Postmortem Redistribution of the Enantiomers of Citalopram and Its Metabolites: An Experimental Study in Rats

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Abstract

A rat model was used to study if postmortem redistribution of the S- and R-enantiomers of citalopram (CIT) and its metabolites demethylcitalopram (DCIT) and didemethylcitalopram (DDCIT) occurs after three different subcutaneous dosing procedures with racemic CIT. Two groups underwent chronic administration (20 mg/kg daily) using osmotic pumps. After 10 days, 1 of these groups received an acute-on-chronic drug challenge with a single injection of 100 mg/kg. The third group received the single 100 mg/kg dose only. Heart blood and brain samples were collected antemortem and 1, 3, or 24 h postmortem for enantioselective HPLC analysis. Increased postmortem blood drug and metabolite concentrations compared with corresponding antemortem concentrations were observed in all groups (p < 0.05 to p < 0.001). At 24 h after death, the ratios between postmortem and antemortem blood concentrations were around 3-4 for CIT as well as for the metabolites. In the brain, no major differences between antemortem and postmortem drug and metabolite concentrations were observed. The enantiomeric ([S]/[R]) concentrations ratios of CIT and metabolites in blood and brain were of similar magnitude before and after death. No differences between antemortem and postmortem parent drug-to-metabolite ([P]/[M]) ratios for CIT/DCIT in blood were observed. Finally, this animal model demonstrates that the S- and R-enantiomers of CIT and its metabolites were redistributed to the same extent postmortem.

Introduction

Citalopram (CIT) is a racemic bicyclic phthalane derivative with the chemical structure (±)-1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile. CIT belongs to the pharmacodynamic class of selective serotonin reuptake inhibitors (SSRIs) and is used for the treatment of various affective psychiatric disorders (1). The S-(+)-enantiomer of CIT (S-CIT) possesses much greater inhibition of serotonin reuptake than the R-(-)-enantiomer of CIT (R-CIT) in vitro (2,3). Further, animal and human in vivo studies have also shown that S-CIT mediates antidepressant-like effects (4,5), and S-CIT has recently been introduced as a separate antidepressant entity (escitalopram) for clinical prescription. The quantitatively most important metabolic pathway of CIT appears to be the N-demethylation step, generating demethylcitalopram (DCIT) and didemethylcitalopram (DDCIT) (6). According to in vitro data, S-DCIT does also possess some SSRI effect (2), although, in general, the metabolites are not considered to play any major role for the overall SSRI effect under normal conditions.

The SSRIs are considered to be less acutely toxic than tricyclic antidepressants in overdose (7). However, several reports of serious clinical intoxications with CIT (8-16) and other SSRIs (17-19) are available. In the majority of scientific communications so far, only the total concentrations of both enantiomers (i.e., [S]+[R]) of CIT, and only sometimes the metabolites, are reported. Accordingly, limited data are available describing the concentrations of the separate enantiomers of CIT and metabolites, particularly in situations in which toxic concentrations prevail. However, we have previously reported on differences in pharmacokinetic properties of the enantiomers of CIT and its metabolites after chronic as well as single administrations of racemic CIT in clinically relevant and high/toxic doses to rats (20,21). To the best of our knowledge, only one study has reported data on the levels of the enantiomers of CIT and its metabolites in human postmortem cases (22). For many years there has been a general awareness that the concentration of a drug in an autopsy blood sample may not necessarily reflect the in vivo concentration just before death. This difference is explained by postmortem drug redistribution, a phenomenon that the forensic pathologists and toxicologists have to consider when interpreting drug concentrations (23). It has previously been shown in controlled animal studies that

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postmortem redistribution may occur after administration of several types of drugs (24). Thus, in order to accurately inter-
pret the postmortem concentrations of CIT a general under-
standing of the changes in the enantiomer concentrations after
death are required. Hence, the aim of the present study was to
investigate if postmortem redistribution of the enantiomers of
CIT and its metabolites DCIT and DDCIT occurs in an experi-
mental rat model after administration of racemic CIT. The ex-
perimental design was chosen to investigate possible changes
in enantiomeric drug disposition and redistribution in blood
and brain after three different types of subcutaneous (s.c.)
closing procedures: acute, chronic, and acute-on-chronic
administration.

Experimental

Animals

Male Sprague-Dawley rats (Taconic M&B A/S, Ry, Denmark)
weighing 250–383 g were used. All animals had free access to
standard laboratory pelleted chow containing 14.5% crude pro-
tein (R70, Lactamin AB, Vadssten, Sweden) and tap water ad li-
bitum. All rats were housed in groups of two animals in
macrolone cages under climate-controlled conditions for reg-
ular 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 a.m.). All experiments were performed in
strict accordance with the guidelines and with the consent of
the Animal Ethics Committee, Linköping, Sweden (permit no.
10-02).

Drugs and chemicals

Citalopram HBr (H. Lundbeck A/S, Copenhagen-Valby, Den-
mark) was dissolved in a mixture of 0.9% NaC1 and propylene
glycol (40:60; v/v) and administered s.c. All reagents used were
of the highest purity commercially available.

Experimental design

The experimental design of the study is illustrated in Figure
1. Three treatment groups with 32 rats in each group were
randomly created. In groups 1 and 2, a bodyweight-adjusted
chronic drug treatment regimen (20 mg/kg daily) was imple-
mented for 10 days using osmotic pumps. On day 10, the rats
of group 2 received an acute-on-chronic drug challenge by a
single injection of 100 mg/kg. The rats of group 3 received the
single 100 mg/kg dose only. To minimize stress in the rats and
other confounding factors, drug administration was performed
under a brief halothane (Fluothane®, Zeneca Ltd., Maccles-
field Cheshire, U.K.) anesthesia. Each of the three groups was
divided into four subgroups. Three of the subgroups consisted
of rats with different postmortem intervals (1, 3, or 24 h),
whereas one subgroup served as antemortem controls. Heart
blood and brain parenchyma samples were collected for subse-
quent drug analyses with an enantioselective high-performance
liquid chromatography (HPLC) assay.

Osmotic pump preparation and implantation

Osmotic pumps (ALZET® model 2ML2, B&K Universal AB,
Sollentuna, Sweden) were filled with 2 mL of a drug solution
containing around 50 mg/mL of CIT such that the rats re-
ceived the dose 20 mg/kg per day. The infusion rate for the so-
lution was 5 μL/h. The pumps were primed by warming them
in a beaker containing 0.9% NaCl in a water-bath (37°C)
overnight. By this procedure, the pumping rate reached steady-
state before the implantation. During halothane anesthesia the
rats were shaved and a minor skin incision was made between
the scapulae. A subcutaneous pocket was formed by blunt dis-
ssection of the connective tissues, whereupon the pumps were
inserted. The skin incision was closed with sutures (Ethilon®II
3/0, Ethicon, Johnson & Johnson AB, Sollentuna, Sweden)
and the total implantation time for each pump was approxi-
mately 10 min. After the chronic drug exposure (i.e., immedi-
ately after sacrifice), the pumps were removed and the incisions
of the postmortem rats were closed using a skin stapler (Prox-
imate® 35W, Ethicon, Johnson & Johnson AB). Thereafter,
the residual amounts of the pumps were assessed by aspirating
with a graduated syringe for checking the delivery profile of the
pumps.

Postmortem and in vivo experiments

After the three different drug administration procedures, the
postmortem rats were sacrificed with CO2 and were left lying on
their backs at room temperature for 1, 3, or 24 h. After clamping inferior vena cava above the diaphragm, postmortem
heart blood samples (0.3–0.5 mL) were drawn from the right
atrium by a polypropylene catheter connected to a syringe.
The control rats were sacrificed under halothane anesthesia by
collection of heart blood (0.5 mL) followed by decapitation.
After collection of blood samples, the brain was removed from
the skull and the neocortical hemisphere and the mesen-
cephalon-pons region were dissected out as previously de-
scribed (25). The choice for these two brain regions was based
on the mesencephalon-pons region being the site for the sero-
tonergic cell bodies, whereas the neocortical region represents
one important projection area in which the serotonergic
synapses are present. The brain tissue samples were weighed
and homogenized in 2 mL Milli-Q® water (Millipore AB, Stock-
Determining of the enantiomers of citalopram and its demethylated metabolites

The concentrations of the S- and R-enantiomers of CIT, DCIT, and DDCIT were determined by using HPLC with fluorescence detection (20,26–28). The extraction of the whole blood samples was carried out according to a previously described method (29) with some modifications (22). A CIT analogue, (z)-1-(3-dimethylaminopropyl)-1-(4-chlorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (Lu 10-202-O; H. Lundbeck A/S), was used as an internal standard. Briefly, 20 μL of the internal standard (2.5 μg/mL) was added to 0.1–0.5 g of blood and diluted with 3 mL acetonitrile/0.025 M phosphate buffer pH 11.5 (5:95, v/v) in a glass tube. After vortex mixing and sonification for 5 min, the solution was centrifuged for 10 min at 3000 rpm. After conditioning of the solid-phase extraction columns, the centrifuged samples was poured on to the columns and then, the residue was washed with 3 mL acetonitrile/0.025 M phosphate buffer pH 11.5 (5:95, v/v) in a glass tube. After vortex mixing and sonification for 5 min, the solution was centrifuged for 10 min at 3000 rpm. After conditioning of the solid-phase extraction columns, the centrifuged samples was poured on to the columns and then, the residue was washed with 3 mL acetonitrile/0.025 M phosphate buffer pH 11.5 (5:95, v/v) in a glass tube. After vortex mixing and sonification for 5 min, the solution was centrifuged for 10 min at 3000 rpm.

The extraction of the brain samples was carried out according to a previously described method (29) with some modifications (20,28). After elution and evaporation, the dried blood and brain samples were redissolved in 100 μL of methanol/100 mM citric acid triethylamine pH 6.3 (55:45; v/v). A volume of 10–50 μL was injected onto a Cyclobond 1 2000 Ac 250-μm × 4.6-mm column (Astec, Whippany, NJ) with a Gynkotek Gina 50 autosampler (Dionex, Sunnyvale, CA). The mobile phase was delivered through a Gynkotek 480 pump (Dionex) at a flowrate of 0.8 mL/min. CIT, DCIT, and DDCIT were detected by 240-nm excitation and 300-nm emission wavelengths, respectively, using a Waters 474 fluorescence detector (Waters Corporation, Milford, MA). The detection signals were recorded and processed using the chromatography data system Chromeleon™ (Version 4.12, Dionex). The limit of detection for the enantiomers of CIT and its metabolites was 0.002 μg/g (S/N = 3:1).

Data analysis

Data are expressed as means ± standard deviation (SD). A probability of less than 5% (p < 0.05) was considered statistical significant. Possible differences between the groups were analyzed by one-factor analysis of variance (ANOVA). When the ANOVA reached statistical significance, Fisher's protected least significant difference (PLSD) post-hoc test was applied. All statistical analyses were performed using StatView® for Windows Version 5.0 (SAS® Institute Inc., Cary, NC).

Results

Body weight and osmotic pump performance

At the time when the osmotic pumps were implanted, the rats weighed 258 ± 26 g. After five days of treatment, the rats weighed 281 ± 25 g. After 10 days of treatment (i.e., the experimental day), the rats weighed 315 ± 23 g. The observed increase in body weight during the study period is expected and in agreement with previous studies (30,31). The 32 rats that were only treated with the single dose weighed 307 ± 22 g on the experimental day.

The amount of residual drug solution in the 64 osmotic pumps was in the range of 0.6–0.8 mL, which is in agreement with previous findings (31), and indicates that the osmotic pump delivery system worked satisfactorily.

Table I. Concentrations (S, R, and S+R) and Enantiomeric Ratios (S/R) of Citalopram (CIT), Demethylcitalopram (DCIT), and Didemethylcitalopram (DDCIT) in Heart Blood (μg/g) at Different Postmortem Intervals (PMI)*

<table>
<thead>
<tr>
<th>Group</th>
<th>PMI 0 h</th>
<th>S (μg/g)</th>
<th>R (μg/g)</th>
<th>S+R (μg/g)</th>
<th>S/R</th>
<th>PMI 1 h</th>
<th>S (μg/g)</th>
<th>R (μg/g)</th>
<th>S+R (μg/g)</th>
<th>S/R</th>
<th>PMI 3 h</th>
<th>S (μg/g)</th>
<th>R (μg/g)</th>
<th>S+R (μg/g)</th>
<th>S/R</th>
<th>PMI 24 h</th>
<th>S (μg/g)</th>
<th>R (μg/g)</th>
<th>S+R (μg/g)</th>
<th>S/R</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIT</td>
<td>0.041 ± 0.01</td>
<td>0.044 ± 0.01</td>
<td>0.085 ± 0.02</td>
<td>0.93 ± 0.05</td>
<td>0.17 ± 0.01</td>
<td>0.024 ± 0.01</td>
<td>0.041 ± 0.02</td>
<td>0.73 ± 0.10</td>
<td>0.11 ± 0.05</td>
<td>0.05</td>
<td>0.15 ± 0.06</td>
<td>0.17 ± 0.06</td>
<td>0.32 ± 0.12</td>
<td>0.93 ± 0.07</td>
<td>0.063 ± 0.03</td>
<td>0.11 ± 0.08</td>
<td>0.17 ± 0.11</td>
<td>0.061 ± 0.17</td>
<td>0.35 ± 0.17</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>DCIT</td>
<td>0.226 ± 0.58</td>
<td>0.274 ± 0.76</td>
<td>0.50 ± 1.34</td>
<td>0.83 ± 0.04</td>
<td>0.44 ± 0.15</td>
<td>0.62 ± 0.18</td>
<td>1.06 ± 0.34</td>
<td>0.70 ± 0.06</td>
<td>0.32 ± 0.08</td>
<td>0.042 ± 0.04</td>
<td>0.17 ± 0.06</td>
<td>0.31 ± 0.11</td>
<td>0.90 ± 0.10</td>
<td>0.074 ± 0.04</td>
<td>0.14 ± 0.09</td>
<td>0.21 ± 0.12</td>
<td>0.60 ± 0.19</td>
<td>0.42 ± 0.17</td>
<td>0.15 ± 0.04</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>DDCIT</td>
<td>0.226 ± 0.58</td>
<td>0.274 ± 0.76</td>
<td>0.50 ± 1.34</td>
<td>0.83 ± 0.04</td>
<td>0.44 ± 0.15</td>
<td>0.62 ± 0.18</td>
<td>1.06 ± 0.34</td>
<td>0.70 ± 0.06</td>
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<td>0.15 ± 0.04</td>
<td>0.15 ± 0.04</td>
</tr>
</tbody>
</table>

* Racemic CIT was administered to rats as follows: Group 1, chronic (20 mg/kg daily; 10 days); Group 2, acute-on-chronic (20 mg/kg daily; 10 days + 100 mg/kg at day 10); Group 3, acute (100 mg/kg). The S-enantiomer of DDCIT was not detectable in any of the groups. All values are mean ± SD.
Concentrations of citalopram and metabolites

The concentrations of the S- and R-enantiomers of CIT are displayed in Table I and Figure 2. Increased postmortem blood drug concentrations compared with antemortem blood drug concentrations were observed in all three groups (p-values ranging from \( p < 0.05 \) to \( p < 0.001 \)). In group 1, the ratios between postmortem and antemortem CIT concentrations were 3.5-3.8 at the three time points after death. In group 2, the corresponding ratios were 1.8, 2.5, and 3.6 at 1, 3, and 24 h postmortem, respectively. The rats of group 3 displayed ratios of postmortem and antemortem CIT concentrations of 2.9, 3.3, and 3.9 at 1, 3, and 24 h after death, respectively. Despite these differences in blood drug concentrations, the enantiomeric (S/R) concentrations ratios of CIT were of similar magnitude at the four investigated time points (group 1, 0.90-0.99; group 2, 0.83-0.85; group 3, 0.90-0.93). In the brain regions cortex and mesencephalon-pons, no major differences between the antemortem and postmortem drug concentrations were observed in any of the three groups investigated. In agreement with the S/R CIT ratios obtained in blood at the four time points, similar S/R CIT ratios were observed in cortex (group 1, 0.88-0.92; group 2, 0.88-0.89; group 3, 0.94-0.95) and mesencephalon-pons (group 1, 0.90-0.96; group 2, 0.87; group 3, 0.93-0.94).

The concentrations of the S- and R-enantiomers of the metabolites DCIT and DDCIT (except for S-DDCIT, which was

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**Figure 2.** Concentrations of the S- plus R-enantiomers of citalopram (CIT) in heart blood and two brain regions: cortex (Ctx) and mesencephalon-pons (Mes). The S-enantiomer of DDCIT was not detectable in any of the groups. Racemic CIT was administered to rats as follows: chronic (20 mg/kg daily; 10 days) (A), acute-on-chronic (20 mg/kg daily; 10 days + 100 mg/kg at day 10) (B), and acute (100 mg/kg) (C). Values are mean ± SD. * = significantly different from antemortem controls (0 h), \( * p < 0.05 \), and \( *** p < 0.001 \).

**Figure 3.** Concentrations of the S- plus R-enantiomers of demethylcitalopram (DCIT) and didemethylcitalopram (DDCIT) in heart blood and two brain regions: cortex (Ctx) and mesencephalon-pons (Mes). The S-enantiomer of DDCIT was not detectable in any of the groups. Racemic CIT was administered to rats as follows: chronic (20 mg/kg daily; 10 days) (A), acute-on-chronic (20 mg/kg daily; 10 days + 100 mg/kg at day 10) (B), and acute (100 mg/kg) (C). Values are mean ± SD. * = significantly different from antemortem controls (0 h), \( * p < 0.05 \), \( ** p < 0.01 \), and \( *** p < 0.001 \).
not detectable) are displayed in Table I and Figure 3. In agreement with the data obtained for CIT, the postmortem blood metabolite concentrations were higher than the antemortem blood metabolite concentrations (p-values ranging from p < 0.05 to p < 0.001). Accordingly, the ratios between postmortem and antemortem blood concentrations of DCIT and DDCIT were found in a similar range (i.e., 2–4) that was observed for CIT. The S/R ratios of DCIT in blood were found in the interval of 0.6–0.7 in all three groups at all three time points. The concentrations of DCIT and DDCIT were lower in the brain than in blood. No statistical differences were observed in brain metabolite levels between the different time points postmortem and antemortem. The brain S/R DCIT ratios were found in the interval of 0.5–0.7 (data not shown), and thus in accordance with the blood S/R DCIT ratios.

At the four time points investigated, the parent drug/metabolite (P/M) ratios for CIT/DCIT in blood were around 2, 5, and 3–4 in group 1, 2, and 3, respectively. In brain, the P/M ratios were around 7–8, 50–80, and 40–50 in group 1, 2, and 3, respectively.

Discussion

The major novel findings from the present study are 1. the postmortem increase of the enantiomers of CIT and its metabolites DCIT and DDCIT in heart blood; 2. the equal redistribution of the S- and R-enantiomers, as reflected by similar enantiomeric (S/R) concentration ratios of CIT and metabolites before and after death; and 3. the similar degree of postmortem redistribution of CIT and its metabolites.

The present study was designed to cover three different types of dosing procedures. The rats of group 1 received a dose that resulted in antemortem steady-state CIT levels that in humans would resemble levels defined as clinically relevant (32). The experimental design of group 2 was chosen to mimic a clinical situation when a chronic dosage is preceding an acute (and commonly intentional) intoxication (referred to acute-on-chronic drug challenge). The rats of group 3 received only a single high/toxic dose to mimic a human acute overdose situation as well as to serve as reference to the acute-on-chronic group data. Based on our previous results (21), the rats of group 2 and 3 were killed 3 h after the single drug injection to cover the time point at which the maximal blood drug concentration appears. The antemortem blood drug concentrations in these rats correlate to toxic concentrations found in humans (10,13), but were obviously non-lethal in rats because all rats survived until termination of the experiments. The postmortem rats were sacrificed with CO₂ asphyxiation, which is suggested to be a suitable model of death by an unrelated cause. Previous studies have shown that CO₂ neither induces histopathological changes (33) nor affects the pH changes that occur postmortem (34). According to pilot experiments, the chosen time points for postmortal examination were 1, 3, and 24 h, because changes in CIT concentrations were observed shortly after death. In human postmortem cases, drug levels from peripheral blood specimens are suggested to better reflect antemortem drug levels than drug levels obtained from central blood (23,35). Therefore, femoral vein blood has been suggested as a standard specimen for postmortem drug analysis (10). However, in the present rat study, pilot experiments revealed that the withdrawn volume of a peripheral blood sample was too small for the enantioselective CIT analysis, and heart blood was used instead.

During recent years, several publications have become available that report citalopram concentrations in human postmortem cases (9,10,13,22,36–38). However, to the best of our knowledge, this is the first controlled experimental study that has investigated the postmortem redistribution of CIT and its metabolites. In all three groups, the concentrations of the enantiomers of CIT, DCIT, and DDCIT increased postmortem compared with the levels observed antemortem. As CIT has a high volume of distribution in both humans (14 L/kg) (32) and rats (21 L/kg) (39), a passive diffusion process from drug depots in solid organs is the most likely explanation of the observed postmortem drug redistribution. Most of the increase in drug levels occurred shortly after death (especially in groups 1 and 3). A similar finding has also been observed in studies on amitriptyline, in which the drug concentration increase in heart blood occurred mainly within the first 2 h postmortem (40,41). When calculating the ratios between postmortem and antemortem blood drug concentrations at 24 h after death, these ratios were between 3 and 4 for CIT and metabolites in all groups investigated. For another type of drug, digoxin, Koren and MacLeod (42) have reported that chronic administration at therapeutic levels favored postmortem redistribution in comparison to acute toxic doses. A resembling phenomenon was not the case in the present study, in which the degree of postmortem redistribution of CIT and metabolites was about the same in all groups. However, it is possible that further drug redistribution occurs after 24 h postmortem in the rats of groups 2 and 3, but according to data by others this possibility seems unlikely at present. For example, in a study by Koren and Klein (43), the heart blood levels of morphine did not change significantly at 96 h postmortem as compared to the levels observed at 24 h postmortem, which also is in agreement with heart blood data on amitriptyline obtained postmortem by Hilberg and coworkers (40,41).

The observation in the present study of similar degree of postmortem redistribution of CIT and its metabolites, irrespective of dose given, is of great importance for the medicolegal casework. The unchanged P/M ratios for CIT and metabolites postmortem indicates that postmortem redistribution does not interfere with the use of P/M ratios in the interpretation of autopsy samples. High P/M ratios may indicate an acute overdose, and accordingly, the P/M ratios were highest in the rats of group 2 and 3 that were treated with an acute high/toxic dose. Interestingly, no major differences in P/M ratios were observed between the rats treated with the acute-on-chronic dosage (P/M ratio ~5) and the rats that only received the acute dose (P/M ratio 3–4). This finding indicates that P/M ratios still can be used to detect an acute intoxication, even in cases in which a high acute intake has been preceded by a chronic dosage.

We have previously reported that R-CIT was present in an increased proportion compared with S-CIT in both serum and brain (S/R CIT ratios ranging from 0.3 to 0.9 depending on dose...
given) when a higher steady-state CIT concentration prevailed (20). Clinical studies have shown that the mean S/R CIT ratios in serum during steady-state are 0.5–0.7 in a therapeutic dose range (26,28,44–47). The comparatively higher levels of R-CIT could be due to a more rapid demethylation of S-CIT by the cytochrome P450 (CYP) enzymes (48,49). It seems that this change in the disposition of the S- and R-enantiomers is established in the elimination phase, because we have previously reported that the S/R CIT ratios decreased during a study period of 20 h following single-dose administration of 100 mg/kg to rats (0.93 at 1 h vs. 0.59 at 20 h) (21). Therefore, the S/R CIT ratios may also give an indication of the time interval between drug intake and death. This statement has actually been verified in a recent study on humans in which Holmgren et al. (22) reported that, besides the use of P/M ratios, enantioselective analysis of CIT and its metabolites could give additional information about the time that has elapsed between drug intake and death. The design of the present study did not allow us to further analyze this issue, but our previously obtained results (21) are most certainly also representative for the postmortem situation because the S/R ratios in the present study did not change after death.

In addition to heart blood, the brain regions cortex and mesencephalon-pons were also analyzed. Around 25% higher drug levels were observed in cortex (a serotonergic synaptic region) than in mesencephalon-pons (a serotonergic cell body region), which is in agreement with previous findings from in vivo studies (20,21). In the present study, the brain concentrations of CIT, DCIT, and DDCIT were about the same before and after death in all three investigated groups. The enantiomers were redistributed to the same extent, indicated by similar S/R ratios of the parent drug and metabolites at the four time points when samples were withdrawn. The literature is scarce concerning reports that describe brain CIT concentrations in humans postmortem. However, Fu et al. (50) have reported a multiple drug intoxication case involving CIT concentrations of 0.88, 1.16, and 5.9 mg/L in femoral blood, heart blood, and brain, respectively. As no major variation in brain drug concentrations were observed in the present study, the brain per se seems to be a suitable organ for drug analysis postmortem. Kristinsson et al. (51) reported that in 9 cases of lethal amitriptyline poisonings, the brain levels of amitriptyline and nortriptyline varied less than 4-fold, whereas in the femoral blood, the variation was more than 14-fold. However, one disadvantage of using the brain as the postmortem sample of choice is that the DCIT and DDCIT levels are generally lower in the brain as in blood in comparison with the CIT levels, probably because it does not pass over the blood-brain barrier as readily, although alternative explanations for this repeated observations may also be possible (20,21). Nevertheless, the relationship between the parent compound and the metabolites in brain is therefore not same as in blood. This finding needs, however, to be clarified in humans.

In conclusion, the present animal study showed that the concentrations of the enantiomers of CIT and its metabolites DCIT and DDCIT increased in heart blood postmortem in comparison with concentrations observed antemortem. Even if the rat has organs that differ from man in proportion to total body weight, similar postmortem changes in drug distribution occurring in man is an obvious possibility at present. The observation of similar redistribution of the S- and R-enantiomers of CIT and metabolites in the presently used rat model is of particular interest because this makes it relevant to propose the use of enantioselective analysis as a tool also for interpretation of human postmortem results. The latter observation is also important in light of the prescription of the pure S-enantiomer of CIT (escitalopram), which has recently been marketed. Because mixed intoxications with racemic CIT and escitalopram may be a common scenario in the future, enantioselective analysis may be necessary for a correct interpretation of the forensic toxicological results in such cases.

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References

4. C. Sanchez, P.B.F. Bergqvist, L.T. Brennum, S. Gupta, S. Hogg, A. Larsen, and O. Wilborg. Citalopram, the (S)-enantiomer of escitalopram, which has recently been marketed. Because mixed intoxications with racemic CIT and escitalopram may be a common scenario in the future, enantioselective analysis may be necessary for a correct interpretation of the forensic toxicological results in such cases.