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Distribution of the methylpiperazinopyridobenzoxazepine derivative JL13, a potential antipsychotic, in rat brain

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Abstract

The brain uptake and distribution of the potential antipsychotic 5-(4-methylpiperazin-1-yl)-8-chloro-pyrido[2,3][1,5]benzoxazepine fumarate (JL13) was examined in rats after neuropharmacologically active doses. Plasma and brain concentrations of the compound were measured by reversed-phase HPLC with UV detection (210 nm). Clozapine was used as an internal standard. After an intraperitoneal dose of 10 mg kg^{-1}, the compound attained mean maximum plasma concentrations within 5 min of dosing, then declined with a mean elimination half-life of approximately 1 h. It rapidly crossed the blood–brain barrier and equilibrated with plasma, achieving mean maximum concentrations and area under the curve approximately 20-times those in plasma, with slight regional differences. Disappearance from whole brain almost paralleled its disappearance from plasma. There was a linear relationship between JL13 concentrations in plasma and brain regions, and in all tissues the concentrations of the compound increased almost linearly with the dose over the range of 5–20 mg kg^{-1}. It thus appears that JL13 brain pharmacokinetics parallels that in plasma, and that plasma concentrations accurately predict brain concentrations in rats.

Introduction

The advantages of clozapine over conventional antipsychotics, in terms of minimal risk of extrapyramidal side-effects and efficacy on both positive and negative symptoms (Fleischhacker & Hummer 1997; Campbell et al 1999; Remington & Kapur 2000), have led to the search for additional atypical agents that are free of its troublesome side-effects (Owens 1996; Barnes & McPhilips 1999; Brown et al 1999). Several compounds, either structurally related to clozapine, or from different chemical classes, have been introduced (Tamminga & Lahti 1996; Fleischhacker & Hummer 1997; Caccia 2000; Remington & Kapur 2000). Others are still in the pipeline, including compound 5-(4-methylpiperazin-1-yl)-8-chloro-pyrido[2,3]-[1,5]benzoxazepine fumarate (JL13; Figure 1) (Liégeois et al 1994).

In receptor-binding studies JL13 has a broad receptor-affinity profile with nanomolar affinities for 5-HT_{2A} and D_4 receptors, and micromolar affinities for D_2, cholinergic muscarinic and 5-HT_{2C} receptors (Liégeois et al 1994; Bruhwyler et al 1997; Goudie & Taylor 1998). Consistent with this in-vitro atypical profile, JL13 is clozapine-like in a variety of behavioural assays indicative of antipsychotic action (Bruhwyler et al 1997), and it generalizes substantially to clozapine in drug

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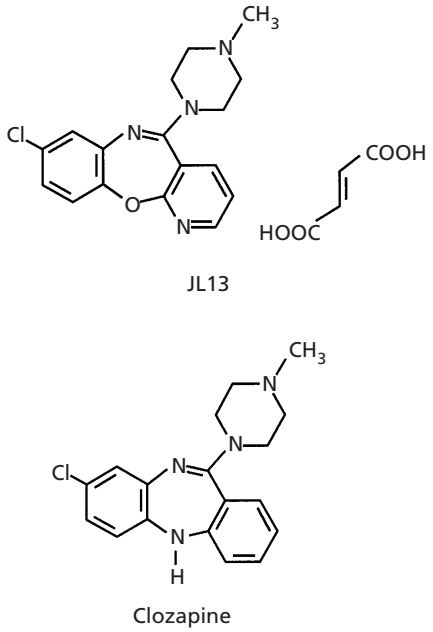

Figure 1 Chemical structures of 5-(4-methylpiperazin-1-yl)-8-chloro-pyrido[2,3][1,5]benzoxazepine fumarate (JL13) and clozapine.

discrimination procedures in rats and monkeys (Bruhwyler et al 1997; Goudie & Taylor 1998; Goudie et al 1998). JL13 selectively raises extracellular dopamine in the rat prefrontal cortex with no effect on dialysate dopamine in striatum and nucleus accumbens (Invernizzi et al 2000), behaving like the prototype drug and other atypical antipsychotics (Moghaddam & Bunney 1990). However, little information is available on its disposition in the species used for neuropharmacological studies. The extent of its brain uptake and distribution is not known. Although most antipsychotics concentrate in brain tissue, wide variation in the brain-to-blood ratio has been observed in animals. This possibly reflects the complex relationships between the protein binding, lipophilicity and other mechanisms that govern the extent of brain uptake (Tsuneizumi et al 1992; Baldessarini et al 1993; Aravagiri et al 1998), making it difficult to predict the effective concentrations of a new antipsychotic achieved at the target site.

The aim of this study was to obtain basic information on the concentrations of JL13 achieved in brain after pharmacologically effective doses, and their relationship with plasma concentrations in rats. Before the brain distribution studies were started, an HPLC method based on a liquid–liquid extraction procedure, separation on a reversed-phase column and UV detection, was developed to quantitate JL13 in plasma and various regions of the rat brain.

Materials and Methods

Animals and treatment

Male CD-COBS rats (Charles River, Italy), 200–225 g, were used. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (EEC Council Directive; Guide for the Care and Use of Laboratory Animals, US National Research Council).

In the first study, rats were intraperitoneally administered 10 mg kg^{-1} JL13 (Therabel Research, Brussels, Belgium), dissolved in 1 M HCl acid which was then neutralized with 1 M sodium hydroxide and brought to a final volume of 4 mL kg^{-1} with water. The rats were killed by decapitation under deep anaesthesia 5, 15, 30, 60, 120, 180 and 240 min after dosing. In subsequent studies JL13 was injected intraperitoneally at doses of 5, 10 and 20 mg kg^{-1} and the rats were killed after 60 min, that is, approximately at the end of the distribution phase. The doses and route of administration were selected on the basis of neuropharmacological studies of JL13 in rats (Bruhwyler et al 1997; Invernizzi et al 2000). Blood samples were collected in heparinized tubes, centrifuged and the plasma was stored at −20°C. Brains were removed immediately after exsanguination, brain areas were dissected (Glowinski & Iversen 1966), blotted with paper to remove excess surface blood and quickly frozen in dry ice.

Chemical analysis

JL13 was determined in plasma and brain samples by HPLC with UV detection (210 nm). Briefly, to 1 mL plasma, 0.01 mL of a solution of the internal standard clozapine (10 μg mL^{-1}) and 0.1 mL 1 M NaOH were added. The samples were shaken with 8 mL hexane–ethyl acetate (90:10, v/v) on an automatic shaker. After centrifugation, the organic phase was separated and evaporated to dryness. After dissolution the residues were injected onto a SPHERI-5 RP8 column (25 cm × 4.6 mm i.d., 5 μm particle size), protected with a New-Guard RP-8 7 μm precolumn (Brown Lee Lab). The mobile phase was 0.01 M aqueous tetramethylammonium perchlorate–CH_3CN (60:40, v/v), adjusted to pH 3.35 with $HClO_4$, at a flow rate of 1 mL min^{-1}. The analysis was run at room temperature.

Brain tissues were homogenized (10 mL g^{-1}) in CH_3OH–0.02 M HCl (20:80, v/v) and samples of 0.5–1 mL were centrifuged at 5000 rev min^{-1} for 10 min. The pellets were re-suspended in 0.5–1 mL CH_3OH–HCl and re-centrifuged. The supernatants were combined,

brought to a volume of 2 mL, and processed as described above.

Pharmacokinetic analysis

The area under the plasma concentration–time curve from zero to the last measurable plasma concentration (AUC_t) was determined by the trapezoidal rule and extrapolated to infinity (AUC) by a conventional method (Gibaldi & Perrier 1983). The terminal slope (β) was determined by non-linear least-squares regression, using the data points of the terminal log-linear region of the plasma concentration–time curves. The elimination half-life ($t_{\frac{1}{2}}$) was determined from the terminal slope by the usual equation ($0.693/\beta$). The maximum plasma concentrations (C_{max}) and the times to reach the maximum plasma concentration reached (t_{max}) were taken directly from the analytical data. Other conventional parameters such as clearance and volume of distribution of JL13 were not calculated because of evidence of a large first-pass effect with intraperitoneal dosing which could result in spurious values.

Regional contents (r) were analysed by two-way analysis of variance, after normalization for dose (d). Post-hoc comparison were made by Tukey-Kramer's test. Least-squares linear regression was used to analyse for significant correlations between dose and tissue concentrations and between brain and plasma concentrations. The plasma and brain concentration data are presented as means $\pm$ s.d.

Results and Discussion

HPLC assay

Under the chromatographic conditions described, retention times were approximately 8.5 min for JL13 and 11 min for the internal standard clozapine, and no interference from drug-free plasma and brain homogenate was observed. The average overall mean recovery was 75% for plasma and 60% for brain tissue, with no significant dependence on concentration.

Daily standard curves containing known concentrations of JL13 were analysed concurrently with each set of unknown samples and quality control samples. The relationships between the peak-height of the compound and the internal standard and the amount of compound added were always linear, with coefficients of correlation exceeding 0.99 over the range of 0.01–0.5 μg mL^{-1} for plasma (1 mL) and 0.1–5 μg g^{-1} for brain tissue (0.1 g). The lowest calibration standard corresponded to the limit of quantification, that is, the lowest concentration that could be measured with acceptable accuracy and precision ($\leqslant 20\%$). At higher concentrations mean coefficients of variation for precision and reproducibility averaged 7% in plasma and 10% in brain for different analyses, with two quality control samples at each compound level and experiment.

The mean accuracy, calculated from the deviation of the mean concentration from the nominal value, indicated inter-assay variability from -1 to 4% for plasma, and from -3 to -4% for brain tissue. It appeared therefore that this was a workable and sensitive enough means of quantitating JL13 in rat plasma and brain regions, and studying their correlations.

Brain distribution studies

The time-course of the mean plasma and brain concentrations of JL13 after intraperitoneal injection of 10 mg kg^{-1} to male rats is shown in Figure 2. The compound appeared rapidly in plasma, attaining mean C_{max} within 5 min of dosing. The decline in plasma concentrations thereafter was apparently biphasic with a mean terminal elimination $t_{\frac{1}{2}}$ of 61 min. This rate of elimination is similar to that of clozapine and its metabolite norclozapine in rodents (approx. 1.5 h) (Baldessarini et al 1993). Consistent with the relatively short elimination $t_{\frac{1}{2}}$, clozapine and its main metabolites do not accumulate in rat plasma and brain with repeated daily dosing (Wilk & Stanley 1978; Baldessarini et al 1993).

Mean brain C_{max} of JL13 occurred 15 min after dosing, only slightly later than in plasma. Disappearance of the

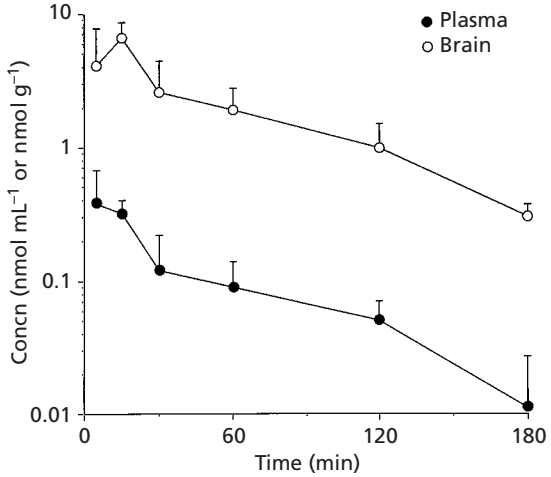

Figure 2 Mean plasma and brain concentration–time curves of JL13 in rats after intraperitoneal injection of 10 mg kg^{-1}. Each value is the mean $\pm$ s.d. of three to five rats.

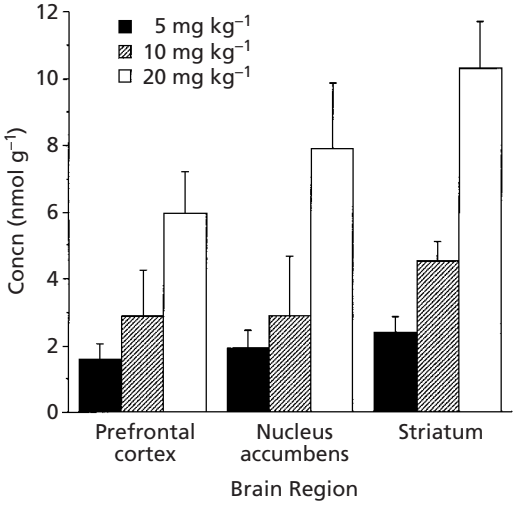

Figure 3 Distribution of JL13 in selected regions of the rat brain after intraperitoneal doses of 5–20 mg kg^{-1}. Rats were killed 1 h after dosing. Results are mean ± s.d. of five rats. Significant differences were observed only at the higher dose and only between striatum and prefrontal cortex ($P < 0.05$).

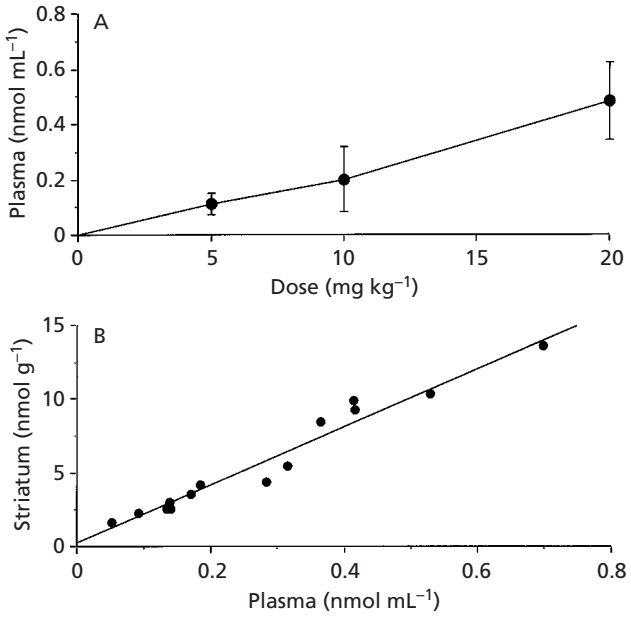

Figure 4 Relationship between dose and plasma concentrations (mean ± s.d.) (A) and between striatum and plasma concentrations (B) of JL13 (individual rats) after intraperitoneal doses of 5–20 mg kg^{-1}.

compound from whole brain paralleled its disappearance from plasma (Figure 2), with comparable mean elimination $t_{\frac{1}{2}}$ of 58 min. However, in this tissue the compound achieved mean C_{max} (6.6 ± 3.7 nmol g^{-1}) and AUC (352 nmol min g^{-1}) approximately 20-times those in plasma (0.4 ± 0.3 nmol mL^{-1} and 16.8 nmol min mL^{-1}, respectively). This brain-to-plasma ratio lies between those of the structurally-related clozapine (⩽ 10 to 24, depending on experimental protocol) (Baldessarini et al 1993; Weigmann et al 1999) and zotepine in the rat (20–30) (Prakash & Lamb 1998). It remains to be clarified whether this ratio reflects the true extent of brain partition, since the plasma protein binding of JL13 was not evaluated in this study. Obviously extrapolation of these results across species should be viewed with caution. Although JL13 should undoubtedly enter the human brain as it and structurally-related drugs do in rodents, there may be differences in the mechanisms that govern the extent of brain uptake between species.

The pattern of distribution of JL13 in the prefrontal cortex, striatum and nucleus accumbens 60 min after intraperitoneal doses of 5–20 mg kg^{-1} is shown in Figure 3. At all doses, JL13 concentrations in striatum tended to be higher than in the other regions but the difference was significant ($Fr(2,36) = 11.4$, $P < 0.001$; $Fd(2,36) = 1.4$, $P < 0.5$; $Frxd(4,36) = 0.25$) only at the higher dose (i.e. striatum = 10.3 ± 1.4 nmol g^{-1} and cortex = 5.9 ± 1.2 nmol g^{-1}; $P < 0.05$). However, these findings rule out preferential localization of JL13 as a mechanism by which it increases dopamine release in cortex but not in other regions of the rat brain (Invernizzi et al 2000). Clozapine also distributes almost evenly in brain regions of the rat (Wilk & Stanley 1978) and rhesus monkey (Hartvig et al 1986).

Over the 5–20 mg kg^{-1} range, plasma concentrations of JL13 increased almost linearly with dose ($[Drug]_{plasma} = 8.2[dose] - 4$; $r^2 = 0.79$). Similar linear relationships were found between the dose and brain concentrations because brain and plasma concentrations of JL13 were closely correlated (e.g. $([Drug]_{striatum} = 19[Drug]_{plasma} + 0.1$, $r^2 = 0.96$; Figure 4), although in all tissue they varied among individual rats at any given dose.

In conclusion, these preliminary pharmacokinetic studies in rats provide evidence that JL13 rapidly diffuses across the blood–brain barrier achieving micromolar concentrations at the site of action after intraperitoneal doses of 5–20 mg kg^{-1}. The compound distributed almost evenly in discrete regions of the rat brain, reaching much higher concentrations than in plasma. Similar to in plasma, however, brain concentrations of JL13 rose almost linearly with the dose and there was a linear relationship between the brain and plasma concentrations. It thus appears that brain concentrations of JL13 parallel the changes in plasma, indicating free and rapid distribution across the blood–brain barrier.

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