

In vitro and in vivo demonstration of risperidone implants in mice

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Received 11 May 2007; received in revised form 30 July 2007; accepted 1 August 2007

Available online 31 August 2007

Abstract

Background: Non-adherence with medication is a critical limitation in current long-term treatment of schizophrenia and a primary factor in poor quality-of-life outcomes. However, few treatments have addressed this shortcoming using an implantable drug delivery approach. The goal of this study was to provide in vitro and in vivo proof of concept for a long-term implantable risperidone delivery system in mice.

Methods: Implantable formulations of risperidone were created using the biodegradable polymer Poly Lactic co Glycolic Acid (PLGA) combined with various drug loads. Implant bioactivity was tested using in vitro release and stability studies, as well as in vivo pharmacokinetic and behavioral studies in mice.

Results: The pattern of risperidone release is influenced by various parameters, including polymer composition and drug load. In vitro measures demonstrate that risperidone is stable in implants under physiological conditions. Behavioral measures demonstrate the bioactivity of risperidone implants delivering 3 mg/kg/day in mice, while pharmacokinetic analyses indicate that reversibility is maintained throughout the delivery interval.

Conclusions: The current report suggests that implantable formulations are a viable approach to providing long-term delivery of antipsychotic medications based on in vivo animal studies and pharmacokinetics. Implantable medications demonstrated here can last two months or longer while maintaining coherence and removability past full release, suggesting a potential paradigm shift in the long-term treatment of schizophrenia.

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Keywords: Treatment adherence; Schizophrenia; Risperidone pharmacokinetics; Drug delivery system; Drug implant; In vitro/in vivo correlation

1. Introduction

Non-adherence with antipsychotic medication remains a principal obstacle undermining better treat-

ment outcomes in schizophrenia (Kane, 2006a; Zygmunt et al., 2002). Although the acceptance of a prescribed pharmacological regimen often dictates treatment outcome, more than 35% of patients demonstrate adherence problems during the first 4–6 weeks of treatment and within two years, up to 74% of patients are unable to adhere to their original prescribed treatment (Gilmer et al., 2004; Lieberman et al., 2005; Valenstein et al., 2004). The short and long-term consequences of non-adherence to pharmacological

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treatment include increased relapses, more frequent and longer hospitalizations, cumulative deteriorations in functioning, and a diminished capacity to maintain employment and relationships. The more relapses a patient has, the more difficult it is to achieve remission after the next episode, creating a negative feedback loop that severely hinders long-term functioning and quality of life (Turner and Stewart, 2006).

In an effort to improve drug adherence, researchers in the 1960s introduced long-acting depot antipsychotic injections. Depot formulations augment the impact of oral formulations by providing further reductions in morbidity and mortality (Kane et al., 2003). Because they bypass the gastrointestinal tract, depot treatments decrease the amount of medication needed and may minimize certain peripheral side effects including hepatotoxicity and hyperprolactinemia (Knox and Stimmel, 2004). However, depot formulations have limitations that restrict more significant improvements in adherence and efficacy (Kane et al., 1998). Decanoate treatment is limited by chemistry, as many compounds are unable to make the required ester linkages. Additionally, depot injections are irreversible and thus lack flexibility in administration and clinical management (Barnes and Curson, 1994). Depot treatments can also result in prolonged pain at the injection site, which increases the likelihood that patients will discontinue treatment (Bloch et al., 2001; Kane et al., 1998).

Understanding the clinical, social, and economic consequences of untreated schizophrenia, we have developed a new method of long-term implantable medication delivery to optimize adherence and convenience in schizophrenia treatment. This long-term drug delivery system may complement the development of new molecules, which are not yet available for clinical application. Furthermore, given that poor adherence is similar across a broad range of medications regardless of their specific side effect or efficacy profiles, there is no guarantee that patients will adhere to newer medications under development. Hence implants provide a complementary approach for improved adherence while bypassing the major limitation of depot formulations — their irreversibility. Specifically, the proposed method of drug delivery uses sterile implants made with a Poly Lactic co Glycolic Acid (PLGA) polymer which is durable and biodegradable, with an anticipated delivery interval of up to four months depending on polymer composition, geometry, and drug load.

The current report provides proof of concept for this new method of implantable drug delivery rather than an introduction of a specific product. This research is sponsored by the Stanley Medical Research Institute and

National Institutes of Health, has no industry sponsorship, and has no inherent limitations regarding future application to a broad array of compounds. Therefore, we have applied the atypical antipsychotic agent risperidone because it is among the most appropriate choices for a long-term delivery system using currently available medications based on efficacy, potency, stability, side effect/risk profile, and local sub-cutaneous in vivo tolerability. In this report, we address the following 4 goals:

- Goal 1) To demonstrate risperidone implant stability following processing and sterilization as well as in a physiologically representative environment.
- Goal 2) To establish in vitro risperidone release as a function of polymer composition, percent drug load and implant geometry.
- Goal 3) To use one set of in vitro parameters to demonstrate in vivo serum concentration and release for implants using a single PLGA polymer in mice.
- Goal 4) To confirm bioactivity of risperidone implants using behavioral testing and electrophysiology in mice.

Accomplishment of these four goals could provide proof of concept for the feasibility of long-term risperidone implants and impetus for further research to transform this novel idea from an academic concept to a meaningful intervention in individuals suffering from schizophrenia.

2. Materials and methods

2.1. *In vitro* studies

2.1.1. *Fabrication of implants*

Implants were fabricated from a PLGA Medisorb biodegradable polymer (Lakeshore Biomaterials, Birmingham, AL) and risperidone (supplied by the NIMH drug synthesis program). Risperidone and polymer were mixed in a common solvent at various ratios between 10 and 60 wt.% drug load and solvent cast by evaporating the solvent at 40 °C in a vacuum oven under light airflow until no solvent remained and the mixture had returned to the constituent mass. The resulting film was either compression molded into pellets at 25,000 lbs, 80 °C for 3 min or melt extruded into rods using a high pressure piston extruder at 100 °C (DACA Instruments, Goleta, CA). Negative control pellets and rods were fabricated in the same manner. PLGA polymer was used because it is biodegradable, physically strong, and

highly biocompatible (Kitchell, 1985). Its non-toxic degradation products are lactic and glycolic acid (Hyon, 2000).

2.2. Experiment 1: implant characterization

Implants were tested after sterilization to ensure that risperidone maintained stability and biological activity throughout the fabrication process. A subset of control and risperidone loaded implants from a single batch were ethylene oxide (EO) sterilized by Steris Isomedix Services (Spartanburg, SC). Sterilization parameters included a chamber temperature of 102 °F, vaporizer water temperature of 165 °F and 60 min humidity dwell time and 240 min sterilant dwell time, to ensure minimal heat and humidity exposure. The resulting implants were analyzed for polymer degradation using inherent viscometry and drug degradation using High Performance Liquid Chromatography (HPLC). Additionally, release patterns from EO sterilized ($n=6$) and control ($n=6$) implants were compared.

Risperidone stability was also evaluated under physiological conditions and in a low pH range of 2.0 to 7.4 based on the hypothesis that the internal implant environment may become more acidic due to PLGA degradation. The standard solution of risperidone was made by dissolving 10 mg of drug in 1000 ml of phosphate buffered saline (PBS) at pH 7.0 (0.9 vol.% NaCl, 0.1 M NaOH, 0.01 M NaH_2PO_4) to yield a final concentration of 10,000 ng/ml. Risperidone solution was then stored in a light safe amber bottle at 37 °C and shaken at 40 rpm. One ml samples were taken weekly and analyzed using HPLC. HPLC was performed using a Waters XTerra RP 18.5 μm , 4.6×150 mm column. Mobile phase was composed of 55 vol.% H_2O with 35 vol.% acetonitrile and 10 vol.% 100 mM ammonium bicarbonate, pH 10. The flow rate was set at 1.0 ml/min and peaks were detected at a wavelength of 280 nm for risperidone and 9-OH-risperidone with a run time of 30 min for each sample. The standard solution of risperidone at pH 7.0 was compared to study samples at pH 2.0, 3.0, 4.4, 5.4, 6.4, and 7.4. Samples of 0.2 ml were taken weekly and analyzed using HPLC as described above.

2.3. Experiment 2: in vitro release studies

We fixed drug load at 20 wt.% and varied polymer composition to evaluate the range of possible delivery intervals as a function of lactide to glycolide ratio. The different polymers used were 50:50, 65:35, and 75:25 (molar ratio of lactide:glycolide) PLGA. In each case,

polymer and drug were mixed in a ratio of 80:20 by mass and solvent cast as described above before being extruded into rod-shaped implants. Negative control implants with 0 wt.% drug load were fabricated in the same manner.

To determine how risperidone release varies as a function of drug load, risperidone release from a constant 85:15 PLGA polymer and different drug loads was simultaneously evaluated. Implants were prepared using a single 85:15 PLGA polymer combined with risperidone at ratios of 10 wt.%, 20 wt.%, 30 wt.%, 40 wt.%, 50 wt.%, and 60 wt.%. Each implant had a mass of about 50 mg, yielding drug mass of 5, 10, 15, 20, 25, and 30 mg respectively.

To assess in vitro release profiles for individual implants of varying lactide to glycolide ratio (polymer composition) and percent drug load, we placed three replicates of each implant type in separate light safe bottles of phosphate-buffered saline on a shaker (500 ml, 37 °C, 40 rpm). One ml aliquots were taken from each bottle three times per week and analyzed by UV spectrophotometry and HPLC. The correlation coefficient for these methods is 0.99 (182 samples), indicating that UV spectrophotometry is an accurate measure of drug level in this particular case when used for a single molecule in an in vitro solution that is devoid of interfering molecules at the specified wavelength. After each aliquot was removed, 1 ml of buffer was introduced to maintain constant volume.

We also examined the effects of implant geometry on the rate of risperidone release by varying surface area to volume ratio. Four rods per condition were fabricated yielding surface area to volume (SA:V) ratios of 1.8, 2.8, and 6.2 cm^{-1} , holding constant 30 wt.% drug load and 75:25 PLGA. Following fabrication, rods were placed in separate bottles of PBS at 37 °C at 40 rpm and 0.2 ml samples were drawn three times per week for HPLC and UV spectrophotometry analysis as described above.

2.4. In vivo studies

2.4.1. Animals

The Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania approved all protocols, and animals were housed in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facilities. A total of 48 C57BL/6J mice were used in these studies. Thirty two mice were used for pharmacokinetic analyses of serum levels over time. Another 16 mice were used in behavioral studies to evaluate the effects of risperidone implants on both pre-pulse inhibition of startle (PPI) and auditory event related

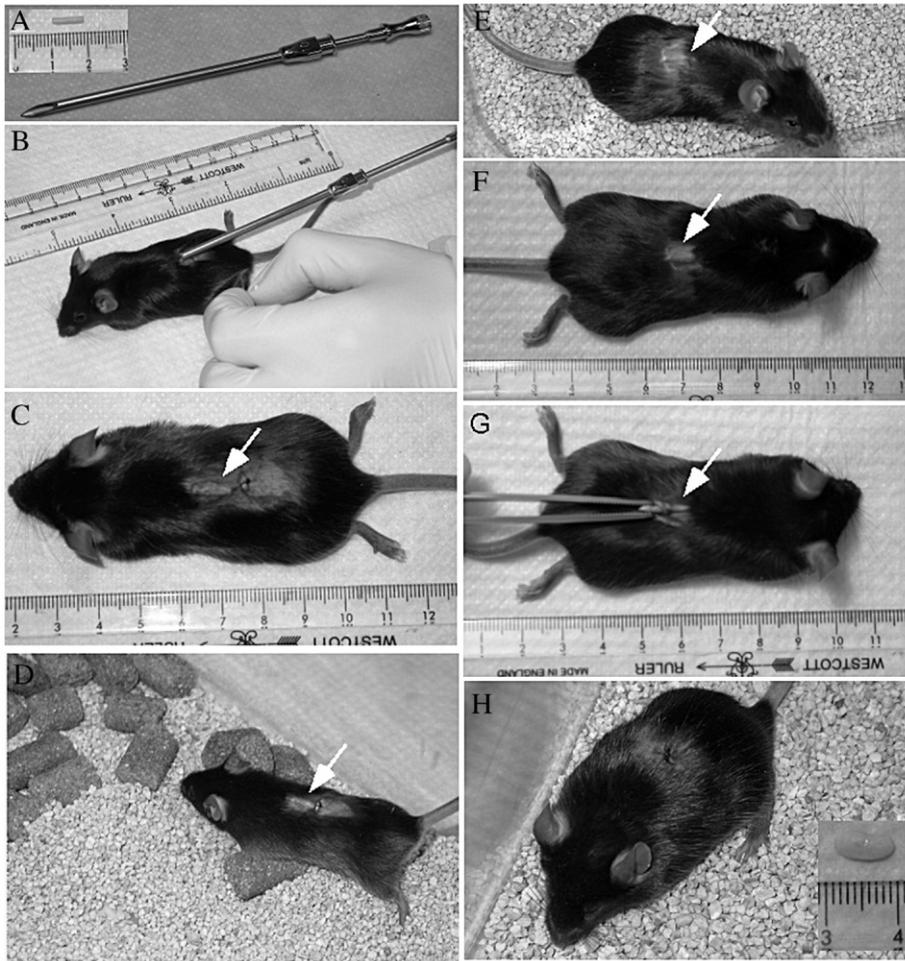


Fig. 1. Insertion and removal of a 1 cm rod-shaped risperidone implant is shown in mouse. A) A 1 cm risperidone implant (inset) and trochar (12 gauge) are shown prior to implantation. B) The mouse was anaesthetized with isoflurane and the skin was shaved prior to cleaning the area with betadine and alcohol. The 1 cm length implant was then inserted through a 3 mm hole using a trochar (similar to a 12 gauge needle). A comparable procedure in humans would utilize a local analgesic, such as lidocaine, to numb the skin prior to insertion. C) The implant site is shown after closing with a single stitch. Note that the implant is visible under the skin (white arrow) and is about the size of a grain of rice. Similar procedures in humans could be closed with steri strips. D) The mouse is shown 10 min later in its home cage with no signs of distress. The implant is seen over the dorsal surface (white arrow). E) A mouse is shown at 2 weeks after implantation. The implant site is completely healed with no signs of distress or adverse events noted. The implant remains palpable and visible (white arrow). F) Mice had implants (white arrow) removed 14, 27, 56 and 83 days after implantation to assess reversibility of the procedure. This panel shows an example of a mouse just prior to implant retrieval. The mouse in the picture is anesthetized with isoflurane. G) Implants were easily removed at all time points without adhesions or local scarring. A small incision was made with a scalpel and the implant was identified under the skin prior to retrieval with forceps. Note, that the retrieval procedure would only be necessary in humans if there was a need to end treatment prematurely. If left in place, these implants are fully biodegradable and would not require retrieval. Also, retrieval in a human would require local analgesia with lidocaine or other similar agent. H) A mouse shown back in its home cage 10 min after implant removal with no signs of distress. An example of a removed implant is shown in the inset. Note, that the implants retained cohesion, fostering removal throughout the study interval. Mice in these groups were then sacrificed and serum risperidone levels obtained.

potentials (ERPs) ($n=8$ with risperidone implants and 8 with polymer alone control implants).

2.5. Experiment 3: pharmacokinetic studies

Groups of mice were sacrificed at 14, 28, 56, and 83 days ($n=4$ per condition at each time point) and

implants were removed for analyses of serum risperidone level using a solid phase extraction protocol. Solid phase extraction (SPE) was performed using the Waters 20-position SPE vacuum manifold and Waters Oasis MCX SPE cartridges (1 ml/30 mg cartridges). SPE cartridges were conditioned with methanol and water, loaded with samples containing 2 vol.% phosphoric

acid, and then washed with 5 vol.% methanol in 0.1 N hydrochloric acid followed by a 100 vol.% acetonitrile wash. The final elution was performed by washing each SPE cartridge with 5 vol.% NH_4OH in 100 vol.% acetonitrile. Each of these samples were then dried under nitrogen in a water bath at 80 °C, reconstituted in 100 ml of mobile phase, vortexed, and centrifuged for 5 min. 75 μl of the reconstituted samples were then loaded into an auto sampler and 50 μl were injected.

Rod-shaped implants were both EO ($n=54$) and surface sterilized ($n=4$) to access the effects of sterilization on serum concentration. Analysis was performed by HPLC with UV detection at 280 nm. 0.5 ml samples of blood were collected at each time point. Blood was centrifuged for 30 min, leaving 200 μl of serum to freeze at -80 °C until analysis. Serum risperidone and 9-OH risperidone concentrations were determined in duplicate at each time point for each animal following solid phase extraction (MCX, Waters) and HPLC/UV detection. In vivo standard solutions of risperidone and 9-OH risperidone were prepared in normal mouse serum (range 1.25–50 ng/ml). Standards were then extracted using the same protocol as the study samples and were included within each run to provide both the standard curve and retention time for each compound. The retention time for risperidone was approximately 8.6 min and the retention time for 9-OH risperidone was approximately 5.9 min. The final in vivo serum concentration profile for each material was determined by plotting serum concentration across time for all animals.

The percent drug load in implants removed from mice was also determined using solid phase extraction. Two ml of acetonitrile was added to each dried rod and was placed on a shaker overnight (37 °C at 40 rpm). Each sample was sonicated for 30 s and 20 μl of sample was placed into 1 ml of mobile phase. The sample was vortexed, centrifuged for 5 min and then 75 μl was removed and placed in a vial. From this vial, 20 μl were injected for HPLC analysis, as explained above.

2.6. Experiment 4: behavioral studies in mice

The behavioral effects of risperidone implants were tested using PPI and ERPs in 16 C57BL/6J mice. Implants were designed to deliver approximately 3.0 mg/kg/day for 90 days using 75:25 PLGA with 40 wt.% risperidone load. This dose was selected based on previous studies using mini-pumps in rodents (Kapur et al., 2003; Naiker et al., 2006). Although no studies to date have established specific D_2 Dopamine receptor occupancy threshold for behavioral effects in mice, the

threshold for motor abnormalities in mice is estimated to be 4.7 mg/kg for an acute dose of risperidone (Hirose et al., 2004). Therefore, we estimated that a dose of approximately 3.0 mg/kg/day would achieve risperidone serum concentration in the appropriate range without inducing motor abnormalities (Olesen et al., 1998).

Implants were placed in the sub-cutaneous space on the dorsal surface under isoflurane anesthesia (Fig. 1). Animals received either risperidone ($n=8$) or blank implants ($n=8$). Mice were tested at 14 and 21 days after implantation using previously described protocols for PPI (Gould et al., 2004; Meztger et al., 2007). Each trial commenced with a 5-min acclimation period containing a 65-dB acoustic background noise followed by a train of five 120-dB startle pulses in an effort to make subsequent startle trials less variable. Startle trials consisted of 40-ms pulses at 0 (no stimulus), 90, 100, 105, 110, 115, and 120 dB. Each stimulus was presented five times in randomized order with interstimulus intervals between 10 and 20 s. Startle trials were followed by PPI trials. Each pre-pulse was 20 ms in duration followed by 40 ms startle stimulus of 120 dB with a 100-ms interstimulus interval. PPI was recorded for pre-pulse intensities of 69, 73, 81 dB and no stimulus. Each pre-pulse trial was administered five times in a random order. Data were collected as 60 1-ms voltage readings, which were averaged over the collection interval to give a single measure for each trial.

Event Related Potential (ERP) studies were performed 28 days after implantation of electrodes and 7 days after the last PPI session. Mice were given 15 min to acclimate to the cage prior to onset of stimulus. Following acclimation, stimuli were generated by Micro1401 hardware and Spike 5 software (CED, Cambridge, England) and delivered through speakers mounted on the cage top. A series of 50 white noise clicks (10 ms duration) were presented in pairs 500 ms apart with a 9 s inter-pair interval at 85 db compared to background of 70 db. Waveforms were filtered between 1 and 500 Hz with individual sweeps rejected for movement artifact based on a criterion of two times the root mean squared amplitude as previously described (Maxwell et al., 2004; Metzger et al., 2006). Average waves were created from 50 ms pre-stimulus to 200 ms post-stimulus.

3. Results

3.1. In vitro studies: experiments 1 and 2

Risperidone was found to be stable under physiological conditions. Initial studies using UV spectrophotometry indicated that the concentration of risperidone

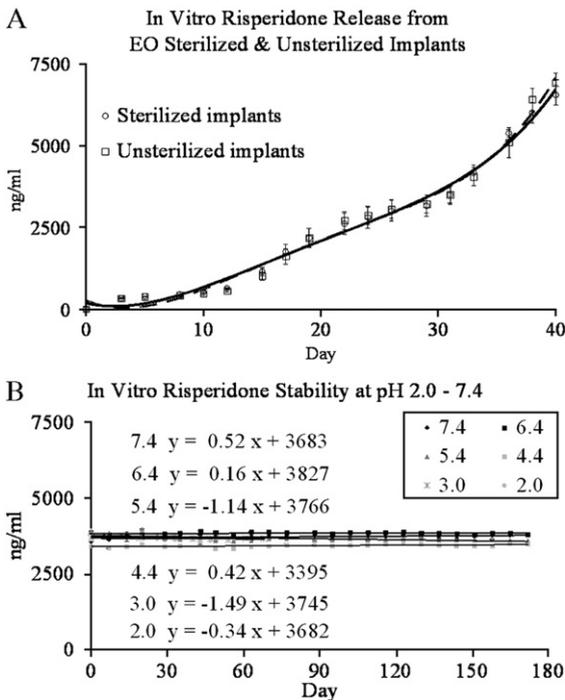


Fig. 2. Risperidone implants remain stable after ethylene oxide (EO) sterilization and after incubation in a low pH environment. A) The patterns of release from four EO sterilized and four surface sterilized implants are shown. The mean \pm SEM for each group is shown at each time point. No significant difference is apparent over six weeks of testing. B) The stability of risperidone in six low pH environments is shown over 180 days. Risperidone is stable at both a neutral pH and low pH as might occur within the local microenvironment of the implant.

remained constant with an overall change of 1.75% over 429 days, equivalent to 0.004% per day (linear trendline: $y = -0.0004x + 9.769$). This finding was then validated and replicated using HPLC analysis.

To ensure that EO sterilization does not interfere with implant characteristics, we measured inherent viscosity to assess polymer stability and performed HPLC analysis to assess risperidone stability before and after EO sterilization. Control implants had an inherent viscosity of 0.42 dl/g in chloroform prior to EO sterilization and a mean inherent viscosity of 0.45 dl/g following sterilization. These data suggest that the molecular weight of the polymer is not substantially altered by the sterilization procedure. Furthermore, sterile implants ($n=6$) were found to be 27 wt.% risperidone by mass and control implants ($n=6$) were 25 wt.% risperidone by mass. These values are not significantly different ($p=0.12$) indicating that risperidone remained stable throughout the sterilization process. Thus, sterilization had no significant effect on drug content in implants. The pattern of release from EO

sterilized and unsterilized risperidone implants are shown in Fig. 2A.

Risperidone also demonstrated stability at low pH levels that mimic possible internal microenvironments during implant degradation. All samples remained stable with negligible daily change in drug mass over 250 days of stability testing: 0.06% for pH 7.4, 0.04% for pH 6.4, 0.10% for pH 5.4, 0.00% for pH 4.4, 0.04% for pH 3.0, and 0.05% for pH 2.0, (Fig. 2B). Thus, risperidone implants met criteria for stability in an acidic environment during the delivery interval (in vitro pH 2.0 to 7.4), indicating that risperidone would not be adversely affected by potentially low pH environments that could exist within implants.

In vitro risperidone release varies with lactide to glycolide ratio (polymer composition), as shown in Fig. 3A. Implants reach full release at 40, 80, and 120 days for polymer compositions of 50:50, 65:35, and 75:25 respectively. Additionally, the pattern of release

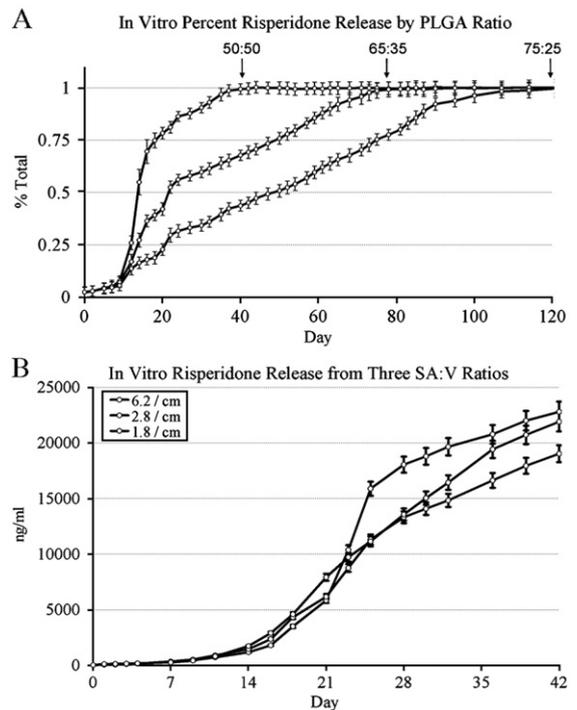


Fig. 3. In vitro risperidone release varies with polymer composition, but not with geometry over the range tested. A) Cumulative in vitro risperidone release from implants containing 50:50, 65:35, or 75:25 PLGA, 20 wt.% drug load. Data are expressed as cumulative % total release over time. Each point represents the mean \pm SEM of 3 implants. Note that implants reach full release at about 40, 80, and 120 days respectively with 20 wt.% initial drug load. B) The release from three implants with different surface area to volume ratios (1.8, 2.8, 6.2 cm^{-1}), tested using 30 wt.% drug load, did not significantly differ over seven weeks of testing ($p=0.90$).

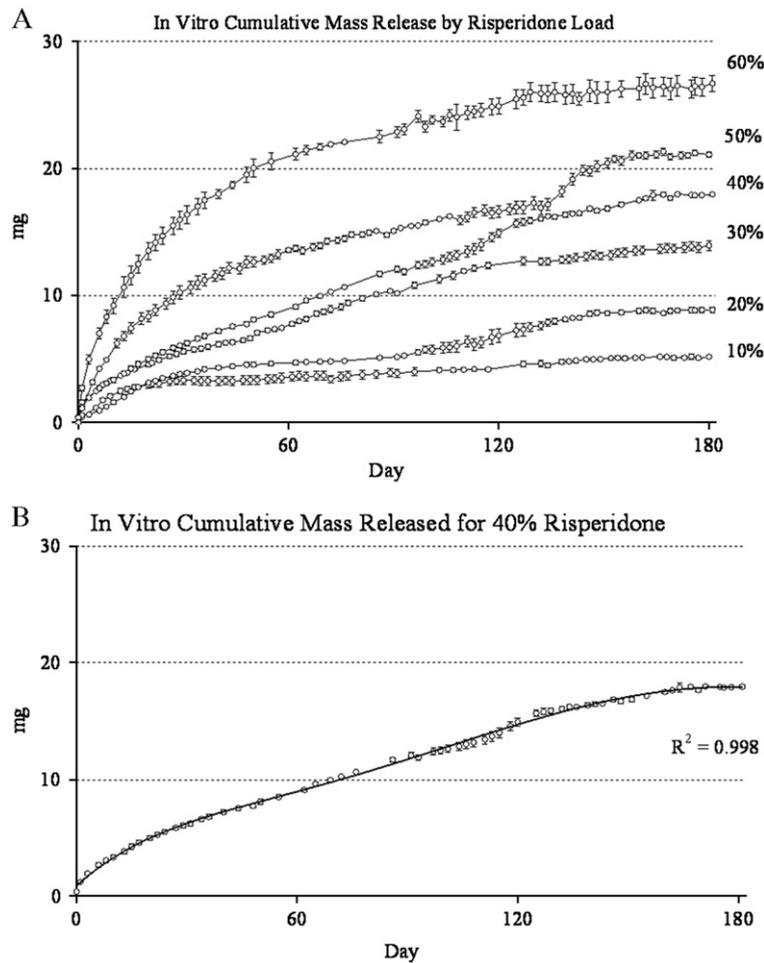


Fig. 4. In vitro risperidone release varies with drug load. In vitro cumulative risperidone release from implants containing 85:15 PLGA with 10, 20, 30, 40, 50, or 60 wt.% drug load by weight are displayed. A) Cumulative mass of risperidone in vitro is shown for each set of three implants (mean \pm SEM). B) The pattern for the 40 wt.% loaded implants is shown alone for clarity with the trend line added to illustrate the correlation coefficient of 0.998.

from three geometries with different SA:V ratios were tested using 30 wt.% drug load in 75:25 PLGA. The pattern of release from implants of different geometric proportions did not significantly differ over eight weeks of testing ($p=0.90$, Fig. 3B). Additionally, we found no significant interaction between implant size and test time on the percent of drug release ($p=0.65$). These results suggest that risperidone kinetics are not significantly affected by SA:V ratio within our limited range of geometries. Future studies could determine the extent to which a larger range of geometries affects release over time.

In vitro risperidone release also varied with wt.% drug load. 85:15 PLGA has an anticipated degradation interval of approximately five months. Risperidone implants using this material demonstrate release intervals of approximately three to four months with the

pattern of release varying across the range of drug loads tested. Our data suggest that the 40 wt.% drug loaded implants display the most linear release for the 85:15 PLGA with similar slopes during both the initial 30 day period of release and throughout subsequent 120 days. The pattern of 10 wt.% loaded implants was similar to that of 60 wt.% load with a large fraction of the total drug load released within the first thirty days. Cumulative mass released from 30 to 60 wt.% risperidone implants is expressed as a percentage of the total drug to facilitate comparison of the pattern of release as a function of drug load (Fig. 4).

3.2. In vivo studies

In vivo studies utilized 75:25 PLGA because it provided a linear release profile with a total in vitro time

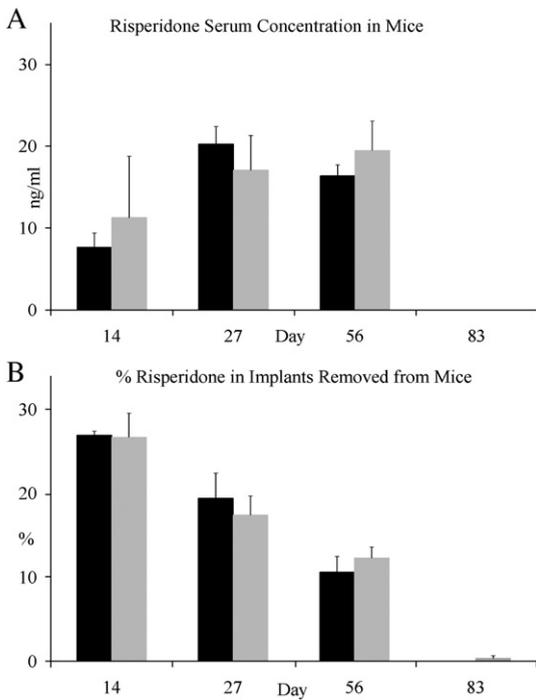


Fig. 5. Pharmacokinetic analyses in mice show that implants release risperidone for greater than 56, but less than 83 days, while retaining coherence past the delivery period. A) Risperidone serum concentrations from mice that received implants show no detectable drug at 83 days, consistent with *in vitro* release patterns. B) Residual risperidone content in implants following removal from mice indicate that implants have no detectable drug at 83 days, consistent with serum concentration at the same time point shown in panel A. Additionally, these data indicate that implants can be removed at a time point beyond the period during which drug is released. However, because PLGA implants are fully biodegradable, they would not require removal as would be the case for minipumps and other non-degradable formulations. The observation that implants removed at 56 days contain 10 wt.% risperidone suggests that *in vivo* degradation and release is faster than the rate observed *in vitro*. Black bars are EO sterilized implants and gray bars are surface sterilized implants.

of approximately three months (Fig. 3A). *In vivo* rod-shaped implants contained 40 wt.% risperidone, yielding 8 mg drug per 20 mg implant designed to deliver approximately 3 mg/kg/day for 90 days based on the expected delivery interval.

3.3. Experiment 3: pharmacokinetic studies

In vivo pharmacokinetic profiles from PLGA implants were tested in mice to assess the prospect of creating a long-term, sterile, drug delivery system for schizophrenia. Mice received either EO sterilized or surface sterilized (betadine wash) 75:25 PLGA-risperidone implants ($n=4$ each per time point) with 40 wt.% drug load. Implants were removed and mice were

sacrificed to assess serum concentration and reversibility at 14, 27, 56, or 83 days. *In vivo* onset was rapid and serum concentration was within the target range of 2–15 ng/ml for a substantial portion of the release interval, though serum levels varied by time (de Oliveira, 1996). Serum levels were approximately 7–10 ng/ml at 14 days and increased to about 15–20 ng/ml at 27 and 56 days

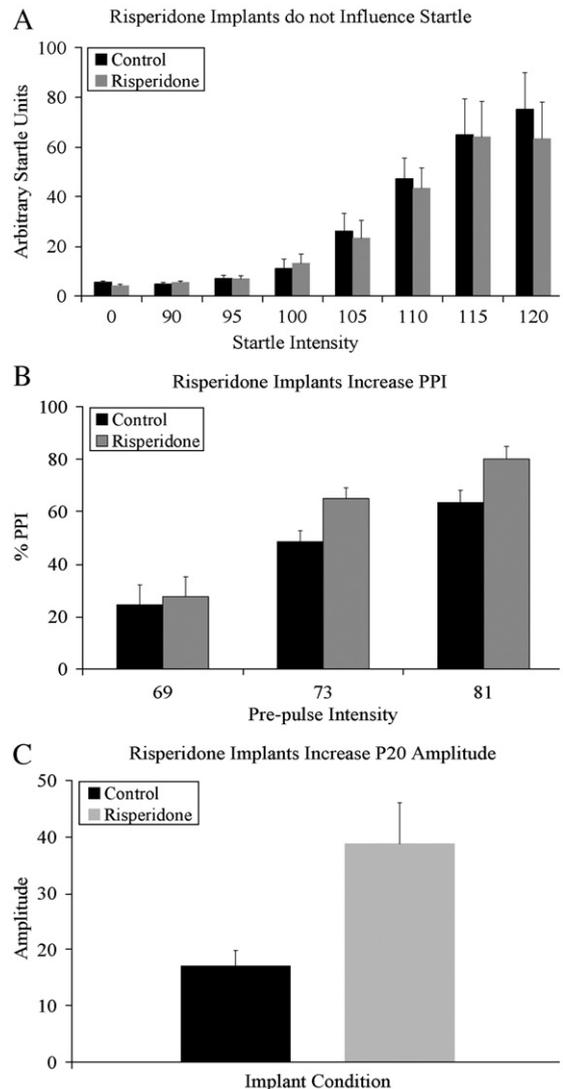


Fig. 6. Behavioral testing in mice demonstrates that bioactivity of risperidone is retained when delivered from PLGA implants. A) Risperidone implants did not alter startle amplitude. B) However, implants marginally increased PPI relative to control implant animals ($p=0.052$) on 14 and 21 days post-implantation. C) Risperidone implants increased the P20 amplitude in C57Bl/6J mice ($p=0.03$) 28 days following implantation, indicating that chronic risperidone administration from implants achieve a comparable biological effect as previously demonstrated using another antipsychotic medication, olanzapine, in osmotic minipumps (Maxwell et al., 2004).

(Fig. 5A). Although implants were removed at 83 days, there was no detectable drug in serum, consistent with residual risperidone content (described below). Thus, implants delivered drug at least 56 days while retaining coherence and reversibility until 83 days.

HPLC/UV spectroscopy confirmed the presence of risperidone in residual sterile implants removed from mice (Fig. 5B). Analyses of drug content in samples extracted from implants show that the percent drug load decreases over time, indicating that drug is released at a faster rate than the polymer is degraded. Implants removed at day 14 show approximately 25 wt.% load, which decreases to 20 wt.% at day 27 and 10 wt.% at day 56. Implants removed at 83 days show 0 wt.% drug load, indicating full release prior to this time point. No local tissue reaction, fibrosis, or erosion was found upon implant removal, suggesting good local biocompatibility throughout the delivery interval at any time point.

3.4. Experiment 4: behavioral studies

Daily qualitative observations of the mice were made for five days immediately following implantation to evaluate for signs of high initial drug release. There were no signs of distress that would have been associated with higher than anticipated levels of drug release. Specifically, mice showed no locomotor impairment, no difficulties with grooming, and no loss of weight, suggesting that they ate and drank appropriately following insertion of implants.

We examined functional measures of risperidone implants using both PPI and ERPs in mice. Risperidone implants did not alter startle amplitude (Fig. 6A), but marginally increased PPI relative to controls ($p=0.052$) at 14 and 21 days post-implantation (Fig. 6B). Risperidone implants increased the P20 amplitude relative to control implant animals ($p=0.03$, Fig. 6C). Risperidone implants did not alter the magnitude of the N40 ERP ($p=0.68$, data not shown). These findings for the P20 are consistent with previous results of increased P20/N40 amplitude following chronic olanzapine (Maxwell et al., 2004) and serve as *in vivo* biomarkers of implant activity in rodents that are analogous to biomarkers of drug activity in human subjects (Umbricht et al., 2004).

4. Discussion

This study demonstrates *in vitro* and *in vivo* proof of concept for risperidone implants using biodegradable PLGA copolymers. Firstly, this study establishes the stability of risperidone across a broad pH range that may

occur inside implants as PLGA degrades to monomer units. Secondly, our results indicate that *in vitro* and *in vivo* release can be controlled based on various parameters, including polymer composition and drug load, while maintaining the bioactivity of the drug. However, release profiles in humans will ultimately depend on the specific combination of polymer composition, risperidone drug load, methods of implant fabrication, and *in vivo* conditions. Current results suggest that implants containing risperidone could achieve appropriate release of medication within approximately two weeks, last between two and three months, and be tailored to patients' needs by altering the polymer and drug parameters mentioned above.

In the current study, functional measures of risperidone implants were examined using PPI and ERPs in mice. Patients with schizophrenia display deficits in PPI of the startle reflex, a cross-species measure of sensorimotor gating (Swerdlow et al., 2006). Moreover, amplitude of the P50 ERP is also impaired in schizophrenia (Boutros et al., 2004). Our results demonstrate that risperidone implants marginally increased PPI relative to controls and increased amplitude of the P20 in mice, which is analogous to the human P50 (Connolly et al., 2004; Maxwell et al., 2004; Metzger et al., 2006). Thus, our results indicate that risperidone implants maintain their biological activity in these widely used animal models of the information processing deficits in schizophrenia.

This study is fundamentally a proof of a new method of drug delivery that could be applied across many drugs. However, we focused on the feasibility, flexibility, and bioactivity of a long-term implantable system using risperidone because it is a strong candidate among current medications based on efficacy, potency, and clinical popularity. The high potency of risperidone results in lower dose requirements and hence smaller and more easily tolerated implants for any duration (Table 1). While our group previously demonstrated a similar proof of concept for haloperidol implants, but many in the psychiatric community view this drug with reservation. We have considered several studies that extol the virtues of newer agents (Kane et al., 2003; Keefe et al., 2004; Lambert and Castle, 2003) and believe that implants are more likely to achieve their translational purpose as a clinical reality if made with risperidone or other new high potency agents. There are additional considerations regarding the side effects of risperidone. The major liabilities for risperidone are related to the dose dependent emergence of motor side effects and prolactin elevation that have been linked to

Table 1
Estimated implant mass for each antipsychotic medication

Agent	Daily oral dose (mg)	Daily parenteral dose (mg/kg/day)	Monthly parenteral dose (mg/kg/mo)	Monthly parenteral dose (mg/mo)	Semiannual implant dose (mg)
Risperidone	4–8	0.03–0.06	1–2	50–100 (consta)	300–600
Haloperidol	6–12	0.03–0.06	1–2	50–100 (decanoate)	300–600
Olanzapine	10–20	0.05–0.10	2–4	100–200	600–1200
Aripiprazole	10–30	0.05–0.15	2–6	100–300	600–1800
Ziprasidone	120–160	0.6–1.2	20–40	1000–2000	6000–12000
Quetiapine	400–600	3–6	100–200	5000–10,000	30,000–60,000

The potency and resulting daily dose of each antipsychotic agent is an important characteristic when considering the feasibility of sequestering a three to six month supply in the sub-cutaneous space through long-term delivery implants. The feasibility for each antipsychotic agent can be assessed based on the comparable oral doses required to achieve symptom remission. Using these ratios, the required parenteral dose in a 6-month implant for each agent can be estimated based on package inserts. As shown, risperidone results in the lowest amount of drug required in an implantable system with resulting reduction in size of the proposed implants. Note, ziprasidone and quetiapine would require implants that are 20 and 100 times larger than those using risperidone. Alternatively, olanzapine and aripiprazole would require only a 2–3 fold increase in size relative to risperidone.

the concentration of the 9-OH risperidone metabolite (Knegtering et al., 2005; Melkersson, 2006). Implants may be able to reduce the ratio of metabolite relative to parent compound because they avoid first-pass metabolism. However, this is a theoretical benefit to parenteral implant formulations and remains a hypothesis that could be explored in future studies.

Although the current formulation utilized risperidone, other high potency antipsychotic medications (currently available or in development) also show promise for this approach. PLGA implants are amenable to many compounds, including devices to control thyrotropin-releasing hormone in maintaining metabolism, deliver L-dopa in the treatment of Parkinson's disease, and manage sustained release of luteinizing hormone releasing hormone (LHRH) antagonist to treat prostate cancer (Du et al., 2006; Okada, 1995; Sabel et al., 1990). Future studies could incorporate various agents to tests their applicability in long-term delivery. The stability of each drug in PLGA polymer implants will vary because the drug is exposed to an aqueous environment as the implant is hydrated. Therefore, antipsychotic agents that are stable in aqueous media for the intended delivery interval are preferable.

Despite rapid advances in the development of antipsychotic drugs, non-adherence remains a pernicious block to long-term improvements in the quality of life and ultimate outcome of patients with schizophrenia (Kane, 2006b; Nasrallah and Lasser, 2006). Furthermore, non-adherence does not appear to be significantly ameliorated by the advent of new agents in clinical trials (Jones et al., 2006). This finding was quantified in the NIH sponsored Clinical Antipsychotic Trials of Intervention Effectiveness, during which 74% of patients discontinued their initial medication despite being enrolled in a clinical trial (Lieberman, 2006). In addition to the clinical conse-

quences of non-adherence, researchers estimate the economic burden of healthcare costs for non-adherence in mental illness is at least \$2.3 billion annually in the United States (Menzin et al., 2003). Thus we believe that this novel method of antipsychotic medication delivery proposed here will complement future development of new molecules in the service of improved adherence and long-term clinical improvement and cost effectiveness.

5. Critical issues and future directions

One issue raised by this study is the finding that in vivo release of risperidone was faster than would be anticipated from our in vitro data. This may be due to the inexact correlation between in vitro release in our assay system and in vivo risperidone serum concentration in mice. Previous studies have examined the in vitro/in vivo correlation (IVIVC) for various other drug delivery systems, suggesting that the IVIVC varies with each compound and polymer matrix (Wang et al., 1996). It is hypothesized that this is due in part to the foreign body response (Fulzele et al., 2007). As other researchers have concluded, more sophisticated in vitro models mimicking drug release under in vivo conditions are necessary for parenteral depot formulations, specifically for risperidone implant configurations (Schliecker et al., 2004). This IVIVC for risperidone will be further explored in a NIH funded study in rats. Thus, while initial studies such as this one can be used to demonstrate the laboratory reality of a long-term implantable approach, subsequent studies will focus on in vivo models to advance translation to humans.

Scaling our results from mice to humans is complicated by many factors such as body mass index, body temperature, drug metabolism, and implant degradation rate (Kapur et al., 2003). There are

considerable interspecies differences in the metabolism of risperidone. Thus, the absolute doses used in our preliminary study approximated the amount of drug needed for a human, despite the difference in body mass and drug metabolism. Because most patients respond to doses of 2–6 mg/day of risperidone in oral form and 25–50 mg/biweekly when given as a depot formulation, a three month implant system would contain approximately 150–300 mg of drug (Lambert and Castle, 2003; Medori et al., 2006). As the concept of using implants moves from pre-clinical animal models to human applications, we would foresee a period of oral or injectable medication prior to initiating implants in a particular individual. In order to understand the conversion from oral and injectable to implantable dosage for comparable serum concentration, definitive studies must be undertaken to address this point in human phase I trials in addition to the pre-clinical animal studies we report here. Furthermore, because the quantitative behavioral testing conducted here was performed 14–28 days after implantation, it may omit possible changes that can occur immediately after implantation. Although neither *in vitro* models nor qualitative *in vivo* behavioral observations indicated that there was an initial burst of drug, future studies should include testing during this critical clinical period. With these proper cautions in scaling and translating results to humans, our study indicates that high potency agents such as risperidone show promise to transform and improve the clinical care of schizophrenia through a novel approach to improve medication adherence.

5.1. Potential advantages

The risperidone formulation described in the current report lasts 2–3 months. However, our group has previously demonstrated proof of concept for haloperidol implants that last 6 months using a similar technology (Metzger et al., 2007). The current report is meant to advance this line of research toward the ultimate goal of year-long delivery intervals. This substantial time period is required for individuals with schizophrenia to stay well long enough to engage in vocational and educational activities, thus optimizing treatment benefits (Ereshefsky and Mannaert, 2005a). It will be crucial that delivery systems lasting 3 months or more are able to be removed in the event of a problem or if the patient so desires. Therefore, the ability to remain reversible is intimately linked to the ability to provide very long-term delivery options. Additionally, the proposed technology is more versatile than current injectable formulations that require chemical modifica-

tions in order to form ester linkages with the active pharmaceutical agent. This versatility makes the proposed implant technology applicable to several typical and atypical antipsychotic compounds as well as non-psychiatric compounds.

5.2. Potential disadvantages

From a clinical perspective, we recognize that implantable delivery systems have disadvantages both similar to and different from depot formulations, primarily arising of the procedure itself. The implantation procedure will likely require numbing of the skin, e.g. lidocaine, and temporary discomfort. Additionally, it is possible that patients will be reluctant to condone the implantation of a foreign body under their skin. However, a 2004 survey of 206 psychiatric patients concluded that almost 50% of individuals would be open to this novel method of drug administration (Irani et al., 2004), suggesting that we cannot assume patients will be unwilling to receive their medication this way.

Ethical considerations related to the use of implantable delivery systems in psychiatric populations must be considered as our concept moves from the lab to the clinic. These include 1) careful attention to protect patient autonomy, 2) safeguards to ensure patients' ability to provide informed consent, and 3) assessment of the patient's comprehension of the intervention and desire to continue or discontinue throughout the course of implantation. By allowing patients to make decisions regarding long-term treatment during periods of relative health, this method of delivery may minimize interruptions in antipsychotic medication. This is especially important because discontinuous antipsychotic treatment is correlated with worse long-term functioning, quality of life, and ability to maintain employment and relationships (Gilmer et al., 2004; Nasrallah and Lasser, 2006).

Because implants are reversible, patients would maintain the ability to discontinue a specific treatment if they chose to stop or switch medications. However, this would be an active decision by the patient, as part of a process involving their physician, rather than a passive or unintentional act of not taking a daily pill. While implantable medication does not guarantee adherence, physicians and caretakers, who share a great deal of the responsibility in treating schizophrenia, would have less doubt that patients were following their treatment regimen. Hence, if a patient misses a scheduled implant visit, the clinical team would be alerted and could intervene appropriately. Furthermore, because long-acting medication offers a gradual decline in blood levels

compared to oral medication, if a patient were to miss an appointment for reimplantation, the result would be a gradual rather than an abrupt reduction in serum level and therefore less likelihood for psychotic relapse (Ereshefsky and Mannaert, 2003, 2005b; Remington et al., 2006).

6. Conclusion

We have demonstrated the behavioral and pharmacokinetic feasibility of a long-term, delivery system providing in vitro and in vivo release of risperidone between two and three months. This implantable delivery system can be designed and engineered to provide considerable flexibility in dosing and delivery interval. We firmly believe that implants represent a potentially beneficial paradigm shift for schizophrenia treatment and have identified critical issues in moving this approach forward. As these technologies move from the laboratory to the clinic, the implications of these results may lead to reduced morbidity and mortality from the ravages of untreated psychosis and perhaps other chronic conditions.

Role of the Funding Source

The SMRI and NIMH had no direct input into the execution, analyses of these experiments or input into the writing of the resulting manuscript.

Contributions

C. Rabin—wrote the manuscript.

Y. Liang—contributed to the experiments within the manuscript.

R.S. Ehrlichman—contributed to the experiments within the manuscript.

Budhian—contributed to the experiments within the manuscript.

K. L. Metzger—contributed to the experiments within the manuscript.

Majewski-Tiedeken—contributed to the experiments within the manuscript.

K. I. Winey—contributed to the conceptual design and plans for experiments within the manuscript.

S.J. Siegel—contributed to the conceptual design and plans for experiments within the manuscript as well as obtaining funding and participated in writing.

The SMRI and NIMH had no direct input into the execution, analyses of these experiments or input into the writing of the resulting manuscript.

Conflict of interest

Steven Siegel has received grant support from AstraZeneca that is unrelated to the content of this manuscript, grant support and consulting income from NuPathe related to drug delivery systems for Migraine attacks and Parkinson's Disease and is an advisor at the CME Institute. Karen Winey has received grant support from NuPathe for drug delivery systems for Parkinson's Disease. All intellectual property related to work in this manuscript is owned by the University of Pennsylvania. Other authors have no financial disclosures that are relevant to this manuscript.

The SMRI and NIMH had no direct input into the execution, analyses of these experiments or input into the writing of the resulting manuscript.

Acknowledgements

The Stanley Medical Research Institute (SJS) funded this research and NIH 1R01MH074672-01. All experiments comply with the "Principles of laboratory animal Care" and conform to University of Pennsylvania IACUC standards.

References

- Barnes, T.R., Curson, D.A., 1994. Long-term depot antipsychotics. A risk-benefit assessment. *Drug Safety* 10, 464–479.
- Bloch, Y.M.S., Strupinsky, S., Altshuler, A., Fennig, S., Ratzoni, G., 2001. Injections of depot antipsychotic medications in patients suffering from schizophrenia: do they hurt? *J. Clin. Psychiatry* 62, 855–859.
- Boutros, N., Korzyukov, O., Jansen, B., Feingold, A., Bell, M., 2004. Sensory gating deficits during the mid-latency phase of information processing in medicated schizophrenia patients. *Psychiatry Res.* 126, 203–215.
- Connolly, P.M., Maxwell, C., Liang, Y., Kahn, J.B., Kanes, S.J., Abel, T., et al., 2004. The effects of ketamine vary among inbred mouse strains and mimic schizophrenia for the P80, but not P20 or N40 auditory ERP components. *Neurochem. Res.* 29, 1179–1188.
- de Oliveria, I.R., Miranda-Scippa, A.M., de Sena, E.P., Pereira, E.L., Ribeiro, M.G., de Castro-e-Silva, E., Bacaltchuk, J., 1996. Risperidone versus haloperidol in the treatment of schizophrenia: a meta-analysis comparing their efficacy and safety. *J. Clin. Pharm. Ther.* 21, 349–358.
- Du, L.J.C., Chi, Qiang, Qie, Jiankun, Liu, Yan, Mei, Xingguo, 2006. Biodegradable PLGA microspheres as a sustained release system for a New Luteinizing Hormone-Releasing Hormone (LHRH) antagonist. *Chem. Pharm. Bull.* 54, 1259.
- Ereshefsky, L., Mannaert, E., 2003. Comparison of the effects of different routes of antipsychotic administration on pharmacokinetics and pharmacodynamics. *J. Clin. Psychiatry* 64, 18–23.
- Ereshefsky, L., Mannaert, E., 2005a. Pharmacokinetic profile and clinical efficacy of long-acting risperidone: potential benefits of combining an atypical antipsychotic and a new delivery system. *Drugs R&D* 6, 129–137.
- Ereshefsky, L., Mannaert, E., 2005b. Pharmacokinetic profile and clinical efficacy of long-acting risperidone: potential benefits of combining an atypical antipsychotic and a new delivery system. *Drugs R&D* 6, 129–137.
- Fulzele, S.V., Satturwar, P.M., Dorle, A.K., 2007. Novel biopolymers as implant matrix for the delivery of ciprofloxacin: biocompatibility, degradation, and in vitro antibiotic release. *J. Pharm. Sci.* 96, 132–144.
- Gilmer, T.P., Dolder, C.R., Lacro, J.P., Folsom, D.P., Lindamer, L., Garcia, P., et al., 2004. Adherence to treatment with antipsychotic medication and health care costs among Medicaid beneficiaries with schizophrenia. *Am. J. Psychiatry* 161, 692–699.
- Gould, T.J., Bizily, S.P., Tokarczyk, J., Kelly, M.P., Siegel, S.J., Kanes, S.J., et al., 2004. Sensorimotor gating deficits in transgenic mice expressing a constitutively active form of Gs alpha. *Neuropsychopharmacology* 29, 494–501.
- Hirose, T., Uwahodo, Y., Yamada, S., Miwa, T., Kikuchi, T., Kitagawa, H., et al., 2004. Mechanism of action of aripiprazole predicts clinical efficacy and a favourable side-effect profile. *J. Psychopharmacol.* 18, 375–383.

- Hyon, S., 2000. Biodegradable poly (lactic acid) microspheres for drug delivery systems. *Yonsei Med. J.* 41, 720–734.
- Irani, F., Dankert, M., Brensing, C., Bilker, W.B., Nair, S.R., Kohler, C.G., et al., 2004. Patient attitudes towards surgically implantable, long-term delivery of psychiatric medicine. *Neuropsychopharmacology* 29, 960–968.
- Jones, P.B., Barnes, T.R., Davies, L., Dunn, G., Lloyd, H., Hayhurst, K.P., et al., 2006. Randomized controlled trial of the effect on Quality of Life of second-vs first-generation antipsychotic drugs in schizophrenia: Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUtLASS 1). *Arch. Gen. Psychiatry* 63, 1079–1087.
- Kane, J.M., 2006a. Review of treatments that can ameliorate nonadherence in patients with schizophrenia. *J. Clin. Psychiatry* 67 (Suppl 5), 9–14.
- Kane, J.M., 2006b. Utilization of long-acting antipsychotic medication in patient care. *CNS Spectr.* 11, 1–7 (quiz 7–8).
- Kane, J.M., Aguglia, E., Altamura, A.C., Ayuso Gutierrez, J.L., Brunello, N., Fleischhacker, W.W., et al., 1998. Guidelines for depot antipsychotic treatment in schizophrenia. European Neuropsychopharmacology Consensus Conference in Siena, Italy. *Eur. Neuropsychopharmacol.* 8, 55–66.
- Kane, J.M., Eerdeken, M., Lindenmayer, J.P., Keith, S.J., Lesem, M., Karcher, K., 2003. Long-acting injectable risperidone: efficacy and safety of the first long-acting atypical antipsychotic. *Am. J. Psychiatry* 160, 1125–1132.
- Kapur, S., VanderSpek, S.C., Brownlee, B.A., Nobrega, J.N., 2003. Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. *J. Pharmacol. Exp. Ther.* 305, 625–631.
- Keefe, R.S., Seidman, L.J., Christensen, B.K., Hamer, R.M., Sharma, T., Sitskoorn, M.M., et al., 2004. Comparative effect of atypical and conventional antipsychotic drugs on neurocognition in first-episode psychosis: a randomized, double-blind trial of olanzapine versus low doses of haloperidol. *Am. J. Psychiatry* 161, 985–995.
- Kitchell, J.W.D., 1985. Poly(lactic/glycolic acid) biodegradable drug–polymer matrix systems. *Methods Enzymol.* 112, 436–448.
- Knegtering, R., Baselmans, P., Castelein, S., Bosker, F., Bruggeman, R., van den Bosch, R.J., 2005. Predominant role of the 9-hydroxy metabolite of risperidone in elevating blood prolactin levels. *Am. J. Psychiatry* 162, 1010–1012.
- Knox, E.D., Stimmel, G.L., 2004. Clinical review of a long-acting, injectable formulation of risperidone. *Clin. Ther.* 26, 1994–2002.
- Lambert, T.J.R., Castle, D.J., 2003. Pharmacological approaches to the management of schizophrenia. *Med. J. Aust.* 178, S57–S61.
- Lieberman, J.A., 2006. Comparative effectiveness of antipsychotic drugs: a commentary on Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUtLASS 1) and Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE). *Arch. Gen. Psychiatry* 63, 1069–1072.
- Lieberman, J.A., Stroup, T.S., McEvoy, J.P., Swartz, M.S., Rosenheck, R.A., Perkins, D.O., et al., 2005. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *N. Engl. J. Med.* 353, 1209–1223.
- Maxwell, C.R., Liang, Y., Weightman, B.D., Kanes, S.J., Abel, T., Gur, R.E., et al., 2004. Effects of chronic olanzapine and haloperidol differ on the mouse N1 auditory evoked potential. *Neuropsychopharmacology* 29, 739–746.
- Medori, R., Mannaert, E., Grunder, G., 2006. Plasma antipsychotic concentration and receptor occupancy, with special focus on risperidone long-acting injectable. *Eur. Neuropsychopharmacol.* 16, 233–240.
- Melkersson, K.I., 2006. Prolactin elevation of the antipsychotic risperidone is predominantly related to its 9-hydroxy metabolite. *Hum. Psychopharmacol.* 21, 529–532.
- Menzin, J., Boulanger, L., Friedman, M., Mackell, J., Lloyd, J.R., 2003. Treatment adherence associated with conventional and atypical antipsychotics in a large state medicaid program. *Psychiatr. Serv.* 54, 719–723.
- Metzger, K.L., Maxwell, C.R., Liang, Y., Siegel, S.J., 2006. Effects of Nicotine Vary Across Two Auditory Evoked Potentials in the Mouse. *Biol. Psychiatry* 61, 23–30.
- Metzger, K.L., Shoemaker, J.M., Kahn, J.B., Maxwell, C.R., Liang, Y., Tokarczyk, J., et al., 2007. Pharmacokinetic and behavioral characterization of a long-term antipsychotic delivery system in rodents and rabbits. *Psychopharmacology (Berl)* 190, 201–211.
- Naiker, D.V., Catts, S.V., Catts, V.S., Bedi, K.S., Bryan-Lluka, L.J., 2006. Dose determination of haloperidol, risperidone and olanzapine using an in vivo dopamine D2-receptor occupancy method in the rat. *Eur. J. Pharmacol.* 540, 87–90.
- Nasrallah, H.A., Lasser, R., 2006. Improving patient outcomes in schizophrenia: achieving remission. *J. Psychopharmacol.* 20, 57–61.
- Okada, H.T.H., 1995. Biodegradable microspheres in drug delivery. *Crit. Rev. Ther. Drug Carr. Syst.* 12, 1–99.
- Olesen, O.V.L., Rasmus, W., Thomsen, Erik, Bruun, Troels, Viftrup, Jens Emil, Linnet, Kristian, 1998. Serum concentrations and side effects in psychiatric patients during risperidone therapy. *Ther. Drug Monit.* 20, 380–384.
- Remington, G., Mamo, D., Labelle, A., Reiss, J., Shammi, C., Mannaert, E., et al., 2006. A PET study evaluating dopamine D2 receptor occupancy for long-acting injectable risperidone. *Am. J. Psychiatry* 163, 396–401.
- Sabel, B.A., Dominiak, P., Hauser, W., During, M.J., Freese, A., 1990. Levodopa delivery from controlled-release polymer matrix: delivery of more than 600 days in vitro and 225 days of elevated plasma levels after subcutaneous implantation in rats. *J. Pharmacol. Exp. Ther.* 255, 914–922.
- Schliecker, G., Schmidt, C., Fuchs, S., Ehinger, A., Sandow, J., Kissel, T., 2004. In vitro and in vivo correlation of buserelin release from biodegradable implants using statistical moment analysis. *J. Control. Release* 94, 25–37.
- Swerdlow, N.R., Geyer, M.A., Shoemaker, J.M., Light, G.A., Braff, D.L., Stevens, K.E., et al., 2006. Convergence and divergence in the neurochemical regulation of prepulse inhibition of startle and N40 suppression in rats. *Neuropsychopharmacology* 31, 506–515.
- Turner, M.S., Stewart, D.W., 2006. Review of the evidence for the long-term efficacy of atypical antipsychotic agents in the treatment of patients with schizophrenia and related psychoses. *J. Psychopharmacol.* 20, 20–37.
- Umbrecht, D., Vysotsky, D., Latanov, A., Nitsch, R., Brambilla, R., D'Adamo, P., et al., 2004. Midlatency auditory event-related potentials in mice: comparison to midlatency auditory ERPs in humans. *Brain Res.* 1019, 189–200.
- Valenstein, M.B.F., Copeland, L.A., McCarthy, J.F., Zeber, J.E., Gillon, L., Bingham, C.R., Stavenger, T., 2004. Poor antipsychotic adherence among patients with schizophrenia: medication and patient factors. *Schizophr. Bull.* 30, 255–264.
- Wang, G., Tucker, I.G., Roberts, M.S., Hirst, L.W., 1996. In vitro and in vivo evaluation in rabbits of a controlled release 5-fluorouracil subconjunctival implant based on poly(D,L-lactide-co-glycolide). *Pharm. Res.* 13, 1059–1064.
- Zygmunt, A., Olfson, M., Boyer, C.A., Mechanic, D., 2002. Interventions to improve medication adherence in schizophrenia. *Am. J. Psychiatry* 159, 1653–1664.