RESEARCH ARTICLE

Rotigotine Polyoxazoline Conjugate SER-214 Provides Robust and Sustained Antiparkinsonian Benefit

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ABSTRACT: Currently available dopaminergic drugs such as levodopa and dopamine (DA) receptor agonists impart considerable improvement in Parkinson's disease (PD) motor symptoms but often lead to significant motor complications including "wearing-*off*" and dyskinesia. Such complications are believed to stem from the pulsatile nature of dopaminergic stimulation with these agents. Continuous dopaminergic drug delivery using polyoxazoline (POZ) polymer conjugation may improve motor symptoms, while avoiding development of side effects. The purposes of the current study are to characterize the in vitro and in vivo pharmacokinetics of POZ conjugation of a U.S. Food and Drug Administration (FDA)-approved DA agonist, rotigotine, and to evaluate their effects in an established rat model of PD. After determination of release profiles of several POZ-conjugated constructs ("fast": SER-212; "moderate": SER-213; and "slow": SER-214) using in vitro hydrolysis, normal male Sprague-Dawley rats were used for determination of the pharmacokinetic profile of both acute and chronic exposure. Finally, a separate group of rats was rendered hemiparkinsonian using intracranial 6-hydroxydopamine (6-OHDA) infusions, treated acutely with POZ-rotigotine, and assessed for rotational behavior and antiparkinsonian benefit using the cylinder test. POZ-rotigotine formulations SER-213 and SER-214 led to substantial pharmacokinetic improvement compared to unconjugated rotigotine. In addition, SER-214 led to antiparkinsonian effects in DA-lesioned rats that persisted up to 5 days posttreatment. Repeated weekly dose administration of SER-214 to normal rats for up to 12 weeks demonstrated highly reproducible pharmacokinetic profiles. The continuous dopaminergic stimulation profile afforded by SER-214 could represent a significant advance in the treatment of PD, with potential to be a viable, once-per-week therapy for PD patients.

Key Words: Parkinson's disease; dopamine; rotigotine; 6-hydroxydopamine; rat

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Most current Parkinson's disease (PD) treatments are based on augmentation of dopamine (DA) stimulation. While this approach can produce considerable improvement in motor symptoms, these therapies are associated with the development of motor complications, including "wearing-*off*" and dyskinesias.[1] Studies suggest that a primary driver of motor complication development is the short duration of action of approved DA replacement strategies, producing oscillations of DA receptor stimulation.[2,3] These behavioral effects are accompanied by long-term changes in molecular and physiological properties of target neurons.[4–6] Although it is unclear whether the mechanisms responsible for dyskinesia are the same as those leading to wearing-*off*, clinical data demonstrate that treatment with longer-acting dopaminergic agents is associated with reduced rates of motor complications and is effective for managing established motor complications.[7,8]

The relationship between fluctuations in DA stimulation and manifestation of motor complications has fueled efforts to develop treatments providing "continuous dopaminergic stimulation" in PD. Commonly used oral levodopa/carbidopa combinations are associated with a high frequency of motor complications, affecting at least 50% of patients after 5 years of treatment.[1] Addition of the catechol-O-methyl transferase (COMT) inhibitor entacapone prolongs the plasma half-life of L-dopa[9] and reduces wearing-*off* in PD patients with established fluctuations. However, in a large clinical trial this combination was insufficient to reduce the risk of developing motor complications when used as initial treatment in early PD.[10] Direct intraduodenal infusion of L-dopa/carbidopa gel has proven highly effective in reducing motor fluctuations,[11–14] but this approach is invasive and carries risks associated with gastrostomy placement.[15] DA agonist drugs such as pramipexole and ropinirole have longer half-lives; although associated with a lower rate of motor complications, wearing-*off* and dyskinesia are still commonly seen with these agents and adverse effects can be problematic.[16] A transdermal system has been developed to provide continuous delivery of the dopamine agonist rotigotine and its use leads to significant improvements in *on* time and decreased dyskinesia intensities, although this approach has its own limitations, which include local skin reactions and difficulties with adhesion leading to early sloughing off.[17–19]

Conjugation of pharmaceuticals to stable biodegradable polymers, such as polyethylene glycol and hyaluronic acid, can improve bioavailability of proteins and small molecule drugs.[20] Polyoxazolines (POZs) are one of the bioconjugate polymers that show early promise in drug delivery.[21–23] In preclinical settings we have shown that POZ-insulin conjugates lower blood glucose levels for up to 8 hours, compared to 2 hours with insulin treatment alone.[24] Here, we describe the development of a POZ-rotigotine conjugate. We have examined its pharmacokinetic profile, and determined the pharmacodynamic effects of POZ-rotigotine treatment in a rat model of PD. We show that subcutaneous POZ-rotigotine produces an exceptionally long and continuous delivery. The use of this technology to ensure continuous dopaminergic stimulation could be valuable in preventing and managing motor complications of dopaminergic therapy.

Subjects and Methods

Animals

Adult male and female Sprague-Dawley rats (250-350 g; Charles River, Raleigh, NC, USA or Sino-British SIPPR/BK Lab Animal Ltd, Shanghai, China) were used. Rats were housed at 19°C to 21°C, under a 12-hour/12-hour light-dark cycle (lights on 7:00 AM) with ad libitum access to standard lab chow and water and were treated in accordance with the National Research Council's "Guide for the Care and Use of Laboratory Animals" or the Canadian Council on Animal Care. An in-house institutional animal care and use committee (IACUC) approved study protocols.

Experiment 1: In Vitro Hydrolysis of Rotigotine From POZ-Rotigotine Compounds

Rotigotine hydrochloride (Chemrich, Andhra Pradesh, India) was used for the development of the POZ-rotigotine conjugates synthesized and characterized by the Serina Therapeutics chemistry group (Fig. 1). The conjugates were prepared with 3 linker chemistries that allowed for different release rates (fast: SER-212; intermediate: SER-213; and slow: SER-214). Each POZ-rotigotine test compound (20 mg) was dissolved in 5% dextrose (D5W) injection according to the U.S. Pharmacopeial (USP) convention. An aliquot was mixed with rat plasma in ratios allowing for dissolution sink conditions. The mixture was incubated in a water bath (37°C) and at different time points, 200-μL aliquots were taken, neutralized with 0.1 N hydrochloric acid, and mixed with acetonitrile. The mixture was centrifuged and the supernatant was filtered and assayed for rotigotine content by reverse-phase chromatography. The cumulative amount of rotigotine released was plotted versus time.

Plasma Analysis of Rotigotine

Rotigotine concentrations in plasma were determined using a high-performance liquid chromatography mass spectrometry method. Separation was performed on a reverse-phase C18 column followed by detection and analysis with a mass spectrometer and an electrospray ionization probe. Standard

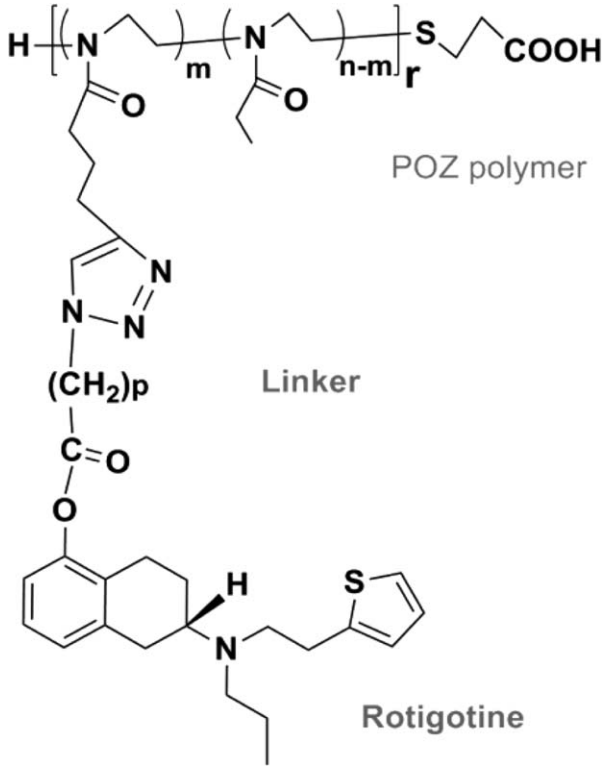

FIG. 1. Structure of POZ-conjugated rotigotine hydrochloride. m = 10, n = 200, p is a value from 1 to 4, and r = "random" arrangement.

solutions of rotigotine in blank rat plasma were prepared at concentrations of 100 to 0.1 ng/mL. Rotigotine-d3 (20 ng/mL; internal standard [IS]; Toronto Research Chemicals, Toronto, ON, Canada) was added to each standard plasma solution. Supernatant was separated and assayed as described earlier in this paragraph. Rotigotine and rotigotine-d3 were detected at m+1 values of 316.2 and 319.2 atomic mass unit (amu), respectively. The linear regression analysis of the rotigotine standards was performed by plotting the peak area ratio of rotigotine over IS (y) against the rotigotine standard concentration (x) in ng/mL. Correlation coefficients obtained by the weighted least squares linear regression method demonstrated the linearity of this analysis.[25] The rotigotine concentration in each unknown plasma sample was calculated.

Experiment 2: Pharmacokinetic Evaluation of Acute and Chronic POZ-Rotigotine

For acute pharmacokinetic evaluation, male rats (n = 3/group) were administered 1 injection of rotigotine (0.5 mg/kg, subcutaneously [sc]), SER-212, or SER-214 (1.6 or 6.4 mg/kg rotigotine equivalent, sc). Rotigotine HCl was dissolved in 1:10 ratio of dimethyl sulfoxide (DMSO) and 5% dextrose. The vehicle for SER-214 was 5% dextrose. Following administration, 6 serial blood samples were obtained from each animal via a jugular vein catheter at time points of 6 hours, 12 hours, 24 hours, 48 hours, 96 hours, and 168 hours. Blood was processed and plasma collected for analysis as explained above in Plasma Analysis of Rotigotine.

Two repeated dose studies were conducted. In the first study, male rats (n = 4/group) were administered SER-214 (1.5 mg/kg rotigotine equivalent, sc) once per week for 6 weeks. In the second study, male and female rats (n = 4/sex/group) were administered SER-214 (1.5 mg/kg rotigotine equivalent, sc) once per week for 12 weeks. Serial blood samples were taken as described in the previous paragraph to determine the effect of repeated exposure to SER-214 on plasma rotigotine levels. Blood was processed and plasma collected for analysis.

Experiment 3: Evaluation of the Effects of POZ-Rotigotine in a Rat Model of PD

Male rats (n = 48) were administered unilateral 6-OHDA nigrostriatal lesions via injection of 12.5 μg of 6-OHDA into a single site of the medial forebrain bundle as described[26] and allowed 2 weeks recovery. Animals were assessed for forelimb asymmetry using cylinder test to eliminate animals lacking overt asymmetry (>85% ipsilateral forelimb use) and delineate 6 equally parkinsonian groups (n = 8/group). One week later, all animals received a single sc injection of vehicle, rotigotine (0.5 or 3 mg/kg), SER-212 (1.6 mg/kg), or SER-214 (1.6 or 6.4 mg/kg). Immediately after administration on day 1 and on days 5 and 9, rotational behavior was assessed using automated rotometers (RotoRat; Med Associates Inc., St. Albans, VT). On days 2, 5, 9, and 13, forelimb asymmetry was assessed using the cylinder test to determine antiparkinsonian effects of the POZ-rotigotine conjugates.

Rotometer

Assessment of rotational activity as a measure of dopaminergic stimulation was accomplished using automated rotometers. Rats fitted with rodent jackets were placed individually in rotometer bowls for 360 minutes. Rotational data was collected via computer software (Med Associates, Inc.).

Cylinder Test

The cylinder test was employed to measure forelimb asymmetry and to assess antiparkinsonian actions of POZ-rotigotine.[27] Rats placed into individual glass cylinders (15-cm diameter [D] × 45-cm height [H]) were recorded until at least 10 independent rearing motions were completed in which the forepaws were in contact with the cylinder wall. Videos were watched for 5 minutes or until 10 independent rears

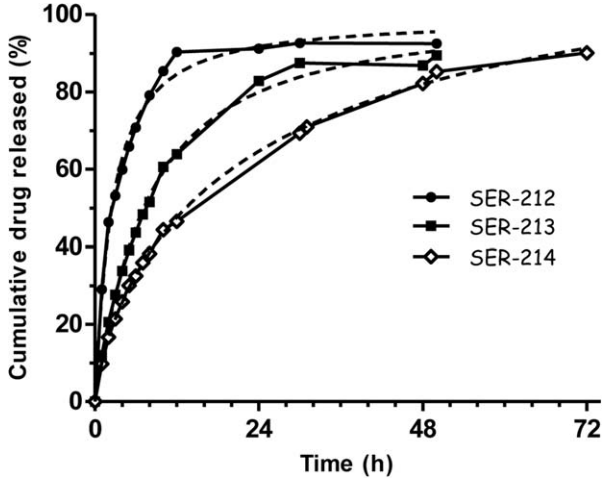

FIG. 2. In vitro release of rotigotine following incubation of SER-212, SER-213, and SER-214 in rat plasma. Solid lines correspond to observed values and hashed lines correspond to a polynomial curve fitted trend. The calculated $t_{1/2}$ for the release of rotigotine are 2.4, 7.1, and 11.9 hours, for SER-212, SER-213, and SER-214, respectively.

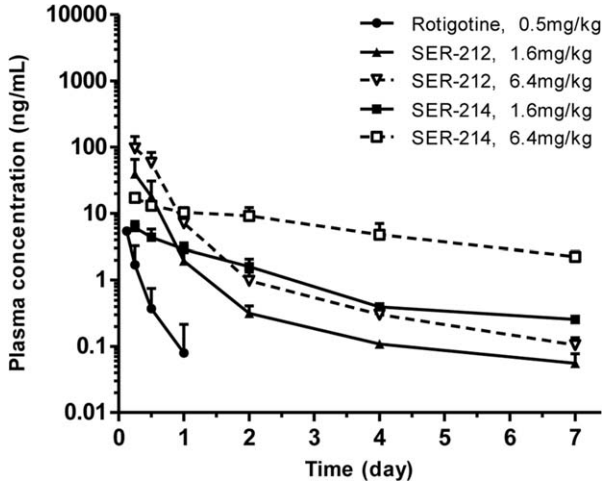

FIG. 3. Pharmacokinetic profiles of SER-212 and SER-214 following a single subcutaneous injection in male Sprague-Dawley rats. Plasma was collected at various time points from 6 to 168 hours postinjection with either rotigotine HCl (0.5 mg/kg), SER-212 (1.6 or 6.4 mg/kg), or SER-214 (1.6 or 6.4 mg/kg).

were completed. Touches were assigned as ipsilateral, contralateral, or both as the first limb(s) that contacted the wall by an observer blinded to treatment. If the other limb was placed within 0.4 seconds of the initial placement, the touch was scored as "both." An overall asymmetry score was calculated as (% ipsilateral + % both) − (% contralateral + % both)/(% ipsilateral + % both) + (% contralateral + % both).

Data Analyses

In experiment 1, cumulative rotigotine release from the POZ conjugates was plotted against time. Slopes were analyzed by curve fitting using polynomial nonlinear regression models and the half-life of drug release was calculated. In experiment 2, all pharmacokinetic parameters for the single and repeat dose studies were calculated using the Phoenix WINNONLIN software version 6.3 (Pharsight Corp., Cary, NC, USA). In repeated dose studies, the weekly partial area under the plasma concentration curve $AUC_{1\text{-}7}$ was calculated for comparison of steady-state drug levels. Statistical analysis of rotational time course data was carried out separately by day using parametric repeated-measures 2-way analysis of variance (ANOVA) with Bonferroni's multiple comparisons post hoc tests. Net contraversive rotations accumulated over the 0-hour to 6-hour period, and mean asymmetry scores from cylinder test analysis were analyzed by day using 1-way ANOVA and Dunnett's multiple comparisons tests. $P < 0.05$ was considered significant.

Results

Characterization of In Vitro Release Profile of Rotigotine POZ Conjugates

Figure 2 shows cumulative amounts of rotigotine released over time in rat plasma from the 3 POZ conjugates SER-212, SER-213, and SER-214. The calculated half-lives for the release of drug are 2.4, 7.1, and 11.9 hours, respectively.

In Vivo Rotigotine Plasma Half-Life Is Prolonged by POZ Conjugation

Figure 3 shows rotigotine plasma concentration over 7 days following a single subcutaneous injection of rotigotine, SER-212 and SER-214. Two of three animals treated with rotigotine (0.5 mg/kg) had undetectable

TABLE 1. Pharmacokinetic parameters of rotigotine, SER-212, and SER-214 after a single subcutaneous injection in rats

Compound	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (hours)	Half-life, $t_{1/2}$ (hours)	AUC_{inf} (ng.hour/mL)	AUC_{inf}/dose (ng.hour/mL)	Increase in exposure
Rotigotine	0.5	5.4	3	2.8	25	50	NA
SER-212	1.6	44.2	6	14.0	453	283	5.64
SER-212	6.4	107.1	6	23.1	1287	201	4.00
SER-214	1.6	6.6	6	40.8	233	146	2.90
SER-214	6.4	17.3	6	60.9	1305	204	4.06

C_{max}, peak plasma concentration; T_{max}, time to reach peak plasma concentration; AUC_{inf}, area under the plasma concentration versus time curve from zero to infinity; NA, not available.

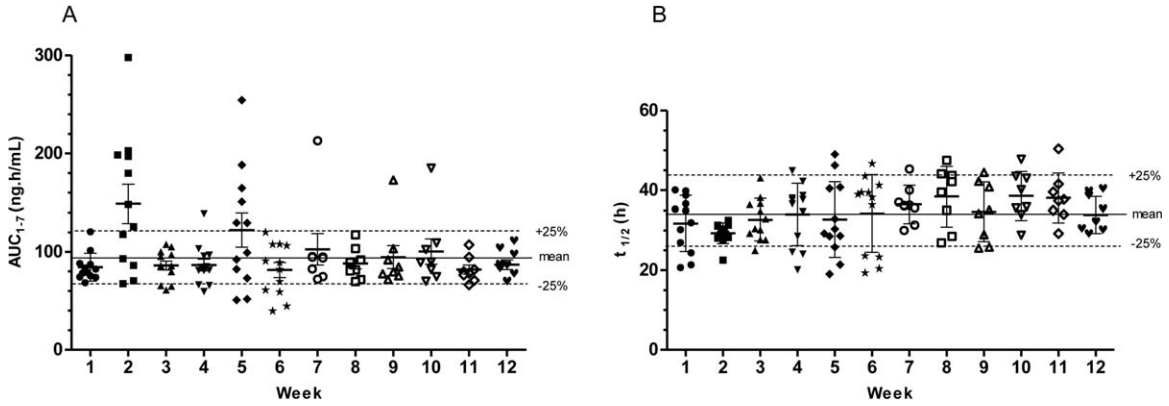

FIG. 4. Comparison of the (**A**) steady-state drug exposure and (**B**) half-life pharmacokinetic profile of SER-214 (1.5 mg/kg) in plasma samples of male Sprague-Dawley rats after weekly subcutaneous dosing. Data is expressed as means (±SD) of pooled data from 6 (n = 12) and 12 (n = 8) weeks of weekly dosing). The "mean" horizontal solid line represents the average of all $t_{1/2}$ or AUC values for all animals and dashed lines are ±25% of the mean.

levels at the 24-hour time point, suggesting rapid metabolism and clearance. The animals treated with SER-212 and SER-214 had prolonged rotigotine exposure over a 7-day period. SER-214 (1.6 mg/kg rotigotine equivalent) had plasma drug concentrations above 0.5 ng/mL for 7 days with a $t_{1/2}$ of 40 hours and an increase in exposure to 2.9 times of rotigotine. The calculated pharmacokinetic parameters are listed (Table 1).

Repeated Treatment Does Not Alter the Pharmacokinetic Profile of POZ-Conjugated Rotigotine

Since the pharmacokinetics of POZ-conjugated rotigotine may change upon repeated administration, the pharmacokinetic profile associated with weekly subcutaneous injection over a period of 6 or 12 weeks was determined in normal rats. Data from these studies were combined due to no change in pharmacokinetics following repeated administration. Figure 4A compares the pooled weekly steady-state drug exposure (AUC) from days 1 to 7 (AUC_{1-7}). Figure 4B compares the pooled weekly terminal half-life of plasma rotigotine following weekly subcutaneous injections of SER-214 (1.5 mg/kg). Treated animals had plasma drug concentrations >0.1 ng/mL and the average $t_{1/2}$ was 34.8 ± 6 hours and 39.2 ± 7 hours for male and female rats, respectively. The results show that interanimal variation in $t_{1/2}$ and AUC_{1-7} values upon repeated dosing is within 25%, suggesting no change in pharmacokinetics.

POZ-Rotigotine Produces Long-Duration Behavioral Effects in the 6-OHDA Rat Model of PD

In vivo efficacy of POZ-rotigotine was assessed in 6-OHDA-lesioned rats. In this model, treatment with dopaminergic drugs leads to contraversive rotation. After a single subcutaneous injection of unmodified or POZ-conjugated rotigotine, rotational effects were assessed on days 1, 5, and 9 (Fig. 5). On day 1, there was a significant effect of time ($F_{35,1470} = 6.6$, $P < 0.001$), treatment ($F_{5,1470} = 4.1$, $P < 0.01$), and the interaction ($F_{175,1470} = 4.0$, $P < 0.001$) on the time course of net contraversive rotations. Lesioned, vehicle-treated animals displayed low levels of ipsiversive rotations. Animals treated with low-dose

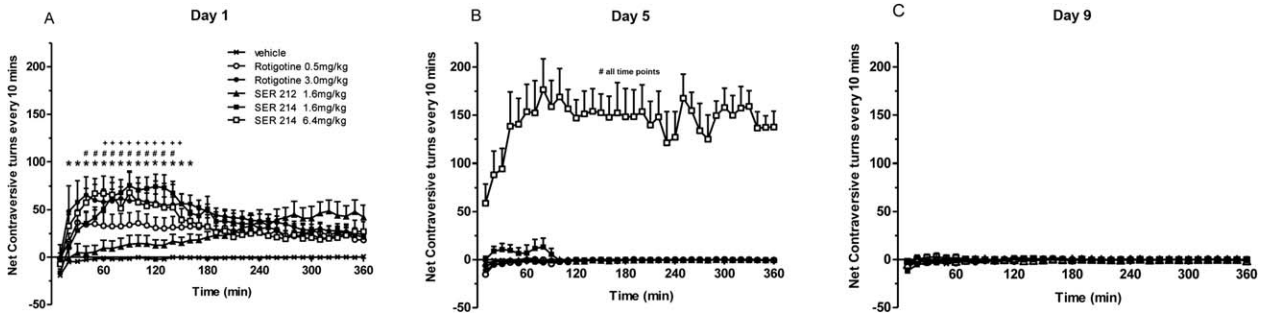

FIG. 5. Time courses of rotational behavior in male 6-OHDA–lesioned Sprague-Dawley rats (n = 8/group) on (**A**) day 1, (**B**) day 5, and (**C**) day 9 after a single subcutaneous injection of rotigotine (0.5 or 3 mg/kg), SER-212 (1.6 mg/kg), or SER-214 (1.6 or 6.4 mg/kg). Net contraversive rotations were collected continuously and cumulated into 10-minute time bins over the 6-hour period of observation. Data are expressed as mean ± SEM, *$P <$ 0.05 rotigotine 3.0 mg/kg versus vehicle, +$P <$ 0.05 SER 214 1.6 mg/kg versus vehicle, #$P <$ 0.05 SER 214 6.4 mg/kg versus vehicle.

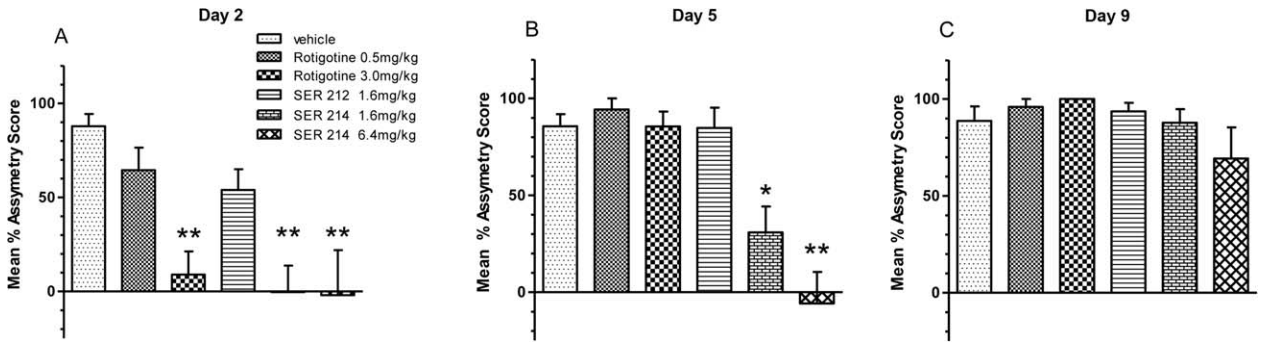

FIG. 6. Cylinder test forelimb asymmetry behavior in male 6-OHDA-lesioned Sprague-Dawley rats (n = 8/group) on (**A**) day 2, (**B**) day 5, and (**C**) day 9 following a single subcutaneous injection of rotigotine (0.5 or 3 mg/kg), SER-212 (1.6 mg/kg), or SER-214 (1.6 or 6.4 mg/kg). Asymmetry scores for each animal were calculated using the following formula: (% ipsilateral + % both) − (% contralateral + % both)/(% ipsilateral + % both) + (% contralateral + % both). Bars denote mean asymmetry score ± SEM, **P* < 0.01 versus vehicle, ***P* < 0.001 versus vehicle (1-way ANOVA, Dunnett's multiple comparisons tests).

rotigotine HCl (0.5 mg/kg) or SER-212 (1.6 mg/kg) did not display elevated rotations compared to vehicle-treated controls. Lesioned animals treated with high-dose rotigotine (3 mg/kg) or either dose of SER-214 (1.6 or 6.4 mg/kg) had increased levels of contraversive rotations compared to controls (all $P < 0.001$; Fig. 5A). At day 5, there was a significant effect of time ($F_{35,1470} = 3.4$, $P < 0.001$), treatment ($F_{5,1470} = 50.0$, $P < 0.01$), and the interaction ($F_{175,1470} = 2.2$, $P < 0.001$; Fig. 5B) No rotational behavior was observed in animals previously treated with either dose of rotigotine (0.5 or 3 mg/kg), SER-212 (1.6 mg/kg) or the low dose of SER-214 (1.6 mg/kg). Interestingly, animals treated with high-dose SER-214 (6.4 mg/kg) continued to show prominent contraversive rotations compared to vehicle-treated rats at all time points (all $P < 0.001$). By day 9, there was a significant effect of time ($F_{35,1470} = 4.8$, $P < 0.001$), but not treatment ($F_{5,1470} = 0.3$, $P > 0.05$) or an interaction ($F_{175,1470} = 0.8$, $P > 0.05$). All rotational activity in each of the treatment groups ceased (Fig. 5C).

Treatment with dopaminergic drugs in 6-OHDA–lesioned rats also reduces deficits in contralateral limb use produced by DA depletion. One day after injection of vehicle, rotigotine, or POZ-rotigotine, rats were tested for forelimb asymmetry using the cylinder test and a significant effect of treatment was observed ($F_{5,46} = 7.1$, $P < 0.001$). Vehicle-treated, 6-OHDA–lesioned animals were characterized by an average forelimb asymmetry score of 88% ± 7%. In animals treated with high-dose (3 mg/kg), but not low-dose (0.5 mg/kg) rotigotine, the forelimb asymmetry score was significantly reduced compared to vehicle (9 ± 13%, $P < 0.01$; Fig. 6A). Treatment with SER-212 (1.6 mg/kg) was ineffective, while SER-214 completely abolished forelimb asymmetry at both doses (1.6 mg/kg; 0% ± 14%, 6.4 mg/kg; −2% ± 26%, both $P < 0.001$). As seen with rotational activity, persistent antiparkinsonian effects of SER-214 were also observed in the cylinder test (Fig. 6B). On day 5, there was a significant effect of treatment on asymmetry score ($F_{5,46} = 14.8$, $P < 0.001$). Control animals remained characterized by a forelimb asymmetry score of 88% ± 7%. There was no improvement of forelimb asymmetry in lesioned rats receiving rotigotine (0.5 or 3 mg/kg) or SER-212 (1.6 mg/kg). However, treatment with either dose of SER-214 (1.6 or 6.4 mg/kg) led to long-duration antiparkinsonian effects 4 days postinjection, by 62% and 100%, respectively, compared to vehicle (1.6 mg/kg; 31% ± 13%, 6.4 mg/kg; −6% ± 17%, $P < 0.01$ and $P < 0.001$, respectively). By day 9, forelimb asymmetry returned to baseline, and there was no effect of treatment ($F_{5,46} = 1.7$; $P > 0.05$; Fig. 6C).

Discussion

Traditional drug therapies for PD are significantly limited by their pharmacokinetic profile. The current study indicates that a novel POZ-rotigotine conjugate, SER-214, alters the pharmacokinetics of rotigotine in a manner likely to be associated with reduced motor complications. Importantly, these benefits were maintained with repeated dosing. In addition, SER-214 led to remarkably long-lasting antiparkinsonian effects in a 6-OHDA–lesioned rat model of PD. The continuous dopaminergic stimulation profile afforded by SER-214 could represent a significant advance in PD treatment, by avoiding motor complication side effects that occur with traditional pharmacotherapeutics.

POZ is a promising polymer currently available for bioconjugate chemistry that provides safe, stable, and easily-soluble drug conjugation.[21,22,24] Additionally, it can be conjugated in a "dose-loading" manner, allowing for control over the speed and amount of drug released from the polymer backbone.[23,24] Release of drug from POZ occurs via simple hydrolysis and POZ

is filtered unchanged by the kidneys (unpublished observations). Rotigotine was chosen as the candidate molecule for development since it is one of the most potent D2/D3 non-ergot agonists currently used clinically with proven safety and efficacy records in PD patients.[17,19,28] Rotigotine has essentially no oral bioavailability and is currently administered through the use of a daily transdermal patch. However, the patch often results in skin reactions and may fall off prematurely, leading to issues with patient compliance.[17,18,29,30] Thus, a new approach that results in a more consistent and accessible treatment option would be ideal for PD patients.

Three POZ-rotigotine conjugates were designed via linker technology to release rotigotine at different rates (Fig. 1). Figure 2 shows the release kinetics in rat plasma of SER-212, SER-213, and SER-214. SER-212 was designated as a "fast release" conjugate ($t_{1/2}$ = 2 hours), while SER-214 was designated as a "slow release" conjugate ($t_{1/2}$ = 12 hours). The in vivo release kinetics mirrored those seen in vitro (Fig. 3) and demonstrated similar extension in pharmacokinetics compared to other POZ-conjugated drugs.[24] When conjugates were administered subcutaneously, SER-214 had a much slower $t_{1/2}$ release rate (~40-50 hours) and did not show an initially high rate of release when compared to SER-212 (Fig. 3). Interestingly, in in vivo studies, the half-life of rotigotine was much longer than expected by in vitro studies, a scenario only explained by depot binding. Studies are ongoing to determine the fate of the drug and the POZ polymer. Additionally, the pharmacokinetic profile of SER-214 was unaltered by repeated weekly administration for up to 12 weeks in normal rats (Fig. 4). Rotigotine levels remained at or above the therapeutic range (0.5 ng/mL) up to 4 days postinjection with little variation in plasma levels, suggesting that SER-214 may be a good candidate for the continuous delivery of rotigotine.

The well-established 6-OHDA–lesioned rat model of PD was used to characterize the antiparkinsonian efficacy of SER-214, using both rotational activity and cylinder test assays. These assays are commonly used to indicate antiparkinsonian effects of other pharmacotherapeutics, including L-dopa, apomorphine, and other DA agonists. In the current study, SER-214 at both doses produced prompt and sustained contraversive rotations.. This effect persisted through day 5 in the high-dose group but was no longer evident by day 9 (Fig. 5). Despite the increase in rotations, animals continued normal behavior (eating/sleeping). The rotational effects were further validated by the cylinder test, a more direct measure of motor benefit.[31] SER-214 dose-dependently restored forelimb activity, and the benefit persisted through day 5 (Fig. 6), indicating the enduring antiparkinsonian effects of POZ-conjugated rotigotine.

In these studies, we have focused on the antiparkinsonian efficacy of SER-214, and have not assessed wearing-*off* or effects on dyskinesia. Though not specifically designed to monitor dyskinesia or wearing-*off* because a single dose was administered, there were no qualitative indications of such behaviors. While a long-duration antiparkinsonian effect alone would have potential value, in a clinical setting the most important characteristics will be reduced development and expression of motor complications due to continuous dopaminergic stimulation. A very extensive body of work suggests that an agent with an extremely long duration of action, such as SER-214, should have low liability to induce motor complications and should be useful in managing patients with established wearing-*off* and dyskinesia.[32] It is interesting that the rotational response at day 5 was greater than that on day 1, despite the lack of an increase in blood level. It is possible that this is a priming effect and will need to be examined through long-term, repeated-dosing studies in animal models both untreated and pretreated with L-dopa to induce wearing-*off* and dyskinesia.

POZ-conjugated rotigotine has potential to be a viable, once-per-week subcutaneous treatment for PD patients. The weekly regimen may have beneficial effects on patient compliance and provide continuous dopaminergic stimulation, believed to be necessary to avoid development of motor complications associated with pulsatile DA receptor stimulation. Future studies are necessary to characterize POZ-conjugated rotigotine with a broader range of doses, its effects in nonhuman primate PD models, and the development and manifestations of motor complications in response to repeated dosing. However, the possibilities for use of POZ-conjugation technology in PD treatment are obvious. More broadly, there is considerable potential for its use with any therapeutic that would benefit from an improved pharmacokinetic profile. ■

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