SER-214, A NOVEL POLYMER-CONJUGATED ROTIGOTINE FORMULATION AFFORDS GREATLY EXTENDED DURATION OF ANTI-PARKINSONIAN EFFECT AND ENHANCED PLASMA EXPOSURE FOLLOWING A SINGLE ADMINISTRATION IN RODENTS AND PRIMATES

R. W. Moreadith[1], T. X. Viegas[1], D. G. Standaert[2], M. D. Bentley[1], Z. Fang[1], B. Dizman[1], K. Yoon[1], R. Weimer[1], J. M. Harris[1], P. Ravenscroft[3],T. H. Johnston[3], M. Hill[3], J. M. Brotchie[3]

[1]Serina Therapeutics Inc., Huntsville, AL, USA; [2]Department of Neurology, University of Alabama, Birmingham, AL, USA; and [3]Atuka Inc., Toronto, ON, Canada.

Background

Dopaminergic neurons in the basal ganglia fire in a continuous manner in normal individuals, thus striatal dopamine concentrations are maintained at a constant level. Degeneration of pre-synaptic neurons in the basal ganglia leads to dopamine deficiency and Parkinson's disease (PD). Pulsatile stimulation of striatal dopamine receptors with short acting dopamine replacement therapies is thought to accelerate molecular and physiological changes that result in development of motor complications in PD, and may accelerate the progression of the disease. A therapeutic strategy that would deliver continuous dopaminergic stimulation (CDS) would represent a significant advance in the treatment of PD. We sought to develop a POZ-rotigotine conjugate that would provide CDS over one week and thereby provide sustained anti-Parkinsonian benefits. This therapy would represent a significant advance in the treatment of PD and may reduce, or avoid, motor complications.

Technology

Polyoxazoline (POZ) polymers are non-ionic, biocompatible and are prepared by the cationic ring opening polymerization method. Molecular weights of 500 to 40,000 Da (Polydispersity Index < 1.10) have been prepared by Serina chemists in linear, branched, and pendent structures as pharmaceutical grade material. Pendent POZ is useful in delivering small drug molecules that may have suboptimal ADME/PK such as limited water solubility, short half-life, limited oral bioavailability or are required at high doses. We have developed a POZ-polymer conjugate of rotigotine for the treatment of PD.

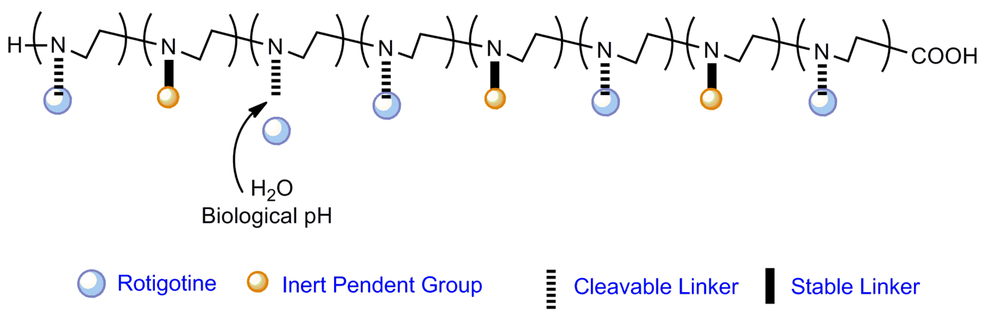

Figure 1: Schematic representation of POZ polymer for drug delivery

Purpose

The target product profile (TPP) for development of a POZ-rotigotine conjugate was as follows :

1. A formulation of lyophilized POZ-rotigotine conjugate that would be water soluble and one dose should fit in a standard insulin syringe (0.5 cc);
2. Prompt onset of dopaminergic tone (within one hour) following *s.c.* administration;
3. Near steady-state levels within a relatively narrow therapeutic window that controls symptoms;
4. Stable dopaminergic tone that would persist for ~ one week; and
5. Repeat dose PK that supports chronic dosing.

We evaluated whether POZ-rotigotine conjugates could provide extended duration of anti-Parkinsonian effect, compared to un-conjugated rotigotine, in the 6-hydroxydopamine (6-OHDA)-lesioned rat model of Parkinson's disease (PD). Additionally, we evaluated the PK of POZ-rotigotine conjugates that would provide therapeutically relevant plasma levels of free rotigotine in both the rat and non-human primate.

Methods

POZ conjugates of rotigotine were prepared with three linker chemistries that allowed for different rates of release, i.e. "fast" (SER-212), "intermediate" (SER-213) and "slow" (SER-214). The rates of release of drug were measured in rat plasma at 37 ºC and the respective half-lives of the conjugates were calculated from the release curves shown in Figure 2.

Male rats (Two groups, n=3 per group) were administered one injection (*s.c.*) of SER-214 (1.6 or 6.4 mg/kg rotigotine eq.). Plasma rotigotine levels were measured at 6 time points for up to 7 days post administration. In another study, male rats received unilateral 6-hydroxydopamine (6-OHDA) injections (12.5 mcg, MFB). Two weeks later, suitably lesioned animals received a single injection (*s.c.*) of vehicle, rotigotine (0.5 or 3 mg/kg), SER-212 or SER-214 (1.6 or 6.4 mg/kg rotigotine eq.) on Day 1 (n=8 per group). Rotational behaviour, forelimb asymmetry (cylinder test) and plasma exposure levels were measured for up to 9 days.

Normal untreated female cynomolgus macaques (n=3 per group, 3.9 ± 0.3 kg, 6.5 ± 0.2 yr age) were administered a single injection (*s.c.*) of SER-213 or SER-214 (1.5 or 4.5 mg/kg rotigotine eq.). Plasma drug levels were assessed prior to and at 13 time points up to 14 days post drug administration.

Male rats (n=4 per group) were administered one injection (*s.c.*) of SER-214 (1.5 mg/kg rotigotine eq.) per week for 6 weeks. Plasma rotigotine levels were measured at 6 time points for up to 7 days post administration. The weekly half-lives ($t_{½}$) and drug exposure levels ($AUC_{0\text{-}inf}$) were calculated.

Rotigotine was measured in plasma using a qualified LC-MS/MS method. An internal standard of deuterated rotigotine was used in the assay and all response *vs* concentration calculations were made by weighted least squares linear regression (WSLR) method. The limit of quantitation (LOQ) was ~ 0.1 ng/mL. All pharmacokinetic calculations were determined using a non-compartmental model (WINNONLIN, Pharsight Corp, CA).

Results

The measured half-lives of SER-212, SER-213 and SER-214 in rat plasma at 37 ºC were 2.4, 7.1 and 11.9 h, respectively.

In normal rats, SER-214 (1.6 mg/kg) provided plasma rotigotine levels above 0.5 ng/ml for 7 days ($t_{½}$ ~ 40 h compared to 2.8 h for *s.c.* rotigotine).

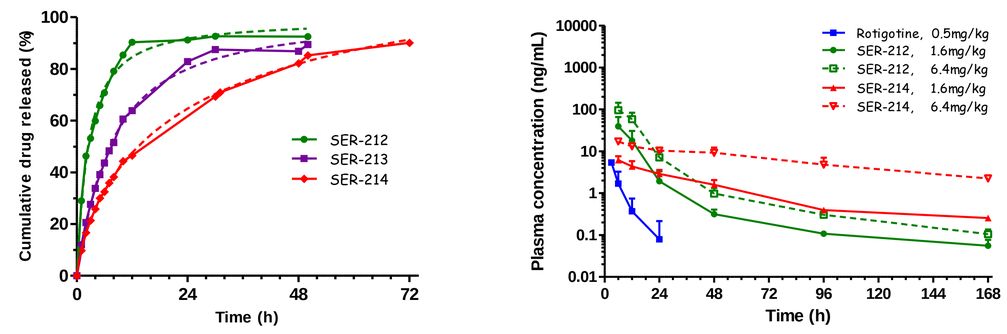

Figure 2: In-vitro hydrolysis of rotigotine from POZ-rotigotine in rat plasma at 37 ºC (*left*) and pharmacokinetics of rotigotine in male rats following subcutaneous administration of rotigotine, SER-212 or SER-214 (n=3, ± SD) (*right*).

In the efficacy study, the following observations were noted as shown in Figure 3

- Day 1, SER-214-treated 6-OHDA-animals showed levels of net contraversive rotations comparable to un-conjugated rotigotine [0-6 h: vehicle -56 ± 20; rotigotine (3 mg/kg) 1570 ± 312; SER-214 (1.6 mg/kg) 1408 ± 286; and (6.4 mg/kg) 1272 ± 405].
- Day 2, un-conjugated rotigotine and SER-214 showed comparable efficacy in reversing forelimb asymmetry, a measure of anti-Parkinsonian potential [asymmetry score: vehicle 88 ± 7%; rotigotine (3 mg/kg) 9 ± 13%; SER-214 (1.6 mg/kg) 0 ± 14%; and (6.4 mg/kg) -2 ± 26%].
- Day 5, only SER-214 and not un-conjugated rotigotine treated animals, continued to display greatly enhanced levels of rotations 6.4 mg/kg (5142 ± 777) (P<0.001 *cf.* vehicle).
- Day 5, only SER-214 and not un-conjugated rotigotine treated animals showed robust reversal of forelimb asymmetry (1.6 mg/kg) 31 ± 13%; and (6.4 mg/kg) -6 ± 16%), (both P<0.01 *cf.* vehicle).

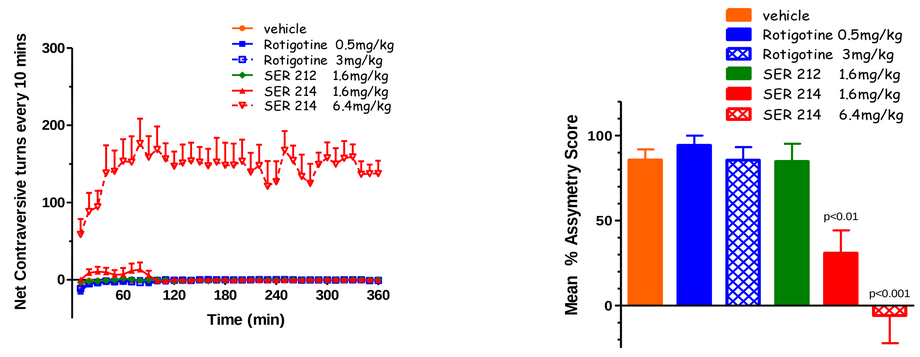

Figure 3: Time-course rotational (left) and forelimb asymmetry (right) behaviour in 6-OHDA-lesioned rats, on Day 5 following a subcutaneous injection of one dose of rotigotine, SER-212 or SER-214 (n=8, ± SEM)

In normal macaques, SER-214 (1.5 mg/kg) was able to provide plasma levels of rotigotine above 1 ng/ml for up to 7 days following a single administration ($t_{½}$ ~ 60 h). When the data was normalized to a dose of 0.5 mg/kg, the predicted profile in humans would achieve the attributes of the TPP.

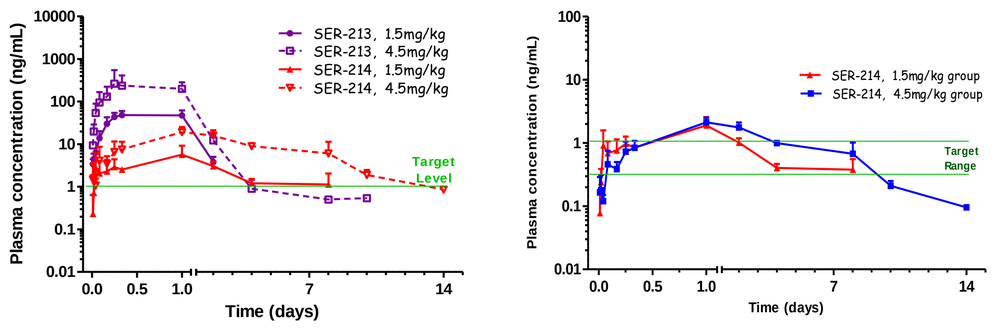

Figure 4: Pharmacokinetic profile rotigotine release in female monkeys following of subcutaneous administration of SER-213 or SER-214 (n=3, ± SD) (left), and a normalized (predicted for humans) profile of SER-214 at 0.5 mg/kg (n=3, ± SD) (right).

In normal rats, repeated weekly *s.c.* administration of SER-214 (1.5 mg/kg) provided plasma rotigotine levels at or above 0.5 ng/ml and with comparable half-lives and drug exposure levels, (within 25% of the 1st dose). No accumulation of drug was observed at this dose of 1.5 mg/kg.

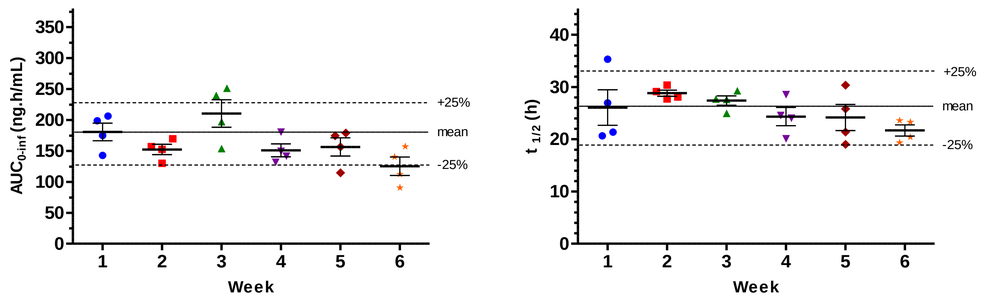

Figure 5: Comparison of $AUC_{0\text{-}inf}$ and $t_{½}$ of rotigotine in male rats following weekly subcutaneous administration of SER-214 for 6 weeks (n=4, ± SD)

Summary

A novel POZ-polymer-conjugated rotigotine, SER-214, is able to provide significantly extended duration of anti-Parkinsonian benefit compared to that afforded by un-conjugated rotigotine or other dopamine therapies. SER-214 met our pre-specified target product profile and is being developed as a once a week injection (s.c.) to treat PD patients. We will advance this into Phase I in humans in 2013. The potential of the POZ platform technology applied to other therapeutic agents that would benefit from enhanced duration of action is considerable.

16th International Conference of Parkinson's Disease and Movement Disorders,
Movement Disorder Society, Dublin, Ireland, June 17-21, 2012, Late Breaking Abstract 5