BL-1020, a novel antipsychotic candidate with GABA-enhancing effects: D2 receptor occupancy study in humans☆

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Abstract

BL-1020 is a potentially novel antipsychotic, which comprises the typical antipsychotic perphenazine linked by an ester bound to γ-aminobutyric acid (GABA), intending a simultaneous dopamine-2 (D2) receptor blockade and GABA facilitation in the brain. This positron emission tomography (PET) study, using [11C]raclopride, assessed the extent and duration of D2 receptor occupancy (D2 RO) and safety for single doses of BL-1020 in healthy male subjects. Overall, this study did not raise any safety concern. Single doses of 16–32 mg BL-1020 caused a dose dependent striatal D2 RO. The 32 mg dose of BL-1020 resulted in an average D2 RO of 44% at 4–6 h post dosing (pd), which declined to 33% at 24 h pd. Equimolar doses of BL-1020 and perphenazine resulted in similar D2 RO at 24 h pd. Pharmacokinetic–pharmacodynamic analysis predicted that oral once daily administration of 32 mg BL-1020 would result in D2 ROs ranging from 52 to 66% at a steady state.

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1. Introduction

Treatment of schizophrenia has been focused towards affecting the dopaminergic system, and in addition the serotonergic system, with drugs classified as typical and atypical antipsychotics. However, a common factor for all antipsychotics is that they often lead to more or less severe side-effects (Lieberman et al., 2005), varying from movement disorders to metabolic disturbances, which limit patient compliance and ultimately their well-being and quality of life. Thus, despite access to various antipsychotics, there is still a clear need to develop alternative therapies for treatment of schizophrenia, which retain the efficacy of current antipsychotics, but decrease the risk for side-effects by involving other neurotransmitter systems.

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BL-1020 is a novel antipsychotic candidate comprising the typical antipsychotic perphenazine linked by an ester bound to γ-aminobutyric acid (GABA) (Nudelman et al., 2008). GABA is the primary inhibitory neurotransmitter in the central nervous system. A large body of evidence from preclinical and clinical studies support that a downregulated GABA system is involved in the pathophysiology of schizophrenia and pharmacological interventions at GABA-ergic pathways have been suggested to resolve the GABA deficits (reviews in Carlsson et al., 2001; Wassef et al., 2003; Guidotti et al., 2005). However, under normal conditions GABA cannot cross the blood–brain barrier (Kuriyama and Sze, 1971). Conjugation of GABA to the dopamine-2 (D2) receptor antagonist perphenazine facilitates brain penetration and upon hydrolysis of BL-1020 in the brain, these two active substances will be released (Nudelman et al., 2008; Geffen et al., 2009). Preclinical studies suggest that GABA agonists may interfere with other neurotransmitter systems, particularly the dopamine system (Shukla et al., 1985, Benes et al., 1993, Karler et al., 1998; Wassef et al., 2003). Therefore, BL-1020 may also potentially reduce anti-parkinsonian side-effects, such as extrapyramidal symptoms (EPS), while retaining its antagonistic effect on dopaminergic receptors.

BL-1020 has been extensively investigated by in vitro and in vivo animal models (Nudelman et al., 2008; Geffen et al., 2009). In receptor binding studies in vitro BL-1020 demonstrated potent dopaminergic, serotonergic, histaminergic, opiate, and specific GABA_A activity. Compared to perphenazine the affinity of BL-1020 was 3-5 fold higher to human D_2 and serotonin-2A (5-HT_2A) receptors (i.e. D_2 K_i = 0.064 nM; 5-HT_2A K_i = 0.211 nM). BL-1020 showed an agonistic activity for the human GABA_A receptor (K_i = 3.74 μM), but no activity on the GABA_B receptor, whereas GABA alone resulted in a K_i of 0.069 μM on the GABA_A receptor. After acute and chronic administration in rats, BL-1020 showed no cataleptic manifestations at doses of 3.31 and 6.63 mg/kg, whereas equimolar doses of perphenazine showed a significant greater and dose dependent increase of catalepsy, indicating inhibitory GABAergic activity after administration of BL-1020. The results were maintained after prolonged administration of BL-1020 during 15 days. In a single dose radiolabelled pharmacokinetic study in rats [14C]BL-1020 revealed a rapid absorption after oral administration. Use of HPLC-radiochromatography showed significant concentrations of [14C]BL-1020 and [14C]GABA in pulverized brain extracts at 15 min post administration, indicating presence of BL-1020 and release of GABA in the brain.

The present study was preceded by one phase I study for BL-1020 in 48 healthy male volunteers to investigate the safety and pharmacokinetics of single oral doses of BL-1020, escalating from 4 to 40 mg. Based on the appearance of significant adverse events (AEs) seen in this study, the maximum tolerated dose was determined to 40 mg. Positron emission tomography (PET), using [11C]raclopride, has demonstrated that D_2 receptor occupancy (D_2 RO) is a reliable pharmacological predictor of response to antipsychotics and EPS (Farde et al., 1992; Nordström et al., 1993; Kapur et al., 1995, 2000). For typical antipsychotics it has been suggested that there may be a narrow therapeutic window between 60 and 80% D_2 RO, which may yield adequate antipsychotic response with low or minimal EPS (Farde et al., 1992; Nordström et al., 1993; Nyberg et al., 1999). Thus the critical question is whether BL-1020 preserved the dopamine antagonist activity and would show relevant D_2 ROs in the human brain without significant side-effects.

This PET study aimed to assess the extent and duration of D_2 RO and safety for different doses of BL-1020 (part A) and to compare D_2 RO of BL-1020 and perphenazine (part B). Here we hypothesized that the effect of BL-1020 and perphenazine on the D_2 receptor were similar after equimolar single doses of both drugs. The results were intended to guide the dose selection in forthcoming phase II studies in schizophrenic patients and hence the knowledge about the D_2 RO would allow elucidating the GABA effect when comparing BL-1020 and perphenazine.

2. Experimental procedures

2.1. Study design

This was a single dose, open-label, 2-panel PET study (parts A and B) investigating the degree of occupancy of D_2 receptors in the human brain after single oral doses of BL-1020 or perphenazine and safety in young healthy male subjects. For part A the rationale was to determine the central D_2 RO and safety to guide the dose selection for part B and following studies. In this part, three cohorts, with four subjects in each, were enrolled and administered single doses of 16–32 mg BL-1020. PET scans were performed at baseline and 6 and 24 h post dosing (pd). The 6 h pd scan aimed to estimate the maximum D_2 RO, whereas the 24 h PET scan was used to assess the duration of D_2 RO. For part B the rationale was to compare the D_2 RO of BL-1020 with the reference compound perphenazine. In this part eight subjects were first exposed to one dose of perphenazine and then, after a washout period of 7 days, to one single dose of BL-1020. Each subject had five PET scans: baseline and two scans in each treatment period following perphenazine and BL-1020 dosing – 4 and 24 h pd. Doses and time points were based on the results of part A. Blood samples were taken to investigate plasma perphenazine and prolactin levels.

As no reproductive toxicological data on BL-1020 were available at the start of the study, the study was performed exclusively on men. Age was taken into account in the study design to avoid a possible confounding of age effects with the D_2 RO results (Nordström et al., 1992). Volunteers were therefore between 21 and 35 years of age. Since perphenazine itself or as a major metabolite of BL-1020, is known to be a substrate of CYP2D6 metabolism (Dahl-Puustinen et al., 1989; Jerling et al., 1996), at screening the subjects were genotyped for CYP2D6 metabolism and the subjects classified as poor metabolizers were excluded to avoid a confounding of drug metabolism and D_2 RO.

The study was performed with approval from the Swedish Medical Products Agency, local ethics and radiation protection committees and FDA (investigational new drug approval) and complied with regulations outlined in the Declaration of Helsinki.

2.2. Subjects

Twenty male volunteers (mean ± SD = 26.0 ± 3.36 years; range = 21–35) with a good health based on medical history, physical examination, electrocardiogram and laboratory profile of both blood and urine, were enrolled in the study. They were non-smokers for at least 1 month, and did not belong to the CYP2D6 poor metabolizer genotype. Body weight of the subjects was on average of 75.7 kg (SD= 11.5; range 50–94). All volunteers signed a document of informed consent before entering the study.

2.3. Study drugs and doses

The study compound BL-1020, 4-aminobutyric acid 2-[4-[3-2-chlorophenothiazine-10-yl propyl]-piperazine-1-yl]-ethyl ester, was...
supplied as hard gelatin capsule with 8 or 16 mg of BL-1020 trimaleate salt, manufactured at CSS (Craigavon, Northern Ireland). BL-1020 trimaleate salt has a molecular weight of 837.29, whereas this is 403.15 for perphenazine. Thus 16 and 32 mg of BL-1020 are equimolar to 7.7 and 15.4 mg of perphenazine, respectively.

In part A, the doses were 16, 24 and 32 mg of BL-1020 for the three cohorts, respectively. The rationale of the starting dose (16 mg) and the highest dose (32 mg) of BL-1020 was based on the performed clinical phase I study and non-clinical studies on toxicology as well as efficacy and safety (induction of catalepsy) using predictive animal models. An interim analysis of the first cohort was performed to guide dose decisions for the following two cohorts.

In part B, subjects were randomized to a combination of doses of perphenazine (8 mg) and BL-1020 (16 or 32 mg). The doses of BL-1020 were based on the preliminary D1 RO data from all groups in part A and 8 mg perphenazine was the maximum allowed dose in this study. Perphenazine is an established, typical antipsychotic with a well documented clinical profile and it has been investigated in many other clinical studies, including PET studies (Farde et al., 1988; Hall et al., 1992; Talvik et al., 2004).

2.4. PET procedures

2.4.1. Radiochemistry and doses

The tracer [11C]raclopride was synthesized at the chemistry section of Uppsala Imanet, GE Healthcare, according to a standard manufacturing procedure. The delivered batches of [11C]raclopride had generally a high amount of radioactivity (n=44; mean±SD=1586±488.5 MBq), which was generally sufficient for administration in two subjects. Specific radioactivity of the tracer batches was on average of 105 GBq/µmol (SD=60.0; range=30–275) and the radiochemical purity was greater than 95%. The average dose of injected radioactivity was 301 MBq (n=76; SD=39; range=210–351). Administered amount of raclopride was less than 10 µg (mean±SD=2.9±1.68 µg), which assures that the tracer concept is valid and not affecting D2 RO estimates.

2.4.2. PET Imaging

The PET scans were performed on two identical ECAT EXACT HR+ scanners (Siemens/CTI, Knoxville, TN), comprising 32 rings of bismuth germanate crystal detectors. These scanners enabled the acquisition of 63 contiguous planes of data with a distance of 2.46 mm and an axial field of view of 15.5 cm.

A 10 min transmission scan (2D-mode) was performed using three retraceable rotating 68Ge line sources. Following the transmission scan, [11C]raclopride was administered as an i.v. bolus in the arm of the subject. Simultaneously, a dynamic emission scan was started (3D-mode), which consisted of 17 time frames with progressive frame duration (5×60 s, 5×120 s, 7×300 s), resulting in a total duration of 50 min. All emission scans were reconstructed with filtered back projection using a 4 mm Hanning filter. The PET data were corrected for photon attenuation, scattered radiation, random coincidences and physical decay of 11C.

PET scans were generally performed according time schedule, but some post dosing scans were rescheduled due to synthesis failures or technical issues. In part A the 24 h PET scans were delayed 1–2 h for two subjects (A1). In part B the scheduled 4 h BL-1020 PET scans were started 1–2 h later for three subjects (B1/B2 2/1) and 4 h later for one subject (B2). After the dosing of perphenazine the 24 h PET scan was delayed 1 h for two subjects (B2).

2.5. Blood sampling

Blood samples (3 mL) were collected for the determination of BL-1020 and perphenazine plasma concentration at pre-dose and 1, 2, 3, 4, 5, 6, 7, 12, 16, 24, 25 and 26 h pd. Plasma samples were analyzed by a validated liquid chromatography method combined with tandem mass spectrometry for the determination of the concentrations of BL-1020 and perphenazine. The lowest limit of quantification for BL-1020 and perphenazine in plasma was 0.05 ng/mL.

Blood samples (3 mL) to determine prolactin concentrations in plasma were taken at pre-dose and 1, 4, 7, 12 and 24 h pd. Prolactin was measured in unextracted human plasma by a sensitive and specific in vitro bioassay. Sensitivity was 5 ng/mL.

2.6. PET data analyses

PET scans were realigned to adjust for subject movements during scans and positions of the subjects in the scanner at the different occasions (Andersson, 1995). Region of interests were delineated manually to represent striatum, including putamen and nucleus caudatus (3 slices, bilaterally) and cerebellum (2 slices). An in-house developed image analysis program generated time–activity data in each scan for each subject, representing the levels of radioactivity over time in striatum and cerebellum, corrected for the subject’s body weight and administered dose of radioactivity (expressed as standardized uptake values – SUV).

Tracer kinetic analysis was applied using a previously described simplified reference tissue model (Lammertsma and Hume, 1996). Striatum was the target region, whereas cerebellum, a region shown to have no specific binding of [11C]raclopride (Hall et al., 1994), was used as reference region. The model assumes the non-specifically bound and free distribution volume of [11C]raclopride to be the same in the reference and target region. Model parameters were estimated by a non-linear least squares method using in-house MATLAB routines. The percentage of D2 receptor occupancy (D2 RO, %) was calculated as the relative difference between the estimated binding potential in the baseline (BPbase) and post-treatment (BPt) experiments as \( \frac{BP_{\text{base}} - BP_{\text{t}}}{BP_{\text{base}}} \times 100\% \).

2.7. Pharmacokinetic–pharmacodynamic modelling

The relationship between plasma perphenazine concentration and striatal D2 RO was evaluated using the WinNonlin non-linear modelling software (version 4.1b, Pharsight, Mountain View, CA). Each data point was treated as a separate observation. A simple \( E_{\text{max}} \) model was fitted to the data as follows,

\[
D_2 \text{RO}(\%) = \left( \frac{E_{\text{max}} \times \text{CONC}_{\text{PET}}}{E_{\text{max}} + \text{CONC}_{\text{PET}}} \right)
\]

where \( E_{\text{max}} \) is the maximum D2 RO, \( \text{CONC}_{\text{PET}} \) is the mean plasma perphenazine concentration from samples immediately before and after a scan and \( E_{\text{C}_{\text{IO}}} \) is the plasma perphenazine concentration required to produce 50% of \( E_{\text{max}} \).

The D2 RO at steady state after repeated once daily dosing of 32 mg BL-1020 was predicted by estimation of steady state perphenazine levels from single dose pharmacokinetics and the pharmacokinetic–pharmacodynamic (PK–PD) results.

2.8. Safety

Safety was evaluated based on spontaneously reported AEs, scheduled physical examinations, vital signs monitoring, blood pressure, 12-lead electrocardiograms, and clinical laboratory results. Clinician-rated assessments for psychic-, neurologic- and autonomic side-effects (UKU side-effect rating scale, section 1–3 (Lingjaerde et al., 1987)) were carried out pre-dose (up to 24 h pre-dose), and 4(±1) h and 24(±1) h pd.

2.9. Statistics

The D2 RO results are expressed as the mean ± SD. Statistical analysis was done using the Student’s t test (D2 RO). Values of \( p < 0.05 \) were considered statistically significant.
3. Results


The average changes in [11C]raclopride uptake over time are shown for striatum in the 4–6 and 24 h pd scans for each cohort in Fig. 1. In part A, a dose dependent reduction in uptake of [11C]raclopride was found at both 6 and 24 h pd. In part B, the same patterns were found for the BL-1020 doses. Following the administration of perphenazine the reduction in [11C]raclopride uptake was larger compared to the equimolar dose of BL-1020 at 4–6 h pd, but the uptake levels were similar at 24 h pd. Fig. 2 depicts [11C]raclopride uptake in baseline and post-treatment scans for one subject from part A and B, whose data were representative for the particular dose group.

3.2. D2 receptor occupancy

Estimated binding potentials and D2 ROs for each cohort are summarized in Table 1. In part A, at 6 h pd the average striatal D2 RO was 47% for the 32 mg dose of BL-1020, which was twice as high and significantly different compared to the average of the 16 mg dose group ($t = 2.939; p = 0.011$). At 24 h pd the differences between the dose groups decreased, but the average D2 RO after 32 mg of BL-1020 (30%) was still significantly different from the average 18% D2 RO after 16 mg BL-1020 ($t = 2.875; p = 0.012$).

In part B, for equimolar doses of BL-1020 (16 mg) and perphenazine (8 mg), the D2 RO was on average 35% for perphenazine at 4–6 h pd, which was somewhat higher, but not significantly different, compared to the average for 16 mg BL-1020 (28%). However, at 24 h pd the average D2 RO was similar for equimolar doses of BL-1020 (22%) and perphenazine (19%). For the comparison of 32 mg BL-1020 and 8 mg perphenazine the average D2 ROs were similar at 4–6 h pd, 42 and 39%, respectively. In contrast at 24 h pd the average D2 RO was significantly higher for 32 mg BL-1020 than for 8 mg perphenazine, 37 and 22%, respectively ($t = 4.453; p = 0.021$).

The pooled data for part A and B (Table 1) confirmed the difference in D2 RO between equimolar doses of BL-1020 (16 mg) and perphenazine (8 mg) at 4–6 h pd, although the differences were not statistically significant at the 5% level ($t = 1.700; p = 0.111$). At 24 h pd the average D2 RO for the equimolar doses was the same. The 32 mg dose of BL-1020 differed significantly from 16 mg BL-1020 at both 4–6 h pd ($t = 2.939; p = 0.011$) and 24 h pd ($t = 2.875; p = 0.012$), but only at 24 h pd compared to 8 mg perphenazine ($t = 3.180; p = 0.007$). A linear relationship was observed between average D2 RO and dose of BL-1020 per kg body weight (Fig. 3) at both 4–6 h ($r = 0.734; p < 0.001$) and 24 h pd ($r = 0.767; p < 0.001$).

3.3. Pharmacokinetics

Average plasma perphenazine profiles are presented in Fig. 4. In part A, the average plasma perphenazine profiles showed a dose dependency following administration of 16–32 mg BL-1020. Part
Figure 2  Uptake of [11C]raclopride in a section through striatum normalized to cerebellum at baseline, and at 4–6 and 24 h post dosing (pd) of 32 mg BL-1020 (part A) and equimolar doses of BL-1020 (16 mg) and perphenazine (8 mg) in part B.

Table 1  Striatal binding potential (BP) and percentage of dopamine D2 receptor occupancy (D2 RO) at baseline and at 4–6 and 24 h after one single oral dose of 16, 24 and 32 mg BL-1020 and 8 mg perphenazine for each cohort and pooled data from part A and B.

<table>
<thead>
<tr>
<th>Cohort and dose</th>
<th>n</th>
<th>Baseline</th>
<th>4–6 h post dose</th>
<th>24 h post dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BP</td>
<td>BP</td>
<td>D2 RO</td>
</tr>
<tr>
<td><strong>Cohort A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1–16 mg BL-1020</td>
<td>4</td>
<td>3.46 ± 0.30</td>
<td>2.67 ± 0.55</td>
<td>23 ± 11.4</td>
</tr>
<tr>
<td>A2–24 mg BL-1020</td>
<td>4</td>
<td>3.38 ± 0.21</td>
<td>2.07 ± 0.27</td>
<td>39 ± 10.9</td>
</tr>
<tr>
<td>A3–32 mg BL-1020</td>
<td>4</td>
<td>3.15 ± 0.36</td>
<td>1.66 ± 0.28</td>
<td>47 ± 6.8*</td>
</tr>
<tr>
<td><strong>Cohort B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1–16 mg BL-1020</td>
<td>4</td>
<td>3.18 ± 0.23</td>
<td>2.26 ± 0.26</td>
<td>28 ± 12.1</td>
</tr>
<tr>
<td>–8 mg perphenazine</td>
<td>4</td>
<td>2.04 ± 0.41</td>
<td>35 ± 16.7</td>
<td>2.57 ± 0.13</td>
</tr>
<tr>
<td>B2–32 mg BL-1020</td>
<td>4</td>
<td>3.55 ± 0.36</td>
<td>2.04 ± 0.58</td>
<td>42 ± 20.2</td>
</tr>
<tr>
<td>–8 mg perphenazine</td>
<td>4</td>
<td>2.18 ± 0.56</td>
<td>39 ± 15.2</td>
<td>2.75 ± 0.25</td>
</tr>
<tr>
<td><strong>Pooled data A &amp; B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 mg BL-1020</td>
<td>8</td>
<td>3.32 ± 0.29</td>
<td>2.36 ± 0.56</td>
<td>26 ± 11.2</td>
</tr>
<tr>
<td>32 mg BL-1020</td>
<td>8</td>
<td>3.35 ± 0.39</td>
<td>1.92 ± 0.50</td>
<td>44 ± 14.4*</td>
</tr>
<tr>
<td>8 mg perphenazine</td>
<td>8</td>
<td>3.37 ± 0.34</td>
<td>2.11 ± 0.46</td>
<td>37 ± 15.0</td>
</tr>
</tbody>
</table>

An independent Student’s t test was applied for part A (df = 6) and the pooled data A & B (df = 14), whereas in part B a dependent Student’s t test was performed (df = 3). Significance levels are expressed as * (p < 0.05) and ** (p < 0.01) compared to 16 mg BL-1020 and if added X compared to the 32 mg BL-1020.
B revealed that dosing of 8 mg perphenazine had different early pharmacokinetics compared to the equimolar dose of BL-1020 (16 mg), but the plasma perphenazine concentrations were at a similar level from 4 h pd. For BL-1020 no plasma profiles over time could be obtained, since there were generally no quantifiable plasma concentrations of BL-1020 (<0.05 ng/ml) from 2 h post dosing. Hence the pharmacokinetic analysis, and its relationship to D$_2$ RO estimates, is restricted to plasma perphenazine concentrations.

The pharmacokinetic analysis, including data of part A and B, showed that the exposure to perphenazine was approximately dose proportional within the studied dose range of 16–32 mg BL-1020, with regard to dose normalized mean values of $C_{\text{max}}$, AUC$_{0-t}$ and AUC$_{0\text{-inf}}$ (Table 2). The mean AUC$_{0-t}$ and mean AUC$_{0\text{-inf}}$ of 8 mg perphenazine after 16 mg BL-1020 were comparable to those obtained for the equimolar dose of 8 mg perphenazine, but $C_{\text{max}}$ was higher and reached earlier for perphenazine. These data also revealed that perphenazine from 16 mg BL-1020 had a slower elimination from plasma than administered perphenazine.

### 3.4. Prolactin levels

Plasma prolactin concentrations were dose dependent at 4 h pd (Fig. 3, part A), where the prolactin concentration was approxi-
mately twice as high for the 32 mg BL-1020 dose compared to the 16 mg BL-1020 dose. The 8 mg dose of perphenazine demonstrated to have a faster and more potent effect on prolactin concentration than the equimolar dose of 16 mg BL-1020, but the prolactin concentrations were at a similar level from 7 h pd and later on (Fig. 3, part B). At 24 h pd, the prolactin levels had returned to normal for both 8 mg perphenazine and 16 mg BL-1020.

### 3.5. Pharmacokinetic–pharmacodynamic modelling

The relationship between plasma perphenazine concentration and striatal D2 RO was well described by a simple $E_{\text{max}}$ model for both the BL-1020 and perphenazine data (Fig. 5). The estimated maximum D2 RO was close to 100% for perphenazine, whereas it was nearly 80% for BL-1020 (Table 3). It was predicted that oral once daily administration of 32 mg BL-1020 would result in D2 RO levels ranging from 52 to 66% at steady state. Estimated steady state perphenazine levels were based on accumulation ratios of AUC$_{0\text{-inf}}$ and AUC$_{0\text{-24 h}}$, assuming linear kinetics, from single dose levels in a subset of the data from part A and B.

### 3.6. Safety

A total of 23 treatment-emergent AEs were reported by 11 subjects in part A (6, 8 and 9 AEs after 16, 24 and 32 mg BL-1020 treatment, respectively). In part B, 31 treatment-emergent AEs were reported by 8 subjects (10, 10 and 11 AEs after 8 mg perphenazine (n=8) and 16 mg and 32 mg BL-1020 treatment (both n=4)). The most frequently occurring AEs after treatment with BL-1020 were fatigue (10 events), polyuria (4 events) nasopharyngitis (3 events), epistaxis, muscle spasms and dizziness (all 2 events). After treatment with perphenazine the most frequent AEs were nasopharyngitis (3 events) and fatigue (2 events). In part A, two of all AEs were reported as moderate and 21 as mild. In part B, one AE was reported as moderate and 30 as mild. The AE with moderate intensity (fatigue) was reported after treatment with 8 mg perphenazine at 5–6 h pd.

No clinically significant changes were observed in ECG, vital signs or UKU side-effect rating scale after BL-1020 or perphenazine treatment. Further, no clinically significant alterations in clinical chemistry or hematology were reported.

### 4. Discussion

To our knowledge this is the first study examining the D$_2$ RO in vivo induced by a compound that conjugates a D$_2$ antagonist with GABA or with any other active endogenous moiety — providing a proof of concept for the pharmacodynamic viability of such an approach. These results allow dose selection for following clinical trials in schizophrenia.

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**Table 2** Summary of PK variables of perphenazine after single oral doses of 16–32 mg BL-1020 and 8 mg perphenazine (part A and part B).

<table>
<thead>
<tr>
<th>PK variable$^2$</th>
<th>BL-1020</th>
<th>Perphenazine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16 mg (n=8)</td>
<td>24 mg (n=4)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>0.184 (0.0659)</td>
<td>0.310 (0.0960)</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>2.00 (1.00–6.00)</td>
<td>1.00 (1.00–2.00)</td>
</tr>
<tr>
<td>AUC$_{0\text{-t}}$ (h×ng/mL)</td>
<td>1.76 (1.09)</td>
<td>2.86 (1.50)</td>
</tr>
<tr>
<td>AUC$_{0\text{-inf}}$ (h×ng/mL)</td>
<td>3.08 (1.51)$^3$</td>
<td>4.00 (1.91)</td>
</tr>
<tr>
<td>$t_{1/2,z}$ (h)</td>
<td>12.3 (3.71)$^2$</td>
<td>12.5 (4.26)</td>
</tr>
</tbody>
</table>

$^1$Mean and SD, except for $t_{\text{max}}$ where median and range are shown.

$^2C_{\text{max}}$ = peak plasma concentration; $t_{\text{max}}$ = time to peak plasma concentration; AUC = area under the curve, extrapolated from 0 to the last quantifiable time point (t) or infinity (inf); $t_{1/2,z}$ = terminal elimination half-life.

$^3n=7$.

$^4n=6$.

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**Figure 5** Relationship between plasma concentrations of perphenazine and striatal D$_2$ receptor occupancy after administration of a single oral dose of 16–32 mg BL-1020 (left; part A and B; n=28) or 8 mg perphenazine (right; part B; n=11).
patients, but more importantly they provide information to test the hypothesis regarding the contributions of the GABA component to antipsychotic efficacy and side-effects.

BL-1020 demonstrated to cause relevant and dose dependent D2 ROs in the human brain. The highest investigated dose of 32 mg BL-1020 resulted in an average D2 RO of 44% at 4–6 h pd, which declined to 34% at 24 h pd. The relatively slow decline in D2 RO may allow for once daily dosing. Based on PK–PD data it is anticipated that once daily dosing of 32 mg BL-1020 will result in a D2 RO between 50–70% at steady state after multiple dosing, which would be at the lower border of the therapeutic window of 60–80% of typical antipsychotics (Farde et al., 1992; Nordström et al., 1993).

Although BL-1020 consists of approximately 50% perphenazine, BL-1020 may not necessarily have the same therapeutic window as conventional antipsychotics and therefore even BL-1020 doses resulting in D2 RO outside this window should be investigated in patients. Besides the GABA properties, receptor binding studies in vitro showed that BL-1020 has also a significant affinity for other neurotransmitters, among others serotonergic receptors (Geffen et al., 2009). Therefore it cannot be excluded that a therapeutic effect of BL-1020 may also be achieved at a relatively low level of D2 RO.

The comparison between equimolar doses of BL-1020 and perphenazine (part B) showed that the dopamine antagonist activity was preserved. The average D2 RO achieved 4 h after administration of 16 mg BL-1020 was marked lower than that from the equimolar dose of 8 mg perphenazine, but the average D2 ROs were similar for 16 mg BL-1020 and 8 mg perphenazine at 24 h pd. For the differences in D2 RO at 4 h pd an explanation was found by differences in plasma perphenazine levels between BL-1020 and perphenazine. The pharmacokinetic data showed that maximum plasma concentrations of perphenazine were attained later for BL-1020 than for perphenazine. As the average concentrations of perphenazine in plasma were similar after administration of equimolar doses of BL-1020 and perphenazine from 4 h pd, these results indicate that most of available BL-1020 in the brain had been cleaved to GABA and perphenazine at this stage. This would imply that the major part of the observed D2 ROs was caused by perphenazine. Further it may explain why there were observed no quantifiable plasma concentrations of BL-1020 at the time of the post dosing PET scans (from 4 h pd). These observations are supported by the [14C]BL-1020 experiment in rats (Geffen et al., 2009), where significant brain concentrations of [14C]BL-1020 could only be detected up to 30 min, although significant amounts [14C]GABA were present until at least 4 h pd.

As a marker of anti-dopaminergic effects, the increasing prolactin levels indicate the ability of BL-1020 to affect the blocking of pituitary dopamine receptors – which are outside the blood–brain barrier. The prolactin data demonstrated clearly that there was a dose dependent change in prolactin levels at 4 h pd following dosing of BL-1020, in both part A and B. Similar effects on prolactin were observed for equimolar doses of BL-1020 and perphenazine, although the peak concentration was higher and earlier observed for perphenazine. Thus, also the prolactin concentrations point towards a delayed profile for BL-1020 compared to perphenazine.

Altogether the comparison of equimolar doses of BL-1020 (16 mg) and perphenazine (8 mg) revealed that there is a delay in achieving maximum central D2 RO and peak in plasma perphenazine and prolactin concentrations for BL-1020 compared to perphenazine. Possible causes are the rate of formation of perphenazine from cleavage of BL-1020 or a delayed absorption phase due to other physical properties of the molecule. A slower release of the active substances resulting in a lower peak concentration in plasma may cause fewer acute side-effects, such as EPS, compared to perphenazine and other typical antipsychotics, which may improve the patient’s compliance to the antipsychotic treatment.

The BL-1020 results of this study did not raise any safety concerns and indicate that administration of 16–32 mg doses of BL-1020 is safe in healthy volunteers. Mild CNS related AEs have been reported for all doses and a connection to the study drug cannot be ruled out. However, none of the AEs were of severe nature and the subjects experienced no significant discomfort. The safety profile confirmed the results of the first single dose escalating, double blind, placebo controlled clinical trial, where no serious or severe adverse events were reported during the course of the study with doses up to 32 mg BL-1020 (unpublished data). These favourable results are also supported by previous findings using a rat model. No undesirable side-effects, such as sedation or other effect on behavior or specific psychological responses, were revealed for doses of up to 20 mg/kg (Geffen et al., 2009). In microdialysis experiments performed in rats, it was found that the increased GABA levels following BL-1020 administration were still within physiological levels (unpublished data). Taking the clinical trials in healthy volunteers together (n=56), it appears that BL-1020 is a safe, well tolerated, effective antipsychotic with potential added benefits of GABA agonism. However, the contributions of GABA to antipsychotic efficacy and prevention of side-effects such as EPS need to be further elucidated in schizophrenia patients. In this aspect knowledge of the D2 RO will be important information to entangle the specific GABA effect.

Our study is not without limitations, which suggests some caution on how the presented work can be interpreted. The number of subjects was relatively small with four subjects in each cohort. Especially the comparison between equimolar doses of BL-1020 and perphenazine (part B) should need more subjects to allow proper pair wise comparisons of both drugs. In this part outliers also showed to have a relatively large impact on the average D2 RO results and its SD. Further, only a limited dose range of BL-1020 and perphenazine could be studied due to safety restrictions in healthy volunteers. As a consequence the range of D2 RO was limited. Certainly it

<table>
<thead>
<tr>
<th>Compound</th>
<th>$E_{\text{max}}$ (%)</th>
<th>$EC_{50}$</th>
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<tbody>
<tr>
<td>BL-1020</td>
<td>77.4 (23)</td>
<td>0.146 (44)</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>97.7 (31)</td>
<td>0.213 (53)</td>
</tr>
</tbody>
</table>

$E_{\text{max}}$ is maximum D2 RO and $EC_{50}$ is the plasma concentration required to produce 50% of $E_{\text{max}}$. Data in parentheses is the coefficient of variation calculated as the standard error divided by the parameter estimate.

$n=28$ for BL-1020 and $n=11$ for perphenazine based on observations with both measurable concentrations of plasma perphenazine before and after the PET scan and estimated D2 RO.
would have been of interest to compare equimolar doses of 32 mg BL-1020 and 16 mg perphenazine, but because of sparse data in healthy volunteers the maximum allowed dose of perphenazine was set to 8 mg. Finally, some deviations of the time schedule occurred, especially for the BL-1020 scans at 4 h pd in part B, which might have affected somewhat the comparisons of BL-1020 and perphenazine. However, as the D2 ROs are related to the plasma concentrations in the PK–PD modeling, this will have no impact on the estimated relationship.

In conclusion, this PET study demonstrates that a conjugate of the D2 antagonist perphenazine and GABA provides relevant D2 RO in the human brain. The results indicated that 32 mg of BL-1020 would be a good starting dose for following trials — and is likely to provide 50–70% D2 RO at a steady state. Further, knowledge of the D2 RO of BL-1020 would allow for more thorough comparisons with other antipsychotics and thus allows entangling more specifically the clinical correlates of concomitant GABA enhancement with antipsychotic efficacy and side-effects. These results prompted further clinical trials with BL-1020 and currently phase 2b trials are ongoing to compare the efficacy, safety and tolerability of BL-1020 as compared to placebo and the atypical antipsychotic risperidone in schizophrenic patients.

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Contributors

Authors LA, YG and GA designed the study and LA, YG were involved in the development of the study protocol and local project management. Authors LA and KH performed the analysis of PET data and CE performed the PK–PD modelling. Author SK was a consultant of BioLineRx during the course of the study and reviewed the study design and outcome of the study. Author LA wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors wish to disclose the following potential conflict of interests. LA, KH and GA are full-time employees of Uppsala Imanet AB, GE Healthcare, CE is employed by Quintiles AB and YG is employed by BioLineRx. SK was a consultant of BioLineRx and in the last 3 years he has received grant support from AstraZeneca, Bristol-Myers Squibb and GlaxoSmithKline, and has served as consultant, scientific advisor or speaker for BioLineRx, AstraZeneca, Bristol-Myers Squibb, Eli Lilly, GlaxoSmithKline, Janssen (Johnson and Johnson), Otsuka, Organon, Pfizer, Servier, Solvay and Wyeth.

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