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Pharmacokinetics of gacyclidine enantiomers in plasma and spinal cord after single enantiomer administration in rats

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Abstract

The purpose of this study was to determine the pharmacokinetics of gacyclidine, a non-competitive NMDA antagonist, in plasma and spinal cord extracellular fluid (ECF) after IV administration of single enantiomers in rats. After implantation of microdialysis probes in spinal cord, concentrations in plasma and ECF dialysates were determined by a chiral GC/MS assay over 5 h after administration of either (+)-gacyclidine or (−)-gacyclidine (1.25 mg/kg). Plasma protein binding was estimated *in vitro* by equilibrium dialysis. Plasma concentrations decayed in parallel in a biphasic manner ($t_{1/2}\alpha$ ~9 min; $t_{1/2}\beta$ ~90 min) with no significant difference between the two enantiomers. Clearance of (+)-gacyclidine and (−)-gacyclidine (291 versus 275 ml/min per kg, respectively), volume of distribution (Vdβ: 38 versus 40 l/kg), and protein binding (90 versus 89%) were not stereoselective. Both gacyclidine enantiomers were quantifiable in spinal cord ECF 10 min after drug administration and their concentrations remained stable over the duration of the experiment in spite of changing blood concentrations. Penetration of the two enantiomers in spinal cord ECF was similar although highly variable between animals. Exposure of spinal cord ECF was comparable for both enantiomers, and not correlated with plasma AUCs. This study showed the absence of any pharmacokinetic difference between the two enantiomers when administered individually, and no enantiomeric inversion. Both gacyclidine enantiomers penetrate rapidly and extensively into spinal cord ECF, and their distribution may involve an active transport system.

Keywords: Enantiomers; Spinal cord; Gacyclidine; Microdialysis

1. Introduction

Rac-gacyclidine (*cis* (pip/Me) 1-[1-(2-thienyl)-2-methylcyclohexyl] piperidine), is a non competitive *N*-methyl-D-aspartate (NMDA) antagonist with neuroprotective properties (Drian et al.,

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1999; Gaviria et al., 2000). It is currently under clinical evaluation for the treatment of spinal cord injuries. Gacyclidine is a racemic mixture of two enantiomers: (−)-(1S;2R)- and (+)-(1R;2S)-gacyclidine (Fig. 1) which possess similar activities as demonstrated in animal studies (Feldblum et al., 2000).

Previous studies performed in our laboratory demonstrated the stereoselective disposition of gacyclidine enantiomers in healthy and spinal cord-injured rats (Hoizey et al., 2000, 2001) after single iv administration of the racemic gacyclidine. However, enantiomers should be considered as separate chemical entities and the presence or absence of any interaction should thus be investigated. Indeed, the disposition of a single isomer may differ when administered alone due to the presence of numerous potentially stereoselective pharmacokinetic processes which can occur in vivo. As such, competition for binding sites and/or stereoselective metabolic pathways have been suggested as underlying mechanisms for several drug enantiomers' interactions. Moreover, studies performed after administration of a racemic mixture do not allow the characterization of an uni- or bi-directional chiral inversion.

Therefore, the present study was performed in order to investigate the pharmacokinetics of gacyclidine enantiomers in plasma and spinal cord extracellular fluid (ECF) after iv administration of single enantiomers in rats. This experiment was carried out by measuring concentrations in plasma using a traditional approach and in spinal cord extracellular fluid by microdialysis. A comparison with our results obtained after iv administration of the racemic mixture is also presented.

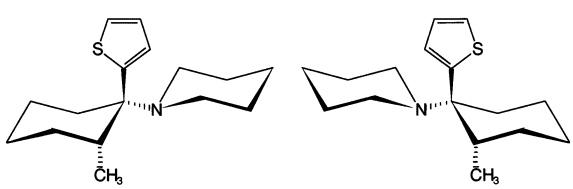

Fig. 1. Chemical structures of gacyclidine enantiomers. Left: (−)-(1S;2R)-gacyclidine; Right: (+)-(1R;2S)-gacyclidine.

2. Materials and methods

2.1. Drugs and chemicals

Rac-gacyclidine (50:50 racemic mixture as well as single (+)-(1R;2S)- and (−)-(1S;2R)-enantiomers) and phencyclidine were supplied by Institut Henri Beaufour (Paris, France). All other chemicals were of reagent grade, obtained from commercial suppliers, and used without further purification.

2.2. Animals

Male Wistar rats weighing 300–340 g were obtained from Elevage Dépré (Saint Doulchard, France). They were housed in conventional facilities in groups of five per cage and maintained in a controlled environment (20 ± 2 °C, 65 ± 15% relative humidity) with a natural light–dark cycle. They were allowed to adapt to the housing environment for at least 1 week prior to study and had access to food (U.A.R., Villemoisson sur Orge, France) and tap water ad libitum. All animal procedures adhered to the 'Principles of laboratory animal care' (NIH publication #85-23, revised 1985).

2.3. Anesthesia

Rats ($n = 10$) were anesthetized with isoflurane (induction: 5% and maintenance: 1–1.5% isoflurane in air) by an Isotec 4 evaporator (Ohmeda, Maurepas, France) and placed onto a heating pad set at 37–37.5 °C (Homeothermic blanket system, Phymep, Paris, France). They were mechanically ventilated at 80 cycles/min with a small animal respirator (Harvard Biosciences, Les Ulis, France) over the duration of the experiment. End-tidal CO_2 was monitored on a CO_2 analyzer (Engström Eliza, Sweden) and maintained between 4.0 and 4.7% through manual adjustment of respirator settings. A polyethylene catheter (No. 3, Biotrol, Paris, France) filled with heparin–saline solution (25,000 iu/l, Choay, Paris, France) was inserted into the right carotid artery and used for blood sampling. A second catheter (No. 1, Biotrol, Paris, France) inserted into the penile vein was used for drug administration.

2.4. Microdialysis

Spinal Cord Microdialysis. After shaving, a dorsal midline incision was made on the skin of the back at T8 extending to T11 level. Paravertebral muscles were detached and adipose tissue separated to expose the dorsal laminae. Laminectomy was performed at a single thoracic level (T9) to expose the corresponding spinal segment, and dura mater opened with a thin injection needle. Before implantation, microdialysis probes (CMA/11, membrane length: 4 mm, cut-off: 6 kDa, O.D.: 240 μm, Phymep) were flushed with Ringer's solution at 15 μl/min to purge membranes and tubing of air bubbles. The flow rate was then reduced to 5 μl/min, the probes inserted into the spinal cord at T8, subsequently moved rostrally up to 5–6 mm above the laminectomy, and allowed to equilibrate for 30 min. Finally, probes were checked for the presence of air bubbles at the end of each experiment.

2.5. Calibration of microdialysis probes

Probe recoveries were estimated by in vivo reverse dialysis (De Lange et al., 1997) in two groups of three rats placed in the same experimental conditions. After implantation in the spinal cord, the probe was perfused with Ringer's solution spiked with either (+)-(1R;2S)- or (−)-(1S;2R)-gacyclidine (10 and 50 ng/ml) at a flow rate of 5 μl/min. Dialysis samples (C_{out}) were serially collected every 20 min over 2 h and frozen (−20 °C) until assayed. The mean recovery was than determined from the ratio of the concentration lost to the initial concentration in the perfusate (C_{in}):

$$\text{Recovery in vivo} = (1 - C_{out}/C_{in}) \times 100$$

2.5.1. Pharmacokinetic studies

This study was performed on two groups of five rats. Each animal received a single iv bolus dose of either (+)-(1R;2S)- or (−)-(1S;2R)-gacyclidine (1.25 mg/kg of base, 0.5 ml/100 g, 1 ml/min) via the penile vein. The catheter was then flushed with 0.2 ml of isotonic saline. Blood (200 μl) was collected through the arterial catheter by means of 1 ml disposable plastic syringes before dosing and at 5, 10, 20, 30, 60, 90, 120, 180, 240 and 300 min after drug administration. After each collection, an equal amount of heparinized saline was injected to flush the catheter and to maintain the fluid volume. Immediately following collection, blood samples were transferred into 1.5 ml microcentrifuge tubes (Eppendorf, Polylabo, Strasbourg, France) containing 5 μl of heparin (25,000 iu/l; Choay) and centrifuged at 5600*g* for 10 min. Spinal cord dialysates were collected by means of a microfraction collector (CMA/140, Phymep). After correction for dead volume (27 μl), samples (100 μl) were continuously collected for periods of 20 min over 300 min post-injection. Plasma samples and dialysates were kept at −20 °C until analysis.

2.5.2. Plasma protein binding

Protein-binding was determined by equilibrium dialysis from rat plasma (200 μl) spiked with either (+)-(1R;2S)- or (−)-(1S;2R)-gacyclidine at concentrations of 10, 50, 100 and 200 ng/ml ($n = 5$). Briefly, plasma samples were dialyzed against 0.15 M, pH: 7.4 phosphate buffer (200 μl) at 37 °C with constant stirring at 8 rpm during 3 h using a Dianorm® equilibrium dialysis system (Braun ScienceTec, Les Ulis, France) equipped with a Spectrapor® (Spectrum Medical Industries, Los Angeles, CA) dialysis membrane (m.w. cut-off = 10 kDa). At the end of each experiment, plasma and buffer samples were collected and frozen at −20 °C until analysis. The bound fraction (f_b) of single gacyclidine enantiomers was calculated according to the general equation:

$$f_b\ (\%) = (1 - C_{Buffer}/C_{Plasma}) \times 100.$$

These values were averaged then used to calculate the concentrations of free gacyclidine enantiomers in plasma from total concentrations.

2.5.3. Drug analysis

Concentrations of gacyclidine enantiomers in plasma and dialysates were determined by an enantioselective GC-MS assay described in details elsewhere (Hoizey et al., 1997, 2000). In short, the inter-assay and intra-assay coefficients of varia-

tion ranged between 1 and 14% ($n = 10$) and between 3 and 15% ($n = 6$) over the concentration range studied (1.5–200 ng/ml). The extraction efficiency of the enantiomers from plasma and Ringer's solution was higher than 90% and the limit of quantitation was 1.5 ng/ml for each enantiomer.

2.5.4. Pharmacokinetic analysis

Individual pharmacokinetic parameters of gacyclidine enantiomers were determined using standard compartmental analysis methods after fitting a two-compartment open model to plasma concentration-time curves by non-linear least-squares regression (MicroPharm, version 5.0, LogInserm, Paris, France). The choice of the model was based on the Akaike Information Criterion. Rate constants (α, β), half-lives ($t_{1/2}\alpha$, $t_{1/2}\beta$), clearance (CL), and volumes of distribution (Vc, Vd_{SS}, and $Vd\beta$) were calculated with standard pharmacokinetic equations (Gibaldi and Perrier, 1982). The area under the concentration-time curve (AUC) was calculated by the trapezoidal rule from time 0 to infinity for plasma data and from time 0 to 300 min for ECF data.Gacyclidine concentrations in dialysates (C_D) were time-averaged over the collection interval, and corrected by the in vivo recovery (R) to yield extracellular concentrations (C_{ECF}):

$$C_{ECF} = (C_D \times 100)/R$$

Penetration of gacyclidine enantiomers into spinal cord was expressed as the AUC_{ECF}/AUC_{Plasma} ratio determined over the duration of the experiment (300 min).

2.5.5. Statistical analysis

All values are reported as mean ± standard deviation. Differences between pharmacokinetic parameters of the two enantiomers were evaluated by the Mann-Whitney U test using GraphPad Prism for Windows (version 3.00, GraphPad Software, San Diego, USA) with the a priori level of significance set at $P < 0.05$. The Pearson product moment correlation coefficient (r) was used to evaluate the strength of the relationship between plasma and spinal cord ECF AUCs.

3. Results

3.1. Protein binding

Protein binding was found to be constant over the concentration range studied (10–200 ng/ml). Both enantiomers were highly bound to plasma proteins (90.2 ± 1.6% and 89.1 ± 1.9% for (+)-(1R;2S)-gacyclidine and (−)-(1S;2R)-gacyclidine, respectively) with no significant difference between the two isomers.

3.2. Gacyclidine pharmacokinetics

Average (± SD) concentration-time profiles for total and free (predicted) (+)-(1R;2S)- and (−)-(1S;2R)-gacyclidine enantiomers in plasma and spinal cord ECF are presented in Fig. 2. Pharmacokinetic parameters determined by non-compartmental and compartmental analysis methods are summarized in Table 1.

In plasma, both enantiomers decayed in parallel with no significant difference between (+)-(1R;2S)-and (−)-(1S;2R)-gacyclidine levels at all time-points. Visual inspection of the concentration versus time profiles indicated a multiexponential decay that could be adequately fitted to a biexponential equation in all animals. Five minutes after the end of injection, maximum plasma concentrations (C_{max}) were in the range of 84.5 to 159.1 ng/ml and 89.7 to 174.7 ng/ml for (+)-(1R;2S)- and (−)-(1S;2R)-gacyclidine, respectively. Concentrations then rapidly declined within the first 30 min after the end of injection. This short distribution phase was followed by an apparent elimination phase where concentrations reached a minimum of 3.0 ± 1.2 ng/ml (range: 1.7–4.3 ng/ml) and 3.3 ± 1.3 ng/ml (range: 2.2–5.3 ng/ml) 300 min post-injection for (+)-(1R;2S)- and (−)-(1S;2R)-gacyclidine, respectively. Differences between the two enantiomers were not, however, statistically significant.

In vivo recoveries of gacyclidine enantiomers became constant within 20 min after the start of the infusion and reached 61.2 ± 2.1% and 58.3 ± 4.4% for (+)-(1R;2S)- and (−)-(1S;2R)-gacyclidine, respectively. In spinal cord ECF, gacyclidine concentrations were characterized by

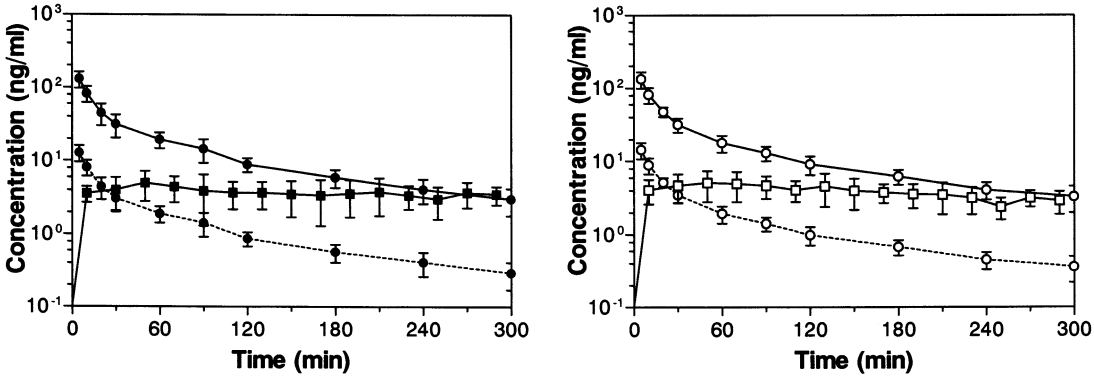

Fig. 2. Concentration-time profiles (mean ± SD) of gacyclidine enantiomers in plasma (●: (+)-(1R;2S)-gacyclidine; ○: (−)-(1S;2R)-gacyclidine) and spinal cord ECF (■: (+)-(1R;2S)-gacyclidine; □: (−)-(1S;2R)-gacyclidine) after intravenous injection of 1.25 mg/kg of (+)-(1R;2S)-gacyclidine (left) or (−)-(1S;2R)-gacyclidine (right) in rats ($n = 5$). Free gacyclidine concentrations in plasma (dotted lines) were predicted from total plasma concentrations.

an initial rapid rise occurring within the first 20 min (range: 1.9 to 5.5 ng/ml) followed by a pseudo-steady-state where they remained constant over the duration of the experiment (overall average value: 3.7 ± 1.6 and 3.9 ± 1.6 ng/ml for (+)-(1R;2S)- and (−)-(1S;2R)-gacyclidine respectively). In light of the low predicted free drug levels in plasma, and in spite of a large inter-individual variability, gacyclidine enantiomers exhibit a very high affinity for spinal cord tissue. Spinal cord ECF levels were not correlated to those determined in plasma ($r = -0.34$ and -0.43 for (+)-(1R;2S)- and (−)-(1S;2R)-gacyclidine, respectively; $P > 0.05$). Finally, penetration in spinal cord ECF, expressed as AUC_{ECF}/AUC_{Plasma}, was not significantly different between the two enantiomers.

4. Discussion

The concentration-time profiles of (+)-(1R;2S)- and (−)-(1S;2R)-gacyclidine in plasma were very similar after IV injection of single enantiomers to rats. Both enantiomers exhibited a high plasma protein binding, an extensive distribution out of the vascular space, and a rapid elimination. None of the differences between the pharmacokinetics parameters of the two enantiomers were statistically significant. Moreover, the fact that the optical antipode of each enantiomer was not detected, under our experimental conditions, in plasma after administration of a single enantiomer suggests that stereochemical inversion does not occur in the rat after iv administration of gacyclidine.

These results, however, are not in full agreement with our previous findings obtained after IV administration of 2.5 mg/kg of the racemate,

Table 1
Pharmacokinetic parameters of gacyclidine following intravenous administration of single (+)-(1R;2S)- and (−)-(1S;2R)-enantiomers in rats (dose: 1.25 mg/kg; five rats in each group)

	(+)-(1R;2S) × -gacyclidine	(−)-(1S;2R) × -gacyclidine
C_{max} (ng/ml)	130.3 ± 33.2	132.0 ± 33.4
$T_{1/2\alpha}$ (min)	8.7 ± 5.0	7.4 ± 2.4
$T_{1/2\beta}$ (min)	89.5 ± 7.6	99.3 ± 37.7
CL (ml/min per kg)	291 ± 77	275 ± 37
V_C (l/kg)	7.4 ± 4.3	7.4 ± 3.0
Vd_{SS} (l/kg)	24.4 ± 5.1	25.6 ± 10.1
Vd_{β} (l/kg)	38.0 ± 12.4	39.6 ± 16.6
AUC_{Plasma} (ng/min/ml)	4510 ± 1012	4591 ± 562
AUC_{ECF} (ng/min/ml)	1071 ± 403	1127 ± 318
AUC_{ECF}/AUC_{Plasma}	0.28 ± 0.16	0.28 ± 0.11

equivalent to 1.25 mg/kg of each enantiomer (Hoizey et al., 2000). Indeed, we found in these studies higher concentrations of (+)-(1R;2S)-gacyclidine, as compared to those of its optical antipode over the duration of the experiment. These concentration profiles resulted in a 25% increase of the AUC for the (+)-(1R;2S)-enantiomer. In addition, total body clearance and volumes of distribution of (+)-(1R;2S)-gacyclidine were significantly lower ($\sim$20%) than that of (−)-(1S;2R)-gacyclidine. These differences, expected to be clinically insignificant, were then suggested to reflect stereoselective distribution and elimination processes when gacyclidine was administered as a racemate.

These small, yet statistically significant, differences between concentrations of gacyclidine enantiomers ($\sim$20%) as determined when the enantiomers were administered concomitantly would tend to suggest the occurrence of an interaction between the enantiomers when injected simultaneously as a racemic mixture. However, the important inter-individual variability found when the enantiomers were administered separately tend to blur the picture somewhat and the presence of statistically significant difference between enantiomers could easily have been masked by the small number of animals and the fact that, in contrast to our previous study, individual enantiomers were given to different animals. Moreover, statistical comparisons performed on such a low number of animals lacks power. In any case, the presence of such an interaction between the enantiomers when injected simultaneously is not expected to have any clinical impact.

The fast penetration of gacyclidine enantiomers into spinal cord ECF led to a rapid attainment of a pseudo steady-state in spinal cord ECF which persisted over the duration of the experiment in all animals. Exposure of spinal cord ECF was high (AUC_{ECF} up to five times higher than $AUC_{Plasma\ Free}$), yet exhibited substantial inter-individual variability and thus could not be predicted from AUC_{Plasma} alone. These findings, which corroborate our results obtained after IV administration of the racemic mixture, further reinforce our hypothesis that gacyclidine enantiomers transport into spinal cord is governed by mechanisms other than passive diffusion (Hammarlund-Udenaes et al., 1997; Hoizey et al., 2000). As such, both enantiomers could be actively transported into spinal cord ECF since spinal cord ECF gacyclidine concentrations did not change over the duration of the experiment. This latter fact could explain why concentrations in spinal cord ECF did not change after repeated administration of constant or increasing doses as performed in one previous study (Hoizey et al., 2001). It may also indicate that the Km of this active exchange system could be below the free gacyclidine concentration estimated in plasma at the end of the experiment (i.e. $\sim$300 pg/ml).

In conclusion, this study demonstrates that the disposition of gacyclidine enantiomers is not stereoselective and that chiral inversion does not occur when the enantiomers are injected separately in the rat. Penetration into spinal cord ECF is characterized by a sustained tissue exposure which seems to involve an active transport system as already suggested following administration of the racemate. Further investigations remain necessary to identify the nature of the transport protein involved in the penetration of gacyclidine in spinal cord ECF, and to address the question concerning the presence of a potential enantiomer/enantiomer interaction when gacyclidine is administered in its racemic form. Preliminary studies based on the use of a variety of metabolic inhibitors of ATP-dependent transport mechanisms (e.g. sodium cyanide) and P-gp modulators (e.g. trifluoperazine) led to conflicting results.

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