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Time Course of 5-HT_{2A} Receptor Occupancy in the Human Brain after a Single Oral Dose of the Putative Antipsychotic Drug MDL 100,907 Measured by Positron Emission Tomography

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MDL 100,907 is a potent and selective antagonist of 5-HT_{2A} serotonin receptors. Animal studies suggest that MDL 100,907 may behave as an atypical antipsychotic drug. Positron emission tomograph (PET) using [^{11}C]NMSP as the radiotracer was used to define the time course of 5-HT_2 receptor occupancy in the human frontal cerebral cortex after a single oral dose of MDL 100,907 (10 or 20 mg) in nine healthy subjects. After the baseline scan each subject was studied three times post dosing at various time points. 5-HT_2 occupancies were in the range of 70 and 90% after each dose. While the occupancy remains in this range over 24 hours after 20 mg MDL 100,907, it decreases by about 20% at 24 hours compared to the timepoint at 8 hours, when only 10 mg are administered ($p < 0.05$). Our results should allow determination of the appropriate dosing regimen for future trials in schizophrenic patients.
[Neuropsychopharmacology 17:175–185, 1997]

KEY WORDS: *Positron Emission Tomography; Human; 5-HT_2 receptors; MDL 100,907; Antipsychotic drugs*

INTRODUCTION

The dopamine hypothesis of schizophrenia, which assumes that at least the positive schizophrenic symptoms are due to an excess of dopaminergic neurotransmission, is based on the observations that typical antipsychotic drugs block D_2-like dopamine receptors, and that dopaminergic agents such as cocaine or amphetamine can induce a psychosis that is indistinguishable from the paranoid subtype of schizophrenia (Carlsson 1988). This hypothesis has been supported by positron emission tomography (PET) studies which show that treatment of schizophrenic patients with neuroleptics at clinically relevant doses leads to a high occupancy of D_2-like dopamine receptors in the range of 65–90% (Farde et al. 1992).

N-methylspiperone (NMSP) has been demonstrated as a radioligand which binds to both D_2 dopamine receptors primarily in the basal ganglia and to 5-HT_2 receptors primarily in cortical regions (Wong et al. 1984; Lyon et al. 1986). With PET and [^{11}C]NMSP as the radiotracer, it has been shown that relatively low doses

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Received August 1, 1996; revised December 30, 1996; accepted January 31, 1997.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/97/$17.00
PII S0893-133X(97)00044-4

(125–200 mg/day) of the prototype atypical antipsychotic clozapine lead to very high 5-HT_2 receptor occupancies ranging between 80% and 90%; only about 20–30% of D_2-like dopamine receptors are occupied by such low doses of clozapine (Nordström et al. 1993). Therefore, clozapine's "atypical" clinical profile may be explained by its relatively low occupancy of D_2-like dopamine receptors combined with its high affinity for 5-HT_2 receptors (Meltzer et al. 1989). Consequently, antipsychotics with combined dopamine and serotonin antagonism such as risperidone were developed to test this hypothesis. Moreover, there is clear evidence from animal studies that the serotonin system plays a modulating role on the function of the nigro-striatal and ventral tegmental-limbic dopamine systems, especially when the dopamine systems are activated (Palfreyman et al. 1993).

The putative atypical antipsychotic MDL 100,907 (R-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)-ethyl]-4-piperidine-methanol) binds with high affinity (K_i = 0.36 nM) and selectivity to the 5-HT_{2A} serotonin receptor. MDL 100,907 is considerably less potent at the 5-HT_{2C} (105 nM) and the α_1 adrenergic receptor (545 nM). The affinity for dopamine D_2-like, muscarinic cholinergic, 5-HT_{1A}, 5-HT_3, GABAergic, histamine H_1, and strychnine sensitive glycine receptors is negligible (K_{is} > 1000 nM) (Palfreyman et al. 1993). In animal studies, MDL 100,907 markedly diminishes the activation of the dopaminergic system produced by amphetamine or 3,4-methylene dioxymethamphetamine (MDMA), whereas basal dopaminergic function is not altered by MDL 100,907 (Palfreyman et al. 1993; Schmidt et al. 1992). MDL 100,907, like clozapine, increases dopamine release in the prefrontal cortex of freely moving rats (Schmidt and Fadayel 1995). Moreover, chronic treatment with MDL 100,907 produces a significant decrease in the number of active ventral tegmental (A10) dopamine neurons, while dopamine neurons in the substantia nigra (A9) are inhibited to a much lesser extent (Palfreyman et al. 1993). MDL 100,907 shares this electrophysiological profile with clozapine. From these results, activity both against positive and negative symptoms of schizophrenia without the liability for extrapyramidal side effects has been predicted for MDL 100,907. However, the selection of a therapeutic dose and dose regimen will be critical for the initial efficacy trial. While attempts at using preclinical models, pharmacokinetic parameters and peripheral surrogate markers may have been used previously to estimate initial dose regimens, these methods are not entirely satisfactory. There are marked differences between species in pharmacological response, absorption, metabolism, and excretion, making suspect the extrapolation of doses and dose regimens between animal models and the clinic.

The objective of this study was to determine, using positron emission tomography (PET) methodology, the time course of 5-HT_2 receptor occupancy in the human brain after a single oral dose of MDL 100,907, and the relationship between plasma levels of MDL 100,907 and the degree of central 5-HT_2 receptor occupancy at various post-dose time points. This should allow determination of the appropriate dosing regimen for future clinical trials in patients with schizophrenia. Therefore, brain 5-HT_2 receptor occupancy, as judged by PET using [^{11}C]NMSP as a radioligand for the 5-HT_2 receptor, was the primary outcome variable in this study. Tolerability was determined on adverse experiences, vital signs, and electrocardiogram.

SUBJECTS AND METHODS

The study was approved by the Johns Hopkins Joint Committee on Clinical Investigation and the Radiation Safety Committee.

Subjects

Nine normal volunteers (three female, six male) gave written informed consent to be included in the study. The age of the subjects ranged from 23 to 43 years (mean: 32.7 years; SD, 7.0 years). The mean height was 174.2 cm (range: 165 to 187.5 cm; SD, 7.4 cm), the mean weight 73.9 kg (range: 61.4 to 86.4 kg; SD, 9.8 kg). They were healthy according to medical history, physical examination, 12-lead electrocardiogram (ECG), fasting clinical laboratory tests, and a magnetic resonance imaging (MRI) of the brain. All subjects were free from any medication for at least 14 days prior to the start of the study, except for subject J.W., who was administered 1 mg of lorazepam intravenously (i.v.) prior to the MRI scan the day before the PET studies due to the experience of claustrophobia in the MRI scanner. Exclusion criteria comprised the presence of a relevant medical illness or a history of, or active, mental disorder according to DSM IV. Subjects with a history of exposure to psychotropic drugs for any indication were also excluded from participation in the study. Females of child-bearing potential had to be using a medically accepted contraceptive method to be eligible for study enrollment. Further, a negative urine pregnancy test had to be documented at screening and immediately prior to the first PET scan. All subjects were non-smokers for at least 60 days prior to the study. Moreover, they had to refrain from the consumption of alcohol from 48 hours prior until the end of the study, and from the consumption of caffeinated beverages from 12 hours prior until the end of the study.

PET Procedure

Four PET experiments were performed on each subject. All subjects were fasting until one hour after drug ad-

ministration, when they received a snack. Six subjects received a single oral dose of 20 mg MDL 100,907 after the baseline scan. For calculation of the 5-HT_2 receptor occupancy, the PET procedure was repeated either 2 hours, 5 hours, and 8 hours post dosing (n = 3), or 2 hours, 8 hours, and 24 hours post dosing (n = 3). Three additional subjects received only 10 mg MDL 100,907; they were studied 2 hours, 8 hours, and 24 hours post dosing. The dose to be administered was determined by a dose-finding PET study. In this study, 10 to 20 mg of MDL 100,907 were determined to displace approximately 80% of total [^{11}C]NMSP bound (unpublished data).

To establish the position of the PET scanner relative to the subject's head, a thermoplastic face mask was molded on the face of the subject prior to the first scan. A MRI scan was then performed with the face mask in place to determine the ac-pc line, which was marked on the face mask by a permanent ink line. The PET scanner was aligned according to this line. PET scans were coregistered with MRI scans for accurate anatomical positioning of regions of interest (frontal cortex and cerebellum) using the computer program "Register" developed at the McGill University, Montreal, Canada (Evans et al. 1991). This program reslices the MRI images to the plane of the PET images. Polygonal regions of interest (ROIs) were drawn on planes, where the frontal cerebral cortex, a region rich in 5-HT_2 receptors, and the cerebellum, which was chosen as a reference area, have maximal areas. The density of 5-HT_2 receptors in the cerebellum was assumed to be negligible (Schotte et al. 1983; Pazos et al. 1987). ROIs were drawn bilaterally, and right and left values were averaged for subsequent analysis.

Radiochemistry and Data Acquisition

The radiotracer [^{11}C]NMSP was synthesized according to the method described by Dannals et al. (1986). The specific activity at the time of injection was 82.3 ± 26.1 GBq/μmole (2224.1 ± 704.7 mCi/μmole, mean ± SD), the range was 50.8 GBq/μmole (1373.7 mCi/μmole) to 163.1 Gbq/μmole (4408.1 mCi/μmole). The injected mass, as determined by high performance liquid chromatography (HPLC), was in the range between 2.1 μg to 6.9 μg (mean 4.4 μg; SD 1.2 μg). Specific activities did not significantly differ between subjects either at baseline or after administration of MDL 100,907.

[^{11}C]NMSP (666–740 MBq (18–20 mCi)) was injected into an antecubital vein over 20–30 seconds for each PET scan. All scans were performed using a GE 4096+ PET scanner with an in plane resolution of 5–6 mm and axial resolution of 6–7 mm. Data acquisition comprised a series of 50 time frames in 15 planes. The scan duration increased progressively from 15 seconds to 6 minutes (9 scans of 15 seconds, 16 scans of 30 seconds, 6 scans of 1 minute, 6 scans of 2 minutes, 11 scans of 4 minutes, 3 scans of 6 minutes), and the total scanning time was 90 minutes. A 10-minute transmission scan using a Ge-68 source was carried out prior to each study for subsequent attenuation correction. Arterialized venous plasma samples for determination of the kinetics of total plasma radioactivity were obtained from the dorsum of a hand warmed by a heating pad to 44° C according to the following protocol: 7–10 second intervals for the first 2 minutes, 30 second intervals up to 3 minutes post injection (p.i.), 1 minute intervals up to 10 minutes p.i., 2 minute intervals up to 20 minutes p.i., and at 25, 30, 45, 60, 75, and 90 minutes p.i. Blood samples were immediately centrifuged and plasma radioactivity counted using a gamma scintillation spectrometer.

MDL 100,907 Pharmacokinetic Analysis

Blood samples for the determination of plasma MDL 100,907 concentrations were drawn immediately before each PET scan. A solid-phase extraction method was used to extract both MDL 100,907, as well as its metabolite MDL 105,725, from plasma prior to quantification by high performance liquid chromatography with mass spectrometry (McElvain et al. 1996). The lower limit of quantification in plasma for both compounds was 100 pg/ml. The between-batch coefficients of variation were 2.6% for MDL 100,907, and 7.4% for MDL 105,725 at the lowest control point on the standard curves. Plasma half-lives of MDL 100,907 were determined for each subject by log-linear regression of the available concentration-time data. Pooled plasma concentration and percent binding data were fit to a simple E_{max} model by nonlinear regression analysis using WINNONLIN, Version 1.0, according to the following equation:

$$\text{Occupancy [\%]} = \frac{E_{\max} * [C]}{EC_{50} + [C]}$$

where Occupancy [%] is the percent receptor occupancy determined by the PET experiments, E_{max} is maximum attainable receptor occupancy, [C] is the plasma concentration of MDL 100,907, and EC_{50} is the plasma concentration predicted to provide 50% of the maximum attainable receptor occupancy.

Calculation of 5-HT_2 Receptor Occupancy

Occupancy calculations were performed by two different methods. Firstly, occupancy was calculated as the percent change in binding potential compared with the baseline. The binding potential itself for each PET scan was measured as the k_3/k_4 ratio determined graphically (Logan et al. 1990) from the time activity curves of the frontal cortex, the cerebellum, and the plasma. Briefly, plots of

$$\int_0^t \mathrm{ROI}(t')dt/\mathrm{ROI}(t') \text{ versus } \int_0^t C_p()t' \, dt/\mathrm{ROI}(t')$$

(where ROI and C_p are functions of time describing the variation of tissue radioactivity and plasma radioactivity, respectively) are generated from the time-activity data in the cerebral cortex, cerebellum, and plasma. The slope of the linear portion of the plot for the cerebellum represents $a = k_1/k_2$, and the slope of the linear portion of the plot for the frontal cortex represents $b = k_1/k_2 (1 + k_3/k_4)$. The binding potential k_3/k_4 is then equal to $b/a - 1$. Plasma radioactivity was corrected for labeled metabolites of [^{11}C]NMSP using the modeling procedure previously described (Wong et al. 1986). The agreement of this model with HPLC correction for metabolites of [^{11}C]NMSP has been demonstrated in various patient populations (Wong et al. 1989). From the k_3/k_4 ratios the 5-HT$_2$ receptor occupancy in the frontal cortex was calculated according to the following equation:

$$\text{Occupancy } [\%]_{\text{kinetic analysis}} = \frac{k_3/k_{4\ Baseline} - k_3/k_{4\ Drug}}{k_3/k_{4\ Baseline}} * 100$$

where $k_3/k_{4Baseline}$ and k_3/k_{4Drug} are the binding potential values from the baseline (drug-free) study and from the study after administration of MDL 100,907, respectively.

Secondly, the occupancy was expressed as the ratio of the radioactivity in the bound compartment (frontal cortex-cerebellum) to that in the free compartment (cerebellum) (B/F ratio) (Farde et al. 1988). Since the ratio analysis assumes that the tissue ratios are obtained at the time of quasi-equilibrium, the radioactivity values at the last three time frames (i.e., at the last 18 minutes) of the 90 minute scanning period were averaged for each time activity curve. This was done because the B/F ratio reached a plateau at that time. The 5-HT$_2$ receptor occupancy in the frontal cortex was then calculated according to the following equation:

$$\text{Occupancy } [\%]_{\text{ratio analysis}} = \frac{B/F_{Baseline} - B/F_{Drug}}{B/F_{Baseline}} * 100$$

where $B/F_{Baseline}$ and B/F_{Drug} are the ratios between radioactivity in the bound compartment B (frontal cortex-cerebellum) and the free compartment F (cerebellum) in the above-mentioned time-window from the baseline (drug-free) study and from the study after administration of MDL 100,907, respectively. This method has been validated by Kim et al. (1994).

Statistical Analyses

A two-sided two-sample Wilcoxon rank analysis for paired data was performed to compare the median of the change in occupancy between two time points after 10 and 20 mg MDL 100,907, respectively. To test the hypothesis that the time-activity curves of the cerebellum, the putamen, and the caudate for the three post-drug studies were all equivalent with the baseline study, we subtracted the baseline study of a given subject from each of the three post-drug studies of that subject, because this removes the random subject effect from the data of each subject. After computing a Principal Components analysis of the variables, we performed a single-sample Hotelling's T square test on the set of differences to test the null hypothesis that their population mean is zero. For all statical evaluations, p values of 0.05 were used as levels of significance.

RESULTS

Regardless of the analytical method used for the occupancy calculation, single oral doses of both 10 mg and 20 mg MDL 100,907 led to a high occupancy of 5-HT$_2$ receptors in the human frontal cortex as measured with Positron Emission Tomography and [^{11}C]NMSP as the radiotracer. Individual and mean occupancy values from both methods are given in Table 1. After a single 20 mg dose, the occupancy did not change significantly over the observation period, remaining in the range of 82–87% or 73–79% as calculated by kinetic analysis (Fig. 1) or ratio analysis (Fig. 2), respectively. After a single 10 mg dose, the 5-HT$_2$ receptor occupancy was in the same range as with the 20 mg dose at the 2 and 8 hour post dosing scan points. However, the occupancy after the 10 mg dose was significantly decreased by 18–21% (depending on the method of calculation) at 24 hours post dosing compared to the 8 hours post dosing timepoint ($p = 0.0495$). Also, with the 10 mg dose, the occupancy was 17% lower at 24 hours compared to the 20 mg dose. While washout rates were similar regardless of the method of calculation, occupancy values calculated with kinetic analysis were consistently 7–12% higher than those calculated with the ratio analysis. In addition to the duration of occupancy as calculated by kinetic analysis, Figure 1 also illustrates the plasma pharmacokinetics.

The plasma elimination half-life of MDL 100,907 calculated from the plasma levels was determined to be 6.56 ± 1.56 hours (mean ± SD; range: 4.51 to 9.78 hours). Mean plasma levels of MDL 100,907 are given in Table 2. Individual plasma concentrations ranged from 0.25 ng/ml to 30 ng/ml. Despite the limited number of samples, a relationship could be observed between plasma concentrations and percent occupancy of 5-HT$_2$ receptors. As concentrations of MDL 100,907 increased in the plasma, percent occupancy increased sharply before reaching an apparent level plateau (Figs. 3 and 4). When the calculations were based on the kinetic analy-

Table 1. Kinetic and Ratio Analysis: Individual Occupancy Values

Subjects	Dose (mg)	2 Hours	5 Hours	8 Hours	24 Hours
		A) Kinetic Analysis			
1	10	74		70	54
2	10	98		98	76
3	10	86		89	65
4	20	77	77	90	
5	20	86	90	97	
6	20	79	83	90	
7	20	89		87	80
8	20	94		83	89
9	20	94		77	76
Mean ± SD	10	86 ± 12		86 ± 14	65 ± 11
Mean ± SD	20	87 ± 7	83 ± 7	87 ± 7	82 ± 7
		B) Ratio Analysis			
1	10	58		54	43
2	10	90		95	64
3	10	75		72	62
4	20	70	69	78	
5	20	79	84	84	
6	20	63	76	80	
7	20	79		78	67
8	20	86		81	88
9	20	77		73	64
Mean ± SD	10	74 ± 16		74 ± 21	56 ± 12
Mean ± SD	20	76 ± 8	76 ± 8	79 ± 4	73 ± 13

Individual and mean occupancy values for the 5-HT_2 receptor in the frontal cortex, calculated as percent decrease in binding potential (k_3/k_4) from baseline (A) or as percent decrease in B/F ratio from baseline (B). Binding potential was calculated with the graphical method described by Logan et al. (1990).

sis, the predicted maximum occupancy was 88.2%, with a predicted 80% occupancy achieved for all plasma concentrations above 1 ng/ml (Fig. 3). The predicted maximum occupancy determined from the ratio analysis was 77.2%, it was effectively achieved for all plasma concentrations above 3 ng/ml (Fig. 4). Final pharmacokinetic parameter estimates are given in Table 3.

When all post-drug PET scans were grouped together and compared to the baseline scan, MDL 100,907 did not lead to detectable displacement of [^{11}C]NMSP

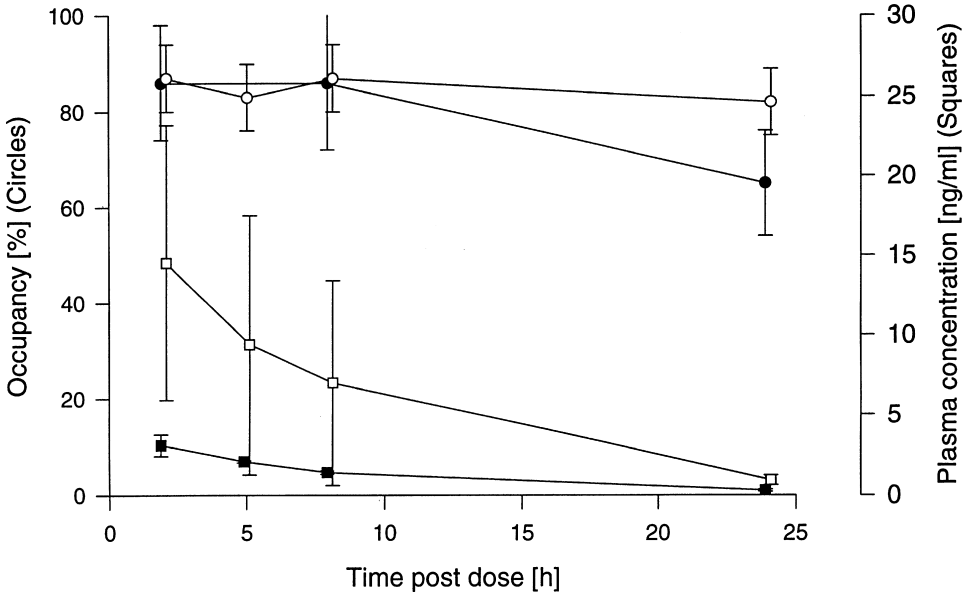

Figure 1. Time course of 5-HT_2 receptor occupancy in the frontal cortex (mean ± SD), expressed in percent decrease in binding potential (k_3/k_4) from baseline (left y-axis, represented by circles), and plasma concentrations for 10 mg and 20 mg oral doses of MDL 100,907 (right y-axis, represented by squares). Binding potential was calculated with the graphical method described by Logan et al. (1990). Black and white symbols represent 10 mg and 20 mg dose groups, respectively.

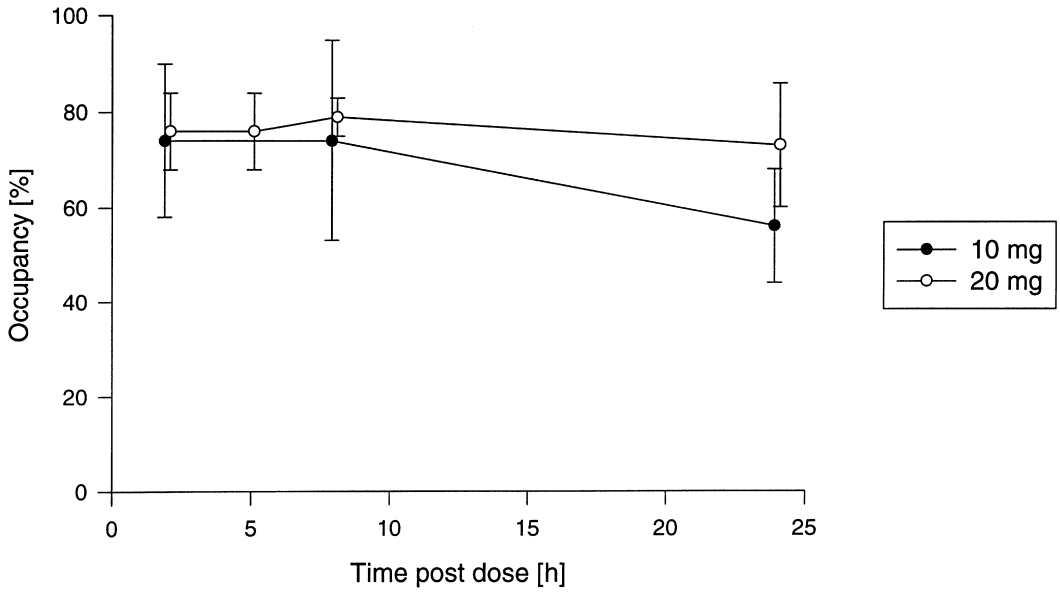

Figure 2. Time course of 5-HT_2 receptor occupancy in the frontal cortex (mean ± SD), expressed in percent decrease in B/F ratio from baseline. Black and white symbols represent 10 mg and 20 mg dose groups, respectively.

binding in the cerebellum ($p = 1.000$) (Fig. 5) or in the basal ganglia (caudate: $p = 1.000$; putamen: $p = 1.000$) (Fig. 6), respectively. This was still true, when only the two-hour post-dose scans were compared to baseline separately (cerebellum: $p = 0.59$; caudate: $p = 0.26$; putamen: $p = 0.45$).

MDL 100,907 was well tolerated by all nine subjects. One subject, who received 20 mg, reported a mild, short-lasting dizziness and dry mouth. One subject on 10 mg reported short-lasting lightheadedness and restlessness, and another one complained about nausea approximately one hour after drug-intake, which was completely and immediately relieved after receiving a snack.

DISCUSSION

This study was designed to determine the time course of the 5-HT_2 serotonin receptor occupancy in the human frontal cerebral cortex after a single oral dose of the putative atypical antipsychotic drug, MDL 100,907. A second objective was to determine the relationship between plasma levels of MDL 100,907 and the degree of central 5-HT_2 receptor occupancy.

Table 2. Plasma Levels (ng/ml) of MDL 100,907 (Mean ± SD)

	2 Hours	5 Hours	8 Hours	24 Hours
10 mg	3.05 ± 0.67		1.39 ± 0.11	0.29 ± 0.07
20 mg	14.53 ± 8.61	9.37 ± 8.12	7.01 ± 6.41	0.93 ± 0.30

Mean plasma levels (mean ± SD) of MDL 100,907 for 10 mg and 20 mg oral doses of MDL 100,907.

We have shown, that both 10 mg and 20 mg MDL 100,907, respectively, lead to a high 5-HT_2 receptor occupancy in the range of 70–90% in the frontal cerebral cortex for a duration of at least eight hours post dose. With 20 mg MDL 100,907, the occupancy at 24 hours post dose is still in the same range as at eight hour post dose; with 10 mg, it decreases by about 20% compared to the eight-hour post dose timepoint.

MDL 100,907 is rapidly absorbed following oral administration with the t_{max} occurring at 1–2.5 hours post dose. The plasma half-life is in the range of 4.5–9.8 hours with a mean half-life of 6.6 hours. Taking into account the half-life of 6.6 hours, it can be concluded that the receptor occupancy half-life of MDL 100,907 is considerably longer than the plasma half-life.

Following administration of MDL 100,907 there appeared to be a plateau of [^{11}C]NMSP displacement that would not exceed 74–87% of total binding. A doubling of the administered dose, from 10 to 20 mg, produced an approximately 3-fold increase in plasma concentration of parent compound but no change in central occupancy. While [^{11}C]NMSP is commonly used for studying central 5-HT_2 receptors, the ligand is not an ideal tracer. NMSP also binds to D_2-like dopamine and α_1 adrenergic receptors (Lyon et al. 1986). While the density of D_2-like dopamine receptors in the cerebral cortex is very low (Lidow et al. 1989), the existence of cortical α_1 receptors may contribute to total cortical binding of the tracer. It has been shown in *in vivo* rodent studies, that about 10% of the cortical binding of [^{3}H]NMSP can not be displaced by high doses of the 5-HT_2 antagonist

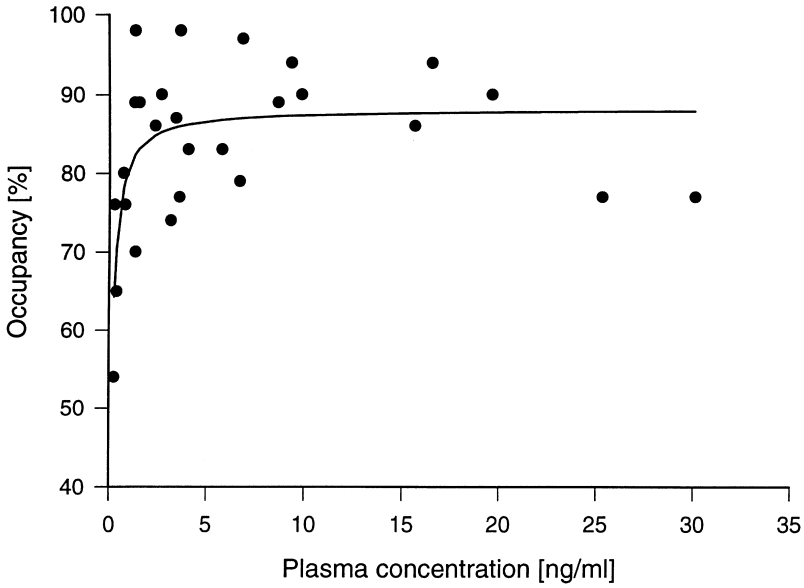

Figure 3. Percent receptor occupancy determined from the kinetic analysis for concentrations of MDL 100,907 in plasma. Each point represents the plasma concentration and percent occupancy data from an individual sample. The predicted maximal occupancy (E_{max}) is 88.2%, and the predicted concentration of half-maximal occupancy is 0.1 ng/ml.

ketanserin (Frost et al. 1987), which may be due to binding of NMSP to cortical α_1 receptors. Since MDL 100,907 is at least 100-fold less potent at the α_1 receptor that at the 5-HT_2 receptor, the binding of [^{11}C]NMSP to cortical α_1 receptors may not be displaceable by MDL 100,907. Also, to a small extent, [^{11}C]NMSP may bind to cortical spirodecanone (Murrin et al. 1985) or 5-HT_{2C} receptors (Canton et al. 1994), although the existence of spirodecanone receptor site in the human brain has been questioned (Camps et al. 1989). In addition, a low but significant density of 5-HT_2 receptors has been demonstrated in the cerebellum *in vitro* (Pazos et al. 1987). As the cerebellum is the reference region for defining nonspecific binding, it has been argued that the 5-HT_2 component may contribute to an apparent overestimation of nonspecifically bound radio ligand. In our study a significant displacement of [^{11}C]NMSP binding in the cerebellum by MDL 100,907 could not be detected (Fig. 5), which means that binding of [^{11}C]NMSP to cerebellar 5-HT_{2A} receptors does not contribute to an overestimation of nonspecific binding. However, it can not be completely ruled out, that binding of [^{11}C]NMSP to other than 5-HT_{2A} receptors (e.g., 5-HT_{2C} receptors) in the cerebellum occurs, although the effect of such binding on the estimation of nonspecific binding should be very small. Consequently, both effects (cortical [^{11}C]NMSP binding to other than 5-HT_2 receptors and overestimation of cerebellar nonspecific binding) could lead to an

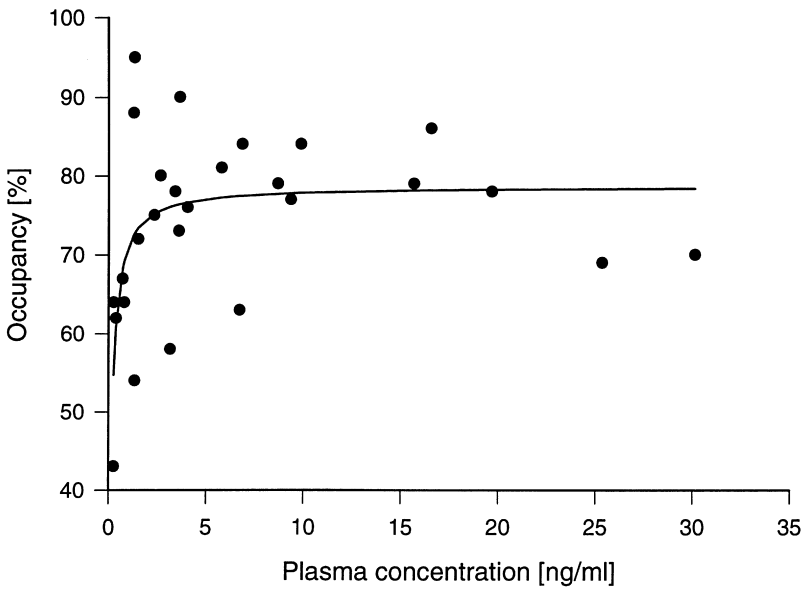

Figure 4. Percent receptor occupancy determined from the ratio analysis for concentrations of MDL 100,907 in plasma. Each point represents the plasma concentration and percent occupancy data from an individual sample. The predicted maximal occupancy (E_{max}) is 77.2%, and the predicted concentration of half-maximal occupancy is 0.1 ng/ml.

Table 3. Final Pharmacokinetic Parameters (Mean ± SEM)

	E_{max}(%)	EC_{50} (ng/ml)
Ratio Analysis	77.2 ± 3.2	0.1 ± 0.1
Kinetic Analysis	88.2 ± 1.72	0.1 ± 0.02

Final pharmacokinetic parameters (mean ± SEM) for the kinetic and the ratio analysis. E_{max} is maximum attainable receptor occupancy and EC_{50} is the plasma concentration predicted to provide 50% of the maximum attainable receptor occupancy.

underestimation of the 5-HT_2 receptor occupancy induced by MDL 100,907. Taking this into account, the "real" 5-HT_2 receptor occupancy by the drug is probably even higher than the calculated occupancy values found in this study. This view is further supported by the apparent discrepancy between central receptor occupancy and peripheral plasma kinetics. This can be expected from the receptor occupancy versus free competitive ligand curve only if one assumes that the 5-HT_2 receptors are completely saturated over a wide range of plasma levels and that the occupancy begins to decrease just after the plasma level of MDL 100,907 has fallen below 1–3 ng/ml, depending on the method of calculation.

While various other ligands have been developed for labeling of 5-HT_2 receptors with positron emission tomography, including [^{11}C]LSD (Wong et al. 1987), [^{18}F]setoperone (Blin et al. 1988), and [^{18}F]altanserin (Lemaire et al. 1991), none of these compounds is specific for the 5-HT_2 receptor. We have recently labeled MDL 100,907 with ^{11}C and carried out human studies with the specific goal of quantifying 5-HT_2 receptors *in vivo* (Wong et al. 1996). Our findings indicate, that the abovementioned methodological issues can be addressed with this new tracer.

Percent receptor occupancy was used as the pharmacodynamic endpoint for this study. While data are limited to only nine subjects, preliminary conclusions may be drawn about the relationship between plasma concentrations of MDL 100,907 and the 5-HT_2 occupancy. The atypical antipsychotic, clozapine, has been shown to occupy more than 80% of 5-HT_2 receptors at clinically active doses (Nordström et al. 1993). Based upon our analyses, approximately 75% or greater receptor occupancies should be observed for plasma concentrations of MDL 100,907 greater than 1 ng/ml (kinetic analysis) or 3 ng/ml (ratio analysis), respectively.

The plasma half-life of MDL 100,907 was determined to be 6.6 hours. Assuming a monoexponential decline of the concentration-time profile and a maximum concentration equal to the mean value of the 2-hour concentration sample for each dose given in this study, one can estimate that concentrations will be below 3 ng/ml at approximately 8 and 12 hours following a single oral dose of 10 and 20mg, respectively. They will be below 1 ng/ml at approximately 12 (10 mg) and 24 hours (20mg). However, since chronic administration of MDL 100,907 is more likely in a clinical setting, definitive multiple dose pharmacokinetic data will be needed for the proper selection of dose and dose regimen. These studies are currently underway.

The method for determination of receptor occupancy is dependent on numerous factors including the physical/chemical properties and pharmacokinetics of the radioligand. Two different methods, kinetic and ratio analysis, were used in this study. The kinetic analysis shows consistently higher occupancy values than that calculated by the ratio analysis. This is in line with earlier findings reported by Kim et al. (1994). Different from the binding of [^{11}C]NMSP to D_2-like dopamine receptors, the binding of this tracer to 5-HT_2 receptors reaches quasi-equilibrium within the duration of a PET study (Kim et al. 1994). The ratio analysis assumes that the tissue ratios are obtained at the time of quasi-equilibrium. Figure 7 gives an example for a typical set of time-activity-curves of the baseline scan in a normal volunteer. It demonstrates, that the specific binding of

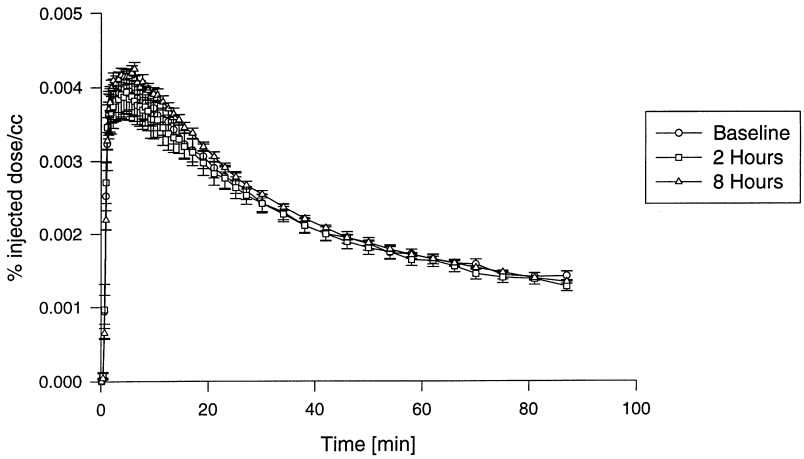

Figure 5. Time-activity-curves from the cerebellum for the baseline scan and the 2-hour and 8-hour post dose scans averaged from all nine normal volunteers (mean ± SEM). MDL 100,907 did not lead to detectable displacement of [^{11}C]NMSP binding in the cerebellum ($p = 1.000$).

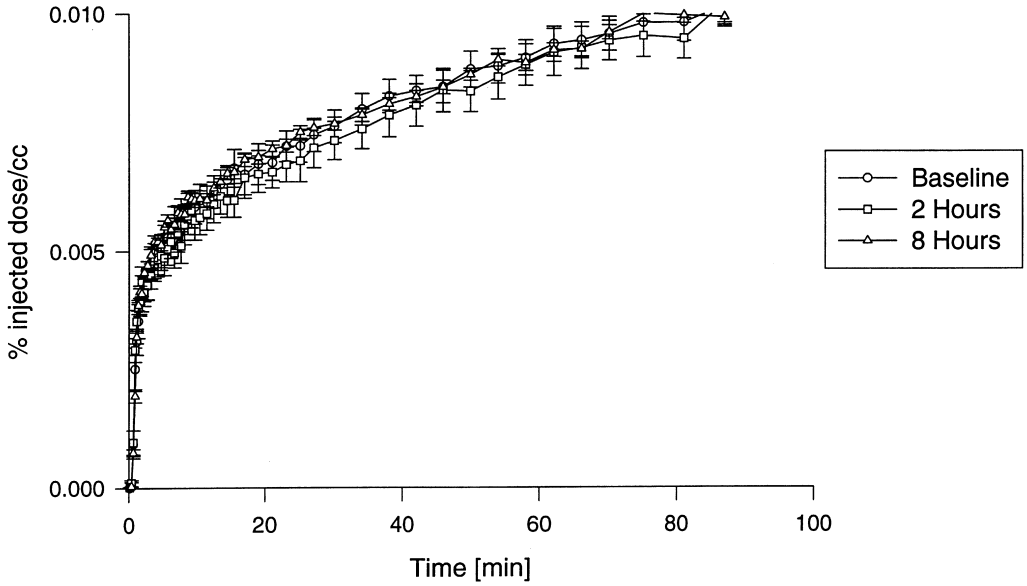

Figure 6. Time-activity-curves from the putamen for the baseline scan and the 2-hour and 8-hour post dose scans averaged from all nine normal volunteers (mean ± SEM). MDL 100,907 did not lead to detectable displacement of [^{11}C]NMSP binding in the putamen ($p = 1.000$).

[^{11}C]NMSP in the frontal cortex reaches a plateau not before 60 minutes after injection, indicating that quasi-equilibrium of frontal cortical [^{11}C]NMSP binding is achieved very late during the time of a PET scan. Kim et al. (1994) reported that a cortex/cerebellum ratio obtained at 45 minutes led consistently to lower 5-HT$_2$ receptor occupancy values than a cortex-cerebellum ratio obtained at 90 minutes. Our observation implies that the duration of a PET scan for the determination of 5-HT$_2$ receptor occupancy with [^{11}C]NMSP should be not less than 90 minutes, if this calculation is based on some kind of simplified ratio analysis.

During early clinical development of psychotherapeutic agents a critical decision will be selection of the dose-regimen. While it may be attractive to select a dose-regimen based upon preclinical observations, this approach can be fraught with false assumptions including the validity of the test model or pharmacologic and

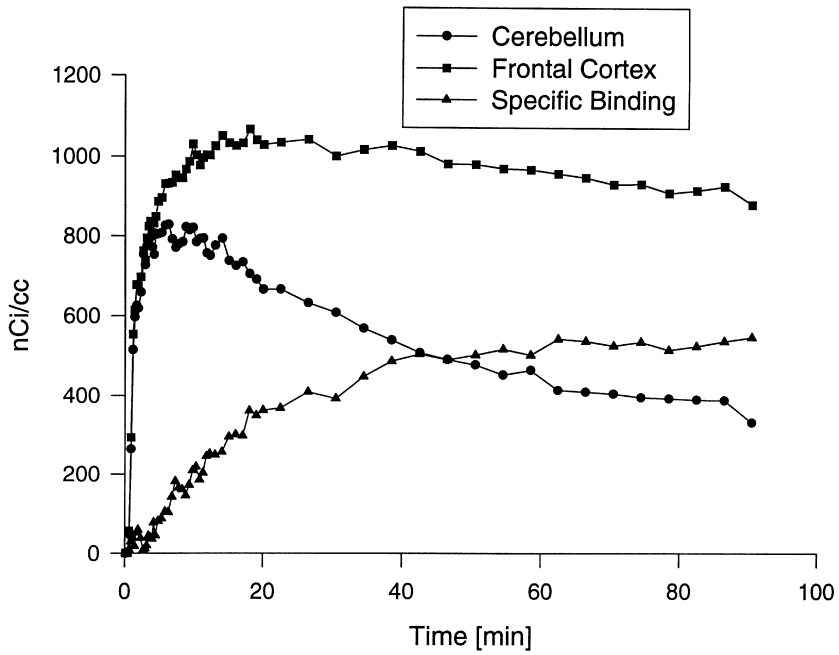

Figure 7. Typical time-activity-curves of the baseline scan in a normal volunteer. Shown are the curves in the frontal cortex (representing the total binding) and in the cerebellum as well as the curve for the specific binding in the frontal cortex, calculated by subtraction of the activity in the cerebellum from the activity in the frontal cortex.

pharmacodynamic dissimilarity between the testing of species and man. An alternative practice is to select the dose-regimen based upon the compound's half-life or effect on peripheral surrogate markers from the Phase I studies. In this study, it was demonstrated that there is a discrepancy between the central pharamacodynamic effects of MDL 100,907, represented by receptor occupancy, and the compound's peripheral pharmacokinetics. This is well illustrated in Figure 1. This finding is consistent with earlier reports investigating the occupancy of a D_2 antagonist antipsychotic (Offord et al. 1993; Wong et al. 1993). Further, prolactin, a measure of D_2 antagonism does not predict the duration of central D_2 receptor occupancy (Seibyl et al. 1996). This suggests the notion that peripheral markers can be poor indicators for central activity. Consequently, the use of central receptor occupancy is an attractive approach for predicting the dose-regimen for initial efficacy studies. The data from this study suggest that MDL 100,907 at 20 mg QID would be appropriate for an initial therapeutic dose and regimen. Studies are currently underway to test this hypothesis.

In conclusion, under the basic assumption that the relevant principle of antipsychotic action of MDL 100,907 is blockade of 5-HT_2 receptors, a single daily dose of 20 mg MDL 100,907 or 10 mg BID should be sufficient for antipsychotic treatment trials. More generally, these studies demonstrate the principle of examination of occupancy changes with time to predict dosing levels and regimens for clinical trials.

ACKNOWLEDGMENTS

We gratefully appreciate the help of Boon Chan, Anquing Chen, Andrew H. Crabb, Abdul Kalaff, David N. Krasenbaum, and Igor Simakhodskiy. The study was supported in part by Hoechst-Marion-Roussel, Cincinnati, Ohio. G.G. was supported by the Deutsche Forschungsgemeinschaft (Gr 1399/1–1).

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