

# Metronidazole and Hydroxymetronidazole Central Nervous System Distribution: 1. Microdialysis Assessment of Brain Extracellular Fluid Concentrations in Patients with Acute Brain Injury

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**The distribution of metronidazole in the central nervous system has only been described based on cerebrospinal fluid data. However, extracellular fluid (ECF) concentrations may better predict its antimicrobial effect and/or side effects. We sought to explore by microdialysis brain ECF metronidazole distribution in patients with acute brain injury. Four brain-injured patients monitored by cerebral microdialysis received 500 mg of metronidazole over 0.5 h every 8 h. Brain dialysates and blood samples were collected at steady state over 8 h. Probe recoveries were evaluated by *in vivo* retrodialysis in each patient for metronidazole. Metronidazole and OH-metronidazole were assayed by high-pressure liquid chromatography, and a noncompartmental pharmacokinetic analysis was performed. Probe recovery was equal to 78.8% ± 1.3% for metronidazole in patients. Unbound brain metronidazole concentration-time curves were delayed compared to unbound plasma concentration-time curves but with a mean metronidazole unbound brain/plasma AUC<sub>0-τ</sub> ratio equal to 102% ± 19% (ranging from 87 to 124%). The unbound plasma concentration-time profiles for OH-metronidazole were flat, with mean average steady-state concentrations equal to 4.0 ± 0.7 μg ml<sup>-1</sup>. This microdialysis study describes the steady-state brain distribution of metronidazole in patients and confirms its extensive distribution.**

Metronidazole, a nitroimidazole antibiotic, is useful for treating infections by *Bacteroides* spp. and many anaerobic bacteria. Since metronidazole is supposed to penetrate extensively into central nervous system (CNS), it has been described in literature as being responsible for both peripheral (1) and central (2–6) neurotoxicity, especially after a prolonged use of metronidazole (7). In both cases, symptoms and lesions on magnetic resonance imaging may spontaneously regress after discontinuation of treatment. While the pathogenesis as yet remains unclear, the most likely hypothesis is an axonal swelling due to metronidazole-induced vasogenic edema, which could be linked to an impairment of vitamin B<sub>1</sub> action, because of metronidazole conversion to a thiamine analog (8). Thus, characterizing the distribution of metronidazole in cerebral tissue may contribute to managing the dosing regimen in order to prevent side effects while preserving maximal antibacterial efficiency.

Differences in anatomy, enzymatic activity or bulk-flow exist between blood-brain-barrier (BBB) and blood-cerebrospinal fluid (CSF) barrier (9), which could result in differences in drug distribution between the CSF and brain extracellular fluid (ECF). Current metronidazole doses rely on a few studies that show an extensive distribution of metronidazole into the CSF (10–12). However, most of the previous CSF pharmacokinetic studies of metronidazole used nonspecific microbiological assays that cannot distinguish parent drug from metabolites (13–16) and at present no study has explored the distribution of metronidazole in the brain ECF.

Intracerebral microdialysis is the state-of-the-art *in vivo* technique allowing ECF sampling to study the distribution of exogenous compounds, such as antibiotics, in the brain (17). The main interest of this technique is to continuously measure brain unbound concentrations as a function of time, thus providing infor-

mation on drug transport across the BBB (17, 18), information that may be in assessing desirable activity and/or neurotoxicity in the CNS. However, one of the crucial issues in the use of quantitative microdialysis for estimating accurate unbound drug concentrations is the determination of probe *in vivo* recovery, which may not be easy in a clinical setting (18). The main goal of the present study was to explore the cerebral ECF distribution of metronidazole in patients with acute brain injury by comparing unbound concentrations in brain and plasma.

## MATERIALS AND METHODS

**Patients.** This study was performed in the neurointensive care unit at the University Hospital of Poitiers (France) and was approved by the local ethics committee (CPP OUEST III, protocol 2008-003311-12). Written informed consent was obtained from a legal representative of the four patients enrolled. The patients (four men), aged 52 to 65 years, were brain injured, sedated with midazolam and fentanyl, and mechanically ventilated. The demographic characteristics are detailed in Table 1. All received metronidazole (B Braun, Boulogne-Billancourt, France) and cefotaxime (Panpharma, Fougères, France) for the clinical management of a lung infection at respective dosing regimens of 500 mg and 2 g three times per day. Routine monitoring for acute brain injury included brain-specific monitoring of the intracranial pressure (Micro-Sensor ICP monitoring

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TABLE 1 Patient demographic characteristics ( $n = 4$ )

Parameter	Patient			
	P1	P2	P3	P4
Age (yrs)	55	64	52	65
Ht (cm)	180	172	175	172
Sex	M	M	M	M
Wt (kg)	90	90	77	79
Creatinine clearance ( $\text{ml min}^{-1}$ ) <sup>a</sup>	171	118	86	144
Serum albumin ( $\text{g liter}^{-1}$ )	22	NA <sup>c</sup>	31	42
Serum total proteins ( $\text{g liter}^{-1}$ )	66	61	64	67
Admission type <sup>b</sup>	TBI	TBI	SAH	TBI
No. of previous administrations	17	6	6	8

<sup>a</sup> Calculated by using the MDRD (modification in diet of renal disease) equation.

<sup>b</sup> TBI, trauma brain injury; SAH, subarachnoid hemorrhage.

<sup>c</sup> NA, not available.

system; Codman & Shurtleff, Inc., Raynham, MA), measurement of the partial pressure of oxygen in brain tissue ( $\text{PbO}_2$ ) measurement (Licox; Integra Neurosciences, Lyon, France), and cerebral microdialysis (CMA-70, polyamide membrane, 20 kDa,  $\mu$ dialysis; Advanced Medical Products, France) for lactate, pyruvate, and glucose concentration determinations.

**Microdialysis probe implantation.** Microdialysis probe implantation and equilibration at a flow rate of  $0.3 \mu\text{l min}^{-1}$  were performed as previously described (17, 19).

**Drug administration and sampling.** The metronidazole brain pharmacokinetic study was conducted at steady state between days 3 and 6, corresponding to 6 to 17 metronidazole administrations. After collection of baseline dialysates and blood samples, 500 mg of metronidazole was infused over 0.5 h. Brain dialysates were collected over the 8-h period at 0.5-h intervals during the first 3 h and at 1-h intervals for the remainder of the experiment. Eight to 13 blood samples were collected over the dosing interval. Blood samples were centrifuged at  $2,000 \times g$  for 15 min at  $4^\circ\text{C}$ , and plasma was collected to determine the total metronidazole and OH-metronidazole concentrations. Two extra plasma samples were collected, one early and one later postdosing, to determine unbound concentrations of both molecules by ultrafiltration at  $2,500 \times g$  for 30 min at  $4^\circ\text{C}$  (Centrifree; Millipore, Billerica, MA). Immediately after collection, dialysates, ultrafiltrates (UF), and plasma samples were kept at  $-80^\circ\text{C}$  until analysis.

**In vivo recovery calculation of metronidazole concentrations.** For each patient, *in vivo* probe recovery was determined using a “retrodialysis-by-drug” method over 2.5 h at the end of the experiment, as previously described (19). The next metronidazole injection was delayed in order to perform recovery estimation. Briefly, microdialysis probe was perfused with a  $50\text{-}\mu\text{g ml}^{-1}$  solution of metronidazole in CNS perfusion fluid and, after a 1-h equilibration period, three 0.5-h interval dialysates were collected. The *in vivo* relative recovery by loss was calculated for each dialysate collected, and the mean value was used to correct the dialysate concentrations (19). The metronidazole residual unbound concentration in brain was taken into consideration to estimate recovery, as previously described (20). Briefly, *in vivo* recovery was calculated for each interval as presented in the following equation: *in vivo* recovery =  $[(C_{\text{in}} + C_{\text{ext}}) - C_{\text{out}}]/C_{\text{in}}$ , where  $C_{\text{in}}$  and  $C_{\text{out}}$  are the perfusate and dialysate metronidazole concentrations and  $C_{\text{ext}}$  is the extrapolated concentration of metronidazole in the dialysate at the midpoint of the retrodialysis interval of collection. We observed that  $C_{\text{ext}}$  was always relatively low (3 to 15%) compared to  $C_{\text{in}}$ .

**Metronidazole and OH-metronidazole assay.** Metronidazole and OH-metronidazole concentrations were determined by high-pressure liquid chromatography with UV detection. The chromatographic system consisted of an Xterra  $C_{18}$  column (150 by 3.8 mm [inner diameter]; Waters, France), a Hitachi L-2130 pump (VWR, Fontenay sous Bois, France), and a Hitachi L-2200 autosampler (VWR) connected to a UV

detector (SPD 10A; Shimadzu, Marne la Vallée, France) at 310 nm. The data were recorded and analyzed on EZ Chrom-Integrator (VWR). The mobile phase consisted of a solution of 0.01 M  $\text{KH}_2\text{PO}_4$  mixed with acetonitrile (86/14 [vol/vol]) at a flow rate of 0.4 ml/min. Brain dialysates and UF samples were injected directly after dilution with an internal standard solution of dimetridazole ( $0.5 \text{ ng ml}^{-1}$ ). For both metronidazole and OH-metronidazole, eight calibration standards using concentrations between 0.25 and  $20 \mu\text{g ml}^{-1}$  were performed. The dialysate and UF metronidazole and OH-metronidazole intra- and interday variabilities were respectively characterized at four (0.25, 0.5, 2, and  $20 \mu\text{g ml}^{-1}$ ) and three (0.5, 2, and  $15 \mu\text{g ml}^{-1}$ ) concentrations, respectively, and were always  $<15\%$ . Plasma samples ( $100 \mu\text{l}$ ) were treated by the addition of  $200 \mu\text{l}$  of acetonitrile containing internal standard ( $2.5 \mu\text{g ml}^{-1}$ ) for deproteinization. In plasma, seven calibration standards were prepared using concentrations between 0.5 and  $40 \mu\text{g ml}^{-1}$ . The plasma intra- and interday variabilities were characterized at four (1, 2, 10, and  $40 \mu\text{g ml}^{-1}$ ) and three (1, 5, and  $30 \mu\text{g ml}^{-1}$ ) concentrations, respectively, and were always  $<15\%$ .

**Pharmacokinetic analysis.** In each patient, two individual unbound fraction values ( $f_u$ ) of metronidazole and OH-metronidazole were calculated as the ratio of metronidazole or OH-metronidazole concentrations in UF to corresponding total plasma concentrations. The mean value was used to convert total concentrations into unbound concentrations. Pharmacokinetic parameters were estimated from unbound plasma and ECF brain unbound concentrations by individual noncompartmental analysis (Phoenix WinNonlin 6.2; Pharsight, St. Louis, MO) as previously described (17, 19). Briefly, areas under the plasma and brain ECF unbound concentration-time curves between two consecutive dosing at steady-state ( $\text{AUC}_{0-\tau}$ ) were calculated by using the linear trapezoidal rule. The elimination rate constant  $k_e$  and corresponding half-life ( $t_{1/2}$ ) were determined in the terminal phase of the decline, and the metronidazole clearance ( $\text{CL}_{\text{ss,u}}$ ) and volume of distribution ( $V_{\text{ss,u}}$ ) were calculated. The average steady-state unbound concentration ( $C_{\text{average}}$ ) was calculated as the ratio of  $\text{AUC}_{0-\tau}$  to  $\tau$ , where  $\tau$  was equal to 8 h.

**Statistical analysis.** Results are expressed as means  $\pm$  the standard deviations. The metronidazole half-life and mean maximum unbound concentration values in plasma and brain were compared by using a non-parametric Wilcoxon test at a significance level of  $P < 0.05$ .

## RESULTS

Estimated *in vivo* metronidazole probe recoveries in patients were relatively high with limited intra and between patients variability (73.9 to 82.9%) (Table 2). Metronidazole and OH-metronidazole plasma protein binding was very limited, with estimated mean unbound fractions in plasma ( $f_u$ ) of  $86.5\% \pm 8.9\%$  and  $79.0\% \pm 13.0\%$ , respectively.

Unbound metronidazole concentration-time curves in the brain were delayed compared to unbound plasma concentration-time curves, with a maximal time peak observed at  $69 \pm 30$  min, but the brain maximal concentration was only slightly and not significantly lower than the corresponding value in plasma ( $C_{\text{max,ub}} = 14.5 \pm 1.2 \mu\text{g ml}^{-1}$  versus  $C_{\text{max,up}} = 17.1 \pm 3.1 \mu\text{g ml}^{-1}$ ) (Fig. 1). Mean metronidazole brain to unbound plasma  $\text{AUC}_{0-\tau}$  ratio was equal to  $102\% \pm 19\%$ . The pharmacokinetic parameters are presented in Table 2.

Unbound OH-metronidazole plasma concentration-versus-time profiles were flat, with corresponding mean average steady-state concentrations of  $4.0 \pm 0.7 \mu\text{g ml}^{-1}$  (Fig. 1). Similar profiles were obtained in brain dialysates (not shown); however, because it was impossible to estimate *in vivo* OH-metronidazole probe recovery, ECF brain concentrations of this metabolite could not be determined.

TABLE 2 *In vivo* probe recovery of metronidazole and pharmacokinetic parameters determined at steady state by noncompartmental analysis in critical care patients receiving 30-min intravenous infusions of 500 mg of metronidazole every 8 h

Patient <sup>d</sup>	Metronidazole pharmacokinetic parameter <sup>b</sup>					Mean % <i>in vivo</i> recovery $\pm$ SD ( $n = 3$ )
	$V_{ss,u}$ (liters)	$CL_{ss,u}$ (liters h <sup>-1</sup> )	$t_{1/2}$ plasma (h)	$t_{1/2}$ brain (h)	Brain ECF/unbound plasma AUC ratio	
Individual patients						
P1	66.7	4.0	6.7	4.2	0.87	82.9 $\pm$ 0.5
P2	56.4	3.4	9.2	4.5	0.83	79.5 $\pm$ 3.4
P3	53.6	3.2	4.9	4.8	1.24	79.1 $\pm$ 1.4
P4	65.6	3.9	4.3	4.4	1.16	73.9 $\pm$ 4.2
Mean data						
Mean	60.6	7.9	6.3	4.5	1.02	
SD	6.6	2.8	2.2	0.2	0.19	

<sup>a</sup> Both individual and mean patient data are presented.

<sup>b</sup> The values for  $t_{1/2}$  plasma and  $t_{1/2}$  brain were not significantly different.

## DISCUSSION

We used intracerebral microdialysis to investigate the CNS distribution of metronidazole in patients. *In vivo* probe recoveries estimated by the retrodialysis-by-drug approach were always >70% in all patients (Table 2), which is 1.5- to 3-fold higher than the recoveries we previously reported for cefotaxime and meropenem in patients (17, 19, 21) and which may be considered optimal. The experimental conditions here, including the flow rate and the selected probes, were identical (0.3  $\mu$ l min<sup>-1</sup>, CMA-70, 20 kDa) to those used in previous cefotaxime studies (17, 19), whereas the same flow rate but larger-cutoff probes (CMA-71, 100 kDa) were used for our meropenem study (21). However, the metronidazole probe recoveries in patient brains were not only particularly high but also virtually identical between individuals (Table 2).

Numerous pharmacokinetic studies of metronidazole have been performed in healthy volunteers (22–24) and in various spe-

cific populations (10, 12), but only two studies have been conducted in critically ill patients (25, 26). In the present study, the mean metronidazole  $f_u$  (86.5%  $\pm$  8.9%) was in accordance with previous results (10). Since the unbound fractions of metronidazole were close to unity, our pharmacokinetic parameters estimated from unbound concentrations can be directly compared to parameters previously reported for total concentrations. Thus, the  $V_{ss,u}$  in the present study (60.6  $\pm$  6.6 liters, Table 2) was close to the value estimated in septic shock patients after a single administration (53.5  $\pm$  4 liters) (26). However, our  $CL_{ss,u}$  (7.9  $\pm$  2.8 liters h<sup>-1</sup>) was 2-fold higher than in septic shock patients (56.2 ml min<sup>-1</sup> or 3.4 liters h<sup>-1</sup>) (26). Consequently, our metronidazole  $t_{1/2}$  (6.3  $\pm$  2.2 h, Table 2) is much lower than previously reported in critical care patients (13.2  $\pm$  5.3 h) and in better agreement with the  $t_{1/2}$  estimated in healthy volunteers (between 7.3 to 7.9 h) (22–24). The most likely explanation for this discrepancy is the

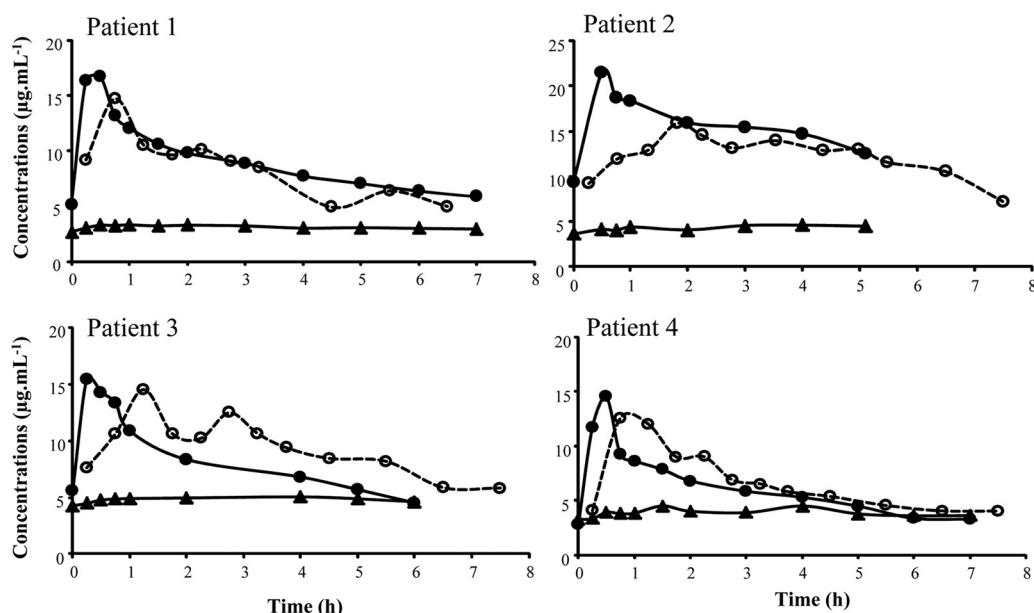


FIG 1 Individual steady-state unbound plasma (●, full line) and brain ECF (○, dashed line) concentrations of metronidazole and unbound plasma concentration of OH-metronidazole (▲, full line) after 500-mg metronidazole infusion over 0.5 h every 8 h in critical care patients.

impaired renal function that is usual in patients with a septic shock (26).

This is the first microdialysis study to explore brain ECF distribution of metronidazole in patients with acute brain injury. We have confirmed the extensive distribution of metronidazole in brain, with a mean AUC ratio close to unity (Table 2). These new data suggest that metronidazole brain ECF distribution is governed by passive diffusion, whereas for cefotaxime the mean brain/plasma AUC ratio was much lower ( $26.1\% \pm 12.1\%$ ) (19), a finding in accordance with the fact that some  $\beta$ -lactam antibiotics are known to be transported by efflux transporters, including organic anion transporter 3, peptide transporter 2, or multidrug-resistant associated protein 4 (27–29).

These findings demonstrate that the extensive distribution of metronidazole within brain ECF contributes to the CNS toxicity (2–6) observed occasionally during treatments with this antibiotic. Although brain ECF concentrations of metronidazole observed here were always  $>4 \mu\text{g/ml}$ , which should be sufficiently high for ensuring proper antimicrobial efficacy against most targeted bacteria, no firm conclusion about antimicrobial efficacy of metronidazole for the treatment of cerebromeningeal infections can be drawn for at least two reasons. First, the bacterial location—i.e., the ECF, the CSF, or both—remains unclear, meaning that CSF concentrations and ECF levels could be complementary for predicting antimicrobial efficacy. Second, our patients did not present with CNS infection, which may alter BBB permeability by opening tight junction or interfering with efflux pump activity (30–32), although such a permeation effect should have greater consequences for antibiotics with limited CNS distribution than for those, such as metronidazole, with extensive and mostly passive diffusion. However, this important issue should be clarified in the future.

This study is the first to describe an extensive distribution of metronidazole in patient brain ECF by using microdialysis. An accompanying paper examines the measurement of metronidazole and hydroxymetronidazole concentrations in the CSF of patients with external ventricular drain (33).

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