Microdialysis Sampling to Determine the Pharmacokinetics of Unbound SDZ ICM 567 in Blood and Brain in Awake, Freely-Moving Rats

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The free concentrations of the serotoninergic 5-HT_3 antagonist SDZ ICM 567 in blood and in the central nervous system were examined in awake, freely-moving rats using blood and brain microdialysis coupled to liquid chromatography. Microdialysis probes were implanted in the jugular vein and in the frontal cortex and dialysis samples were simultaneously collected from both sites. Pharmacokinetic parameters were calculated after a 10 mg/kg intravenous dose of [^{14}C]SDZ ICM 567. The elimination half lives measured in whole blood, brain and blood microdialysates were similar (≈1.7 h). The AUC_{0-5h} corresponding to the unbound drug was 462 ± 142 ng · ml^{-1} · h in blood dialysate, not significantly different from the AUC corresponding to the free concentration in whole blood, i.e. 586 ± 63 ng · ml^{-1} · h. The free fraction in blood obtained *in vitro* by equilibrium dialysis (21%) or by microdialysis (19%) was not statistically different from that obtained *in vivo* (17%) in microdialysis experiments. The unbound concentrations (AUC_{0-5h}) of SDZ ICM 567 in the brain cortex were 86 ± 24 ng · ml^{-1} · h, lower than those expected from unbound blood concentrations, suggesting an active transport out of the central nervous system. Finally, microdialysis sampling allowed the determination of pharmacokinetic parameters of SDZ ICM 567 in blood and brain as well as the estimation of the free fraction of drug in blood.

KEY WORDS: brain microdialysis; blood microdialysis; pharmacokinetics; free drug concentration; SDZ ICM 567.

INTRODUCTION

Microdialysis (1) as an *in vivo* sampling technique is a powerful tool to monitor continuously the blood and tissue of animals for metabolic and pharmacokinetic purposes (2–5). Because there is no net fluid exchange, continuous sampling for long periods of time without disturbing the pharmacokinetics is possible; in addition, this technique allows the possibility of estimating unbound drug concentrations in blood and other tissues (6). Thus, the time course of the free concentration of a variety of drugs was simultaneously followed in the blood and in the brain of animals (7,8).

The aim of this study was to use microdialysis for determining the pharmacokinetics of SDZ ICM 567, a 5-HT_3 antagonist, in brain cortex and blood from awake, freely-moving rats. The knowledge of the central nervous system (CNS) distribution for 5-HT_3 antagonists is essential because of their potential efficacy in multiple CNS disorders, including anxiety, schizophrenia, cognition and drug dependence, as well as their ability to inhibit the cancer chemotherapy-induced emesis. SDZ ICM 567 (Sandoz Pharma, Basel, Switzerland), the 7-methoxy derivative of tropisetron (Fig. 1), stems from the rationale to devise a compound with a controlled biotransformation pathway, avoiding the phenotypic differences in biotransformation encountered with tropisetron in human studies.

It is known that anesthetics can have an important effect on the observed pharmacokinetics of drugs (8,9). Moreover, it is well accepted that the unbound concentration in biological media is pharmacologically more relevant than the total concentration. In addition, a number of drugs are bound not only to the plasma proteins but also to the red cells (10); this fact should be considered when the binding properties of a given compound are analyzed. These concepts strongly support the use of brain and blood microdialysate samples for pharmacokinetic determinations in awake, freely-moving rats.

MATERIAL AND METHODS

Microdialysis Probe Characterization

In order to determine the *in vivo* concentration of SDZ ICM 567 in the particular fluid surrounding the dialysis probes, the efficiency or recovery of the probe must be calculated. Relative recovery is expressed as the ratio of the concentration in the perfusion solution to that in the sample solution. For this purpose, the "zero net flux" or "difference" method was used (11,12) under steady-state conditions in separate experiments. Steady state was established in four animals by constant infusion through the femoral vein of [^{14}C]SDZ ICM 567 at an infusion rate of 0.3 mg/h. In some experiments steady state was achieved by infusing unlabelled SDZ ICM 567 (13). Four different solutions (10, 25, 50 and 100 ng/ml) of [^{14}C]SDZ ICM 567 were perfused through the probes, at a flow rate of 2 μl/min, and the concentration of [^{14}C]SDZ ICM 567 in the dialysate was analyzed. Linear regression analysis was performed on a plot of net transport versus perfusate concentrations to determine the point of no net flux and the recovery.

Blood Binding

Because microdialysis only measures the free concentration in blood, the extent of binding of the drug to blood components must be known to compare results from microdialysis to those from whole blood. The extent of binding of [^{14}C]SDZ ICM 567 in rat blood was determined *in vitro* by equilibrium dialysis and microdialysis. Equilibrium dialysis was performed using a Dianorm apparatus with 1 ml Teflon cells. One side of the dialysis cell contained isotonic phosphate buffer (16 mM $Na_2HPO_4 \cdot 2H_2O$, 4 mM KH_2PO_4, 100 mM NaCl, pH = 7.4) equal in volume to that of the blood in the opposite side of a Visking cellulose membrane 27/32. Drug was added to the blood at concentrations of 20,500 and 4000 ng/ml. The dialysis was carried out at 37°C for 2 h

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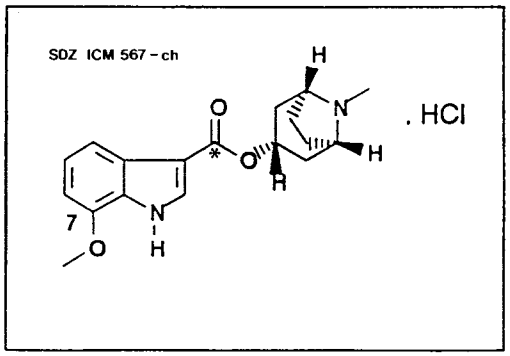

Fig. 1. Structure of SDZ ICM 567 labelled with carbon 14 (*).

under constant stirring at 8 r.p.m.; preliminary diffusion kinetic experiments had indicated that equilibrium was reached at this time. The fraction unbound f_u of drug was calculated as: f_u (%) = buffer concentration/blood concentration · 100.

The binding of SDZ ICM 567 to blood components was also measured using *in vitro* blood microdialysis in two steps. In the first step the relative recovery of SDZ ICM 567 in the absence of blood components was estimated in a buffer solution containing a known concentration of [^{14}C]SDZ ICM 567. The second step involved the estimation of the recovery in blood containing the same concentration of [^{14}C]SDZ ICM 567. The blood or buffer reservoir was thermostated at 37°C and stirred by Teflon stirring bars at a constant speed of 200 rpm (IKA Labortechnik, Staufen, Germany). In both cases the recovery was calculated as C_{out}/C_m, where C_{out} is the concentration in the dialysate and C_m is the medium concentration, the medium being either buffer or blood. The free fraction (f_u) was estimated as the ratio of the recovery in blood to the relative recovery in buffer.

In vivo Pharmacokinetic Experiments

Four male Hanover Wistar rats weighing 250–300 g were used in this study. One week prior to drug administration, rats were anesthetized with ketamine HCl/xylazine, maintained with methoxyflurane and then placed in a stereotaxic apparatus. Guide cannulas and matching probes were of the concentric tube type obtained from Carnegie Medicine (Stockholm, Sweden). Guide cannulas (CMA 12) were implanted into the frontal cortex (stereotaxic coordinates from bregma: anterior, 3.2 mm; laterally, 1.2 mm; and ventrally to the skull surface, 1.5 mm). The day before the experiment, a blood microdialysis probe (CMA 20, 0.5 mm diameter, 10 mm length membrane) was placed in the jugular vein. For this, a small incision through the skin was made and the external jugular vein exposed. The microdialysis probe was inserted through the pectoral muscle into the vein in a direction opposite blood flow. For the intravenous (i.v.) drug administration, a silastic catheter was implanted into femoral vein and fixed with suture.

On the day of the experiment, the obturator in the guide cannula was removed, the brain probe (CMA 12, 0.5 mm diameter, 4 mm length membrane) was inserted manually through its guide cannula, and the animal was placed in a cage for microdialysis studies with freely moving rats (Europhor Instruments, Toulouse, France). The brain and blood microdialysis probes were perfused using a syringe pump (CMA 100) with artificial cerebrospinal fluid (0.8 mM Mg^{2+}, 1.1 mM Ca^{2+}, 2.9 mM K^+, 155 mM Na^+, 13.7 mM HCO_3^-, 0.249 mM $H_2PO_4^-$, 132 mM Cl^-, 0.249 mM SO_4^{2-}, 5.92 mM glucose, pH = 7.4) and phosphate buffer (16 mM $Na_2HPO_4 \cdot 2H_2O$, 4 mM KH_2PO_4, 100 mM NaCl, pH = 7.4), respectively, at a flow rate of 2 µl/min. Ninety minutes after the brain probe introduction, an i.v. bolus dose of 10 mg/kg [^{14}C]SDZ ICM 567 (specific radioactivity, 162 µCi/mg), prepared in isotonic saline/ethanol 97.5:2.5 v/v, was given. Brain and blood microdialysis perfusates were collected every 30 and 15 minutes, respectively, for 5 hours. To validate the microdialysis sampling technique, whole-blood samples were simultaneously taken from the tail vein at 2, 15, 30 and 60 minutes and every hour thereafter. The *in vivo* free concentrations for microdialysis samples were calculated by dividing the dialysate concentration by the probe recovery values. Whole blood concentrations were corrected for blood binding using the values determined *in vitro*. Disposition half-lives ($t_{1/2}$) of SDZ ICM 567 were calculated from the slope of the terminal straight line when using semilogarithmic coordinates. In the case of blood samples, AUC was calculated by the linear trapezoidal rule, whereas for the dialysate, the AUC was obtained as the sum of the products of the measured concentrations and the collection time interval

$$AUC = \Sigma Ci\Delta ti$$

Analysis of [^{14}C]SDZ ICM 567

The procedure for the measurement of the unchanged [^{14}C]SDZ ICM 567 involved the addition of non radiolabelled SDZ ICM 567 to each sample as an internal standard. Prior to HPLC analysis, whole blood samples were extracted with diethylether, whereas microdialysate samples were directly injected into the HPLC. Chromatography was performed on an HPLC system (MT2, Kontron Instruments, Zurich, Switzerland). Separation was conducted on a Spheri-5 RP18 column (220 × 4.6 mm) (Brownleelabs) at 50°C. The mobile phase consisted of acetonitrile/tetramethylammonium hydrogensulphate 0.1% (65:35 v/v). The flow rate was 1 ml/min; the effluent was monitorized at 250 nm and collected in polyethylene vials by a fraction collector (SuperFrac, Pharmacia LKB, Uppsala, Sweden). The concentration of [^{14}C]SDZ ICM 567 in each sample was calculated from the ratio of the amount of radioactivity in the eluate fraction corresponding to SDZ ICM 567 relative to the area of the ultraviolet absorbance of the non-radiolabelled SDZ ICM 567 used as an internal standard. Radioactivity measurements in the eluate fractions were carried out by direct scintillation counting. After adding 10 ml of scintillation cocktail (Lumasafe, Lumac, Landgraaf, the Netherlands), the samples were counted in a Tri-carb liquid scintillation analyzer (Canberra Packard, IL).

RESULTS AND DISCUSSION

Blood Binding and Microdialysis Probe Calibration

Because microdialysis only measures the unbound concentration of a given drug whereas the blood sampling tech-

nique will give the total concentration, it is necessary to know the extent of blood binding of SDZ ICM 567 in order to compare the two sampling methods. This study describes the use of the microdialysis technique to determine the blood binding of SDZ ICM 567 in rats in comparison with the equilibrium dialysis method. Unlike ultrafiltration or ultracentrifugation, both methods allow the measurement of binding to plasma proteins and blood cells.

The free fraction of SDZ ICM 567 determined *in vitro* by equilibrium dialysis was 21 ± 3% (n = 14). The percentage bound was independent of the concentration of SDZ ICM 567 over the range studied (20–4000 ng/ml). By using *in vitro* microdialysis the unbound fraction at a concentration of 1000 ng/ml was 19 ± 6% (n = 9); this value was not statistically different of that obtained from the equilibrium dialysis method ($p < 0.05$). These results suggest that microdialysis is a valuable method for *in vitro* estimation of unbound concentration of drugs in blood.

Similar correlations between microdialysis and other separation methods like equilibrium dialysis and ultrafiltration were found by other authors (2,5,6). However, all these *in vitro* studies concern plasma protein binding and not blood binding. Since the drug can be taken up by the blood cells (10), the free fraction in blood should rather be estimated. Moreover, the *in vitro* estimation of the free fraction in blood allowed us an easy comparison with the *in vivo* free fraction obtained during the blood microdialysis study (see pharmacokinetic experiments).

To relate the concentration of SDZ ICM 567 in the dialysate to that in the particular fluid surrounding the dialysis membrane, the efficiency or recovery of the microdialysis probes was determined. The *in vitro* recovery of probes can easily be estimated using a solution of known concentration in which the probe is introduced and the resulting dialysate concentration measured. However, it is well known that *in vitro* recovery of a probe is not a reliable estimator of *in vivo* recovery because of factors in the biological fluids that modify the transport of the compound from the external medium to the probe (11). To overcome the limitation of *in vitro* probe calibration, different approaches have been proposed to estimate the true tissue concentration. In this study the "point of zero net flux" or "difference" method (11,12), to determine *in vivo* relative recovery for the brain and blood microdialysis probes was used. This method is based on determining mass transport of the drug across the microdialysis membrane as a function of perfusate concentrations. When the concentration in the dialysate is lower than in the medium surrounding the membrane, the diffusion of the drug is from the medium to the probe; the opposite occurs when the concentration in the medium is lower than in the perfusate. The point of no net flux is that at which perfusate and medium concentrations are identical. This point can be determined by linear regression of the net transport for different perfused concentrations. In this method recovery is given by the slope of the regression line. After steady state has been reached in both blood and brain compartments by constant infusion of 0.3 mg/h [^{14}C]SDZ ICM 567 for 16–18 h through the femoral vein, the radioactivity in both brain and blood dialysate samples was measured for four different concentrations of [^{14}C]SDZ ICM 567 in the perfusate (10, 25, 50 and 100 ng/ml) and the concentration of SDZ ICM 567 calculated. The *in vivo* recovery using this method was given by the slope of the regression line of the plot of net transport versus perfusate concentrations, which was 0.48 ± 0.44 (r = 0.995± 0.004, n = 4 probes) for brain probes and of 0.46 ± 0.03 (r = 0.998 ± 0.001, n = 4 probes) for blood probes. In some experiments, the rat was infused with non-labelled SDZ ICM 567; the recovery values obtained in this condition was not significantly different from that obtained with the labelled compound (data not shown), similar to that found for lactate (13).

Pharmacokinetic Experiments

The calculated pharmacokinetic parameters for SDZ ICM 567, mean values and standard deviations (n = 4) are summarized in Table I. SDZ ICM 567 intravenously administered to awake freely-moving rats is rapidly distributed showing a short initial distribution phase and a terminal elimination phase with a half-life of 1.7 ± 0.7 h. The time course for blood concentrations is shown in Fig. 2. The AUC_{0-5h} in whole blood was 2768 ± 297 ng · ml^{-1} · h, whereas that corresponding to free concentration (corrected for the *in vitro* blood binding determination) was 586 ± 63 ng · ml^{-1} · h. Free concentrations in blood obtained using blood microdialysis were similar to those obtained from whole blood sampling corrected for blood binding (Fig. 2). In the blood microdialysate the AUC_{0-5h} was 462 ± 142 ng · ml^{-1} · h (after *in vivo* recovery correction) and the elimination half-life 1.3 ± 0.5 h, values statistically did not differ from those calculated from whole blood (t-test, $p > 0.05$). The pharmacokinetic parameters in rats obtained from blood microdialysis agree very well with those obtained from the whole blood measurements. This fact suggests that microdialysis is an appropriate method for the study of pharmacokinetics in blood as has been found by other investigators (2, 9, 14, 15), providing results comparable to classical serial blood removal methods. Because microdialysis measures only the unbound concentration of a given drug, the *in vivo* free fraction can be estimated from free blood concentrations obtained with blood microdialysis and total blood concentrations. Indeed, the *in vivo* free fraction for SDZ ICM 567 calculated from the microdialysis experiments is 16.7%, i.e. in the same range as values obtained *in vitro* by both equilibrium dialysis and microdialysis. These results validate the *in vitro* measurements of the free fraction of SDZ ICM 567 in blood; they also demonstrate the usefulness of blood microdialysis for measuring *in vivo* blood binding of a drug (15,16).

Fig. 3 shows the time dependence of brain concentrations of SDZ ICM 567 after intravenous administration. The

Table I. Pharmacokinetic parameters for SDZ ICM 567 in the blood and brain cortex of awake, freely-moving rats. Values are mean ± S.D. of n = 4 rats

	Whole blood	Blood MD	Brain MD
$t_{1/2}$ (h)	1.7 ± 0.7	1.3 ± 0.5	2.0 ± 0.9
AUC_{0-5h} (ng · ml^{-1} · h)	586 ± 63	462 ± 142	86 ± 24

MD: microdialysis experiments.

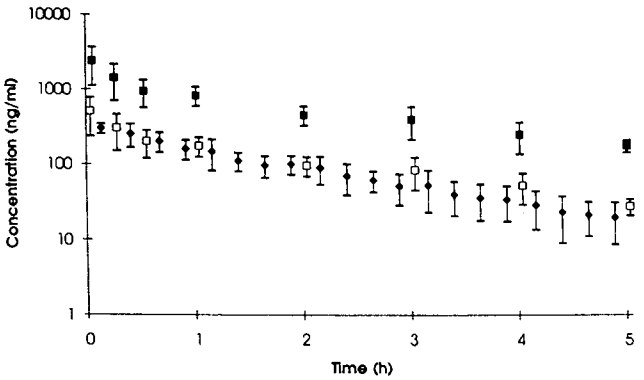

Fig. 2. Concentration-vs-time profile for SDZ ICM 567 in whole blood (■), blood dialysate (corrected for recovery, ♦) and free (calculated) concentration in whole blood (□) of awake freely moving rats (mean ± SD of 4 rats) after an i.v. bolus of 10 mg/kg.

terminal elimination half-life was 2.0 ± 0.9 h, similar to that found in blood (t-test, $p > 0.05$); however, the AUC_{0-5h} after correction for the corresponding *in vivo* recovery value, was 86 ± 24 ng · ml^{-1} · h; thus, the free concentration of SDZ ICM 567 in the brain of rats represented approximately 19% of the free concentration in blood. The results do not agree with a pharmacokinetic compartmental model of distribution corresponding to passive diffusion, since under equilibrium conditions the unbound concentration in brain should be equal to that at the systemic blood level. However, several mechanisms may explain the difference obtained in our study such as a phenomenon of transport out the brain or some uptake and metabolism processes in brain cells. Differences in unbound brain and blood concentrations have been also found in rats for acyclovir and H2G, an acyclic guanosine analog (14) and for caffeine and theophylline (4,17), as well as for morphine in the striatum (5), suggesting that passive transport does not account for the distribution of these agents to and from the central nervous system.

In conclusion, the microdialysis technique used in this study allows a continuous and simultaneous measurement of the free drug concentration in two different body compartments. Such analysis represents an important additional information to classical blood sample measurements and should find a large use in future pharmacokinetic studies.

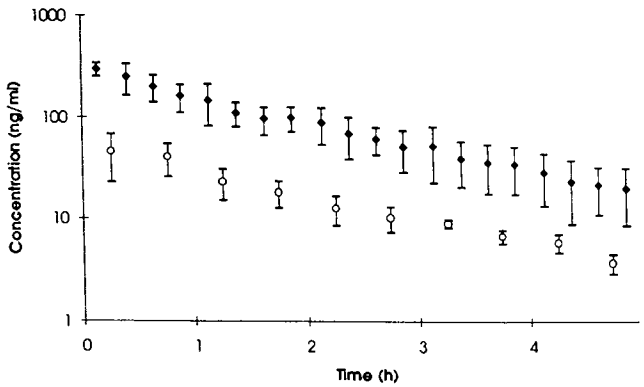

Fig. 3. Free concentration-vs-time profile for SDZ ICM 567 in blood (♦, corrected for recovery) and brain cortex (○, corrected for recovery) of rats (mean ± SD of 4 rats) after an i.v. bolus of 10 mg/kg.

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