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Reversible Inhibitors of Monoamine Oxidase-A (RIMAs): Robust, Reversible Inhibition of Human Brain MAO-A by CX157

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Reversible inhibitors of monoamine oxidase-A (RIMA) inhibit the breakdown of three major neurotransmitters, serotonin, norepinephrine and dopamine, offering a multi-neurotransmitter strategy for the treatment of depression. CX157 (3-fluoro-7-(2,2,2-trifluoroethoxy)phenoxathiin-10,10-dioxide) is a RIMA, which is currently in development for the treatment of major depressive disorder. We examined the degree and reversibility of the inhibition of brain monoamine oxidase-A (MAO-A) and plasma CX157 levels at different times after oral dosing to establish a dosing paradigm for future clinical efficacy studies, and to determine whether plasma CX157 levels reflect the degree of brain MAO-A inhibition. Brain MAO-A levels were measured with positron emission tomography (PET) imaging and [^{11}C]clorgyline in 15 normal men after oral dosing of CX157 (20–80 mg). PET imaging was conducted after single and repeated doses of CX157 over a 24-h time course. We found that 60 and 80 mg doses of CX157 produced a robust dose-related inhibition (47–72%) of [^{11}C]clorgyline binding to brain MAO-A at 2 h after administration and that brain MAO-A recovered completely by 24 h post drug. Plasma CX157 concentration was highly correlated with the inhibition of brain MAO-A (EC_{50}: 19.3 ng/ml). Thus, CX157 is the first agent in the RIMA class with documented reversible inhibition of human brain MAO-A, supporting its classification as a RIMA, and the first RIMA with observed plasma levels that can serve as a biomarker for the degree of brain MAO-A inhibition. These data were used to establish the dosing regimen for a current clinical efficacy trial with CX157.
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INTRODUCTION

According to the World Health Organization, major depressive disorder (MDD) is predicted to become the second leading cause of disease burden worldwide (after HIV) by 2030 (Mathers and Loncar, 2006). This creates a sense of urgency to develop more effective antidepressant medications (Sen and Senacora, 2008). Monoamine oxidase inhibitors (MAOIs), such as tranylcypromine and phenelzine, were among the first drugs used to treat MDD (Yamada and Yasuhara, 2004). MAOIs are believed to act by increasing the brain levels of the mood-elevating neurotransmitters serotonin, norepinephrine and dopamine by blocking their catabolism. However, because of their non-selective and irreversible binding profile, these early MAOIs caused severe hypertensive events when patients consumed foods and beverages high in tyramine (a vasoactive metabolite of tyrosine, which is normally metabolized by MAO) (Yamada and Yasuhara, 2004). This well-known dietary interaction is now referred to as the *cheese effect* because of the high concentration of tyramine found in aged cheese (Blackwell *et al*, 1967; Anderson *et al*, 1993; DaPrada *et al*, 1988). Because of this safety concern, MAOIs have been largely supplanted by serotonin or serotonin/norepinephrine reuptake inhibitor drugs for the treatment of MDD (Sen and Senacora, 2008).

CX157 (3-fluoro-7-(2,2,2-trifluoroethoxy)phenoxathiin-10,10-dioxide, Figure 1) is an investigational compound currently in development for the treatment of MDD. It is a unique molecule with potent and specific MAO-A inhibiting

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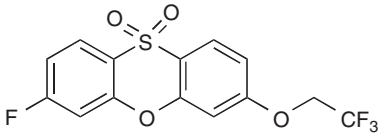

Figure 1 Chemical structure of CX157.

activity in mammalian tissues (3.3 nM in human brain) (data on file, CeNeRx Biopharma, Cary, NC). Mechanistic studies in animals have shown that CX157 acts to inhibit MAO-A activity in a reversible and competitive manner; elevating brain levels of all three monoamine neurotransmitters (data on file, CeNeRx Biopharma). Molecules showing this mechanism of action are classified as RIMAs, or reversible inhibitors of MAO-A, to distinguish them from the traditional, nonselective MAOIs (eg, tranylcypromine or phenelzine) that act irreversibly. Because serotonin, norepinephrine and dopamine are primarily metabolized by MAO-A (Bortolato *et al*, 2008), the RIMAs offer the potential for the mood-elevating effects of these three brain neurotransmitters, but without the safety concerns associated with dietary tyramine (Da Prada *et al*, 1988; Patat *et al*, 1995). In this regard, the reversible and competitive nature of the RIMAs on MAO-A allows for their displacement from binding to intestinal and hepatic MAO-A in the presence of increasing concentrations of dietary tyramine. In addition, because of the preference of these molecules for MAO-A, the inactivation of dietary tyramine and many other bioactive amines can still proceed through deamination by MAO-B (Anderson *et al*, 1993; DaPrada *et al*, 1988).

In this study, we used positron emission tomography (PET) to measure the *in vivo* inhibition of brain MAO-A by CX157 in normal human subjects. These experiments were conducted along a time course with several doses of an oral formulation of CX157, administered in single and repeated dosing regimens in order to determine the dose and time-related characteristics of brain MAO-A inhibition by CX157. Earlier another RIMA, moclobemide, had been shown to inhibit brain MAO-A (69–74%) at its therapeutically effective dose using [^{11}C]harmine (Ginovart *et al*, 2006). We used [^{11}C]clorgyline, a MAO-A-specific radiotracer previously used for measuring brain MAO-A activity in humans (Fowler *et al*, 1987, 1996, 2001a). Clorgyline is a mechanism-based inhibitor of MAO-A, which binds covalently to the flavin cofactor of the enzyme while it is in the enzyme-substrate complex. Thus, when clorgyline is labeled with carbon-11 and administered *in vivo*, the PET image represents functionally active MAO-A (Fowler *et al*, 1987). Plasma levels of CX157 at the time of the PET scans were also obtained to determine its relationship to the inhibition of [^{11}C]clorgyline binding to brain MAO-A. This study formed the basis for the CX157 dosing regimen in a recent clinical efficacy trial in the treatment of MDD (http://www.clinicaltrials.gov).

MATERIALS AND METHODS

Subjects

This study was carried out at Brookhaven National Laboratory and approved by the local Institutional Review Board (Committee on Research Involving Human Subjects, Stony Brook University) and by the Brookhaven Radioactive Drug Research Committee for compliance with 21CFR 361.1 radiation exposure limits for a nonpharmacological study. More specifically, an average dose of 7 μg mass clorgyline was administered for the PET scans, with the maximum dose being 10 μg (corresponding to a specific activity of 0.2 Ci/μmol at time of injection). We note that the pharmacological dose of clorgyline for treatment of depression is 2.5–10 mg/day (Potter *et al*, 1982); thus, the average and the maximum dose (7 and 10 μg, respectively), which we administered in each PET study, was far below the pharmacological dose of clorgyline. Fifteen normal healthy men whose average age was 33.5 ± 8.8 years (range: 23–50) and average BMI 25.4 ± 1.8 kg/m^2 (range: 25.4–28.5) were enrolled after informed consent was obtained and confidentiality of information was assured. A complete medical history was obtained on all subjects on the day of screening. Inclusion criteria allowed for healthy nonsmoking males who were able to understand and give informed consent, aged 18–64 years, with BMI $\geqslant 21$ and $\leqslant 30$ kg/m^2. To minimize confounds from prior drug exposures or comorbidity, we excluded subjects with a prior history of substance abuse or with positive urine drug screens, prior or current treatment with psychotropic medications, psychiatric comorbidities, neurological disease, medical conditions that may alter cerebral function (ie, cardiovascular, endocrinological, oncological, or autoimmune diseases), or head trauma with loss of consciousness (>30 min). All subjects had a urine drug screen (Biosite, San Diego, CA) on the day of each PET scan.

CX157 Administration

CX157 is not available commercially and is not a controlled substance. CX157 was provided to Brookhaven National Laboratory (Upton, NY) by CeNeRx BioPharma (Cary, NC), which were to be administered orally as CX157 Liquid Filled Capsules (AAIPharma, Wilmington, NC) on the day of dosing. CX157 Liquid Filled Capsules were packaged in HDPE bottles containing 30 liquid-filled gelatin Licaps capsules, each with 5 mg of active ingredient, fully solubilized in Labrasol at a concentration of 12.5 mg/ml (ie, each capsule contained 0.4 ml of solution). Each capsule was sealed with a layer of a gelatin/polysorbate-80 to prevent leakage. Capsules were re-weighed on the day of administration to assure that there was no leakage. For dosing at the 80 mg level, a 10 mg/ml solution of CX157 was prepared on-site within 1 h of use. An 8-ml aliquot was diluted and administered orally in 12 ml (0.4 oz) of a 67% aqueous Labrasol solution, followed by 8 oz of water.

Study Design

A sequential experimental design paradigm, the up-and-down rule (Rosenberger, 1996), was used to conduct this study. A dose range of 20–80 mg was chosen for these studies. PET data were analyzed after the initial two subjects for each dose, and this information was used to adjust the dosing and timing parameters for subsequent scans (ie, moving the dose up or down and/or changing the timing of the scans; see 'Results' for more details). Subjects either

received a single dose/24 h of CX157 or repeated b.i.d. dosing of CX157 for a week before scanning. All of the dose administrations were supervised by a nurse to ensure compliance. Plasma samples were obtained for the analysis of CX157 concentration (see below for details of the pharmacokinetic sampling). To capture all of the time points, some of the subjects were dosed and scanned on 2 different days, at least 1 week apart.

Single-dose studies. Each subject received a baseline scan and a scan at 2 h after a single oral dose of CX157 (20 ($n=2$), 40 ($n=3$), 60 ($n=3$) or 80 mg ($n=4$) and then two or three more scans over the next 24 h.

Repeated dose studies. Three subjects were scanned at baseline and then received oral doses of 40 mg of CX157 b.i.d. for 7 days (1030 and 2230 hours). At 12 h after the penultimate dose, they received a 12-h post-dose scan. After 1 h, they received another 40 mg dose of CX157 and three more scans at 2, 5 and 8 h post dose.

[^{11}C]Clorgyline PET Scans

[^{11}C]Clorgyline was synthesized as described previously (MacGregor *et al*, 1988). PET scans were obtained on a whole body, high-resolution positron emission tomograph (Siemens HR + $4.5 \times 4.5 \times 4.8\,mm^3$ at the center of the field of view) in a 3D dynamic acquisition mode for 60 min, as described previously (Fowler *et al*, 2001b; Alia-Klein *et al*, 2008). Each subject had 2–5 PET scans with [^{11}C]clorgyline (7.1 ± 0.6 mCi/scan; specific activity $\sim$250 mCi/μmol at the time of injection), one at baseline and the remaining within 24 h after a single dose of CX157 (20, 40, 60 or 80 mg of CX157) or after 1 week of supervised dosing of 40 mg of CX157 b.i.d.. Studies performed on the same day were separated by at least 2 h, and scans carried out on separate days were separated by at least 1 week. An arterial plasma input function for [^{11}C]clorgyline was measured using the same solid-phase extraction procedure, as reported previously (Alexoff *et al*, 1995). As a standard operating procedure, we carried out Allen's test in both wrists before arterial line insertion. Because subjects were studied on more than 1 day, we typically alternated wrists on different days of study. However, on some occasions, when the alternative wrist could not be catheterized, we inserted the arterial line in the same wrist but at a different site. One of the subjects received a repeat scan of his 2-h 60 mg dose 1 week after the first dose because of a very low level of brain MAO-A inhibition relative to the those who had been studied up to that point.

PET Image Analysis

Emission data were attenuation-corrected and reconstructed using filtered back projection. For the purpose of region identification for [^{11}C]clorgyline scans, time frames from dynamic images taken from 0 to 60 min were summed and then manually re-sliced along the AC-PC line. Planes were added in groups of two, placing the thalamus at plane 12, to obtain 23 planes for region of interest (ROI) placement. ROIs were drawn directly on PET scans using an atlas for reference. The regions were then projected to dynamic scans in order to obtain time–activity data. ROIs for the following brain areas were obtained: frontal cortex, parietal cortex, occipital cortex, visual cortex, temporal cortex, insula, orbitofrontal cortex, anterior cingulate gyrus, rectal gyrus, thalamus, caudate nucleus and putamen. ROIs were identified in at least two contiguous slices and the weighted average was obtained. For the thalamus, caudate, putamen, frontal, parietal and temporal cortices, the right and left regions were averaged.

For quantification of MAO-A binding, PET time–activity data for [^{11}C]clorgyline from different brain regions and time–activity data in arterial plasma were used to calculate the model term K_1, the plasma-to-brain transfer constant, which is related to blood flow, and λk_3 (a function of the concentration of catalytically active MAO-A molecules) (Fowler *et al*, 1996). The model term k_3 is related to the binding or trapping of [^{11}C]clorgyline by MAO-A; λ is defined as K_1/k_2 and is independent of blood flow (Logan *et al*, 1991) and k_2 is related to the efflux of tracer from brain to blood.

Estimation of the Degree of Inhibition of [^{11}C]Clorgyline Binding to MAO-A by CX157

For each subject, a λk_3 value for each of the 12 ROIs was determined at baseline and at different times after CX157 administration. A composite global λk_3 value was also determined from an average of the individual λk_3 values at baseline and at each time after CX157 administration. In order to determine the percent inhibition of MAO-A, the percent change in λk_3 relative to baseline for each of the ROIs was determined and then these values were averaged to give an average percent change at each CX157 dose and each time after CX157 administration. These values were used to determine the percent change (mean ± SD) at each time point for each dose group. Because the irreversible MAO inhibitor clorgyline inhibits the activity of MAO by binding at or near the flavin portion (the active site) of the enzyme (De Colibus *et al*, 2005), so that the CX157 interaction was impaired, the inhibiting action of CX157 on [^{11}C]clorgyline binding to MAO-A was used as a 'surrogate' measure of its pharmacodynamic effects as a RIMA.

We calculated the coefficient of variation (CV) (Carson, 1986) for our parameter λk_3 for three studies with injected doses of 6.7, 7.3 and 6.6 mCi. The average CVs for λk_3 over all ROIs were 0.057 ± 0.033, 0.055 ± 0.034 and 0.048 ± 0.03 for the three studies, respectively. CVs and the test–retest reproducibility of [^{11}C]clorgyline binding showed in earlier studies (Fowler *et al*, 1996, 2001a) indicate that that our model parameter λk_3 is sufficiently well determined from ROI data.

Statistical Analysis

A one-way ANOVA analysis was used to examine whether there was a significant dose-related inhibition of [^{11}C]clorgyline binding at 2 h for the 20 *vs* 40 *vs* 60 *vs* 80 mg single dosage. Multiple comparisons determined by the Tukey method at the family-wise error rate of 0.05 (two-sided) were used to examine pair-wise differences. One-way repeated-measures ANOVA with *post hoc* paired sample *t*-test were used to determine whether there was a time-related

inhibition of [^{11}C]clorgyline binding for each dose regime. A two-way repeated-measures ANOVA was used with group (single 40 mg *vs* b.i.d. 40 mg) as a fixed factor and repeated measures on time (2, 5, 8 and 12 h) to examine whether MAO-A inhibition differs after single 40 mg *vs* b.i.d. 40 mg dosing.

Pharmacokinetic Sampling and Analysis

Plasma samples for pharmacokinetic analysis were obtained from each subject before CX157 administration (baseline), at the beginning of each PET scan and at several additional time points between 5 min and 24 h after dose administration. Blood samples (10–16 ml) were drawn into K_2 EDTA-containing tubes, which were centrifuged to obtain plasma. Plasma was frozen at -70°C before analysis. Concentrations of CX157 in plasma were determined using a validated LC/MS method with a lower limit of quantification of 0.5 ng/ml. To investigate the pharmacodynamic relationship between plasma concentrations of CX157 and the inhibition of [^{11}C]clorgyline binding to MAO-A in the brain, the observed binding inhibition (percent inhibition of λk_3 values) was plotted against the plasma concentration observed at the beginning of each PET scan measurement, using the data from all subjects and time points. The mean value of MAO-A inhibition (over all individual ROIs) from each PET scan was plotted against the plasma CX157 concentration obtained at the time of the scan. The resulting plot was fit to an E_{max} response model:

$$\%\,\text{Inhibition} = (E_{max} \times \text{concentration})/(\text{EC}_{50} + \text{concentration})$$

where EC_{50} is the concentration producing a 50% inhibiting response and E_{max} the maximum inhibiting response. All available data pairs were fit by nonlinear regression (Levenberg–Marquardt algorithm) to obtain estimates of the parameters EC_{50} and E_{max}. Reasonable fits to the data set were also obtained using the Hill equation, the E_{max} model with an E_0 term and the Hill equation with an E_0 term. The models were compared by determining the Akaike Information Criteria (AIC) (Akaike, 1976) for each model, and the simple E_{max} model was selected because it had the lowest value of the AIC. Pharmacokinetic and pharmacodynamic calculations were determined using SigmaPlot 9.0 software (San Jose, CA).

RESULTS

A total of 16 subjects were enrolled and 15 completed the study and were included in the data analysis. No serious adverse events (AEs) or deaths occurred during the study. The overall incidence of AEs was 13.3%. Two subjects experienced AEs. One subject received a 40-mg dose of CX157 b.i.d. for 8 days and reported chest pain, palpitations, upper abdominal pain and abnormal spermatozoa progressive motility (the latter was an incidental finding reported by the subject and not a test performed as part of the trial). The other subject received an 80 mg dose of CX157 emulsion with Labrasol and vomited shortly after dosing. None of the AEs were serious and the events were reported as mild to moderate in intensity and resolved without intervention.

By choosing the up-and-down rule (Rosenberger, 1996), we were able to rapidly assess the dose of CX157 and the timing for the scans from the first few subjects. For example, the first two subjects received a 40 mg dose of CX157 and PET scans were conducted over a 24-h period. The PET scans from these two initial subjects were rapidly analyzed in order to decide whether to move the dose up or down and whether the performance of a scan 24-h post drug would be useful. After finding a robust inhibition ($\sim$60%) at 40 mg CX157 and complete recovery of MAO-A activity at 24 h, we moved the dose of CX157 down to 20 mg to assure that we were not on a plateau. We also learned that a 24-h scan was unnecessary. Results from the two subjects who received a dose of 20 mg CX157 showed a lower inhibition of [^{11}C]clorgyline binding to MAO-A, and thus the subsequent doses of CX157 were $\geqslant$40 mg. We also note that the ability to rapidly obtain PK analysis for plasma drug levels proved very valuable in these initial studies.

Single-Dose Studies

Initial PET scans were conducted after single doses of CX157 to determine the extent of inhibition of [^{11}C]clorgyline binding to MAO-A. Baseline K_1 values averaged 0.41 ± 0.05, 0.41 ± 0.05 and 0.42 ± 0.05 ml/min/cc for the 2, 5 and 8 time points, respectively, and there was no significant difference after CX157 ($p > 0.08$, paired *t*-test). The baseline λk_3, which is the measure of MAO-A binding in the absence of CX157, averaged 0.34 ± 0.044 cc_{brain}/ml_{plasma}/min (range 0.27–0.42) for all subjects. Intersubject variability in the percent (%) inhibition of [^{11}C]clorgyline binding was observed at each dose of CX157 (see Table 1). With the exception of the 20 mg dose group, the inhibition of [^{11}C]clorgyline binding by CX157 was robust, with a peak effect (47–72%) observed at the 2-h time point (the earliest time point examined; Figure 2).

There was a significant dose-related inhibition of [^{11}C]clorgyline binding at 2 h for the 20 *vs* 40 *vs* 60 *vs* 80 mg single dosages (one-way ANOVA, $F = 5.76$; d.f.s = 3,7; $p = 0.03$). Multiple comparisons by Tukey's method at the family-wise error rate of 0.05 (two-sided) indicated that the only significant pairwise difference occurred between 80 mg dose and 20 mg dose of Cx157 (mean difference = 53.8%; 95% CI: (10.4, 97.3)).

One-way repeated-measures ANOVA with *post hoc* paired samples *t*-test indicated a significant decrease ($p < 0.05$) in λk_3 relative to baseline at all time points examined for the 80 mg single-dose group, but only a significant decrease ($p < 0.05$) at the 2-h time point for the 40 and 60 mg single-dose group (Table 1). Inhibition of [^{11}C]clorgyline binding by CX157 was rapidly reversible with only 6.8 ± 14.6 and 17.6 ± 17.9% inhibition remaining by 12 h for the 40 and 60 mg dose groups, respectively ($p > 0.05$ *vs* baseline). Complete recovery to baseline values of the inhibition of MAO-A binding was observed by 24 h after the 40 mg dose of CX157 (see Figure 2 for average % inhibition at different times after CX157 for each dose group). Representative brain images at baseline and at 2 and 12 h after oral administration of a single dose of 60 mg of CX157 for one of the subjects are shown in Figure 3.

Table 1 Average Percent Inhibition of [^{11}C]Clorgyline Binding (λk_3) for Different Doses and Different Times after CX157 along with Average Plasma CX157 (ng/ml) and Range

Time (h)	% MAO-A inhibition (CX157; range)				
	Single dose (20 mg, $n=2$)	**Single dose (40 mg, $n=3$)**	**Single dose (60 mg, $n=3$)**	**Single dose (80 mg, $n=4$)**	**Multi-dose (40 mg BID. $n=3$)**
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
2	18.5 ± 10.6 (6.1 ± 3.3; 3.8–8.1)	54.7 ± 8.9 (38.3 ± 18.5; 18.4–55.1)	46.8 ± 22.6[a] (60.6 ± 84.4; 7.6–158)	72.3 ± 8.7 (137 ± 58.8; 70.7–183)	48.3 ± 9.5 (37.5 ± 11.3; 24.5–45)
5	18.8 ± 6.7 (2.7 ± 1.1; 1.9–3.4)	34.5[b] (8.4)	31.6 ± 27.4 (24.1 ± 37.4; 3.6–67.3)	48.6 ± 12.1 (37.1 ± 25; 13.5–70.2)	34.1 ± 7.8 (19.2 ± 5.8; 13.1–24.7)
8	ND	6.5[b] (1.8)	23.4 ± 30.0 (9.1 ± 8.0; 3.4–14.7)	30.4 ± 8.9 (11.7 ± 5.2; 5.7–15.4)	32.2 ± 6.1 (15.5 ± 4.4; 10.8–19.5)
10	ND	ND	ND	ND	27.3[b] (6.9)
12	12.4 ± 10.8 (1.1 ± 0.7; 0.6–1.6)	6.8 ± 14.6 (2.2 ± 1.5; 0.9–3.9)	17.6 ± 17.9 (6.5 ± 6.6; 1.8–11.1)	43.1[b] (16.6)	23.1 ± 3.7 (10.0 ± 1.4; 9–11)
24	ND	−1.8 ± 0.7 (2.8 ± 1.3; 1.8–3.7)	ND	ND	ND

ND, not determined.
[a]Includes only the re-scanned λk_3 (subject 789).
[b]Single subject value.

Repeated Dose Studies

The level of [^{11}C]clorgyline binding to MAO-A was also examined after repeated oral dosing of CX157. Three subjects were scanned at baseline, dosed orally for 1 week with 40 mg of CX157 b.i.d., and then re-scanned at 2, 5, 8 and 12 h after the last dose of CX157. Average data for changes in MAO-A inhibition for these three subjects along with average plasma CX157 levels are presented in Table 1 and in Figure 2e. Two-way repeated-measures ANOVA with group (single 40 mg *vs* b.i.d. 40 mg) as a fixed factor and repeated measures on time (2, 5, 8 and 12 h) showed that the percent [^{11}C]clorgyline inhibition did not differ significantly between the single-dose 40 mg group and the b.i.d. 40 mg group at 2 h (54.7 ± 8.9 *vs* 48.3 ± 9.5%); $F=0.14$; d.f.s = 1,2; $p=0.75$) nor at 5 h ($F=0.00$; d.f.s = 1,2; $p=0.97$). However, there was a trend of a difference at 8 h ($F=13.12$; d.f.s = 1,2; $p=0.07$), and at 12 h, we evidenced a significant difference in percent inhibition, which seems to support the notion of an extended biological half-life with repeated dosing (23.1 ± 3.7 *vs* 6.8 ± 14.6%; $F=60.27$; d.f.s = 1,2; $p=0.02$). This observation was also supported by higher plasma CX157 levels at 12 h with repeated dosing (Table 1). As expected, we also observed a significant difference in time ($F=77.86$; d.f.s = 3,6; $p<0.0001$), and also a significant time and group interaction ($F=17.21$; d.f.s = 3,6; $p=0.002$).

Plasma CX157 Concentration

Plasma concentration *vs* time profiles in subjects receiving single or repeated doses of CX157 showed that CX157 was rapidly absorbed after oral administration, with an overall median T_{max} (time of maximum plasma concentration) of 0.5 h (range: 5 min to 1 h). After reaching peak levels, plasma concentrations declined in a multi-exponential manner, with most of the plasma exposure occurring within the first 4–6 h after dosing. Plasma concentrations were generally proportional to the administered dose, with some intersubject variability. The half-life of CX157 in plasma averaged 6.0 ± 3.4 h.

Brain MAO-A Inhibition *vs* Plasma CX157 Concentration

A plot of percent inhibition of [^{11}C]clorgyline binding to brain MAO-A by CX157 at each dose and at each time point *vs* CX157 concentration obtained at the beginning of each scan showed that these two parameters were highly correlated ($R^2=0.9353$; $p\leqslant0.05$), and when the data were fit to a sigmoid E_{max} model (Figure 4), using non-linear regression, the following parameter estimates (and the 90% CIs) were obtained: $E_{max}=80.7\%$, $EC_{50}=19.3$ ng/ml and $n=1.0$. The estimated E_{max} value (80.7%) suggests that a majority of the [^{11}C]clorgyline bound to MAO-A at baseline could be inhibited by CX157. However, because E_{max} was $<100\%$, it is possible that a small fraction of the PET signal resulted from [^{11}C]clorgyline that was not bound to MAO-A.

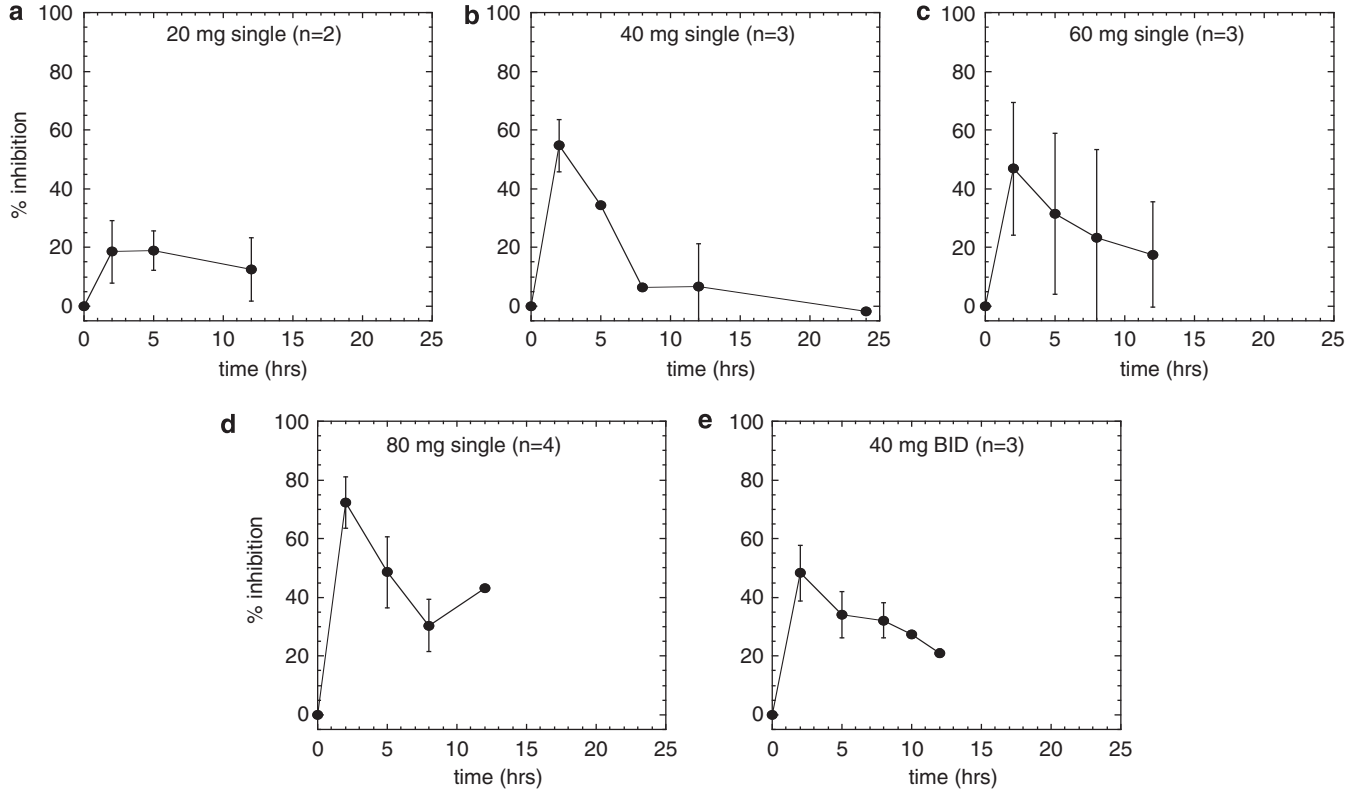

Figure 2 (a–e). Percent inhibition of [^{11}C]clorgyline binding to brain MAO-A ($\Delta\lambda k_3 \times 100$) for different dose groups at different times after a single dose (20, 40, 60 and 80 mg) of CX157 (a–d) and after repeated CX157 doses of 40 mg b.i.d. for 7 days (e).

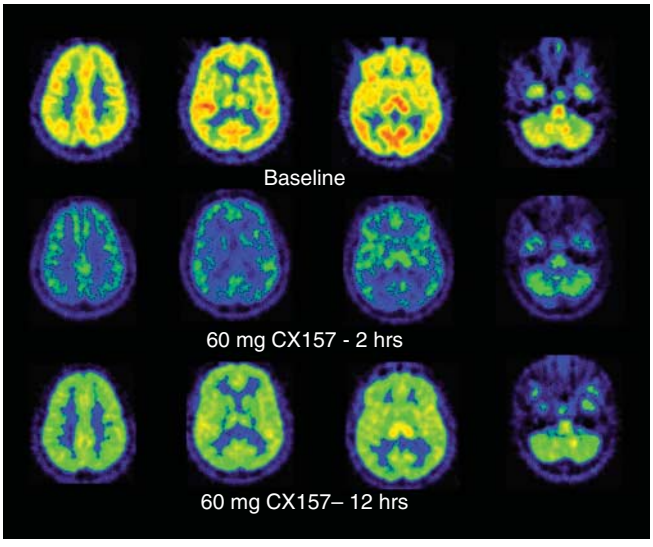

Figure 3 PET images of [^{11}C]clorgyline binding in the brain in one of the subjects showing four different planes in the transaxial view at baseline (top row), 2 h (middle row) and 12 h (bottom row) after a single 60 mg dose of CX157. Images were obtained by summing the time frames from 30 to 60 min and normalizing to the concentration of [^{11}C]clorgyline in the arterial plasma. A rainbow color scale is used, where red represents the highest level of [^{11}C]clorgyline binding.

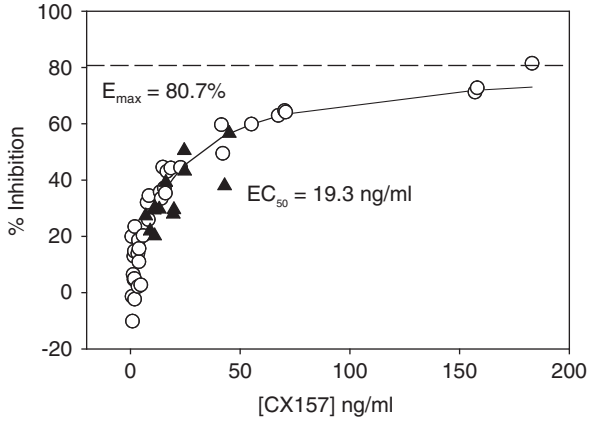

Figure 4 Plot of percent inhibition of [^{11}C]clorgyline binding to brain MAO-A ($\Delta\lambda k_3 \times 100$) *vs* plasma CX157 concentration measured at the beginning of each PET scan. Open circles indicate individual data points from subjects receiving a single dose of CX157. Data points from subjects who received repeated dosing with CX157 are shown as solid triangle. The line shows the data fit to an E_{max} model: percent inhibition $= (E_{max} \times \text{concentration}^n)/(EC_{50}^n + \text{concentration}^n)$, where $E_{max} = 80.7\%$, $n = 1.0$, and $EC_{50} = 19.3$ ng/ml.

DISCUSSION

The use of PET imaging, with highly selective radiotracers, is increasing in the area of pharmaceutical research to determine the *in vivo* human pharmacokinetics and/or pharmacodynamics of new chemical entities, and even approved drugs, that act directly in the brain (Fowler *et al*, 1999; Nutt *et al*, 2007; Kegeles *et al*, 2008; Volkow *et al*, 2009). PET imaging is a safe and relatively noninvasive method to determine CNS penetration and distribution, and for targeting pharmacodynamic activity at molecular brain

sites. Carbon-11, because of its short half-life (20.4 min), permits serial studies in the same individual (baseline PET scan and up to three more PET scans in a day), allowing an individual to serve as his/her own control and reducing the effect of inter- and intrasubject variability. In addition, plasma drug concentrations obtained at the time of PET imaging can serve as a means of showing whether there is a relationship between plasma drug levels and pharmacodynamic effects in the brain. When these two measures are positively correlated, plasma drug levels can serve as a biomarker for pharmacodynamic effects of a drug in the brain and assist in the selection of dosing regimens for future efficacy trials, thereby reducing the length and cost of drug development.

PET studies have been carried out with different MAO subtype-selective [^{11}C]radiotracers to measure the degree, duration and specificity of brain MAO inhibition by various drugs in humans and to determine dosing intervals. These studies have been conducted with both reversibly binding radiotracers, such as the MAO-A radiotracer [^{11}C]harmine (Bergström *et al*, 1997; Ginovart *et al*, 2006), and irreversibly binding radiotracers such as the MAO-A and MAO-B radiotracers [^{11}C]clorgyline and [^{11}C]L-deprenyl-D2, respectively (Fowler *et al*, 1993, 1994, 1996, 2001a; Bench *et al*, 1991). Irreversibly binding radiotracers such as [^{11}C]N-methylspiperone have also been used to measure dopamine D2/D3 receptor occupancy by neuroleptic drugs (Wong *et al*, 1986). When quantifying data with irreversibly binding radiotracers such as [^{11}C]clorgyline, the potential of flow limitation in brain regions with high enzyme activity and low blood flow must be considered. However, the dynamic range of values for DV and for λk_3 for the thalamus–cerebellum for [^{11}C]harmine and for [^{11}C]clorgyline are 2.1 and 2.5, respectively (data from Ginovart *et al*, 2006 and Fowler *et al*, 1996). This data comparison suggests that the effect of blood flow is minimal for [^{11}C]clorgyline using the modeling strategy described in this study.

Using [^{11}C]clorgyline as the radiotracer, we have shown that CX157 enters the brain rapidly after oral dosing, producing a dose- and time-related inhibition of brain MAO-A in human subjects. The effect of CX157 was greatest at 2 h and recovered to baseline within 24 h after a single dose. This indicates the reversible nature of the inhibition of brain MAO-A by CX157 and supports its classification as a RIMA. After repeated b.i.d. dosing with CX157, a more sustained inhibition of MAO-A over the 12-h period was observed. These latter data suggest that continuous therapeutic levels of MAO-A inhibition could be achieved with multiple dosing regimens of CX157.

Intersubject variability in the percent MAO-A inhibition was observed in some of the subjects receiving the same dose of CX157 measured at the same specific time point. This variability appeared related to observed individual differences in CX157 pharmacokinetics, as supported by the high correlation between the plasma concentration of CX157 and the percent inhibition of [^{11}C]clorgyline binding for all subjects, at all doses and at each time point examined (Figure 4). This strong correlation also suggests that a rapid equilibrium exists in the distribution of CX157 between plasma and CNS compartments, and that the activity of CX157 at the effector site (brain MAO-A) is proportional to its concentration in plasma at any given time.

When the data were fit to a sigmoid E_{max} model using nonlinear regression, it showed that plasma concentration of CX157 could serve as a biomarker of the pharmacodynamic inhibition of brain MAO-A, supporting the use of this relationship for modeling therapeutic dosing regimens of CX157, on the basis of the existing pharmacokinetic database. In future efficacy trials, it may also be possible to determine more accurately the therapeutic level of brain MAO-A inhibition through a comparison of individual clinical outcome measures and corresponding steady-state plasma concentrations of CX157. In this regard, traditional MAO inhibitor drugs (eg, tranylcypromine and phenelzine) have been prescribed for decades, but the degree of MAO-A inhibition required to produce an antidepressant effect has never been directly determined by comparing brain MAO-A inhibition and clinical response in patients. Peripheral measures of monoamine metabolites (eg, 3,4-dihydroxyphenylglycol (DHPG) or 3-methoxy-4-hydroxyphenylglycol (MHPG)) in individuals taking MAOIs have provided a broad range of estimates (ie, 20–80% of inhibition of MAO-A) for therapeutic efficacy with these drugs (McDaniel, 1986; Berlin *et al*, 1990, 1995; Holford *et al*, 1994; Radat *et al*, 1996; Zimmer, 1990), leaving investigators unsure of a therapeutic dose range for clinical trials.

The most widely studied RIMAs are moclobemide, toloxatone, befloxatone and brofaromine. These RIMAs appear to be comparable with other antidepressant drugs in terms of efficacy and tolerability (Lotufo-Neto *et al*, 1999). However, none of the RIMAs are approved in the United States and are therefore without an effect on the treatment of MDD. For most of the RIMAs studied, therapeutic dose ranges were established by traditional methods. Initially, moclobemide and esuprone were evaluated *in vivo* using PET imaging with [^{11}C]harmine, although a kinetic modeling analysis was not applied (Bergström *et al*, 1997). Similar to CX157, both of these drugs showed their peak effect on MAO-A at early times after the administration of the last dose. But unlike CX157, moclobemide and esuprone showed only a slight tendency for reversal of MAO-A inhibition by 11 and 23 h, respectively, after treatment cessation. In addition, there was no correlation observed between plasma drug levels and brain MAO-A inhibition for esuprone, and plasma pharmacokinetic values were not reported for moclobemide. More recently, Ginovart *et al* (2006) developed a kinetic modeling approach to quantify [^{11}C]harmine binding after treatment with moclobemide. In that study, they were able to analyze that a therapeutic dose of moclobemide (300 mg b.i.d.) inhibited between 64 and 79% of [^{11}C]harmine binding, but no moclobemide plasma pharmacokinetics were reported. Therefore, it would appear that CX157 is the first agent in the RIMA class with documented reversible inhibition of brain MAO-A, which is correlated to its plasma concentration. Given the observed correlation between inhibition of brain MAO-A binding and plasma pharmacokinetics of CX157, it is likely that these data will assist in optimizing the therapeutic dose and obtaining regulatory approval for the clinical use of CX157 in this therapeutic area. In this regard, the pharmacodynamic/pharmacokinetic relationship observed using CX157 may provide a means of establishing a therapeutic dose in MDD that would minimize side effects for this agent and further reduce the risk of dietary interactions.

Because serotonin, norepinephrine and dopamine are metabolized by MAO-A, drugs that inhibit MAO-A cause an elevation in these three monoamine neurotransmitters in the brain (Da Prada *et al*, 1989). These neurotransmitters are involved in mood, arousal and the sense of well-being, and thus their elevation in the CNS is believed to be the main mechanism underlying the efficacy of MAO-A inhibitor drugs in the treatment of MDD (Chen *et al*, 2008). The neurobiological rationale for the efficacy of MAO-A inhibitors and other drugs that increase these neurotransmitters received strong support by the recent discovery that individuals with MDD have elevated levels of brain MAO-A (Meyer *et al*, 2006). This finding is consistent with a low monoamine synaptic activity in depressed patients and with the efficacy of MAO-A inhibitors and other drugs that increase these neurotransmitters.

CONCLUSION

CX157 is the first RIMA with demonstrated reversible inhibition of MAO-A in the human brain (as assessed through its inhibition [^{11}C]clorgyline binding to MAO-A), for which brain MAO-A inhibition has been shown to be proportional to and predictable from plasma drug levels. This is significant because of the need to optimize drug dosing and timing for clinical efficacy trials based up the knowledge of its pharmacodynamics in the brain. This study validates the use of plasma CX157 concentrations as a surrogate biomarker for brain MAO-A inhibition. In this regard, plasma concentrations of CX157 can be used without PET scan to model dosing regimens for future efficacy trials, optimize the therapeutic dose and determine the optimal degree of MAO-A inhibition required for clinical efficacy.

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DISCLOSURE

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