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Stereoselective Single-Dose Kinetics of Citalopram and Its Metabolites in Rats

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ABSTRACT The single-dose kinetics of the enantiomers of citalopram (CIT) and its metabolites, demethylcitalopram (DCIT) and didemethylcitalopram (DDCIT), were investigated after administration of 10, 20, or 100 mg/kg (s.c.) *rac*-CIT to rats. Samples from serum and two brain regions were collected 1, 3, 10, or 20 h postdose for HPLC analysis. In the 100 mg/kg rats, the enantiomeric (S/R) serum concentration ratios of CIT decreased during the study period (0.93 at 1 h vs. 0.59 at 20 h; $P < 0.001$). In the 10 and 20 mg/kg rats, the decrease in serum S/R CIT ratios was not so evident as in the 100 mg/kg rats. In all three groups the S/R CIT ratio was almost the same in the brain as in serum, although both CIT enantiomer levels in the brain were found to be 5–10 times higher than the levels in serum. The serum and brain metabolite levels were low in the 10 and 20 mg/kg rats, whereas the levels increased during the study period in the 100 mg/kg rats. In conclusion, the CIT enantiomers were shown for the first time to be stereoselectively metabolized after single-dose administration to rats, as previously shown in steady-state dosing studies in humans and rats. *Chirality 15:622–629, 2003.*
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KEY WORDS: citalopram; enantiomer; citalopram; HPLC; metabolites; pharmacokinetics; rat; toxicokinetics

The selective serotonin reuptake inhibitors (SSRIs) have contributed to the effective pharmacotherapy of different mood disorders.[1] One of the drugs of the SSRI class is citalopram (CIT), used for the treatment of various affective disorders.[2,3] CIT is a racemic mixture of the (+)-(S)-enantiomer and the (–)-(R)-enantiomer. The major CIT metabolites, demethylcitalopram (DCIT) and didemethylcitalopram (DDCIT), are also chiral compounds, but they are less potent than the parent compound with regard to SSRI properties.[4] Pharmacological in vitro and animal in vivo studies suggest that the antidepressant effect of CIT is mediated by (+)-(S)-CIT.[5,6] (+)-(S)-CIT is a potent antagonist of the serotonin transport mechanism and possesses the greatest serotonin transporter selectivity among the SSRIs.[7] (+)-(S)-CIT (escitalopram) has recently been introduced for general prescription in major depression.[8]

In comparison with the tricyclic antidepressants, CIT is regarded as safer and having fewer adverse effects at therapeutic doses. However, in recent years several severe side effects in different organ systems have been reported after intentional, severe overdosing of *rac*-CIT in surviving patients.[9–12] Furthermore, fatal overdoses linked with *rac*-CIT have also been reported.[13–15] To date, however, only sparse data are available describing the pharmacokinetic properties of the separate enantiomers following in vivo systemic exposure of such high/toxic doses of *rac*-CIT and further scrutiny of this issue is therefore warranted. We have previously reported on differences in pharmacokinetic properties of the CIT enantiomers after *rac*-CIT treatment with two different, clinically relevant doses and one high/toxic dose administered chronically to rats.[16] It was concluded that the (–)-(R)-enantiomer was present in an increased proportion compared with the (+)-(S)-enantiomer in both serum and brain, when a higher steady-state CIT concentration prevailed. In that study,[16] the 10–20 mg/kg doses and the 100 mg/kg dose resulted in steady-state drug levels that in humans would be defined as clinically relevant and high/toxic, respectively. As most clinical intoxications due to antidepressant drugs are not reflected by a steady-state condition, the aim of the present study was to follow up previous observations by determining whether differences in the disposition of the enantiomers also occur if single doses of *rac*-CIT are administered from clinically relevant to high/toxic doses to rats. Therefore, the previously investigated doses in steady-state of

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rac-CIT (10, 20 or 100 mg/kg) were administered as a single s.c. injection to rats and serum as well as brain concentrations of the enantiomers of CIT and its main metabolites, DCIT and DDCIT, were analyzed 1–20 h postdose.

MATERIALS AND METHODS

Animals

Sixty-six male Sprague-Dawley rats (M&B A/S, Ry, Denmark), ~250–300 g, were used. All animals had free access to standard laboratory pelleted chow containing 14.5% crude protein (R70; Lactamin AB, Vadstena, Sweden) and tap water ad libitum. All rats were housed in groups of two animals in macrolone cages under climate-controlled conditions for a normal indoor temperature and humidity. The rats were kept in a constant 12:12 h light:dark cycle synchronous with daylight (lights on at 8:00 AM). The study was approved by the Animal Ethics Committee, Linköping, Sweden (Permit Nos. 47-00 and 15-01).

Drugs and Chemicals

Rac-Citalopram HBr (*rac*-CIT; H. Lundbeck A/S, Copenhagen-Valby, Denmark) was dissolved in a mixture of 0.9% NaCl and propylene glycol (40:60; vol/vol) at a concentration such that the rats received 3 ml/kg s.c. All reagents used were of the highest purity commercially available.

Experimental Design

The rats were allowed to recuperate for at least 1 week from transport-induced stress before the start of the experiments. *Rac*-CIT was administered systemically to the upper part of the back of the rat by a single s.c. injection of 10, 20, or 100 mg/kg. To minimize stress and other confounding factors, drug administration was performed using a short halothane (Fluothane®, Zeneca, Macclesfield Cheshire, UK) anesthetic procedure. Thus, three experimental groups of rats denoted as the 10 mg/kg group (n = 18), the 20 mg/kg group (n = 24) and the 100 mg/kg group (n = 24) were investigated separately. For collection of samples for drug analyses the rats were sacrificed by decapitation under halothane anesthesia (for details, see below) 1, 3, 10, or 20 h after drug administration; six rats from each group at each time point (except rats treated with 10 mg/kg, which were not analyzed at the 20-h time point). Thereafter, the concentrations of CIT and major metabolites in blood serum and two brain regions were analyzed by an enantioselective high-performance liquid chromatography (HPLC) assay (for details, see below).

Termination of In Vivo Experimentation

At the time of sacrifice, the rats were decapitated under halothane anesthesia with a guillotine and mixed arteriovenous blood was collected from the neck wound. The blood samples were left for 30 min to allow clotting, followed by centrifugation (2,000*g* for 10 min) for collection of the supernatant serum which was transferred to a new test tube for subsequent drug analyses. Immediately after sacrifice the brains were removed from the skulls and the neocortical hemispheres as well as the mesencephalon-pons region were dissected out as previously described.[17] The two brain regions chosen represent an important terminal region and a cell body region where the serotonin transporter protein is found within the serotonin-innervated system in the CNS. The brain tissue specimens were weighed and homogenized in 2 ml of Milli-Q® water (Millipore AB, Stockholm, Sweden) by means of a sonifier (Model B-30; Branson Sonic Power Company, Danbury, CT) and centrifuged at 2,000*g* for 15 min. The brain and serum supernatants were stored at –70°C until the drug analyses were performed.

Determination of the Enantiomers of Citalopram and Metabolites

The concentrations of the (+)-(S)- and (–)-(R)-enantiomers of CIT, DCIT, and DDCIT in serum and brain homogenate were determined by using HPLC with fluorescence detection according to a previously described procedure[18] with some modifications.[16,19] The extraction of the samples was carried out according to a previously described method.[16,19] A CIT analog, (±)-1-(3-dimethylaminopropyl)-1-(4-chlorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile, was used as an internal standard. Briefly, CIT, DCIT, DDCIT and the internal standard were extracted from calibration standards, control solutions, brain supernatants, and rat serum by solid-phase extraction. After elution and evaporation, the dried samples were redissolved in 100 µl of the mobile phase (55:45; methanol:10 mmol/l citrate:triethylamine buffer, pH 6.3). A volume of 25 µl (the samples from the 100 mg/kg group) or 50 µl (the samples from the 10 and 20 mg/kg groups) was injected onto a Cyclobond I 2000 Ac 250*4.6 mm column (Astec, Whippany, NJ). Detection was with a Waters 474 fluorescence detector (Waters, Milford, MA) at an excitation wavelength of 240 nm and an emission wavelength of 300 nm. The temperature of the column was set to 10°C using a Jones Chromatography Model 7955 column chiller/heater (Hengoed, UK). The detection signals were recorded and processed using the chromatography data system Chromeleon™ (v. 4.12; Dionex, Sunnyvale, CA). The limits of detection for the enantiomers of CIT and its metabolites were 2 nmol/l (S:N; 3:1). Figure 1 shows representative chromatograms of (Fig. 1A) an extracted spiked serum sample and (Fig. 1B) an extracted serum sample from a rat treated with 100 mg/kg of *rac*-CIT. The identification of the (+)-(S)- and (–)-(R)-enantiomers of CIT, DCIT, and DDCIT was confirmed by injecting control solutions spiked with the pure enantiomers in different proportions (e.g., S/R ratio 1:1, S/R ratio 2:1, and S/R ratio 1:4). In a previous study using the same methodology, the analytical recoveries for the six enantiomers from spiked drug-free plasma were between 87% and 110%.[19]

Pharmacokinetic Analysis

The area under the drug concentration–time curve (AUC) based on the mean concentration of each CIT enantiomer was calculated by the linear trapezoidal rule. The area from the last concentration point (C_{last}) to infinity was calculated as C_{last}/β, where β was the terminal elimination rate constant calculated by regression through the three

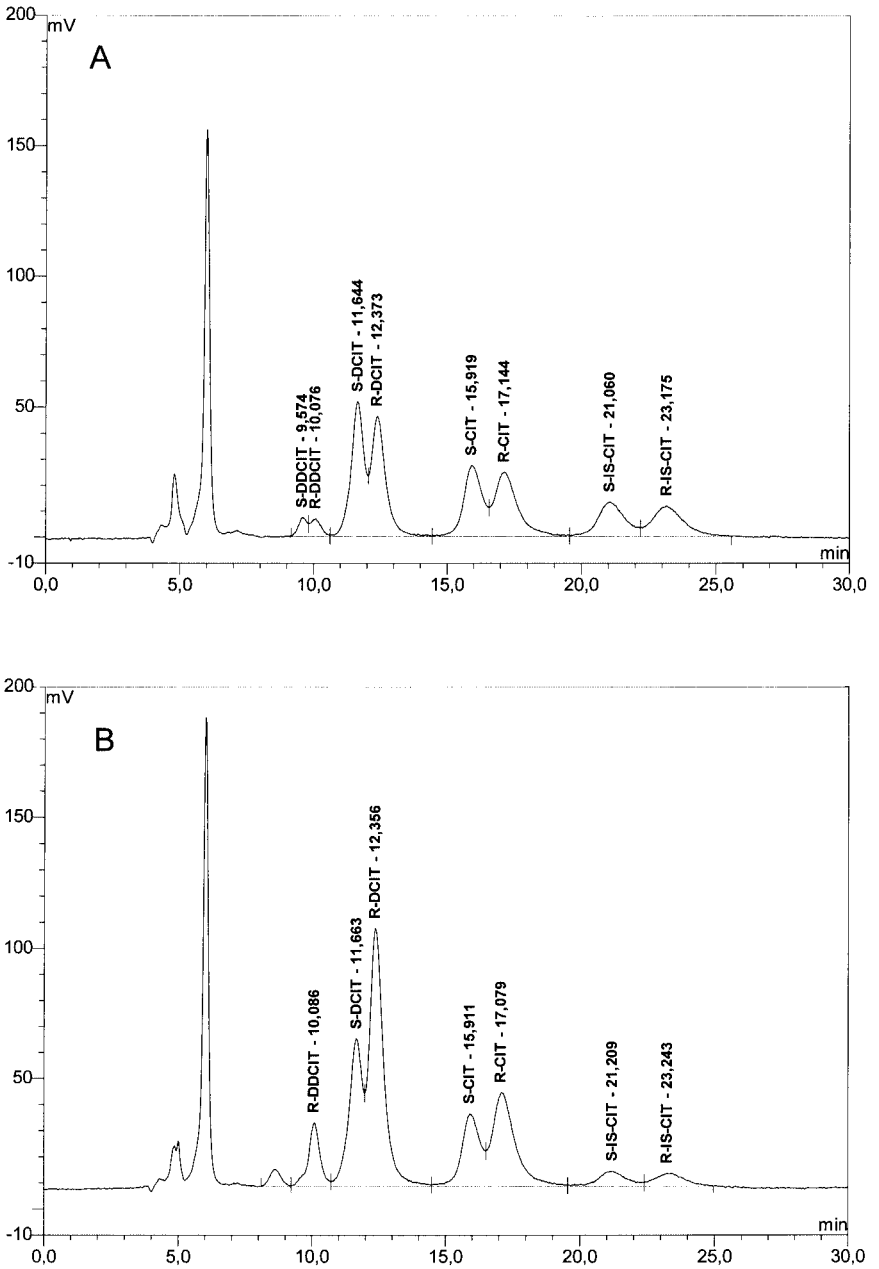

Fig. 1. Chromatograms showing the separation of the (+)-(S)- and (–)-(R)-enantiomers of citalopram (CIT), demethylcitalopram (DCIT), didemethylcitalopram (DDCIT), and the internal standard (IS-CIT). **A:** Extracted spiked serum sample containing 616, 322, and 67 nmol/l of CIT, DCIT, and DDCIT, respectively. **B:** Extracted serum sample from a rat treated with 100 mg/kg obtained 10 h after the drug administration.

data points in the terminal elimination phase (i.e., 3, 10, and 20 h postdose). Thus, the AUC extrapolated to infinity was calculated ($AUC_{0-\infty}$).

Statistical Analysis

Data are expressed as means ± the standard error of the means (SEM). All statistical analyses were performed using StatView® for Windows v. 5.0 (SAS Institute, Cary, NC). The enantiomeric (S/R) concentration ratios of CIT at the different time points after drug administration were analyzed by one-factor analysis of variance (ANOVA). A probability of less than 5% ($P < 0.05$) was considered statistically significant. When significance was reached with ANOVA, Fisher's protected least significant difference (PLSD) post-hoc test was applied.

RESULTS

Concentrations of the Enantiomers of Citalopram

Concentration–time profiles of the (+)-(S)- and (–)-(R)-enantiomers of CIT in serum, cortex, and mesencephalon-pons are displayed in Figure 2. The highest serum levels of (+)-(S)- and (–)-(R)-CIT in the 100 mg/kg rats were found in the interval 2,000–3,000 nmol/l seen 3 h after drug administration. At 20 h postdose, the (+)-(S)- and (–)-(R)-CIT levels ranged between 500–1,000 nmol/l. In contrast to the 100 mg/kg rats, the highest serum levels of (+)-(S)- and (–)-(R)-CIT in the 10 and 20 mg/kg groups were observed at 1 h postdosing. Thereafter, the (+)-(S)- and (–)-(R)-CIT levels declined in an almost identical manner and 10–20 h after drug administration the (+)-(S)- and (–)-(R)-CIT levels ranged between 10–100 nmol/l.

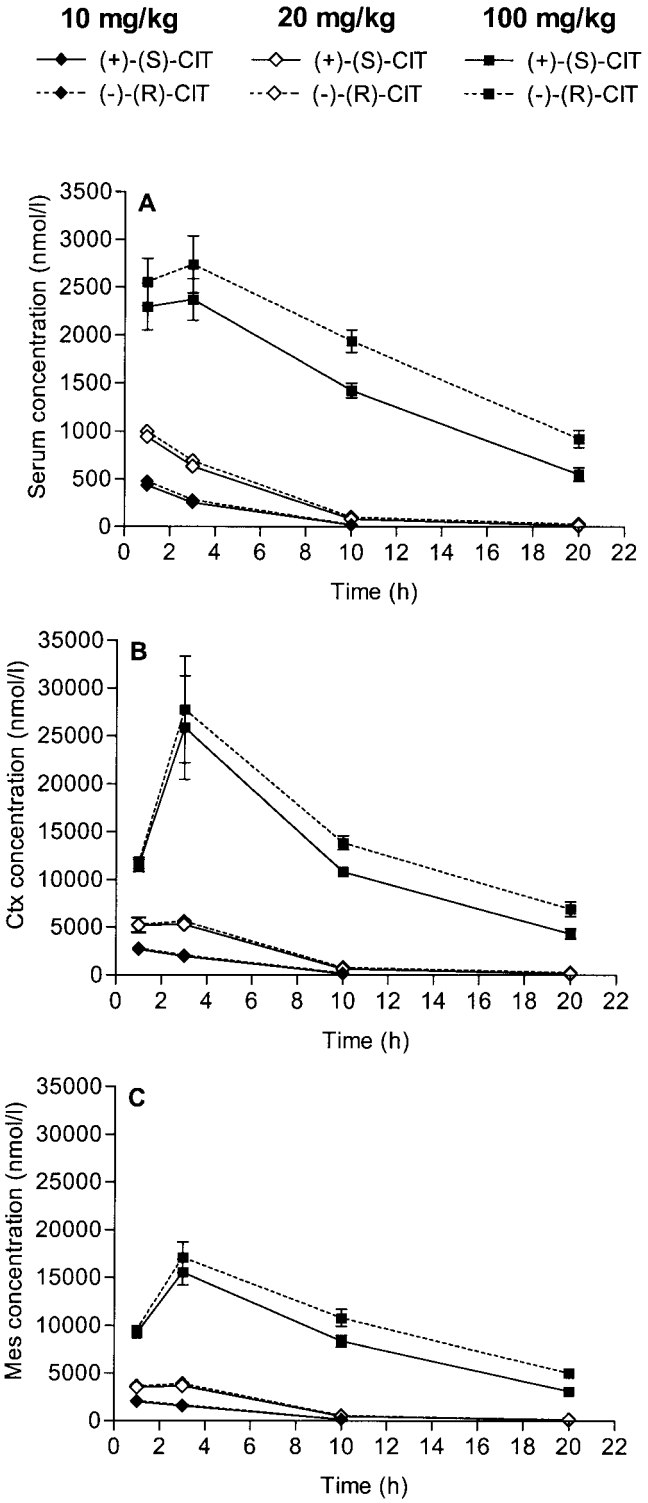

Fig. 2. Concentration–time profiles of the (+)-(S)- and (–)-(R)-enantiomers of citalopram (CIT) in (**A**) serum, (**B**) cortex (Ctx) and (**C**) mesencephalon-pons (Mes) following single administration of *rac*-CIT (10, 20, or 100 mg/kg, s.c.) to rats. All values are means ± SEM.

At each time point the (+)-(S)- and (–)-(R)-CIT concentrations were markedly higher in the two brain regions than in serum following all three doses. Thus, both CIT enantiomer levels in the brain were found to be 5–10 times higher than the levels in serum. Within the brain there was also a regional difference, the concentrations being 25–40% higher in the cortex region than in the mesencephalon-pons at the 3-h time point. Furthermore, it is noteworthy that a 5-fold increase in dose (i.e., 20 mg/kg to 100 mg/kg) also resulted in a 5-fold increase in (+)-(S)- and (–)-(R)-CIT concentrations in cortex at 3 h postdose.

Mean values of $AUC_{0-\infty}$ were calculated for the rats treated with the *rac*-CIT doses of 20 and 100 mg/kg. The 10 mg/kg rats were excluded, as this group was not analyzed at the 20-h time point. In serum, the mean $AUC_{0-\infty}$ values of (+)-(S)- and (–)-(R)-CIT were 5,045 and 5,739 nmol × h/l (20 mg/kg group) and 35,298 and 51,434 nmol × h/l (100 mg/kg group), respectively. In cortex, the mean $AUC_{0-\infty}$ values of (+)-(S)- and (–)-(R)-CIT were 37,951 and 42,105 nmol × h/l (20 mg/kg group) and 287,963 and 380,104 nmol × h/l (100 mg/kg group), respectively. In mesencephalon-pons, the mean $AUC_{0-\infty}$ values of (+)-(S)- and (–)-(R)-CIT were 27,114 and 28,967 nmol × h/l (20 mg/kg group) and 202,592 and 276,932 nmol × h/l (100 mg/kg group), respectively.

Enantiomeric (S/R) Concentration Ratios of Citalopram

The enantiomeric (S/R) concentration ratios of CIT in serum, cortex, and mesencephalon-pons at four different time points after drug administration are shown in Table 1. In the rats treated with 100 mg/kg, no statistically significant differences were observed between the serum and brain S/R ratios observed at 1 h compared with the S/R ratios observed at 3 h. In serum and both brain regions the S/R ratios were lower ($P < 0.001$) at the 10-h time point compared with the S/R ratios seen at 1 and 3 h postdose. Twenty hours after drug administration, the decrease in S/R ratios was even more pronounced ($P < 0.001$ in comparison with the S/R ratios at 1, 3, and 10 h).

In the rats treated with 10 and 20 mg/kg, S/R ratios of similar magnitude were observed in both serum and brain 1 and 3 h postdose. In the 10 mg/kg rats, the S/R ratios in cortex were lower ($P < 0.001$) at the 10-h time point compared with the S/R ratios at the 1-h time point, but it should be noted that a rather large variation in concentration of (+)-(S)- and (–)-(R)-CIT was observed in the rats 10 h postdose (CV for (+)-(S)- and (–)-(R)-CIT was 31% and 16%, respectively). In the 20 mg/kg rats, the S/R ratios in serum and cortex were lower ($P < 0.001$) at the 10-h time point compared with the corresponding S/R ratios at the 1- and 3-h time points. At 20 h postdose the decrease over time in the serum S/R ratios was further evidenced in the 20 mg/kg rats ($P < 0.001$ in comparison with the S/R ratios at 1 and 3 h), but it should be noted that (+)-(S)- and (–)-(R)-CIT were only quantified in two out of six rats at this time point.

S/R ratios of the mean values of $AUC_{0-\infty}$ in serum and brain were also calculated. The serum mean S/R ratios were 0.88 and 0.69 in the 20 and 100 mg/kg rats, respectively. In cortex, the mean S/R ratios were 0.90 and 0.76 in the 20 and 100 mg/kg rats, respectively. In mesencephalon-pons, the mean S/R ratios were 0.94 and 0.73 in the 20 and 100 mg/kg rats, respectively.

TABLE 1. Enantiomeric (S/R) concentration ratios of citalopram (CIT) in serum, cortex (Ctx), and mesencephalon-pons (Mes) after single administration of *rac*-CIT (10, 20, or 100 mg/kg, s.c.) to rats (means ± SEM)[a]

	S/R CIT ratios (10 mg/kg)			S/R CIT ratios (20 mg/kg)			S/R CIT ratios (100 mg/kg)		
Time	Serum	Ctx	Mes	Serum	Ctx	Mes	Serum	Ctx	Mes
1 h	0.91 ± 0.01	0.97 ± 0.01	0.96 ± 0.01	0.94 ± 0.01	0.99 ± 0.01	0.97 ± 0.01	0.93 ± 0.01	0.98 ± 0.01	0.97 ± 0.01
3 h	0.90 ± 0.01	0.93 ± 0.01	0.95 ± 0.01	0.91 ± 0.01	0.95 ± 0.01	0.94 ± 0.01	0.87 ± 0.01	0.93 ± 0.01	0.91 ± 0.01
10 h	0.89 (n = 3)	0.77 ± 0.10	0.96 ± 0.10	0.77 ± 0.02	0.81 ± 0.01	0.91 ± 0.02	0.74 ± 0.01	0.78 ± 0.01	0.78 ± 0.01
20 h	N.I.	N.I.	N.I.	0.60 (n = 2)	0.50 (n = 1)	0.91 (n = 1)	0.59 ± 0.02	0.62 ± 0.03	0.61 ± 0.02

[a]Samples were collected 1, 3, 10, and 20 h after drug administration; six rats from each group at each time point (except the rats treated with 10 mg/kg which were not investigated (N.I.) at the 20-h time point). For statistical comparisons, see Results.

Concentrations of the Enantiomers of Demethylcitalopram and Didemethylcitalopram

Concentration–time profiles of the (+)-(S)- and (–)-(R)-enantiomers of DCIT and DDCIT (except for (+)-(S)-DDCIT, which was not detectable) in serum, cortex, and mesencephalon-pons are displayed in Figure 3. In the 100 mg/kg group, a slow, but substantial accumulation of (+)-(S)-DCIT, (–)-(R)-DCIT, and (–)-(R)-DDCIT was observed in serum. In the 10 mg/kg group, mean (+)-(S)- and (–)-(R)-DCIT serum levels of 10–70 nmol/l were observed 1–10 h postdosing. The (–)-(R)-DDCIT levels were somewhat higher than the (+)-(S)- and (–)-(R)-DCIT levels (mean values, 30–110 nmol/l). In the 20 mg/kg rats the (–)-(R)-DCIT levels were twice as high as the (+)-(S)-DCIT levels 1–10 h after drug administration ((–)-(R)-DCIT, 100–200 nmol/l; (+)-(S)-DCIT, 50–100 nmol/l). The concentrations of (–)-(R)-DDCIT were slightly higher at the 10-h time point and were also detectable 20 h postdosing.

In contrast to the observation of much higher brain than serum concentrations of the parent compound, the concentrations of the metabolites were relatively lower in brain than in serum. Furthermore, the metabolites were not detectable in any of the rats 1 h after drug administration. In the 100 mg/kg rats the time profiles of (+)-(S)-DCIT, (–)-(R)-DCIT, and (–)-(R)-DDCIT were in conformity with the metabolite profiles observed in serum, with the exception that the levels of (–)-(R)-DCIT increased between 10 and 20 h postdose. It should also be noted that (–)-(R)-DDCIT was not detected at the 3-h time point. In the 10 mg/kg rats, only (–)-(R)-DCIT and (–)-(R)-DDCIT were measurable (mean values, 40–120 nmol/l). In the 20 mg/kg group the (–)-(R)-DCIT and (–)-(R)-DDCIT levels were higher than the (+)-(S)-DCIT levels 1–20 h postdosing.

DISCUSSION

The major novel finding of the present study is that an acute administration of *rac*-CIT resulted in the stereoselective disposition of the (+)-(S)- and (–)-(R)-enantiomers of CIT and its main metabolites. This finding was evidenced by the enantiomeric (S/R) concentration ratios of CIT that decreased during the study period, which was most evident in the rats treated with the high/toxic dose of 100 mg/kg. The S/R ratios of $AUC_{0-\infty}$ verified the stereoselective kinetics and indicated differences in the enantiomeric disposition following administration of different doses. Irrespective of the dose given, the levels of both enantiomers of CIT were higher in the brain parenchyma than in serum. Furthermore, higher concentrations of CIT and its metabolites were observed in the neocortical region than in the mesencephalon-pons region, indicating a regional difference in drug disposition in the brain.

Despite the obvious species differences between the rat and man regarding pharmacokinetics and the toxicology of compounds such as CIT, the design of the present investigation is relevant to the clinical situation. Thus, among patients prescribed antidepressant agents, intentional intoxication by means of a high dose intake for suicidal purposes is one of the main hazards. However, it is not possible to investigate this clinical situation in patients in a controlled trial setting, nor is the detailed outlining of the toxicokinetic results easy to survey in this nonsteady-state condition in humans, although accidental reports are available in the literature. Thus, the present data on the enantiomeric pharmacokinetic outcome of CIT and its metabolites DCIT and DDCIT cannot be easily compared with data from other clinical or animal experimental studies conducted to date.

To the best of our knowledge, only one published single-dose study has reported data concerning the S/R CIT ratios.[20] In that study, 20 mg of *rac*-CIT was administered by i.v. infusion for 30 min to eight healthy male volunteers, which resulted in S/R CIT ratios between 0.9 and 1.2 in plasma. However, it should be noted that the sampling time in that study was shortly after the start of the infusion (i.e., 20–160 min), which may explain the equal proportion between the two enantiomers. In the present study, a significant decrease in S/R CIT ratios was observed over time at 10 h and 20 h after administration, which was most evident in the rats treated with 100 mg/kg. At 20 h postdose, the mean serum S/R CIT ratio was 0.59, which, however, was higher than the mean steady-state S/R ratio of 0.34 previously observed when the same CIT dose of 100 mg/kg was given chronically for 10 consecutive days.[16] Although species differences should be taken into account, the serum and brain S/R CIT ratios in the 100 mg/kg group 20 h after the single drug administration in the present investigation are in fair agreement with S/R CIT ratios reported for patients in steady-state treated with *rac*-CIT in a therapeutic dose range: 10–80 mg/day.[18,19,21–24] However, since the sampling time is a crucial factor in single-dose studies, we also calculated the S/R ratios of $AUC_{0-\infty}$ to verify the enantiomeric (S/R) concentration ratios of CIT.

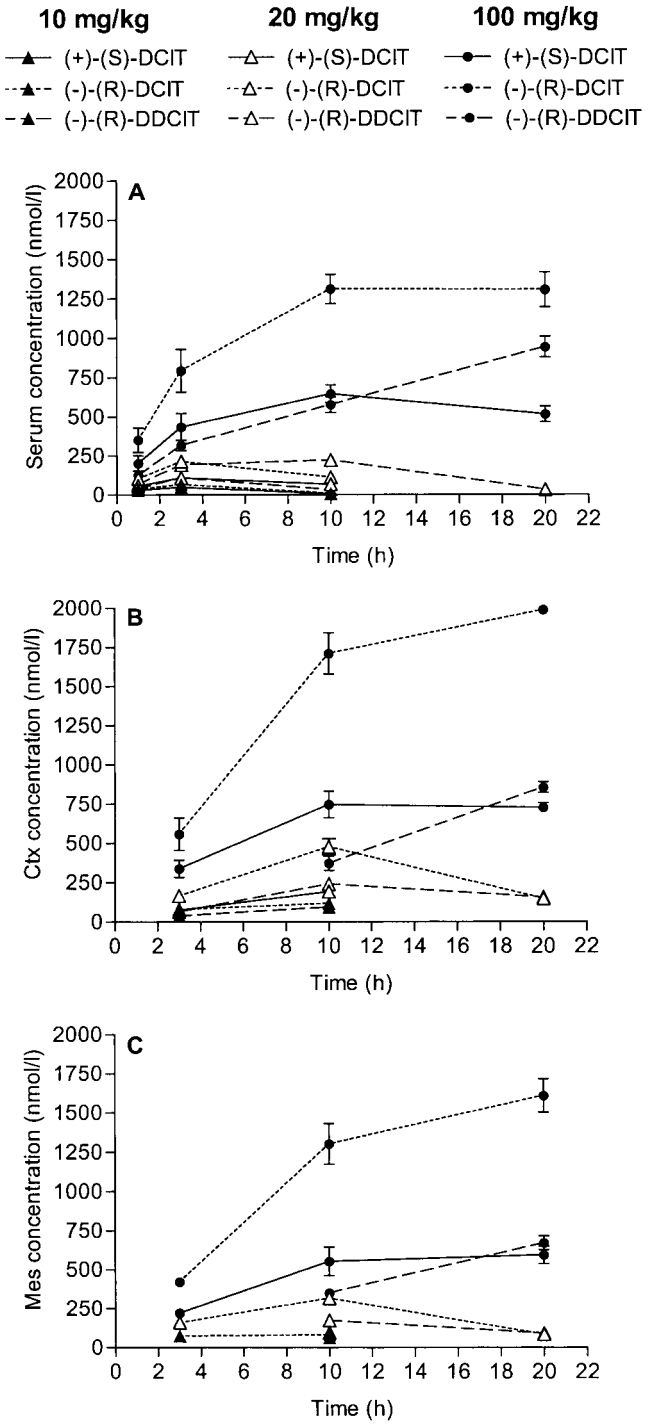

Fig. 3. Concentration–time profiles of the (+)-(S)- and (–)-(R)-enantiomers of demethylcitalopram (DCIT) and didemethylcitalopram (DDCIT) in (**A**) serum, (**B**) cortex (Ctx) and (**C**) mesencephalon-pons (Mes) following single administration of *rac*-CIT (10, 20, or 100 mg/kg, s.c.) to rats. The (+)-(S)-enantiomer of DDCIT was not detectable in any of the groups. All values are means ± SEM.

There may be several explanations for the observed differences in the enantiomeric pharmacokinetics of CIT. Plasma protein binding and membrane permeability may play a role. However, stereoselective plasma protein binding seems unlikely in the present study, as the initial levels of the separate enantiomers were about the same. Therefore, the observed stereoselective pharmacokinetic outcome is most likely established in the elimination phase through a higher clearance of (+)-(S)-CIT than of (–)-(R)-CIT.[23,25] In this context, it is noteworthy that in vitro studies have shown that (+)-(S)-CIT is more rapidly demethylated than (–)-(R)-CIT in human liver microsomes.[26,27] Also in the rat, it seems that (+)-(S)-CIT has greater affinity than (–)-(R)-CIT for the enzymes involved in the metabolism of CIT. However, if these enzymes are the same as in humans (i.e., the cytochrome P-450 (CYP) enzymes CYP3A4, CYP2C19, and CYP2D6[26,27]) is not exactly known today. In line with the expected stereoselective metabolism of (+)-(S)- and (–)-(R)-CIT, it is anticipated that the (+)-(S)-enantiomers of the metabolites also would undergo more rapid elimination than their optical antipodes. This fact was found to be clearly evident in the rats treated with 100 mg/kg in the present investigation, where the S/R DCIT ratios at all time points were found to be significantly lower than the corresponding S/R CIT ratios. The (+)-(S)-enantiomer of DDCIT was not detectable in any of the rats, which was also the case in several other studies previously conducted by us in rats given chronic doses of *rac*-CIT.[16,28,29] In comparison with the levels of the parent compound, the metabolite levels were generally lower in the brain as in serum, which may signify that the metabolites cross the blood–brain barrier less readily than CIT does. This phenomenon has also been confirmed in clinically relevant doses in other reports.[16,28,30,31] It should also be noted that CIT and its metabolites are stereoselectively metabolized in the brain, but this process occurs mainly through monoamine oxidases and not, as in liver, through cytochrome P-450.[32]

Since the half-life of *rac*-CIT in the rat is 3–5 h in the dose range 8–20 mg/kg,[33,34] low levels of CIT were detected 10 h after administration of the 10 and 20 mg/kg doses. On taking into account first-pass metabolism after oral administration (around 50%), our data on the CIT concentrations in rats treated with 10 and 20 mg/kg were in general agreement with other rat studies in which *rac*-CIT has been administered orally in single doses of both 8 mg/kg[33] and 20 mg/kg.[34] Furthermore, Cremers et al.[35] reported serum CIT concentrations of 300 nmol/l 1 h after *rac*-CIT given s.c. to rats in a dose of 10 µmol/kg (≈3 mg/kg), which corresponds quite well with the serum CIT levels found in the 10 mg/kg rats in the present study (CIT levels around 1,000 nmol/l).

In agreement with previous single-dose studies,[34,36] the present investigation clearly displayed a distribution of CIT with a marked gradient between blood and brain even after single *rac*-CIT administration in clinically relevant as well as high/toxic doses. The existence of such a gradient is also in accordance with earlier observations on rats undergoing long-term treatment with different *rac*-CIT doses.[16,28,29,34,37] As CIT has a high volume of distribution in both man[38,39] and animals,[33] this finding was not unexpected. Despite these differences in absolute concentrations of CIT in serum and brain, no major alterations in S/R CIT ratios were found between the two matrices. This finding is in agreement with data from chronic dosing of *rac*-

CIT to rats[16] and is further supported by Rochat et al.,[40] who showed that CIT, within a regular dose and concentration span, crosses the blood–brain barrier via a nonstereoselective carrier-mediated mechanism. In conclusion, the CIT enantiomers were shown for the first time to be stereoselectively metabolized after single-dose administration to rats, as previously shown in steady-state dosing studies in humans and rats.

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