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The D_2 Receptor Occupancy Profile of Loxapine Determined Using PET

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Positron emission tomography (PET) studies of typical neuroleptics suggest that 60% to 80% of striatal D_2 occupancy may be sufficient for optimal clinical treatment of psychosis. Therefore, striatal D_2 occupancy may be used as an index to determine the optimal dose range. Toward this end, we determined the in vivo *D_2 profile of loxapine, using [^{11}C]-raclopride and PET. Seven patients selected from a clinical population were scanned while taking steady-state oral loxapine from 10 to 100 mg/day. Their D_2 receptor occupancy was estimated by comparing them to age-matched data from neuroleptic-naive patients. The D_2 receptor occupancy ranged from 52% to 90%, and there was a very strong relationship between dose and D_2 occupancy, suggesting that 15 to 30 mg/day of loxapine would produce, the putatively optimal, 60% to 80% striatal D_2 blockade. This dose range is much lower than that used in most clinical settings and points to the potential efficacy of loxapine at lower doses.* **© 1996 American College of Neuropsychopharmacology**
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Clinical efficacy of typical neuroleptics, as well as their extrapyramidal side effects, are best explained by their dopamine D_2 receptor blocking properties. This is supported by recent positron emission tomography (PET) imaging data showing that optimal therapeutic response is obtained when D_2 occupancy exceeds 60% to 70% (Nordstrom et al. 1993). However, beyond 80% D_2 occupancy extrapyramidal side effects become prominent (Farde et al. 1992). Therefore, the range of 60% to 80% D_2 occupancy may constitute an optimal window for treatment and may provide a rational basis for determining the optimal dose of a typical neuroleptic.

Loxapine is a midpotency neuroleptic and is of particular interest as some authors have claimed a superior therapeutic efficacy and a lesser propensity for extrapyramidal symptoms (EPS) when loxapine is compared with other typical neuroleptics (Bishop et al. 1977; Fruensgaard et al. 1977). More recently it has been shown to have a higher affinity for the 5-HT_2 and D_4 receptors as compared to the D_2 receptors (K_i values: D_2 = 24 nmol/L, 5-HT_2 = 6.2 nmol/L, D_4 = 7.5 nmol/L) suggesting an "atypical" pharmacological profile (Singh et al. 1996). When loxapine was introduced two decades ago, few dose-finding studies were done. In the absence of firm dosing guidelines, loxapine has been used clinically in doses as low as 5 mg/day and as high as 800 mg/day (Bezchlibnyk-Butler and Jeffries 1991). Such a range is probably unwarranted, as it has recently been shown that low doses of haloperidol (equivalent of 2 to 5 mg/day) give clinically sufficient D_2 blockade

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(Nyberg et al. 1995; Kapur et al. 1996). Furthermore, Kapur et al. (1995) have recently shown that drugs with combined 5-HT_2 and D_2 profiles must be used at the lowest dose required for adequate D_2 occupancy, because higher doses may mitigate the potential benefits of 5-HT_2 antagonism. Therefore, determining the lowest doses of loxapine that produce 60% to 80% D_2 occupancy would provide a rational basis for determining optimal clinical dose. Toward this end, we have determined the D_2 occupancy profile of loxapine in a cross-sectional series of patients receiving doses ranging from 10 to 100 mg/day.

METHODS

Seven patients (six men, one woman; age 21–38 years) participated in this study carried out within the Schizophrenia Division at the Clarke Institute of Psychiatry. The patients were selected if they: (i) were on a fixed dose of loxapine for 7 days or more; (ii) had a DSM-III-R diagnosis of a schizophrenia ($n = 6$) or delusional disorder ($n = 1$); (iii) had not taken any other oral or depot neuroleptic for 6 months; were not taking any concurrent psychotropics *except* a benzodiazepine or benztropine; and (iv) had no concurrent substance abuse or dependence. Written consent was obtained from each subject, using forms approved by the University of Toronto Review Committee on the Use of Human Subjects.

The D_2 receptor status was assessed using [^{11}C]-raclopride and PET imaging in the morning, 12 to 14 hours after the last nightly dose of loxapine. An EPS rating was obtained at the time of scanning. The PET scans were obtained using a GEMS-2048-15B head scanner after injection of 10 mCi of high specific activity [^{11}C]-raclopride (300 to 1600 Ci/mmol), using a protocol that is described in detail elsewhere (Kapur et al. 1995; Houle et al. 1996). Striatal and cerebellar regions of interest (ROI) were drawn on two contiguous PET slices with reference to a coregistered MRI scan (GE Signa 1.5 T scanner, spin-echo sequence T_2 weighted).

To estimate the D_2 dopamine receptor binding potential of ^{11}C-raclopride, we used a two-tissue compartment model, which partitions the counts obtained from the striatum into those that are specifically bound to the dopamine D_2 receptor and those that reflect the free radioligand and nonspecific binding (Farde et al. 1992). The counts from the cerebellum are used as an estimate of the free and nonspecific binding in the striatum. For scans done with high specific activity of raclopride, the rate equations for this model are:

$$\frac{dC_f}{dt} = K_1 C_p - (k_2 + k_3) C_f + k_4 C_b \quad (1)$$

$$\frac{dC_b}{dt} = k_3 C_f - k_4 C_b \quad (2)$$

where $C_p(t)$ is the arterial plasma concentration of the radiotracer, $C_f(t)$ the concentration of free ligand in the brain, and $C_b(t)$ the concentration specifically bound to dopamine receptors. The rate constant k_3 is proportional to the free receptor density ($B_{max} - B$), where B is the concentration of receptors occupied either by endogenous dopamine or a drug, which is low enough to be disregarded when high-specific activity radioligand is used. The ratio k_3/k_4, which is equal to $(B_{max} - B)/K_d$, is referred to as the binding potential (BP) and provides a convenient index of the available receptor density (Farde et al. 1992).

To calculate the binding potential, we first fit a biexponential function to the cerebellar time activity curve using a nonlinear least-squares fit. We do not include the data points from the first 10 minutes of the study when there are rapid variations in the time activity curves as well as significant contribution from the blood in the first minute or two after the bolus injection. By not including these rapidly varying segments of the curves, we can use simpler fitting functions and achieve more reliable estimates of the fit parameters (Houle et al. 1996). We subtract the fitted cerebellar curve from the striatal curves to obtain the time activity curve for the specifically bound ligand, which is then fitted with the following function:

$$b(t) = b_0(1 - \exp(-b_1 t))(1 + b_2 t) \quad (3)$$

where $b_2 << b_1$ for a bolus plus constant infusion. If true equilibrium is achieved, then $b_2 = 0$. The fitted curve for the bound ligand is differentiated analytically to obtain $dC_b(t)/dt$. We then have a set of linear equations for the time points t_i:

$$\frac{dC_b(t_i)}{dt} = k_3 C_f(t_i) - k_4 C_b(t_i) \quad (4)$$

We solve this set of equations for k_3 and k_4 and obtain the BP without assuming that the derivative $dC_b(t)/dt$ reaches zero at some point during the scan.

Some previous authors have used the ratio of striatal to cerebellar [^{11}C]-raclopride uptake, between two time points during imaging, as an estimate of BP. Difficulty with this approach lies in that different groups have used mutually exclusive time intervals, e.g., 21 to 33 minutes (Nordstrom et al. 1993) and 33 to 58 minutes after injection of [^{11}C]-raclopride (Antonini et al. 1993). Our analytic method avoids this arbitrary choice of points. However, it must be acknowledged, that the ratio method applied to our data yielded essentially identical results ($r = 0.99$, $F_{1,17} = 3095$, $p < .0001$), and the resulting values of D_2 occupancy differed by less than 2% between the two methods.

Because these patients were already on treatment, it was not possible to obtain their baseline D_2 data. Therefore drug-free occupancy was estimated using BP data

from 12 age-matched (19 to 38 years) neuroleptic-naive patients with a diagnosis of schizophrenia. Controlling for age effects is important as aging is associated with a decline in the number of D_2 receptors (Antonini et al. 1993). Our data in neuroleptic-naive patients showed a BP ratio from 2.3 to 3.5 (mean 3.02 ± 0.34) with a very significant decline with age (13% per decade with 95% CI of 5% to 21%; $F_{1,10} = 12.96$; $p = .0048$; multiple R = 0.75). Age corrected baseline esimates from neuroleptic-naive patients were used to determine the loxaprine-induced D_2 receptor occupancy as: $(R_{Bas} - R_{On})/(R_{Bas})$ where R_{Bas} is the age-corrected BP obtained from the neuroleptic naive data, and R_{On} is the BP ratio for patients on loxapine. The absence of subject's own baseline values introduces a potential error in estimation of occupancy—based on the variance in our age-corrected data it appears that the potential error in estimation of receptor occupancy is no more than 6% to 9% for patients with 50% occupancy and 3% to 4% for patients who have 80% occupancy—similar to Farde's estimate of ±3% (Farde et al. 1992).

RESULTS

The receptor occupancy in the seven patients ranged from 52% to 90% on doses of 10 to 100 mg/day. The relationship between dose and D_2 occupancy was modeled by a saturation curve as depicted in Figure 1. The curve fit the data very well with an $R^2 = 0.94$ for an $ED_{50} = 8.9$ mg/day (95% confidence limits of $ED_{50} = 7.4$ to 10.6). The curve demonstrates that, on average, 50% of the D_2 receptors would be occupied by 8.9 mg/day of loxapine (ED_{50}); and that 15 mg/day of loxapine would provide 63% occupancy (95% CI: 59% to 67%) and 30 mg/day would provide 77% occupancy (95% CI: 74% to 80%).

The patients were selected in a nonrandom fashion, and treatment was not controlled; therefore, no systematic inferences regarding the efficacy of loxapine should drawn from this sample. EPS ratings at the time of scanning showed clinically significant Parkinson's symptoms in only one patient—on 40 mg/day of loxapine with 83% D_2 occupancy. He experienced and exhibited mainly stiffness in his legs. Another patient, the one on 100 mg/day with 90% D_2 occupancy was receiving benztropine as an anti-Parkinsonian agent for EPS experienced in the weeks before the PET study. Therefore the absence of EPS at the time of PET scanning in this patient, despite high D_2 occupancy, is most likely due to the action of benztropine.

DISCUSSION

This is the first report of in vivo D_2 receptor binding for loxapine. The data unequivocally show that low doses of loxapine lead to high D_2 occupancy as measured with PET using [^{11}C]-raclopride. In an attempt to characterize the relationship between dose and occupancy, we have modeled the relationship as a saturation curve—and the curve fits the data very well. Obviously, a curve generated from seven data points can only be regarded as a rough guide. Nonetheless, this curve does represents the only such data available on loxapine and shows that 15 mg of loxapine may be the required dose to reach 60% occupancy in most patients; whereas 30 mg/day may be the upper margin before the D_2 occupancy crosses the 80% threshold.

In vitro comparisons suggest that approximately 15 mg of loxapine is equipotent with 2 mg of haloperidol (evidence reviewed in Bezchlibnyk-Butler & Jeffries 1991). Comparison of our PET data on loxapine with previous PET data on haloperidol confirms this relative potency of 15:2. We have recently reported that 2 mg of

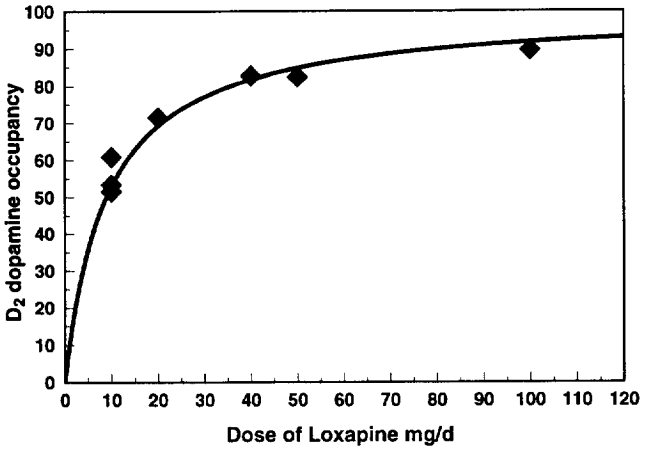

Figure 1. D_2 dopamine receptor occupancy as measured with [^{11}C]-raclopride and PET, in seven patients on different doses of loxapine [10 mg/day: 52%, 53%, 61%; 20 mg/day: 71%; 40 mg/day: 83%; 50 mg/day: 82% and 100 mg/day: 90%]. The occupation of receptor by a competitive antagonist can be modeled by an equation:

$$\%\ D_2\ R_{Occ} = 100 \times \frac{D_{\text{Level}}}{ED_{50} + D_{\text{Level}}}$$

where $\%D_2\ R_{Occ}$ is the percentage of available D_2 receptors occupied, ED_{50} is a constant, D_{Level} is the concentration of the antagonist in the compartment impinging directly on the receptors, and the multiplication by 100 expresses the proportion as a percentage. However, the extravascular tissue concentration of loxapine is not known and in its absence plasma levels would have provided a useful measure. However, we do not have plasma levels on these patients. We have used dose as a surrogate, recognizing full well that dose provides only an imperfect measure of D_{Level} and does not account for individual pharmacokinetic variability. The plotted curve represents the best least-squares fit to the data (R^2 of 0.94) with an ED_{50} of 8.9 mg/day, suggesting that a dose of 8.9 mg/day of loxapine provides 50% D_2 receptor occupancy.

haloperidol induces 60% to 70% occupancy (Kapur et al. 1996), and similar figures have been obtained by Nyberg et al. (1995). Therefore, the relative potency relationship of 15:2 for loxapine:haloperidol holds in vitro as well as in vivo.

Loxapine is currently used in doses much higher than those needed to provide 60% to 80% D_2 blockade (Bezchlibnyk-Butler and Jeffries 1991). Doses up to 800 mg/day have been reported and even controlled clinical trials have used loxapine in doses as high as 80 to 150 mg/day (Bishop et al. 1977). Our PET data suggests the use of loxapine in lower doses. This claim in favor of low dose loxapine is buttressed by recent low trials of haloperidol. A series of double-blind randomized clinical trials have shown that low doses of haloperidol [average 3.7 mg (McEvoy et al. 1991), 4 mg (Stone et al. 1995), and 3.3 mg (Janicak et al. 1995) for chronic patients and 2.1 mg for first episode patients (McEvoy et al. 1991)] are as effective as much higher doses (10 to 60 mg/day) of haloperidol in acute psychosis. Whereas data on the comparative efficacy of low dose loxapine are lacking, extrapolating from these haloperidol studies using the 15:2 potency ratio obtained in our PET study, would also suggest an initial therapeutic dose range of 15 to 30 mg/day of loxapine.

Recent in vitro studies have shown that loxapine has a preferential affinity for 5-HT_2 and the D_4 receptor compared with the D_2 receptor—properties that supposedly are responsible for risperidone's and clozapine's unique clinical efficacy (Singh et al. 1996). We have suggested elsewhere (Kapur et al. 1995; Kapur and Remington 1996) that the drugs with 5-HT_2/D_2 properties may lose their differential therapeutic profiles (compared with D_2-only drugs) if they are used in doses that saturate the D_2 system. It is yet to be shown that loxapine shows prominent 5-HT_2 blockade in vivo, as has been shown for clozapine and risperidone. We are currently investigating this question. However, regardless of its action on the 5-HT_2 receptors, information regarding loxapine's D_2 occupancy profile presented here should encourage use of the drug in lower doses.

In summary, in a small cross-sectional study we have demonstrated that doses of loxapine much lower than those used conventionally result in 60% to 80% D_2 occupancy. In light of the emerging relationship between D_2 occupancy and clinical outcome, and empirical trials showing the efficacy of low dose neuroleptic treatment, our findings suggest that the optimal therapeutic dose of loxapine, for most patients, may lie in the range of 15 to 30 mg/day.

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