Antipsychotic Dosing in Preclinical Models Is Often Unrepresentative of the Clinical Condition: A Suggested Solution Based on in Vivo Occupancy

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ABSTRACT
What is the appropriate dose of an antipsychotic in an animal model? The literature reveals no standard rationale across studies. This study was designed to use in vivo dopamine D2 receptor occupancy as a cross-species principle for deriving clinically comparable doses for animal models. The relationship between dose, plasma levels, and in vivo dopamine D2 receptor occupancy was established in rats for a range of doses administered as a single dose or multiple doses (daily injections or osmotic minipump infusions) for five of the most commonly used antipsychotics. As a single dose, haloperidol (0.04–0.08 mg/kg), clozapine (5–15 mg/kg), olanzapine (1–2 mg/kg), risperidone (0.5–1 mg/kg), and quetiapine (10–25 mg/kg) reached clinically comparable occupancies. However, when these “optimal” single doses were administered as multiple doses, either by injection or by a mini-pump, it led to no or inappropriately low trough (24-h) occupancies. This discrepancy arises because the half-life of antipsychotics in rodents is 4 to 6 times faster than in humans. Only when doses 5 times higher than the optimal single dose were administered by pump were clinically comparable occupancies obtained (e.g., haloperidol, 0.25 mg/kg/day; olanzapine, 7.5 mg/kg/day). This could not be achieved for clozapine or quetiapine due to solubility and administration constraints. The study provides a rationale as well as clinically comparable dosing regimens for animal studies and raises questions about the inferences drawn from previous studies that have used doses unrepresentative of the clinical situation.

A search of the Medline database for the terms “antipsychotic or neuroleptic” and “human” reveals 38,000 articles since the 1960s. When the term human is replaced with “animal,” the search returns an almost equal number of articles, 34,000 articles. Studies of antipsychotics in animal models are almost as important a scientific enterprise as their study in humans (Lipska and Weinberger, 2000). In humans, the antipsychotics are effective in treating psychosis in a very narrow therapeutic dose range, a dose range that can be determined only in careful clinical trials of psychosis. Since psychosis per se cannot be modeled or measured in animals, what is the representative dose of antipsychotics in animal models?

A sampling of published papers shows three different approaches to this question (Kapur et al., 2000a). Most commonly, the dose of an antipsychotic is listed in the methods section without any justification for the particular choice. Less commonly, authors refer to previous papers (which may have been as arbitrary in their choice) as their reason to choose a particular dose. Least commonly, one finds a reasoned discussion of the choice of dose or finds a complete dose-response experiment. Thus, despite the critical importance of dosing, most of the studies in the literature have neglected this issue, leading in some instances to confounding and misleading conclusions (Kapur et al., 2000a).

Determination of the correct antipsychotic dose in animals is complicated by several factors. First, the symptoms that these drugs treat in patients, delusions and hallucinations, cannot be modeled by themselves in animals. What is used instead are a series of paradigms with predictive validity (e.g., amphetamine-induced hyperlocomotion, stereotypy, sensorimotor gating, etc.) (Arnt et al., 1997). However, just because an antipsychotic “works” in one of these predictive models, it does not mean that the dose at which it does so is representative of the clinical condition. Second, most of the currently popular atypical antipsychotics block multiple receptors with affinities

ABBREVIATION: D2BP, D2 receptor binding potential.
varying by two orders of magnitude (Schotte et al., 1996). A classic example is clozapine, which blocks more than a dozen different receptors with affinities ranging from <1 nM to >100 nM. As a result of this nonspecificity, when used at different doses, the drug produces a very different pharmacodynamic profile. Finally, antipsychotics in the clinical situation are used under multiple-dosing conditions. Whereas the half-life of these antipsychotics in humans is usually 12 to 24 h, their half-life in rodents tends to be 2 to 4 h. Thus, if the human practice of once-a-day or twice-a-day dosing is emulated in rodents, the rat receives a dose only once every four to eight half-lives. As a result, whereas a steady state in humans leads to a substantial presence of the drug at the active site throughout the 24 h, in rats the drug is almost completely eliminated at trough.

Given that a true anti-"psychotic" effect cannot be achieved in an animal, how does one choose representative doses for animal studies? Perhaps the most valid approach is to find doses that produce patient-equivalent effects on a critical target within the brains of experimental animals. This is now possible for most antipsychotics. With the advent of positron emission tomography and single photon emission computerized tomography imaging, it has been possible to measure the effects of most antipsychotics on dopamine D₂ receptors in vivo, in patients, at clinically relevant doses in the "therapeutic window" (Kapur and Remington, 2001). These findings are now well replicated across different laboratories, seem to correlate with clinical efficacy and side effects, and also help understand clinical differences between the available antipsychotics (Kapur et al., 1999; Nyberg and Farde, 2000; Bressan et al., 2001). We do not wish to suggest that action at D₂ receptors is the exclusive or central mechanism of action of these antipsychotics. However, it does provide an empirical and plausible marker that can be measured reliably and analogously in both species, and thus provides one basis for choosing clinically comparable doses of drugs for studies in animal models.

To obtain such clinically comparable doses for five of the most common antipsychotics in use currently (haloperidol, risperidone, olanzapine, quetiapine, and clozapine), we chose to measure their dopamine D₂ receptor occupancy using a validated method of calculating in vivo receptor occupancy that is similar to that used in clinical studies. Once the clinically comparable single doses were identified, we carried out studies using multiple-dosing regimens, by daily injections or continuous-infusion pumps, to obtain the clinically comparable doses for multiple-dosing studies.

Materials and Methods

Animals. Male Sprague-Dawley rats (Charles River Canada, Montreal, PQ, Canada) with initial body weight of 250 to 275 g were used. All animals were housed two per cage with food and water available ad libitum.

Drugs. Haloperidol (Sabex Inc., Boucherville, QC, Canada) and risperidone (Sigma-Aldrich, St. Louis, MO) were dissolved in distilled water, whereas olanzapine (gift from Eli Lilly & Co., Indianapolis, IN), quetiapine (gift from AstraZeneca, Mississauga, ON, Canada), and clozapine (ANAWA Trading SA, Wangen, Zurich, Switzerland) were dissolved in 1 to 2% glacial acetic acid in distilled water. All drugs were administered subcutaneously. [3H]Raclopride (PerkinElmer Life Sciences, Boston, MA) was used as the radioligand for occupancy studies and given intravenously via the tail.

Single-Dose Conditions. Animals (n = 150) were allocated to one of five drug conditions, and then further subdivided to one of five or six doses per drug or corresponding vehicle: haloperidol (0.025–1 mg/kg), clozapine (2.5–60 mg/kg), olanzapine (0.1–2 mg/kg), risperidone (0.05–2 mg/kg), and quetiapine (5–100 mg/kg). Rats that received a subcutaneous injection of haloperidol, olanzapine, or risperidone were sacrificed 2 h after drug administration (Campbell et al., 1980; Schotte et al., 1996). Those rats that received clozapine or quetiapine were sacrificed 1 h after drug administration since these drugs have been shown to have peak effects in the brain earlier than the other antipsychotics (Burki, 1986; Saller and Salama, 1993). It should be pointed out that the "peak" times were chosen based on previous studies relating to pharmacokinetics and functional effects and therefore should be regarded as approximations as opposed to definitive periods of maximal occupancy.

Ten minutes before sacrifice, the animals were tested for catalepsy. Animals were placed on an inclined (60°) grid and observed by a rater blind to the treatment assignment status of the animals. To establish a reliable baseline, the first 30 s were excluded from the actual rating time. The time the rat remained in the same position was then measured for a maximum of 2.5 min. The catalepsy was scored from 0 to 5 according to the time (square root transformation) the animal remained immobile (minutes): 0 = 0 to 0.08, 1 = 0.09 to 0.35, 2 = 0.36 to 0.80, 3 = 0.81 to 1.42, 4 = 1.43 to 2.24, and 5 = >2.25 min. An animal was considered cataleptic with a score greater than or equal to 2.

Multiple-Dosing Conditions. Animals (n = 144) were allocated to one of five antipsychotic drug groups to receive daily doses of antipsychotic either by subcutaneous injection or by Alzet osmotic minipump at a rate of 5.0 µl/h (model 2ML2, DURECT Corp., Cupertino, CA) for 7 days. Since the half-life of all antipsychotics is of the order of 2 to 4 h, 7 days is more than sufficient to obtain steady-state kinetics. All animals underwent surgery for implantation of a minipump or sham procedures. The rats were anesthetized with 1 to 2% isoflurane, and pumps were inserted into the subcutaneous space through a small incision on the back. All animals with drug-releasing minipumps received a daily injection of vehicle, whereas those that had undergone sham surgery received a daily injection of drug. The doses chosen for this multiple-dosing experiment were based on the dose that gave rise to clinically comparable D₂ occupancy under acute conditions and, where feasible, 5 times this dose. Although this was possible for haloperidol and olanzapine, we were not able to do it for risperidone due to nonavailability of sufficient quantities of the compound. With clozapine and quetiapine we ran into a dissolution/tolerability problem. To administer the 5-times single dose required concentrations in excess of 100 mg/ml drug in solution to be administered via the Alzet pump. Although such concentrations could be achieved by lowering the pH of the solvent into the range of 2 to 3, these low pH solutions led to tolerability problems when implanted subcutaneously for long periods of time. Thus, animals received pump delivery or daily injections of haloperidol (0.05 or 0.25 mg/kg), olanzapine (1.5 or 7.5 mg/kg), risperidone (1 mg/kg), quetiapine (10 or 25 mg/kg) or clozapine (7.5 or 15 mg/kg). Corresponding controls received sham surgery and daily vehicle injections. The experimental design is summarized in Fig. 1.

D₂ Occupancy Measurement. On the day of occupancy determination, the animals receiving daily injections of antipsychotic for 7 days were further split into two groups to examine the trough and peak occupancy levels. Animals that were assigned to the "trough" group were sacrificed at the time that they would have usually received their next injection (i.e., 24 h after last injection), while the animals in the "peak" group received a final injection on the seventh day and were sacrificed 1 h (clozapine and quetiapine groups) or 2 h (haloperidol, olanzapine, and risperidone groups) after drug administration.

Thirty minutes before sacrifice all animals received an i.v. injection of [3H]Raclopride (7.5 µCi/rat, in a volume of 0.4 ml of 0.9% NaCl solution) via the lateral tail vein. Animals were sacrificed by decap-
itation, and plasma was collected and stored at −80°C until drug levels could be assayed. The brains were immediately removed, and striata and cerebellum were rapidly dissected. The cerebellum was homogenized with a small spatula, and approximately one-third (50–100 mg) of this was sampled. The left and right striata were pooled into a single sample (60 mg). Tissue samples were collected in previously weighed 20-ml glass scintillation vials. The vials were then weighed with tissue, and 2 ml of Solvable (Canberra Packard Canada, Montreal, QC, Canada) was added. The vials were kept on an automated shaking tray and gently agitated for 24 h at room temperature. Thereafter, 5 ml of Aquasure (Canberra Packard Canada) scintillation fluid was added and allowed to mix for another 24 h. [3H]Raclopride radioactivity was determined by liquid scintillation spectrometry using a Beckman Coulter LS5000 CE liquid scintillation counting system (Beckman Coulter, Inc., Fullerton, CA). Striatal and cerebellar counts were obtained and expressed as dpm/mg.

The D2 receptor binding potential (D2BP) was obtained for each of the animals as (striatum dpm/mg − cerebellum dpm/mg)/(cerebellum dpm/mg). The receptor occupancy in each rat was determined with reference to the D2BP in the control group using the same formula as used in human studies (Farde et al., 1988; Kapur et al., 1999): % Occupancy = 100 × (D2BPcontrol − D2BPindiv/D2BPcontrol). For further details on the protocol for receptor occupancy assessment and its validation versus [11C]raclopride, see Wadenberg et al. (2000).

Drug Plasma Level Measurement. Plasma obtained from animals at the time of sacrifice for the occupancy studies detailed above was stored for analysis for drug levels. In general, drug levels were quantified using a liquid-liquid extraction to prepare the specimen for analysis. The samples obtained from liquid extraction were separated using liquid chromatography and then introduced into the mass spectrometer using electrospray ionization implemented using an HP 1100 LC-DAD-MSD system controlled by HP LC-MSD Chemstation software (Hewlett-Packard, Palo Alto, CA). As applied to haloperidol, the method has a lower limit of quantitation of 1 nM and a linearity limit of 212 nM with CV ranging from 3 to 10% across the dose range. Clozapine is detected with a lower limit of quantitation of 10 nM and a linearity limit of 6100 nM with CV ranging from 2 to 7% across the dose range. Risperidone is quantified with a lower limit of 1 nM and a linearity limit of 7200 nM with CV ranging from 2 to 7% across the dose range. Quetiapine levels were quantified with a lower limit of 5 nM and a linearity limit of 2265 nM with quality control samples within ±10%.

Results

In single-dose models, the doses that were required to achieve 50% D2 receptor occupancy (ED50) are listed in Table 1. Based on these data, the estimated doses that would approximate the clinically comparable D2 receptor occupancy are: haloperidol, 0.04 to 0.08 mg/kg s.c.; olanzapine, 1 to 2 mg/kg s.c.; risperidone, 0.5 to 1 mg/kg s.c.; quetiapine, 10 to 25 mg/kg s.c.; and clozapine, 5 to 15 mg/kg s.c. (Table 1 and Fig. 2).

The same doses administered repeatedly by injection achieved peak occupancy similar to human levels, but the trough occupancy at the end of the day was minimal and not at all comparable with what is seen in patients (Fig. 3). If the single therapeutic dose was administered daily via
minipump, the occupancies were stable through the day, but
the average occupancies were much lower than clinically
comparable therapeutic levels (see Fig. 3 for details). When
administered by pump, a dose approximately 5 times higher
than the single doses achieved stable therapeutic occupan-
cies comparable with that seen in humans (Fig. 3 and Table
2): 0.25 mg/kg haloperidol, 7.5 mg/kg olanzapine. We were
unable to examine the doses for quetiapine and clozapine
that were 5 times higher than the representative single dose
because it was not feasible to dissolve these concentrations
without making the solutions too acidic for prolonged admin-
istration (for 100 mg/kg/day, the concentration required
would be approximately 292 mg/ml).

Drug plasma levels reflected the same overall pattern as
the occupancy data (Table 2). With the injection approach,
the peak levels were very high, often multiple times higher
than that seen in clinical conditions. However, this was ac-
companied by nearly undetectable trough levels, multiply
lower than that seen in patients. When the “optimal” single
dose was administered as a continuous infusion, the levels
were often undetectable or unrepresentatively low (see Table
2 for details). Only when the drug was administered with a
pump, at levels 5 times the representative single dose, were
the levels close to that seen in clinical conditions at steady
state.

Discussion

There are few studies that have systematically addressed the
issue of antipsychotic dosing in animal models previously. Al-

<table>
<thead>
<tr>
<th>Drug</th>
<th>% D₂ Occupancy in Patients</th>
<th>Dose for 50% D₂ Occupancy In Rats (mg/kg s.c.)</th>
<th>Dose for Clinically Comparable D₂ Occupancy (mg/kg s.c.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haloperidol</td>
<td>65–80</td>
<td>0.02 (0.016–0.024)</td>
<td>0.04–0.08</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>65–80</td>
<td>0.57 (0.42–0.72)</td>
<td>1–2</td>
</tr>
<tr>
<td>Risperidone</td>
<td>65–80</td>
<td>0.32 (0.16–0.48)</td>
<td>0.5–1</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>30–60</td>
<td>11.81 (8.59–15.03)</td>
<td>10–20</td>
</tr>
<tr>
<td>Clozapine</td>
<td>45–65</td>
<td>7.26 (2.24–12.27)</td>
<td>5–1</td>
</tr>
</tbody>
</table>

Fig. 2. Relationship between drug dose and D₂ receptor occupancy after single-dose subcutane-
ous administration. Shaded box depicts the occupancy range that corresponds to clinically com-
parable conditions. A, haloperidol; B, olanzapine; C, risperidone; D, quetiapine; E, clozapine.

Fig. 3. D₂ occupancy levels produced by daily
injections (at trough or peak) or osmotic
minipump. Intermittent daily injection does not
result in sustained clinically relevant D₂ occu-
pancy. D₂ occupancy comparable to the clinical
condition is achieved with the minipump at ap-
proximately 5 times the clinically comparable
single dose. Shaded box depicts the occupancy
range that corresponds to clinically comparable
conditions. A, haloperidol; B, olanzapine; C, ris-
peridone; D, quetiapine; E, clozapine.
D2 occupancy in our study is 0.06 mg/kg, whereas it was 0.36 mg/kg in Zhang and Bymaster (1999). However, the dose of haloperidol required to produce 75% D2 occupancy was 0.05 mg/kg in our study. Both of these studies find the same relative potency for D2 occupancy (Olsson and Farde, 2001). The plasma levels noted here are those observed at the usual clinical doses, but they have not all been independently tested as being "optimal." A simple approach using just the same milligram per kilogram dose in animals and humans would not work well (Tables 1 and 3). For example, in patients at steady state, a dose of 0.2 mg/kg/day olanzapine provides plasma levels in the range of 50 nM and a receptor occupancy of about 70%. Such a dose in the rats would provide neither adequate plasma levels nor significant occupancy. Conversely, in rats, 7.5 mg/kg/day, which leads to 430 nM in the plasma, is subtherapeutically low. Thus, differences in absorption, distribution, metabolism/excretion, and perhaps brain penetration preclude the use of simple dose or plasma level parity as an optimal approach.

### Table 2

<table>
<thead>
<tr>
<th>Drug Group</th>
<th>Delivery Condition</th>
<th>D&lt;sub&gt;2&lt;/sub&gt; Occupancy (Mean ± S.D.)</th>
<th>Plasma Levels (Mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haloperidol</td>
<td>Injection, trough</td>
<td>19 ± 31</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>Injection, peak</td>
<td>74 ± 7</td>
<td>1.57 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>Minipump</td>
<td>41 ± 16</td>
<td>&lt;1</td>
</tr>
<tr>
<td>0.25 mg/kg</td>
<td>Injection, trough</td>
<td>5 ± 2</td>
<td>11 ± 2.08</td>
</tr>
<tr>
<td></td>
<td>Injection, peak</td>
<td>92 ± 1</td>
<td>2.40 ± 1.01</td>
</tr>
<tr>
<td></td>
<td>Minipump</td>
<td>69 ± 14</td>
<td></td>
</tr>
<tr>
<td>Olanzapine</td>
<td>Injection, trough</td>
<td>16 ± 24</td>
<td>6.67 ± 2.89</td>
</tr>
<tr>
<td>1.5 mg/kg</td>
<td>Injection, peak</td>
<td>74 ± 7</td>
<td>25 ± 88.39</td>
</tr>
<tr>
<td></td>
<td>Minipump</td>
<td>92 ± 1</td>
<td>3124.3 ± 823.29</td>
</tr>
<tr>
<td>Risperidone</td>
<td>Injection, trough</td>
<td>38 ± 16</td>
<td>130.83 ± 118.9</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>Injection, peak</td>
<td>59 ± 4</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>Minipump</td>
<td>92 ± 1</td>
<td></td>
</tr>
<tr>
<td>Quetiapine</td>
<td>Injection, trough</td>
<td>75 ± 7</td>
<td>434.3 ± 291.19</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>Injection, peak</td>
<td>ND</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>Minipump</td>
<td>61 ± 3</td>
<td>99.97 ± 33.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31 ± 9</td>
<td>39.36 ± 24.36</td>
</tr>
<tr>
<td>Clozapine</td>
<td>Injection, trough</td>
<td>ND</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>7.5 mg/kg</td>
<td>Injection, peak</td>
<td>29 ± 15</td>
<td>1240 ± 438.42</td>
</tr>
<tr>
<td></td>
<td>Minipump</td>
<td>2 ± 22</td>
<td>98 ± 128.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2 ± 20</td>
<td>15 ± 6.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 ± 12</td>
<td>2969 ± 809.60</td>
</tr>
<tr>
<td></td>
<td>Minipump</td>
<td>ND</td>
<td>98 ± 45.30</td>
</tr>
</tbody>
</table>

ND, no detectable occupancy.

### Table 3

Usual clinical dosing parameters

Dosing parameters were compiled from the following sources: Bezchlibnyk-Butler and Jeffries (1999), Kapur et al. (1999, 2000b,c), Tauscher and Kapur (2001), and Citrome and Volavka (2002).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Usual Clinical Dose</th>
<th>Daily Dose for a 65-kg Man</th>
<th>Peak % D&lt;sub&gt;2&lt;/sub&gt; Occupancy In Patients</th>
<th>Plasma Levels at Steady State&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haloperidol</td>
<td>2–4 mg/day</td>
<td>0.05 mg/kg/day</td>
<td>65–80</td>
<td>5–10</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>10–20 mg/day</td>
<td>0.20 mg/kg/day</td>
<td>65–80</td>
<td>40–80</td>
</tr>
<tr>
<td>Risperidone</td>
<td>2–6 mg/day</td>
<td>0.06 mg/kg/day</td>
<td>25–50</td>
<td>25–50</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>300–800 mg/day</td>
<td>7.0 mg/kg/day</td>
<td>30–60</td>
<td>550–1100</td>
</tr>
<tr>
<td>Clozapine</td>
<td>300–500 mg/day</td>
<td>6.0 mg/kg/day</td>
<td>45–65</td>
<td>1050</td>
</tr>
</tbody>
</table>

<sup>a</sup>The plasma levels noted here are those observed at the usual clinical doses, but they have not all been independently tested as being ‘optimal.’
effective means to obtain valid and representative antipsy- 
chotic doses in animals.

Most importantly, even if one uses the dose that provides 
comparable single-dose effects, it does not lead to clinically 
comparable occupancies with multiple dosing. The half-life of 
the drugs is, on average, 4 to 6 times faster in rodents than in 
humans: haloperidol, rodent 1.5 h versus human 12 to 36 h 
(Cheng and Paalzow, 1992; Bezchlibnyk-Butler and Jeffries, 1999); 
risperidone, rodent 1 h versus human 20 to 24 h (van 
Beijsterveldt et al., 1994; Bezchlibnyk-Butler and Jeffries, 
1999); olanzapine, rodent 2.5 h versus human 21 to 54 h (Ara-
vagiri et al., 1997; Bezchlibnyk-Butler and Jeffries, 1999); 
quetiapine, rodent 0.5 h versus human 6 to 7 h (Saller and 
Salama, 1993; Bezchlibnyk-Butler and Jeffries, 1999); and clo-
zapine, rodent 1.5 h versus human 5 to 16 h (Baldessarini et al., 
1993; Bezchlibnyk-Butler and Jeffries, 1999). For a certain 
peak concentration (occupancy), the trough is inversely propor-
tional to the exponent of the half-life of the drug (Rowland 
and Tozer, 1995). Since animals have a 4 to 6 times shorter half-life, 
it is only to be expected that for similar peak levels/occupancies, 
the animals would show significantly lower trough levels/occup-
cancies than those seen in humans.

Thus if one wishes to capture the same pattern of diurnal 
occupancies as in patients, a single daily injection cannot suf-
fice. Since the human dosing intervals are of the order of one 
half-life, to obtain peak/trough effects similar to those in hu-
mans, the drugs would have to be administered to animals in 
six to eight equally spaced injections through the day. Since this 
is not a practical approach, we examined whether administ-
ering the drug through the pump may more closely approximate 
the human occupancies. However, if just the optimal single dose 
was administered only via the pump through the day, the oc-
cupancy level was much lower (and in several cases undetect-
able) than those associated with routine clinical treatment (Ta-
ble 2). The ratio of the maintenance daily dose versus the single 
loading dose to obtain a certain plasma level is inversely pro-
portional to the plasma half-life (Rowland and Tozer, 1995); this 
may explain why the average maintenance dose required is 
about 5 to 6 times the single dose.

Based on the foregoing considerations, we propose that the 
dooses presented in Table 1 form a reasonably valid approxima-
tion of the clinical situation in single-dose rat models. Insofar 
as multiple dosing is concerned, we propose that only administra-
tion by pump (or administration more than four times a day) 
can provide clinical-like occupancies for haloperidol, olanzap-
ine, and risperidone. Due to sheer administration limitations, it 
is hard to achieve clinically comparable effects for quetiapine 
and clozapine, and indeed, none of the previous preclinical 
studies may have achieved them. We are only aware of one 
study that expressly addressed this issue through the use of 
repeated dosing via the addition of clozapine to drinking water 
(Schmitt et al., 1999). In this study, 40 mg/kg/day were deliv-
ered via drinking water, yet the serum concentrations for clo-
zapine were only 22 ng/ml (whereas clinically comparable concen-
trations are in the range of 350 ng/ml, 12 h after last dose), 
thus pointing to the limitation of this method of delivery, even 
though the nominal dose may seem reasonably high. Therefore, 
multiple-dosing studies of quetiapine and clozapine should be 
interpreted with caution, especially where they claim to be a 
representative model for the clinical condition.

Limitations of our data as well as its implications are out-
lined next. First, we observed that animals treated with 10 
mg/kg/day quetiapine multiple dosing using the daily injection 
approach showed no detectable D2 occupancy, even at peak 
(Table 2). This is a surprising finding given that when a single 
injection of the same dose is administered, it leads to 50% 
occupancy at peak. Furthermore, this dose showed a plasma 
level consistent with expectation (469 nM), and the next higher 
dose (25 mg/kg/day) showed occupancy (78%) and plasma levels 
(913 nM) also consistent with expectation. Although it is con-
ceivable that multiple dosing may lead to changes in disposition 
that may account for this, a more likely explanation is that this 
result represents experimental error.

In a strict sense, the doses proposed here (Table 1) are only 
valid for the species studied (young Sprague-Dawley male 
rats) and the route of administration (subcutaneous). One 
would have to be cautious in extrapolating across species and 
strains, since pharmacokinetic differences are noted between 
them. Furthermore, one would have to be cautious in extrap-
olating across routes (s.c. versus oral or i.p.) because different 
first-pass and other metabolic considerations could change 
these optimal doses. Nonetheless, the general approach out-
lined here could be used for deriving the right estimates for 
these different strains and routes of administration. A limita-
tion to this method of deriving optimal preclinical dosing of 
antipsychotics is that it is (and will be) relevant only for the 
current generation of atypical and typical antipsychotics, 
which have a significant level of D2 occupancy in patients. As 
new drugs are developed, which totally avoid the D2 recep-
tors, obviously such an approach would not work. However, 
one could hope that once such new targets are developed, 
similar equivalent occupancy studies could be done across 
humans and animals to confirm the valid doses. Finally, we 
do not see the restricted optimal ranges we provide in Table 
1 as a substitute for doing complete dose-response relation-
ship studies. The ideal way to understand the findings in 
animal models still remains the complete dose-response rela-
tionship study; however, the ranges presented here will be 
important in interpreting the dose-response relationships 
in a clinical context.

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References

Aravagiri M, Ames D, Wirshing WC, and Marder SR (1997) Plasma level monitoring of 
olanzapine in patients with schizophrenia: determination by high-performance liquid 
chromatography with electroschemical detection. Ther Drug Monit 19:307–313.

Arnt J, Skarnes TF, and Hyyti J (1997) Differentiation of classical and novel antipsy-

Baldessarini RJ, Centorrino F, Flood JG, Volpicelli SA, Huston-Lyons D, and Cohen 

Bezchlibnyk-Butler KZ and Jeffries JJ (1999) Clinical Handbook of Psychotropic 

Bressan RA, Jones HM, EJ PJ, and Pilowsky LS (2001) Dopamine D2 receptor 

Psychopharmacology 89:77–84.

Campbell A, Herschel M, Cohen BM, and Baldessarini RJ (1980) Tissue levels of 
haloperidol by radioreceptor assay and behavioral effects of haloperidol in the rat. 


Citrome L and Volavka J (2002) Optimal dosing of atypical antipsychotics in adults: 

occupancy in schizophrenic patients treated with antipsychotic drugs. Arch Gen 
Psychiatry 45:71–76.


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