Distribution of Gacyclidine Enantiomers in Spinal Cord Extracellular Fluid

Guillaume Hoizey,1,5 Matthieu L. Kaltenbach,1 Sylvain Dukic,1 Denis Lamiable,2 Aude Lallemand,3 Pierre D’Arbigny,4 Hervé Millart,2 and Richard Vistelle1

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Purpose. Determination of the pharmacokinetics of gacyclidine enantiomers, a non-competitive NMDA antagonist, in plasma and spinal cord extracellular fluid (ECF) of rats.

Methods. Implantation of microdialysis probes in spinal cord (T9). Serial collection of plasma samples and ECF dialysates over 5 hours after IV bolus administration of (±)-gacyclidine (2.5 mg/kg). Plasma protein binding determined in vivo by equilibrium dialysis. Chiral GC/MS assay.

Results. Plasma concentrations of (+)-gacyclidine were ~25% higher than those of (−)-gacyclidine over the duration of the experiment in all animals. Plasma concentrations decayed in parallel in a biphasic manner (t1/2α: ~9 min; t1/2β ~90 min) with no significant difference between enantiomers. Clearance and volume of distribution of (−)-gacyclidine were approximately 20% higher than that of its optical antipode (CL: 248 ± 19 ml/kg.min−1; Vd: 31.6 vs 23.5 l/kg). Protein binding (~90%) was not stereoselective. Both gacyclidine enantiomers were quantifiable in spinal cord ECF 10 min after drug administration and remained stable over the duration of the experiment in spite of changing blood concentrations. Penetration of (−)-gacyclidine was significantly higher (~40%) than that of (+)-gacyclidine in all animals. Yet, exposure of spinal cord ECF was similar for both enantiomers, and not correlated with plasma AUCs.

Conclusions. The disposition of gacyclidine enantiomers is stereoselective. Both enantiomers exhibit a high affinity for spinal cord tissue and their distribution may involve a stereoselective and active transport system. This hypothesis could also explain the discrepancy between drug concentrations in plasma and spinal cord ECF.

KEY WORDS: enantiomers; extracellular fluid; gacyclidine; microdialysis; spinal cord.

INTRODUCTION

One major problem in interpreting in vivo data in terms of receptor interactions is the interposition of pharmacokinetic processes that control drug availability in the biophase. This is especially true for centrally acting drugs, due to the presence of cerebral barriers, and for chiral compounds which can exhibit stereoselective pharmacokinetics (1–3). Thus, monitoring free drug concentrations in tissue is crucial to better understand the time course of drug effects, and to optimize drug dosing regimens.

Gacyclidine (cis (pip/Me) 1-[1-(2-thienyl)-2-methylcyclohexyl] piperidine), a non competitive N-methyl-D-aspartate (NMDA) antagonist, is a chiral drug (Fig.1) with neuroprotective properties (4,5). Proposed in the treatment of central nervous system injuries, its pharmacokinetic properties in the target tissue remain unknown, yet are essential to better understand the time course of its pharmacological effects and should contribute to improve its clinical efficacy.

Consequently, the purpose of this study was to determine the pharmacokinetics of gacyclidine enantiomers in rats by using the traditional approach of plasma data analysis together with the determination of the concentration-time profiles of free gacyclidine enantiomers in spinal cord extracellular fluid (ECF) by microdialysis. This work also highlights the potential value of microdialysis in the study of drug pharmacokinetics at its site of action. Indeed, this approach has not yet been applied to the determination of free interstitial drug levels in this specific tissue.

MATERIALS AND METHODS

Drugs and Chemicals

Gacyclidine (racemic mixture, (+) and (−) 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.

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 one 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).

Anesthesia

Rats (n = 6) 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 CO₂ was monitored with a CO₂ analyzer (Engström Eliza, Sweden) and maintained between 4.0 and 4.7% through manual adjustment of respirator settings. A polyethylene catheter (N°3, Biotrol, Paris, France) filled with heparin-saline solution (25000 UI/l, Héparine Choay, Paris, France) was inserted into the right carotid artery and used for blood sampling. A second catheter (N°1, Biotrol, Paris, France) inserted into the left jugular vein was used for drug administration.

**Microdialysis**

**Spinal Cord Microdialysis**

After shaving, a dorsal midline incision was made on the skin of the back at the T8 to T11 level. Paravertebral muscles were detached and adipose tissue separated to expose the dorsal laminæ. Laminctomy 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, Carnegie, Phymep, France) were flushed with Ringer’s solution at 15 ml/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 laminctomy, and allowed to equilibrate for 30 min. Finally, probes were checked for the presence of air bubbles at the end of each experiment. Calibration of Microdialysis Probes. Relative gacyclidine enantiomer microdialysis probe recovery was estimated by in vivo reverse dialysis. In this approach, the substance of interest is introduced into the perfusate and one assumes that its relative loss during the perfusion (delivery) is an estimate of the recovery (6). Recoveries were determined in a dedicated group of rats (n = 4) using the same experimental protocol as described above. After implantation in spinal cord, the probe was perfused with Ringer’s solution spiked with gacyclidine (80 ng/ml) at a flow rate of 5 μl/min. Dialysates were serially collected every 20 min over 3 h and frozen (−20°C) until assayed. The mean in vivo recovery computed from all recovery ratios calculated as shown below:

\[
\text{Recovery}_{\text{in vivo}} = \left(1 - \frac{C_{\text{out}}}{C_{\text{in}}}ight) \times 100
\]

where \(C_{\text{in}}\) and \(C_{\text{out}}\) are the drug concentrations in the perfusate inflow and outflow, respectively.

**Histology**

At the end of each experiment, animals were deeply anesthetized. All blood from the circulation was removed by perfusing the heart with saline (200 ml) and severing the inferior vena cava. Subsequently, in situ fixation was performed by perfusing 200 ml of 10% neutral buffered formalin, and the spinal column from T7 to T10 removed. The spinal cord was carefully dissected out of the vertebrae and placed in 4% buffered formalin for at least 7 days for tissue fixation. Spinal cord tissue was later embedded in paraffin, and serially cut with a microtome. Every 10th 5 μm section was routinely stained with hematoxylin pholine saffron (HPS) and used for microscopic examination. The whole path of the semipermeable part of the microdialysis membrane and the implantation site could thus be screened for morphological changes.

**Pharmacokinetic Studies**

Each animal received a single IV bolus dose of racemic gacyclidine (2.5 mg/kg of base) via the jugular 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; Héparine Choay) and centrifuged at 5 600 g for 10 min. Dialysates (100 μl) were continuously collected for periods of 20 min over 300 min post-injection by means of a microfraction collector (CMA/140, Carnegie, Phymep, France). Plasma samples and dialysates were kept at −20°C until analysis.

**Plasma Protein Binding**

Binding of gacyclidine enantiomers was determined by equilibrium dialysis from plasma of individual rats (n = 5) spiked with racemic gacyclidine at concentrations of 20, 50 and 200 ng/ml. Briefly, plasma samples (200 μl) 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 an equilibrium dialysis system (Dianorm®, Braun ScienceTec, Les Ulis, France). The fluids in the dialysis cells were separated by Spectrapor® dialysis membranes (m.w. cutoff: 10 kDa; Spectrum Medical Industries, Los Angeles, USA). At the end of dialysis, plasma and buffer samples were collected and frozen at −20°C until analysis. The bound fraction (\(f_b\)) of each gacyclidine enantiomer was then calculated according to the general equation:

\[
f_b (%) = \left(1 - \frac{\text{Conc}_{\text{buffer}}}{\text{Conc}_{\text{plasma}}}ight) \times 100
\]

Non-specific drug adsorption onto the dialysis membrane was determined for each concentration in triplicate to provide a correction factor taken into account, when appropriate, in the calculation of the bound fraction.

Finally, the average plasma protein binding was used to calculate the concentrations of free gacyclidine enantiomers in plasma from total concentrations.

![Fig. 1. Chemical structures of gacyclidine enantiomers. Left: (-)-1S, 2R-gacyclidine; Right: (+)-1R, 2S-gacyclidine.](image-url)