/kg)	20	20	40	40

by particles remained unchanged. The final volume was recorded as bulk volume, V_b, and the tapped bulk density (g/cc) was calculated as M/V_b, where "M" was the weight of microspheres employed.

2.3.4. Drug Content. Risperidone content in the microspheres was analyzed by a reverse phase HPLC method using a Nucleosil C-18 column (Phenomenex, Torrance, CA) at a flow rate of 1 mL/min. The mobile phase consisted of 30% v/v acetonitrile and 0.1% (v/v) trifluoroacetic acid in water. Drug content (%) was expressed as the "weight of drug in microspheres/weight of microspheres × 100."

2.3.5. In Vivo Studies. In accordance with Institutional Guidelines and an in-house developed and an approved protocol, four groups of male Sprague-Dawley rats (Harlan Inc., Indianapolis, IN) weighing approximately 300 gm were used in the *in vivo* study. *Group 1* received *Formulation A*, *Group 2* received *Formulation B*, *Group 3* received *Formulation C*, and *Group 4* received *Formulation D*.

Briefly, vials containing freeze dried microspheres along with diluent were reconstituted with WFI (water for injection) and injected subcutaneously at the base of the rat neck at a dose of 20 or 40 mg/kg Risperidone (Table 1). Blood was sampled from the rat tail vein at predetermined intervals, after which the samples were centrifuged in Microtainer tubes (Becton Dickinson & Co., Franklin Lakes, NJ) and serum was collected. Serum samples for each of *Group 1* (*Formulation A*), *Group 2* (*Formulation B*), *Group 3* (*Formulation C*), and *Group 4* (*Formulation D*) were frozen and stored at −20°C until analysis. Subsequently, serum levels were assessed at Medtox Labs, USA, using a validated analytical method.

3. Results and Discussion

3.1. Polymer Selection. Properties of the four formulations used in this study are shown in Table 1. *Formulations A and B* were prepared with 50 : 50 PLGA at molecular weights 45 and 74 kDa, respectively, while *Formulations C and D* were manufactured from 54 and 65 kDa PLGA having a 75 : 25 lactide : glycolide ratio. Based on the molecular weight and copolymer ratio, *Formulations A and B* were expected to have a shorter duration of action while *Formulations C and D* would provide a more prolonged *in vivo* drug release profile due to a higher lactide content in the 75 : 25 copolymer.

3.1.1. Morphology of Risperidone Microspheres. The scanning electron micrographs revealed a spherical shape with a smooth surface and homogeneous particle size distribution (Figure 1) that would be appropriate for subcutaneous administration to rats. Additionally, the microspheres could not be fractured suggesting that the interior of all four formulations was not hollow. When viewed at the same magnification (Figure 1), the particle size of *Formulation A* appeared marginally larger than *Formulation B*, while the particle size of *Formulation C* was slightly smaller than *Formulation D*. A glance at Table 1 confirms these observations as the mean particle sizes for *Formulations A–D* were 24.6, 18.9, 17.1, and 21.9 μm, respectively. For dosage forms like drug loaded microspheres, measurement of particle size is important as it impacts "initial burst" release [49]. A smaller particle size confers a higher surface area to volume ratio to the dosage form. It follows that a larger surface area allows for rapid water incursion and consequently, faster dissolution of drug molecules that are associated with the outer surface or accessible pores. Hence, an initial burst is expected with smaller sized microspheres.

From literature, the particle size of the commercial long acting Risperidone microsphere formulation has been reported to be between 25 and 150 μm [50], significantly larger than *Formulations A, B, C, and D*. Hence, the SEM results in Figure 1 indicated that the release profiles from the four formulations would be vastly different from the marketed preparation. For instance, an "initial burst" of drug release was expected for all the formulations. Given that the particle sizes for *Formulations A–D* are quite similar overall, the extent of "initial burst" was expected to be broadly similar.

3.1.2. Bulk Density. Bulk density values for PLGA microsphere formulations are routinely measured as they provide information on the porous network in these dosage forms. Density is inversely proportional to porosity, and a change in this parameter indicates inefficient packing due to the presence of nonspherical microspheres or the formation of hollow microspheres [51]. A relationship between bulk density, surface area, and onset of mass loss has also been reported by Mehta et al. [33]. Hence, a low bulk density is indicative of highly porous microspheres, since porosity correlates well with polymer hydration, and thereby, degradation; bulk density values are an indicator of drug release rates [52, 53].

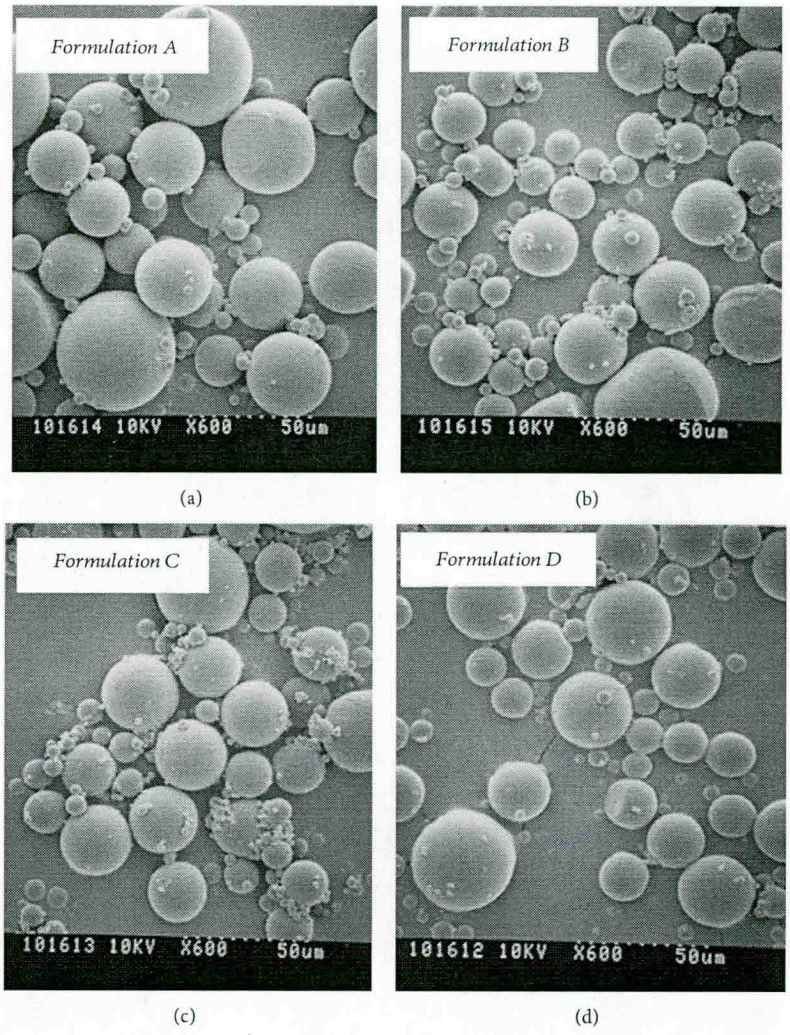

FIGURE 1: SEMs of Risperidone PLGA microspheres.

Table 1 summarizes the results of bulk density measurements. Values for all the formulations ranged from 0.65 to 0.76 g/cc. The high bulk density values were indicative of a low degree of internal porosity with similar pore volumes for *Formulations A–D*. Given that particle sizes for all four formulations are similar and bulk density is high, both parameters were expected to contribute equally to the initial burst release from the microspheres.

3.1.3. Drug Content. Drug content is an important property of the microsphere dosage form as it provides information related to the amount of drug available for release from the dosage form. Results of drug content, as determined by HPLC, are presented in Table 1. For the purposes of the current study, high drug loadings were targeted in part to mimic the loading level of 38.1% in the marketed Risperidone depot formulation [50]. Therefore, *Formulations A–D* were prepared at loadings between 25 and 34% (Table 1). These values suggest a high drug : polymer ratio for the four formulations, at a value higher than the drug solubility in the polymer. This situation avors the initial burst release phenomenon. Hence, a high value of initial burst was expected for all four formulations.

Based on the morphology, particle size, and drug content data, the formulations were expected to behave in the following manner: (a) High initial burst was expected from all the formulations, and (b) *Formulations A and B*, manufactured using 50 : 50 PLGA, were expected to exhibit a shorter duration of action than *Formulations C and D*, where the duration of action was expected to be prolonged.

3.2. In Vivo Results

3.2.1. Serum Levels of Risperidone and Its Metabolite for Formulations A, B, C, and D. In vivo, Risperidone is extensively metabolized in the liver by CYP2D6 to form 9-hydroxyrisperidone, a pharmacologically active metabolite. Serum levels of Risperidone and its metabolite for each

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