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Pharmacokinetic evaluation of LASSBio-579: an *N*-phenylpiperazine antipsychotic prototype

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Abstract

This work aimed to investigate the pharmacokinetics of the *N*-phenylpiperazine antipsychotic prototype LASSBio-579 and to compare the results with those described for its bioisosteric derivative LASSBio-581. LASSBio-579 was administered to male Wistar rats as a 10 mg kg^{-1} intravenous bolus and 30 and 60 mg kg^{-1} intraperitoneal and 60 mg kg^{-1} oral doses, and plasma concentrations were determined by a validated LC-MS/MS method. Individual plasma concentration–time profiles were evaluated by non-compartmental and compartmental analysis, using WinNonlin. LASSBio-579 plasma protein binding was 93 ± 4%. After intravenous administration of 10 mg kg^{-1}, the Vd_{ss} (0.6 ± 0.2 L kg^{-1}) and the t½ (5.2 ± 1.1 h) determined were smaller than those obtained after extravascular routes, but the CL_{tot} (0.23 ± 0.05 L h^{-1} kg^{-1}) was statistically similar ($\alpha = 0.05$). The intraperitoneal and oral bioavailability was around 1.7% and 0.6%, respectively. The plasma profiles obtained after intravenous and intraperitoneal administration of the compound were best fitted to a three-compartment and two-compartment lag-time open model, respectively. Brain tissue showed low penetration (6.3%) and t½ of 1.1 h. Both the limited bioavailability and the lower brain penetration of LASSBio-579, in comparison with the LASSBio-581, suggest that its CNS activity may be due to a high receptor binding affinity or to a specific distribution into brain structures.

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Introduction

Dopamine constitutes about 80% of the catecholamine neurotransmitter content in the brain (Vallone et al 2000) and it plays an important role in locomotion, emotion, cognition and hormone regulation (Weinshenker & Szot 2002; Elhwuegi 2004). The antipsychotic agents used to treat schizophrenia are among the dopaminergic drugs. Schizophrenia is a chronic psychiatric disorder and approximately 1% of the world population suffers from its severe symptoms, occupying more than half of the beds in psychiatric clinics. Schizophrenia is distributed over the whole population independent of gender, location, social class or race (Kandel et al 2000; Stahl 2000).

The therapeutic action of the conventional antipsychotic drugs has been attributed to their ability to block D_2 dopamine receptors in the mesolimbocortical dopamine systems. Although these agents are important for treating the positive symptoms of this disorder (delusions, hallucinations, hostility), they are less efficient in treating the negative symptoms (apathy, attention impairment, anhedonia). Moreover, conventional antipsychotics produce extrapyramidal side effects due to blockage of D_2 receptors in the nigrostriatal pathway (Farde et al 1992; Kapur et al 2000; Westerink 2002; Kapur & Mamo 2003).

Clozapine was introduced for treatment-resistant schizophrenia, leading a new group of atypical antipsychotics with potent activity on multiple receptor subtypes, including dopamine and serotonin receptors (Van Tol et al 1991; Ichikawa & Meltzer 1998). Clinical usage, however, has shown that some patients are still refractory to treatment and that these new drugs induce important side effects (Owens 1996; Svensson 2003; Gaszner & Makkos 2004).

In this context, the search for new atypical antipsychotics led to design and synthesis of new *N*-phenylpiperazine derivatives by molecular hybridization of clozapine and L-741 (3-[[4-(4-chlorophenyl)piperazin-1-yl]methyl]-1*H*-pyrrolo[2,3]pyridine), a selective ligand for D_2 dopamine receptors, aiming to obtain drugs with therapeutic efficacy

similar to clozapine, devoid of its haematological side effects. Among the 22 compounds obtained, LASSBio-581 (1-[1-(4-chloro-phenyl)-1*H*-[1,2,3]triazol-4-ylmethyl]-4-phenyl-piperazine) and LASSBio-579 (1-[1-(4-chloro-phenyl)-1*H*-pyrazol-4-ylmethyl]-4-phenyl-piperazine) (Figure 1) (Menegatti et al 2003) are under pharmacological investigation.

LASSBio-579 and LASSBio-581 were able to displace YM-09151-2 (cis-*N*-(1-benzyl-2-methylpyrrolidine-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide) binding to D_2 (IC50 of 0.3 μM and 0.6 μM, respectively) but did not alter SCH 23390 (R-[+]-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) binding to D_1 receptors (Menegatti et al 2003). Electrophysiological assays demonstrated that these derivatives act as agonists on pre-synaptic dopamine D_2 receptors (Menegatti et al 2003). As a result, these compounds were active in some antipsychotic activity predictive tests: LASSBio-579 and LASSBio-581 caused a discrete catalepsy in mice; LASSBio-579 presented an inhibitory effect on amphetamine-induced stereotypy in rats and partially reversed the apomorphine hypothermic effect in mice (Neves et al 2003).

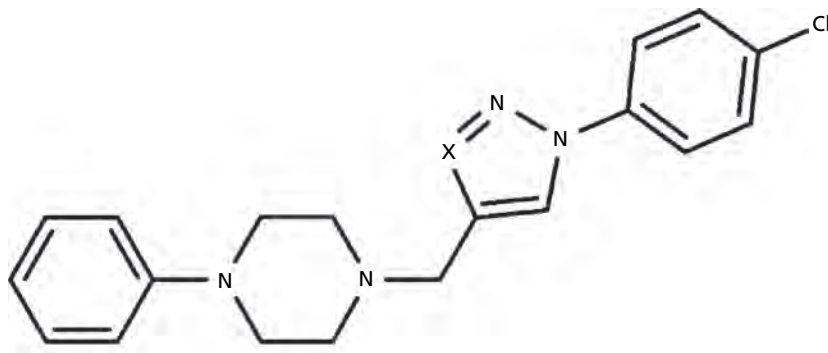
These derivatives also caused a reduction on mice core temperature (Neves et al 2003). However, this hypothermic effect was not blockaded by haloperidol 0.5 mg kg^{-1} given intraperitoneally, indicating that it is not mediated by direct dopamine D_2 receptor activation and pointing to a potential participation of other neurotransmitter systems in the *N*-phenylpiperazine derivative's hypothermic effect. In this context, because literature data point to serotonin (5-HT) as one of the major neurotransmitters involved in both central and peripheral aspects of thermoregulation (Schwartz et al 1995) and the atypical antipsychotics, such as clozapine and risperidone, produce a reduction in rodent's corporal temperature, which has been associated to 5-HT_2 receptor blockade (Ninan & Kulkarni 1999; Oerther & Ahlenius 2000), the involvement of the serotonergic system in LASSBio-579 and LASSBio-581 actions was investigated in rodents (Neves et al 2007).

In fact, Neves et al (2007) provided evidence that LASSBio-579 and LASSBio-581 may act as 5-HT_{1A} agonists and 5-HT_{2A} antagonists. It is important to point out that the 5-HT_2 receptor antagonism may provide an atypical profile for antipsychotic drugs that also present weak anti-dopaminergic action (Meltzer et al 2003). Moreover, the combination of D_2 antagonism and 5-HT_{1A} agonism induces neurochemical and behavioural changes that may be useful to treat negative and cognitive symptoms of schizophrenia with minor extrapyramidal side effects and perhaps enhanced efficacy in refractory patients. For instance, atypical antipsychotic drugs such as clozapine, quetiapine and ziprasidone have weak D_2 and potent 5-HT_{2A} receptor antagonist, as well as 5-HT_{1A} receptor agonist, properties (Ichikawa & Meltzer 1998; Prinssen et al 1999; Millan 2000; Assié et al 2005).

The acute toxicological evaluation of the compounds (30 mg kg^{-1} i.p.) did not reveal haematological side effects. In mice, the LD50 determined for LASSBio-581 and LASSBio-579 was 708 and 1409 mg kg^{-1}, respectively (Neves et al 2003).

The pharmacodynamic results obtained for LASSBio-581 and LASSBio-579, which are potential prototype candidates to treat schizophrenia, led to their pre-clinical pharmacokinetic evaluation. In 2005, Tasso and co-workers described LASSBio-581 pharmacokinetic profile and tissue distribution in a pre-clinical study (Tasso et al 2005). The drug was absorbed by the oral (~25%) and intraperitoneal (~47%) routes of administration, showing a two-phase pharmacokinetic disposition. Protein binding by ultrafiltration was 71 ± 4%. After intravenous administration, the volume of distribution, the clearance and the half-life were 0.8 ± 0.4 L kg^{-1}, 0.6 ± 0.2 $Lh^{-1}kg^{-1}$ and 1.2 ± 0.4 h, respectively. The compound distribution into different organs was evaluated in tissue homogenates after intravenous administration and showed a high penetration in lungs (51.0%), followed by the brain (39.2%).

Considering that LASSBio-579 also showed an important pharmacodynamic profile, this work aimed to determine the pharmacokinetic profile of LASSBio-579 after administration of various doses by different routes, to investigate the compound tissue distribution as well as to compare the results obtained for LASSBio-579 with those reported for LASSBio-581 by Tasso et al (2005).



X = CH (LASSBio-579) or N (LASSBio-581)

Figure 1 Chemical structure of LASSBio-581 and LASSBio-579.

Materials and methods

Chemicals and solvents

LASSBio-579 (hydrochloride) was synthesized by the Laboratório de Avaliação e Síntese de Substâncias Bioativas (LASSBio, Rio de Janeiro, Brazil). Fluconazole was purchased from Delaware (Porto Alegre, Brazil). Analytical grade ammonium hydroxide and glacial acetic acid were obtained from Merck (Darmstat, Germany). HPLC grade acetonitrile and methanol were purchased from Merck (Darmstat, Germany). HPLC water was obtained from Millipore's Milli-Q System and used throughout the analysis.

Animals

Male Wistar rats, 270–300 g, were purchased from Fundação Estadual de Produção e Pesquisa em Saúde (Porto Alegre, Brazil). The rats were kept at constant room temperature (22 ± 1°C) and 60% relative humidity, under controlled 12-h light–dark cycle (light on: 0700–1900 h) during the acclimation period and had free access to water and food. Rats that received LASSBio-579 by the oral route were fasted 12 h before and 4 h after administration of the compound.

The protocol of this animal study was approved by the Ethics in Research Committee of the Universidade Federal do Rio Grande do Sul (No. 2003239, UFRGS, Porto Alegre, Brazil).

Pharmacokinetic design

To describe the LASSBio-579 pharmacokinetic profile, four groups were studied: G1 (n = 9) received LASSBio-579 10 mg kg^{-1} as a single intravenous bolus dose into the lateral tail vein; G2 and G3 (n = 7/group) received 30 and 60 mg kg^{-1} of the compound, respectively, by the intraperitoneal route; G4 (n = 8) received a single dose of 60 mg kg^{-1} of the LASSBio-579 by oral gavage. The evaluated doses were based on previously conducted pharmacodynamic experiments (Neves et al 2003).

Dosage form preparations

The intravenous dosage form was prepared by dissolution of LASSBio-579 in 5% glucose solution under heating at 60°C, resulting in a concentration of 2 mg mL^{-1}. The 30 and 60 mg kg^{-1} doses for intraperitoneal and oral administration were prepared by dispersing LASSBio-579 in sterile 0.9% NaCl solution, containing 3% (v/v) polysorbate 80, followed by sonication, resulting in suspensions with a concentration of 3 and 6 mg mL^{-1}, respectively. The dosage forms were prepared in the same manner as in the pharmacodynamic studies (Neves et al 2003).

Plasma sampling

Fifteen minutes before the LASSBio-579 administration, blank blood samples were withdrawn from the rats. After intravenous administration, blood samples were harvested from the lateral tail vein at 0.08, 0.25, 0.50, 1, 1.5, 2, 4, 6, 9, 12, 18 and 24 h. After oral administration, sampling took place at 0.17, 0.33, 0.50, 0.75, 1, 2, 4, 6, 9, 12, 18 and 24 h; and after intraperitoneal administration at 0.25, 0.50, 0.75, 1, 1.5, 2, 4, 6, 12, 18, 24, 30 and 36 h. Blood samples were approximately 200 μL in volume and were collected into heparinized reaction tubes. They were centrifuged at 6800 *g* at 21°C for 15 min to obtain plasma, which was stored at −20°C until analysis by an LC-MS/MS method.

LC-MS/MS analysis

LASSBio-579 plasma concentrations were determined by a previously validated LC-MS/MS method (Conrado et al 2007). This method was validated in the concentration range of 30–2000 ng mL^{-1} and the calibration curves were linear with determination coefficient > 0.98. The lower limit of quantification was 30 ng mL^{-1}. The accuracy of method was within 15%. Intra- and inter-day relative standard deviations were $< 13.5\%$ and $< 6.5\%$, respectively.

Chromatographic analysis was performed on a Shimadzu liquid chromatograph using a Micromass Quattro LC mass spectrometer as detector. The system was controlled by MassLynx software (version 3.5). The mobile phase consisted of acetonitrile–water (80:20, v/v) containing 0.4 mM ammonium hydroxide and 0.2 mM glacial acetic acid; it was delivered isocratically at a flow rate of 1.0 mL min^{-1} (split ratio 1:5). The analysis was carried out at 40°C using a Shimadzu Shim-pack HPLC column (150 mm × 4.6 mm i.d., 5 μm ODS stationary phase), which was protected by a Waters Novapak RP18 guard column (4 μm).

Before the chromatographic analysis, 100-μL plasma samples were deproteinized by addition of 200 μL of acetonitrile containing 3 μg mL^{-1} of the internal standard (fluconazole), vortexed for 30 s and centrifuged at 6800 *g*, 21°C for 15 min. Fifty microlitres of the supernatant were injected into the LC-MS/MS system. The analyte was monitored using a Micromass triple quadrupole mass spectrometer, equipped with an electrospray ionization interface, operated in the positive mode. The spectrometer was programmed in the multiple reaction monitoring (MRM) mode to allow the specific transition of precursor ion to fragment for each compound. Detection of the ions was performed by monitoring the decay of the mass-to-charge (m/z) ratio 353 precursor ion to the m/z 191 product ion for LASSBio-579 and the decay of the m/z 307 precursor ion to the m/z 220 product ion for internal standard.

Plasma protein binding

LASSBio-579 plasma protein binding was determined by ultrafiltration. The experiments were performed using 1 mL of pooled Wistar rat plasma, which was spiked with appropriate standard working solution to obtain LASSBio-579 final concentrations of 25, 50 and 150 μg mL^{-1}. After vortexing for 30 s, a 100-μL volume was taken to determine the LASSBio-579 total concentration in each sample. Concentrations higher than the upper limit of the calibration curve were diluted in blank plasma and vortexed for 30 s. The remaining volumes were introduced into Centrifree ultrafiltration devices

(Millipore) and allowed to equilibrate at 21°C for 10 min before centrifugation. The ultrafiltrate (<11% of the total plasma volume) was obtained by centrifugation at 2000 *g* for 15 min. The total and unbound concentrations were determined, and then the samples were processed as described in the previous section. Samples of LASSBio-579 in aqueous solution, at the same spiked concentrations, were run together to assess the binding of the compound to the membrane of the filtration device. All experiments were performed in triplicate. The difference between the plasma and aqueous solution binding was assumed to be LASSBio-579 protein binding.

Pharmacokinetic modeling

LASSBio-579 pharmacokinetic parameters were obtained from the individual plasma profiles by non-compartmental approach, using WinNonlin v. 1.0 software (North Carolina, USA). The peak plasma concentration (C_{max}) and the corresponding time (t_{max}) were directly read from the experimental data.

Compartmental analysis of individual concentration–time curves was also performed by WinNonlin software. The plasma profiles obtained after the intravenous and intraperitoneal administrations of the compound were best fitted to a three-compartment (equation 1) and two-compartment lag-time (equation 2) open model, respectively.

$$C = A_1 \cdot e^{-\lambda_1 t} + A_2 \cdot e^{-\lambda_2 t} + A_3 \cdot e^{-\lambda_3 t} \quad (1)$$

where C is the total plasma concentration over time t; A_1, A_2 and A_3 are the intercepts of the distribution to the shallow peripheral compartment, distribution to the deep peripheral compartment and elimination phase, respectively; and λ_1, λ_2 and λ_3 are the distribution rate constants for the shallow peripheral compartment, deep peripheral compartment and the elimination rate constant, respectively.

$$C = a \cdot e^{-\alpha(t-t_0)} + b \cdot e^{-\beta(t-t_0)} + c \cdot e^{-ka(t-t_0)} \quad (2)$$

where C is the total plasma concentration over time t; a, b and c the intercepts of the distribution, elimination and absorption phases, respectively; α and β are the distribution and elimination rate constants, respectively; ka is the first-order absorption rate constant; and t_0 is the delay between the dosing time and the appearance of concentration in the sampling compartment.

To obtain the best fitting of the intravenous plasma profiles, weighted non-linear regression (1/concentration) was used. Non-weighted data were used for intraperitoneal modelling. Model selection was guided using visual inspection of the observed versus estimated concentrations. Additionally, the Akaike's information criterion (AIC) and the correlation coefficient provided by the software were used.

Tissue distribution

LASSBio-579 penetration was investigated in homogenates of brain, heart, liver, kidney, spleen, lung and adipose tissue up to 12 h after a single 10 mg kg^{-1} intravenous bolus dose into the lateral tail vein. The rats (3/time point) were sacrificed by decapitation at 0.08, 0.17, 0.25, 1, 3, 6 and 12 h after LASSBio-579 intravenous bolus administration and the tissues and blood samples were quickly collected. Tissues were gently blotted with absorbent paper to remove the surface blood and frozen at −80°C until LC-MS/MS analysis. For the analysis, tissue samples were thawed and weighed (0.4–2 g) and sterile 0.9% NaCl solution (2–4 mL) was added to facilitate homogenization, which was performed in an ice bath using a PowerGen125 apparatus. The homogenized samples were introduced into Falcon tubes, sonicated for 5 min in an ice bath and centrifuged at 3500 *g* for 15 min at 21°C. One-hundred microlitres of the supernatants were transferred into Eppendorf tubes and processed as described for plasma samples in the section on LC-MS/MS analysis.

Tissue samples from untreated rats were used as blanks. Homogeneized tissue samples from untreated rats were spiked with LASSBio-579 standard solutions to prepare calibration curves for each evaluated tissue. Therefore, the respective tissue homogenate was used as sample matrix for the LC-MS/MS analysis. The results of LASSBio-579 tissue penetration were expressed as μg (g tissue)$^{-1}$ and calculated by the equation (Nix et al 1991):

$$C_t = C_S \cdot V_S / P \quad (3)$$

where C_t is the tissue concentration (μg g^{-1}), C_s the supernatant concentration, V_s the supernatant volume and P the weight of the tissue sample.

Mean tissue concentration–time curves were used to determine pharmacokinetic parameters (AUC, t½, MRT, C_{max} and t_{max}) using the same equations described for plasma. LASSBio-579 tissue penetration was determined as the $AUC_{0-\infty}$ tissue to $AUC_{0-\infty}$ plasma ratio.

Statistical analysis

The pharmacokinetic parameters determined by the non-compartmental and compartmental analysis for the evaluated doses and administration routes were statistically compared by analysis of variance ($\alpha = 0.05$).

Results

Plasma protein binding

LASSBio-579 (25–150 μg mL^{-1}) binding to the ultrafiltration membrane was 6.6 ± 0.1%, regardless of the compound concentration investigated. This nonspecific binding was taken into consideration when determining the protein binding. LASSBio-579 plasma protein binding was 93.2 ± 3.7%, regardless of drug concentration.

LASSBio-579 pharmacokinetic profile in plasma

The mean plasma concentration versus time curve obtained after intravenous administration of LASSBio-579 (10 mg kg^{-1}, G1) to Wistar rats is shown in Figure 2. The experi-

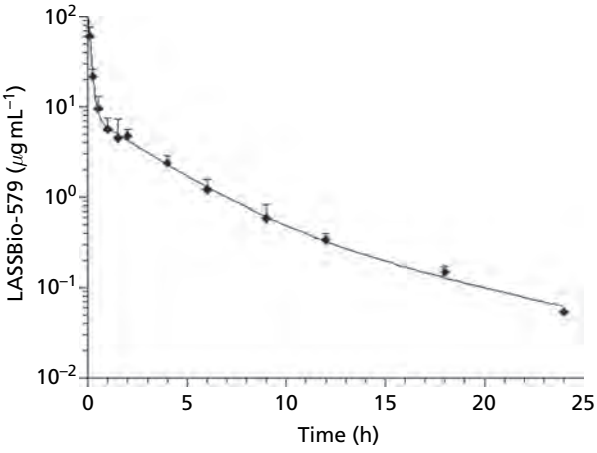


Figure 2 Mean plasma concentration–time profile after intravenous bolus administration of LASSBio-579 10 mg kg^{-1} (n = 9) to rats. Data represent mean + s.d.

mental data were best fitted to a three-compartment model resulting in an AIC of −21.6 and correlation coefficient of 0.9999.

The pharmacokinetic parameters determined by model-independent and compartmental analysis after intravenous administration of the compound are summarized in Table 1. The pharmacokinetic parameters estimated by the compartmental analysis were not statistically different from those determined by the model-independent analysis, confirming the suitability of the three-compartment open model to describe the experimental data.

Table 1 Pharmacokinetic parameters after single intravenous administration of LASSBio-579 (10 mg kg^{-1}) to Wistar rats (n = 9)

Parameter	Model-independent	Three-compartment
t½ λ_3 (h)	—	7.7 ± 4.2
t½ (h)	5.2 ± 1.1	—
$AUC_{0-\infty}$ ($\mu g\,mL^{-1}h^{-1}$)	44.8 ± 10.0	39.8 ± 10.8
CL_{tot} ($Lh^{-1}kg^{-1}$)	0.23 ± 0.05	0.27 ± 0.09
Vc (Lkg^{-1})	—	0.09 ± 0.06
Vd_{ss} (Lkg^{-1})	0.6 ± 0.2	0.86 ± 0.34
Vd_{area} (Lkg^{-1})	—	3.28 ± 2.83
MRT (h)	2.8 ± 0.7	3.2 ± 0.7
A_1 ($\mu g\,mL^{-1}$)	—	177.3 ± 127.7
A_2 ($\mu g\,mL^{-1}$)	—	7.4 ± 5.6
A_3 ($\mu g\,mL^{-1}$)	—	1.1 ± 1.1
λ_1 (h^{-1})	—	12.1 ± 7.9
λ_2 (h^{-1})	—	0.6 ± 0.5
λ_3 (h^{-1})	—	0.12 ± 0.06

t½ λ_3, terminal half-life in three compartment model; t½, terminal half-life; $AUC_{0-\infty}$, area under the plasma concentration–time curve from time zero to infinity; CL_{tot}, total clearance; Vc, volume of distribution of central compartment; Vd_{ss}, volume of distribution at steady state; Vd_{area}, volume of distribution during elimination phase; MRT, mean residence time; A_1, A_2 and A_3, intercepts of the distribution to the shallow peripheral compartment, distribution to the deep peripheral compartment and elimination phases, respectively; λ_1, λ_2 and λ_3, distribution rate constants for the shallow peripheral compartment, deep peripheral compartment and elimination rate constant, respectively. Data are means ± s.d., n = 9.

The obtained LASSBio-579 pharmacokinetic profiles for intraperitoneal administration of 30 and 60 mg kg^{-1} doses and oral administration of 60 mg kg^{-1} dose to Wistar rats are shown in Figure 3 (A, B and C, respectively).

After intraperitoneal administration, LASSBio-579 plasma profiles exhibited biexponential decay. These experimental data were best fitted to a two-compartmental lag-time open model with AIC values ranging from −48.0 to −64.2 and correlation coefficients of 0.9886–0.9946 for 30 and 60 mg kg^{-1} doses, respectively. This model considers the delay from the dosing time to the drug appearance in the sampling compartment (t_0).

After oral administration of LASSBio-579 60 mg kg^{-1} (G4), two plasma peaks, C_{max1} of 66 ± 12 ng mL^{-1} at 0.33 h and C_{max2} of 69 ± 45 ng mL^{-1}, less evident, at 12 h, were observed. Due to this observation, the compartmental modelling of the oral profile was not successful using WinNonlin.

The calculated LASSBio-579 pharmacokinetic parameters after intraperitoneal and oral administration are shown in Table 2. The comparison between the parameters calculated through the compartmental analysis (t½, $AUC_{0-\infty}$, MRT, CL_{tot} and Vd_{ss}) and those obtained from the model-independent analysis for both intraperitoneal doses (G2 and G3) did not show significant statistical differences.

The comparison between G2 and G3 showed that the $AUC_{0-\infty}$ increased from 2.1 ± 0.8 to 4.7 ± 1.3 $\mu g\,mL^{-1}h^{-1}$ when the intraperitoneal dose was doubled, resulting in similar bioavailability, around 1.7% for this administration route. For both doses, the CL_{tot} was similar, around 0.25 L $h^{-1}kg^{-1}$, but the Vd_{ss} and t½ were statistically different (Table 2).

The lower plasma levels after oral administration of LASSBio-579 60 mg kg^{-1} approached the limit of quantification of the analytical method used, hence a good characterization of the $AUC_{0-\infty}$ was not possible and an average extrapolation of 40 ± 16% was obtained. The estimated bioavailability of this route was approximately 0.6%. All the determined pharmacokinetic parameters, in this way, were approximations of the actual disposition of the compound after oral administration.

LASSBio-579 tissue distribution

The obtained tissue profiles after LASSBio-579 intravenous dosing of 10 mg kg^{-1} were evaluated by non-compartmental approach. The determined mean pharmacokinetic parameters are reported in Table 3.

All investigated tissues presented peak levels at 5 min, except for the adipose tissue, which showed two peaks, at 10 min and at 3 h after dosing. The highest tissue concentrations were reached in the lungs (33.0 $\mu g\,g^{-1}$), in the adipose tissue (10.2 and 11.7 $\mu g\,g^{-1}$) and in the kidneys (11.6 $\mu g\,g^{-1}$). The adipose tissue displayed the highest LASSBio-579 exposure (AUC_{0-t} of 92.9 $\mu g\ g^{-1}\ h^{-1}$), followed by the lungs ($AUC_{0-\infty}$ of 23.8 $\mu g\ g^{-1}\ h^{-1}$) and the spleen ($AUC_{0-\infty}$ of 5.9 $\mu g\ g^{-1}h^{-1}$). The lowest exposure was observed in the liver ($AUC_{0-\infty}$ of 2.3 $\mu g\,g^{-1}h^{-1}$). A reduced brain penetration (6.3%) was observed, lower than the penetration determined

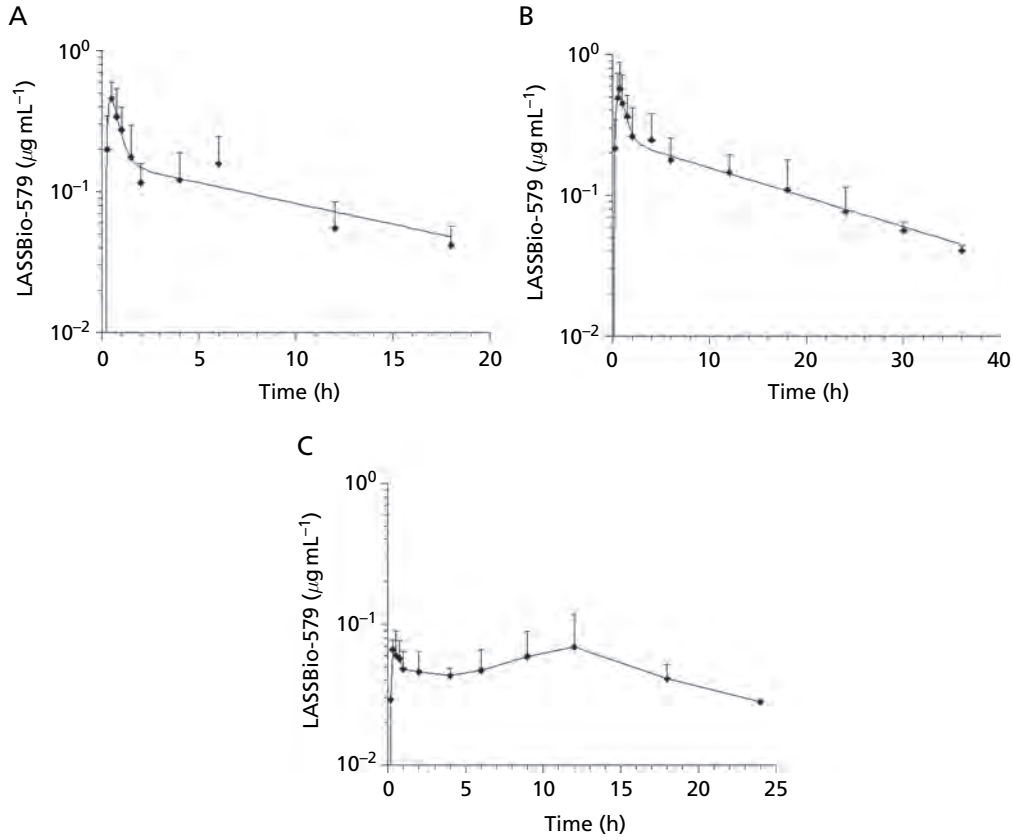


Figure 3 Mean plasma concentration–time profiles after LASSBio-579 administration of 30 mg kg^{-1} (n = 7) (A) or 60 mg kg^{-1} (n = 7) (B) intraperitoneally and 60 mg kg^{-1} (n = 8) Corally. Data represent mean + s.d.

Table 2 Pharmacokinetic parameters obtained after single intraperitoneal and oral administration of LASSBio-579 to Wistar rats

Parameter	30 mg kg^{-1} i.p.		60 mg kg^{-1} i.p.		60 mg kg^{-1} oral
	Model-independent (G2A)	Two-compartment (G2B)	Model-independent (G3A)	Two-compartment (G3B)	Model-independent (G4)
t½ β (h)	—	9.5 ± 5.8	—	17.9 ± 10.1	—
t½ (h)	6.2 ± 1.2	—	9.7 ± 3.2[a]	—	11.5 ± 4.2
$AUC_{0-\infty}$ ($\mu g\,mL^{-1}h^{-1}$)	2.1 ± 0.8	2.3 ± 1.1	4.7 ± 1.3[a]	5.5 ± 1.5[b]	1.5 ± 0.5
CL_{tot} (L h^{-1} kg^{-1})	0.26 ± 0.10	0.25 ± 0.13	0.24 ± 0.08	0.20 ± 0.05	0.25 ± 0.11
Vd_{ss} (L kg^{-1})	1.9 ± 0.9	2.6 ± 1.1	3.5 ± 1.6[a]	4.6 ± 2.1[b]	—
MRT (h)	8.2 ± 1.5	12.7 ± 7.7	15.5 ± 6.5[a]	24.3 ± 13.7	18.9 ± 5.0
a ($\mu g\,mL^{-1}$)	—	1.4 ± 1.3	—	4.07 ± 3.03[b]	—
b ($\mu g\,mL^{-1}$)	—	0.17 ± 0.06	—	0.26 ± 0.14	—
α (h^{-1})	—	2.53 ± 0.68	—	2.70 ± 1.98	—
β (h^{-1})	—	0.10 ± 0.05	—	0.05 ± 0.03	—
k_a (h^{-1})	—	8.96 ± 7.61	—	3.80 ± 2.04	—
t_0 (h)	—	0.16 ± 0.12	—	0.15 ± 0.10	—
f (%)	1.6	—	1.8	—	0.6

[a]Statistical difference between G2A and G3A ($P < 0.05$); [b]statistical difference between G2B and G3B ($P < 0.05$). t½ β, terminal half-life in two-compartment model; t½, terminal half-life; $AUC_{0-\infty}$, area under the plasma concentration–time curve from time zero to infinity; CL_{tot}, total clearance; Vd_{ss}, volume of distribution at steady state; MRT, mean residence time; a and b, intercepts of the distribution and elimination phases, respectively; α and β, distribution and elimination rate constants, respectively; ka, first-order absorption rate constant; t_0, delay between the dosing time and the appearance of concentration in the sampling compartment; f, bioavailability.

Table 3 Tissue pharmacokinetic parameters of LASSBio-579 after intravenous administration of 10 mg kg^{-1} dose

Tissue	$AUC_{0-\infty}$ ($\mu g\ g^{-1}\ h^{-1}$)	t_{max} (h)	C_{max} ($\mu g\ g^{-1}$)	t½ (h)	MRT (h)	Tissue-to-plasma ratio (%)
Adipose	92.9[a]	0.17	10.2	NC	NC	224.0[b]
		3.00	11.7			
Brain	2.7	0.08	9.5	1.1	0.6	6.3
Heart	3.1	0.08	7.4	1.3	1.2	7.3
Kidneys	5.4	0.08	11.6	1.2	1.2	12.5
Liver	2.3	0.08	5.1	2.4	2.0	5.3
Lungs	23.8	0.08	33.0	8.1	3.9	55.7
Spleen	5.9	0.08	10.4	0.9	0.7	13.8
Plasma	42.8	NC	NC	2.7	2.5	—

[a]AUC_{0-t}, where t = 12 h; [b]calculated as (AUC_{0-t} tissue/AUC_{0-t} plasma) × 100 due to extrapolated AUC of 43%. NC, not calculated; $AUC_{0-\infty}$, area under the tissue concentration–time curve from time zero to infinity; t_{max}, time to peak concentration; C_{max}, peak concentration; t½, terminal half-life; MRT, mean residence time; tissue-to-plasma ratio, tissue fraction calculated as ($AUC_{0-\infty}$ tissue/$AUC_{0-\infty}$ plasma) × 100. Data are means, n = 21.

for other tissues. The tissues half-lives were similar among brain, spleen, heart and kidneys, around 1 h. The lungs and the liver presented longer t½ of 8.1 and 2.4 h, respectively.

Discussion

In the search for drugs that have better therapeutic efficacy and less side effects than those currently used for treating schizophrenic patients, new *N*-phenylpiperazine derivatives were originally designed to have a therapeutic efficacy similar to clozapine and to be devoid of its haematological side effects. Among these compounds, LASSBio-581 and LASSBio-579 are being studied in the pre-clinical phase (Neves et al 2003, 2007; Tasso et al 2005).

To achieve optimum therapeutic efficacy, drugs or drug candidates should present high potency and selectivity for interacting with a specific biological target as well as the ability to attain target tissue concentrations at the necessary levels to produce the effect. Absorption, distribution, metabolism and elimination (ADME) processes play a fundamental role in defining the body disposition of a drug candidate, and therefore its therapeutic potential. In this context, the determination of the LASSBio-579 pharmacokinetic parameters followed by their comparison with those determined for its bioisosteric derivative LASSBio-581 (Tasso et al 2005) may contribute to the understanding of this bioisosteric exchange with respect to ADME processes.

Similar total clearance was observed for all evaluated groups after LASSBio-579 dosing. The volume of distribution at steady state, however, was statistically lower after intravenous administration (0.6 ± 0.2 L kg^{-1}) than extravascular routes (>1.9 L kg^{-1}). If active transport was the driving force for drug distribution from the bloodstream to the extravascular space, its saturation would explain the smaller Vd_{ss} after intravenous administration due to higher plasma levels observed (around 130 μg mL^{-1}) in relation to the levels reached after intraperitoneal and oral routes (<600 ng mL^{-1}).

Statistical differences were observed for the parameters t½ and Vd_{ss} between the evaluated intraperitoneal doses. The absorption rate constant had a tendency to reduce from $8.96 \pm 7.61\ h^{-1}$ to $3.80 \pm 2.04\ h^{-1}$ for the 30 and 60 mg kg^{-1} intraperitoneal doses, respectively. This variation is probably related to the higher (2×) concentration of the suspension used to administer the higher dose, which could have more dissolution problems. Thus, the mean residence time of 12.7 ± 7.7 h and 24.3 ± 13.7 h for the respective doses would be mainly attributed to the slower absorption process of the 60 mg kg^{-1} dose. The lag-time to start the absorption process (around 0.15 h), however, was dose independent.

The determined pharmacokinetic parameters for LASSBio-579 after intravenous administration of 10 mg kg^{-1} are different to those reported for LASSBio-581, after administration of the same intravenous dose (Tasso et al 2005). While a two-compartment model was enough to describe the body disposition of LASSBio-581 after intravenous dosing, a three-compartment model was necessary to describe LASSBio-579 plasma profile, showing the differences in tissue affinity between the two compounds.

LASSBio-579 total clearance (0.23 ± 0.05 L h^{-1} kg^{-1}) was smaller than that described for LASSBio-581 (0.6 ± 0.2 L h^{-1} kg^{-1}), consequently its half-life (5.2 ± 1.1 h) was 4.3 times longer than that of LASSBio-581 (1.2 ± 0.4 h). The AUC of LASSBio-579 (44.8 ± 10.0 μg mL^{-1} h^{-1}) was found to be bigger than that obtained for LASSBio-581 (26.5 ± 13.2 μg mL^{-1} h^{-1}) (Tasso et al 2005); this is related to the smaller LASSBio-579 total clearance. In addition, it was observed that the volume of distribution at steady state of LASSBio-579 (0.6 ± 0.2 L kg^{-1}) was not statistically different to that determined for LASSBio-581 (0.8 ± 0.4 L kg^{-1}) (Tasso et al 2005).

LASSBio-579 plasma protein binding ($93 \pm 4\%$) was larger than that reported for LASSBio-581 ($71 \pm 4\%$) (Tasso et al 2005). Besides the specific molecular recognition, this difference may be attributed, at least in part, to the distinct lipophilicity of LASSBio-579 and LASSBio-581, which are determined in-silico as 3.48 and 2.91, respectively, by using the program Pallas 3.1 (Compudrug Int.) and expressed as the theoretical logarithm of the n-octanol-to-water partition coefficient (*c*log P) (Menegatti 2001). The relationship between the decrease of lipophilicity and plasma protein binding was reported by Toon & Rowland (1983) for a homologous series of nine 5-n-alkyl-5-ethyl barbituric acids.

In addition, a lower plasma concentration was obtained after intraperitoneal and oral administration of LASSBio-579, resulting in very a low bioavailability (around 1.7 and 0.6%, respectively), in comparison with those reported for LASSBio-581 (around 47% and 26%, respectively) (Tasso et al 2005). This result could be related not only to the high lipophilicity of the LASSBio-579, which would be the limiting step of the absorption process, but also to a different first-pass metabolism possible in these routes of administration. Besides that, the concentration plateau observed for the mean plasma profile of LASSBio-579 after an oral dose could be related to the slow dissolution of the compound, considering its low solubility in water.

Tissue distribution of LASSBio-579

After systemic administration, LASSBio-579 was rapidly distributed into all organs investigated. The high lipophilicity of the compound suggests that tissue perfusion is the limiting step of the distribution process, resulting in slow tissue uptake and elimination under conditions of low tissue perfusion.

The tissue concentration–time profiles showed a fast distribution of LASSBio-579. For brain, heart, liver, kidneys, spleen and lungs the concentration peak took place 5 min after administration, followed by concentration decay up to 3 h after dosing. The adipose tissue displays two peaks: the first peak at 10 min may be attributed to the multilocular or brown adipose tissue fraction in the samples; the second peak, at 3 h, coincides with the disappearance of the compound from the other tissues, suggesting its redistribution to the adipose tissue. A similar phenomenon was described by Blatt et al (2001) for the lipid soluble vitamin E, justifying the loss of 90% of the drug in the body to the adipose tissue. The high penetration of LASSBio-579 into the adipose tissue indicates that it behaves as a drug deposit. As a result, the longer half-life observed in plasma in comparison with the half-lives observed in other tissues could be attributed to the slow movement of the drug from the adipose tissue back into plasma for elimination.

As observed for LASSBio-581 (Tasso et al 2005), high tissue concentrations of LASSBio-579 were reached in the lungs, which could be explained by its basic and lipophilic characteristics leading to accumulation in the lung mitochondria after administration to animals, as previously reported for other drugs with similar characteristics (Hori et al 1987). Furthermore, blood contamination in lung and liver homogenates is high (Triplett et al 1985) and could also explain the high levels observed. The liver displayed the lowest tissue-to-plasma ratio among the tissues investigated. Enzymatic degradation of LASSBio-579 in the liver could explain this finding.

LASSBio-579 brain penetration was lower than that reported for LASSBio-581 (6.3% vs 39.2%). Its elimination half-life was also smaller in brain (1.1 vs 1.9 h). The short half-life observed for LASSBio-579 in brain could be considered inappropriate and prevent its utility as a treatment for schizophrenia. However, pharmacokinetic parameters determined in brain using tissue homogenates have to be viewed carefully. Drug concentrations in brain homogenates are, in fact, the average of concentration in all structures. The drug, on the other hand, can have a distinct profile in specific structures such as the regions rich in dopaminergic and serotonergic neurons, which are believed to be involved in the schizophrenia disorder. Furthermore, the half-life observed in rats will probably be increased in man, considering that drug elimination is always faster in small animals.

Neves et al (2003) reported that LASSBio-579 displayed an improved pharmacodynamic profile in comparison with its biososteric derivative. Considering that the pharmacodynamic studies were conducted following extravascular administrations (intraperitoneal and oral) of both compounds, and the limited bioavailability of LASSBio-579, beside its shorter brain half-life, it is plausible to assume that the levels of this compound at the biophase during those experiments were very low. In this context, the results suggest that the central nervous system pharmacological activity of LASSBio-579 may be due to a high receptor binding affinity or to a specific distribution into the brain structures.

Conclusion

The obtained results give a new perspective on the bioisosteric relation between pyrazole and 1,2,3-triazole rings. In fact, the exchange of these rings in *N*-phenylpiperazine derivatives resulted in important pharmacokinetic differences. LASSBio-579 presented a different body disposition, its intravenous profile being characterized by a three-compartment open model. Moreover, a higher plasma protein binding, a lower brain tissue penetration, a longer plasma half-life and a limited bioavailability were observed in comparison with those of its bioisosteric derivative LASSBio-581.

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