Cytochrome P450-Dependent Disposition of the Enantiomers of Citalopram and Its Metabolites: In Vivo Studies in Sprague-Dawley and Dark Agouti Rats

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ABSTRACT The female Sprague-Dawley (SD) and Dark Agouti (DA) rats are considered the animal counterparts of the human extensive and poor metabolizer cytochrome P450 (CYP) 2D6 phenotypes, respectively. The aim of this work was to study possible rat strain differences in the steady-state pharmacokinetics of the (+)-(*S*)- and (−)-(*R*)-enantiomers of citalopram and its demethylated metabolites. A chronic drug treatment regimen (15 mg/kg daily) was implemented for 13 days in separate groups of SD ($n = 9$) and DA ($n = 9$) rats by using osmotic pumps. The concentrations of citalopram and two major metabolites in serum and two brain regions were analyzed by an enantioselective high-performance liquid chromatography assay. Higher serum and brain levels of citalopram and demethylcitalopram, but lower levels of didemethylcitalopram, were observed in DA rats when compared with SD rats. The enantiomeric (*S/R*) concentrations ratios of citalopram were lower in the DA rats when compared with the SD rats (0.53 ± 0.05 vs. 0.80 ± 0.03, $P < 0.001$), indicating a possibly decreased capacity in the metabolism of the (−)-(*R*)-enantiomer in the DA rats. This study shows that CYP2D deficiency results in steady-state pharmacokinetic differences of the enantiomers of citalopram and its metabolites. *Chirality 23:172–177, 2011.*

KEY WORDS: citalopram; CYP2D6; Dark Agouti; enantiomer; pharmacokinetics; Sprague-Dawley

INTRODUCTION

The selective serotonin reuptake inhibitor (SSRI) citalopram is effective for the treatment of various affective psychiatric disorders, such as major depressive disorder, panic disorder, and obsessive compulsive disorder.[1] Citalopram is a racemic mixture of the (+)-(*S*)- and (−)-(*R*)-enantiomers. The SSRI activity of citalopram resides in (+)-(*S*)-citalopram, whereas (−)-(*R*)-citalopram is practically devoid of serotonin reuptake potency.[2,3] Due to this fact, (+)-(*S*)-citalopram has been developed as a single enantiomer drug (escitalopram). However, *rac*-citalopram is still one of the most prescribed antidepressants worldwide, which motivates further detailed studies of the separate enantiomers of the parent compound and its major metabolites.

The quantitatively most important metabolic pathway of citalopram appears to be the formation of demethylcitalopram (dm-citalopram) and didemethylcitalopram (ddm-citalopram).[4] In vivo studies, performed in relation to sparteine and mephenytoin oxidation polymorphisms in humans, have revealed that the N-demethylation of citalopram to dm-citalopram is partially mediated by the cytochrome P450 (CYP) enzymes CYP2D6 and CYP2C19, whereas the formation of ddm-citalopram from dm-citalopram is largely under the control of CYP2D6.[5,6] In vitro studies in human liver microsomes have further shown that the formation of citalopram to dm-citalopram is mediated mainly by CYP3A4 and CYP2C19, with an additional contribution of CYP2D6.[7–9] Furthermore, Olesen and Linnet[9] reported that CYP2D6 exclusively mediated the formation of ddm-citalopram from dm-citalopram. It has been shown both in vitro and in vivo that the CYP enzymes favor a more rapid demethylation of (+)-(*S*)-citalopram than of (−)-(*R*)-citalopram.[9–11]

Interindividual variability in drug metabolism and response is a clinically important problem in the use of many drugs, including citalopram. A major cause of this problem is the variability of activity of the drug metabolizing CYP enzymes in the liver. One example is CYP2D6, which is a highly polymorphic enzyme. Approximately 7–10% of all Caucasians lack the functional activity of CYP2D6, and they are classified as poor metabolizers for substrates of this enzyme, whereas the remainder is termed extensive metabolizers.[12] The implications of the CYP2D6 polymorphism for exogenous compounds can be difficult to study in a controlled manner in clinical studies in humans. Therefore, different animal models can be valuable complements to such studies. The female Sprague-Dawley (SD) and Dark Agouti (DA) rats are considered the animal counterparts of the human extensive and poor metabolizer phenotype, respectively.[13–15] However, the CYP2D subfamily has evolved differently in humans and rats. Isoenzymes of the human CYP2D subfamily are encoded by one active CYP2D6 gene and two pseudogenes, whereas in the rat, six genes, CYP2D1-5 and CYP2D18, have been identified.[12,16] It is still unclear which of these six genes that is/are homologous to the human

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CYP2D6. It has long been assumed that CYP2D1 corresponds well with the human CYP2D6.[17,18] However, Schultz-Utermoehl et al.[15] have shown that impaired debrisoquine 4-hydroxylase activity in the female DA rat was due to low levels of CYP2D2.

Several studies have reported that the female DA rat displays an impaired metabolism for a number of CYP2D6 substrates.[13–15] However, the literature is scarce concerning experimental data on how CYP2D deficiency may influence the in vivo pharmacokinetics of antidepressants, such as citalopram, especially when the enantiomeric drug disposition is taken into account. Thus, the aim of this work was to study possible differences in the steady-state pharmacokinetics of citalopram in female DA rats when compared with female SD rats to evaluate the role of CYP2D6 in the metabolism of citalopram. For this purpose, all rats underwent chronic drug exposure with racemic citalopram and the disposition of the (+)-(*S*)- and (−)-(*R*)-enantiomers of citalopram and its metabolites, dm-citalopram and ddm-citalopram, in serum and brain was investigated.

MATERIALS AND METHODS

Animals

Aged-matched 8-wk-old female SD (222–251 g) and DA (123–140 g) rats were obtained from Scanbur BK AB, Sollentuna, Sweden. 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 or three animals in macrolone cages under climate-controlled conditions for regular in-door 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.

Drugs and Chemicals

Rac-Citalopram HBr (kindly provided by H. Lundbeck A/S, Copenhagen-Valby, Denmark) was dissolved in a mixture of 0.9% NaCl and propylene glycol (40:60; v/v) and administered subcutaneously using osmotic pumps. All reagents used were of the highest purity commercially available.

Experimental Design

The rats were allowed to recuperate for at least 1 wk from transport-induced stress before the start of the experiments. A bodyweight-adjusted chronic drug treatment regimen (15 mg/kg daily) was implemented for 13 days in separate groups of SD ($n = 9$) and DA ($n = 9$) rats by using osmotic pumps (for details, see below). The chosen citalopram dose of 15 mg/kg is within the range of 10–20 mg/kg that previously has been shown to produce "therapeutic" plasma concentrations in rats.[19–23] Body weight measurement was performed on the day of the pump implantation, 3, 6, and 10 days after the implantation, and on the day the rats were sacrificed (i.e., day 13). At the end of the study, the rats were sacrificed by decapitation under halothane anesthesia (for details, see below). Thereafter, the concentrations of citalopram and two major metabolites in blood serum and two brain regions were analyzed by an enantioselective high-performance liquid chromatography (HPLC) assay (for details, see below).

Osmotic Pump Implantation

Osmotic pumps (ALZET® model 2ML2; Scanbur BK AB, Sollentuna, Sweden) were filled with 2 ml of a drug solution corresponding to the citalopram dose 15 mg/kg daily. The concentration of the drug solution was adjusted to allow delivery of a similar dose/kg body weight to both rat strains at a rate of 5 μl/h for 13 days. During halothane (Fluothane®, Zeneca, Macclesfield Cheshire, UK) anesthesia, the rats were shaved and a minor skin incision was made between the scapulae. A subcutaneous pocket was formed by blunt dissection 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 ∼10 min. The pumps were left in place throughout the entire study (i.e., for 13 days), and hence, there was no washout period before the rats were sacrificed. After sacrifice, the residual amounts of the pumps were assessed by aspirating with a graduated syringe for checking the delivery profile of the pumps.

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 of the blood, followed by centrifugation (2000*g* for 10 min) for collection of the supernatant serum that was transferred to a new, empty test tube for subsequent drug analyses. After collection of blood samples, the brain was removed from the scull and the neocortical hemisphere as well as the mesencephalon-pons region was dissected out. The brain tissue samples were weighed and homogenized in 2-ml Milli-Q® water (Millipore AB, Stockholm, Sweden) by the use of a sonifier (Sonics Vibra-Cell VC 130; Chemical Instruments AB, Lidingö, Sweden) and centrifuged at 2000*g* for 15 min. All samples were stored at −70°C until analysis.

Determination of the Enantiomers of Citalopram and Metabolites

The concentrations of the (+)-(*S*)- and (−)-(*R*)-enantiomers of citalopram, dm-citalopram, and ddm-citalopram in serum (nmol/l) and brain homogenate supernatant (pmol/g ≈ nmol/l) were determined by using HPLC with fluorescence detection according to a previously described procedure[24] with some modifications.[21,25] The extraction of the samples was carried out according to a previously described method.[21,25] Briefly, the enantiomers of citalopram, dm-citalopram, ddm-citalopram, and an internal standard (a chlorinated version of citalopram with the substance name Lu 10-202-O; H. Lundbeck A/S) were extracted from calibration standards, control solutions, rat brain supernatants, and serum by solid-phase extraction. After elution and evaporation, the dried samples were redissolved in 100 μl of methanol:100 mmol/l citrate triethylamine buffer, pH 6.3 (55:45; v/v). A volume of 50 μl was injected on to a Cyclobond I 2000 Ac 250 × 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. Detection was done with a Waters 474 fluorescence detector (Waters Corporation, Milford, MA) at an excitation wavelength of 240 nm and an emission wavelength of 300 nm. The temperature of the column was set to 30°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™ (Version 6.40; Dionex, Sunnyvale, CA). The limits of detection for the enantiomers of citalopram and its metabolites were 2 nmol/l (S:N; 3:1), respectively. The absolute recoveries from spiked drug-free plasma were between 87 and 110%.[25] The brain tissue extraction recoveries for citalopram and metabolites were found to be around 40%.[19]

Statistics

Data are expressed as means ± SEM. A probability of less than 5% ($P < 0.05$) was considered statistical significant. When two dependent groups were compared, a two-tailed Student's *t*-test for paired observations was applied. When two independent groups were compared, a two-tailed Student's *t*-test for unpaired observations was applied. All statistical analyses were performed using StatView® for Windows Version 5.0 (SAS® Institute, Cary, NC).

RESULTS

Body Weight and Osmotic Pump Performance

At the time when the osmotic pumps were implanted, the SD and DA rats weighed 235 ± 3 g and 131 ± 2 g, respec-

TABLE 1. Concentrations (nmol/l) and enantiomeric ratios (*S/R*) of citalopram, demethylcitalopram (dm-citalopram), and didemethylcitalopram (ddm-citalopram) in serum and two brain regions (cortex and mesencephalon-pons) following chronic administration with citalopram (15 mg/kg daily, 13 days) to Sprague-Dawley (SD; *n* = 9) and Dark Agouti (DA; *n* = 9) rats

	Serum			Cortex			Mesencephalon-pons		
	SD	DA	*P*-value	SD	DA	*P*-value	SD	DA	*P*-value
Citalopram	296 ± 46.1	647 ± 115	0.0123	1817 ± 182	2037 ± 194	0.4208	1770 ± 182	2518 ± 281	0.0403
S/R-ratio	0.80 ± 0.03	0.53 ± 0.05	0.0003	0.85 ± 0.03	0.36 ± 0.03	<0.0001	0.88 ± 0.02	0.50 ± 0.03	<0.0001
dm-Citalopram	151 ± 26.0	305 ± 40.8	0.0059	225 ± 22.3	260 ± 23.2	0.2976	239 ± 33.3	280 ± 37.0	0.4354
S/R-ratio	0.28 ± 0.01	0.33 ± 0.01	0.0010	0.17 ± 0.03	0.26 ± 0.03	0.0486	0.19 ± 0.04	0.26 ± 0.02	0.1092
ddm-Citalopram	142 ± 19.2	78.3 ± 4.48	0.0084	130 ± 9.30	77.2 ± 5.63	0.0003	102 ± 14.1	51.0 ± 7.69	0.0076
S/R-ratio	0.12 ± 0.01	0.19 ± 0.02	0.0004	0.21 ± 0.03	0.41 ± 0.03	0.0002	0.21 ± 0.08	0.50 ± 0.03	0.0441

All values are means ± SEM.

tively. At the last day of drug treatment (i.e., day 13) the SD and DA rats weighed 255 ± 4 g and 140 ± 3 g, respectively. The amount of residual drug solutions of citalopram in the osmotic pumps was in the range of 0.5–0.7 ml, evidencing that the rats received the programmed daily dose.[26]

Concentrations of the Enantiomers of Citalopram and Its Metabolites

The pharmacokinetic outcome of the study is shown in Table 1 and Figure 1. In both serum and mesencephalon-pons, the citalopram concentrations were found to be higher in the DA rats compared with the SD rats ($P < 0.05$). The citalopram levels in the cortex region were also slightly higher in the DA rats, but however, it was not statistically significant. No significant differences in citalopram concentrations could be found between the two brain regions. In comparison with serum, the levels of citalopram were about three to six times higher in the brain in both rat strains. The serum and brain levels of (−)-(*R*)-citalopram were markedly higher in the DA rats than in the SD rats ($P < 0.01$). Consequently, the *S/R* ratios of citalopram were lower in the DA rats as compared to the SD rats in serum and in the two brain regions ($P < 0.001$).

The dm-citalopram concentrations in serum were found to be higher in the DA rats when compared with the SD rats ($P < 0.001$). In comparison with the SD rats, the DA rats displayed slightly higher *S/R* ratios of dm-citalopram. The ddm-citalopram levels were found to be clearly lower in the DA rats when compared with the SD rats ($P < 0.01$). As the (−)-(*R*)-ddm-citalopram concentrations were lower in the DA rats than in the SD rats ($P < 0.01$), the *S/R* ratios were higher in the DA rats when compared with the SD rats. In relation to the parent compound, less metabolite concentrations were found in the brain when compared with serum.

The *P/M* ratios (ratio between concentration of parent drug and metabolite) for dm-citalopram and ddm-citalopram in serum were 2.1 and 2.3 in the SD rats, and 2.1 and 9.1 in the DA rats. The *P/M* ratios for dm-citalopram and ddm-citalopram in brain were 8.5 and 15–19 in the SD rats, and 8.0–9.5 and 28–65 in the DA rats.

DISCUSSION

To our knowledge, this is the first study to show that significant quantitative strain-related differences in the steady-state pharmacokinetics of citalopram and metabolites are present between DA and SD rats. Higher serum and brain levels of citalopram and dm-citalopram, but lower levels of ddm-citalopram, were observed in DA rats when compared with SD rats. Interestingly, the enantiomeric (*S/R*) concentrations ratios of citalopram were lower in the DA rats when compared with the SD rats, indicating a decreased capacity in the metabolism of the (−)-(*R*)-enantiomer in the DA rats. Hence, it is plausible that the present results are due to differences in CYP2D enzyme activity of the two rat strains.

In spite of the fact that a body weight adjusted chronic drug treatment was conducted, the concentrations of citalopram and dm-citalopram in serum were found to be markedly higher in the DA rats when compared with the SD rats. In contrast, the DA rats displayed clearly lower levels of ddm-citalopram than the SD rats. Similar differences were observed in the brain. Citalopram is metabolized to dm-citalopram by CYP3A4, CYP2C19, and CYP2D6, whereas dm-citalopram is further metabolized to ddm-citalopram by CYP2D6.[5–8] Accordingly, the present results of higher levels of citalopram and dm-citalopram, and lower levels of ddm-citalopram, in the DA versus the SD rats are due to the well-documented CYP2D deficiency of the DA rats.

Differences were also observed between the DA and SD rats with respect to the levels of the (+)-(*S*)- and (−)-(*R*)-enantiomers of citalopram and its metabolites. In serum, as well as in the brain, the concentrations of (−)-(*R*)-citalopram and (−)-(*R*)-dm-citalopram were found to be higher in the DA rats when compared with the SD rats. In addition, the levels of (+)-(*S*)-dm-citalopram were higher in the DA rats than in the SD rats in serum, but not in the brain. Furthermore, the concentrations of (−)-(*R*)-ddm-citalopram were found to be lower in the DA rats as compared to the SD rats in both serum and brain. Consequently, lower *S/R* ratios of citalopram were observed in the DA rats than in the SD rats, and the *S/R* ratios for ddm-citalopram were found to be higher in the DA rats. These results can be compared with a human study including different panels of mephenytoin and debrisoquine hydroxylation phenotypes, that is, poor metabolizers and extensive metabolizers of CYP2C19 and CYP2D6.[11] Herrlin and co-workers[11] reported that poor metabolizers of CYP2D6 displayed lower *S/R*-citalopram ratios compared with extensive metabolizers of CYP2D6, which is in agreement with the lower *S/R*-citalopram ratios in the CYP2D deficient DA rats when compared with the SD rats. In vitro studies in human liver microsomes have shown that CYP2C19, CYP2D6, and CYP3A4 favor a more rapid demethylation of the (+)-(*S*)-enantiomer than of the (−)-(*R*)-enantiomer of citalopram.[9,10] In contrast, CYP2D6 has been shown to favor (−)-(*R*)-dm-citalopram over (+)-(*S*)-dm-citalo-

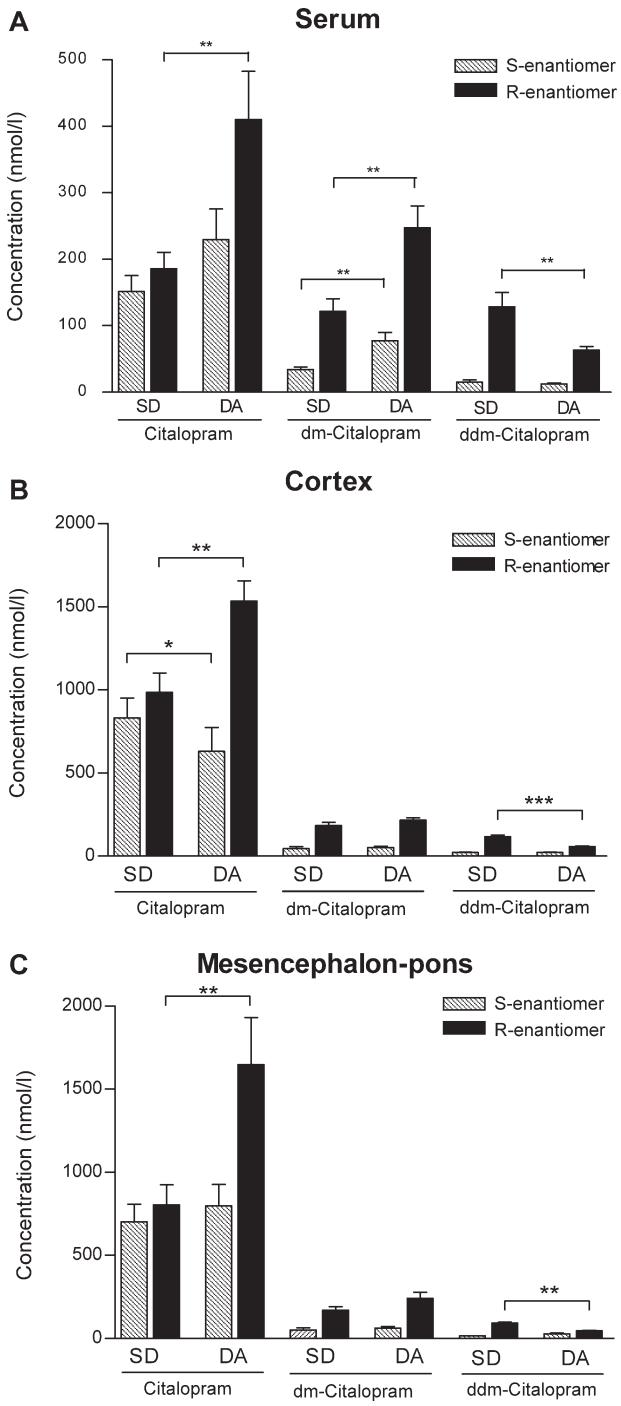

Fig. 1. Concentrations (nmol/l) of the *S*- and *R*-enantiomers of citalopram, demethylcitalopram (dm-citalopram), and didemethylcitalopram (ddm-citalopram) in (**A**) serum, (**B**) cortex, and (**C**) mesencephalon-pons following chronic administration with citalopram (15 mg/kg daily, 13 days) to Sprague-Dawley (SD; $n = 9$) and Dark Agouti (DA; $n = 9$) rats. Values are means ± SEM. * denotes significant difference between SD and DA (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

pram in the second demethylation step.[9] The present results indicate that the metabolism of the (−)-(*R*)-enantiomeric forms of citalopram and its metabolites was more affected by the CYP2D deficiency than the (+)-(*S*)-enantiomeric forms. One explanation for this observation might be that one, or several, of the CYP2D enzymes has different affinity for the enantiomers of citalopram. Interestingly, using a variety of in vivo and in vitro paradigms, it has been shown that (−)-(*R*)-citalopram counteracts the serotonin enhancing properties of (+)-(*S*)-citalopram.[27–31] This finding of a negative effect of (−)-(*R*)-citalopram emphasizes the relevance of the results from this study, in which (−)-(*R*)-citalopram was present in a higher proportion than (+)-(*S*)-citalopram in the DA rats when compared with the SD rats. Hence, the present data tend to suggest that the pharmacodynamic SSRI effect might be less pronounced in the CYP2D-deficient DA rats when compared with the CYP2D-replete SD rats. Given the limitations of extrapolating animal data to the clinical situation, our findings indicate that a dose reduction to poor metabolizers of CYP2D6 might not be sufficient to "normalize" the pharmacokinetics and pharmacodynamics of citalopram. Accordingly, this possible scenario also highlights the importance of enantioselective drug analysis, especially in depressive patients not responding to citalopram treatment. Notably, Baumann and Eap[32] have also stated that an appreciation of the stereoselective differences between enantiomers will facilitate improvements in the benefit:risk ratio of drugs used in the management of depression.

The concentrations of citalopram and its metabolites were measured in two different brain regions; the frontal cortex, which is an important projection area where the serotonergic synapses are present, and in the mesencephalon-pons, the site for serotonergic cell bodies. In both brain regions, higher concentrations of citalopram were seen when compared with in serum, which is in agreement with previous studies from our group.[21,33] Compared with the concentrations of the parent compound, the metabolite levels of citalopram were found to be lower in brain than in serum. This can be explained by the fact that the metabolites enter the blood-brain barrier less readily than the parent compound does.[34] The *S*/*R* ratios for citalopram and its metabolites were found to be similar in serum and brain, which are in agreement with previous studies.[21,33] The CYP enzymes, including CYP2D, are except from the liver also present in the brain in both humans and rats. The highest expression of CYP2D in brain has been observed in the cerebral cortex, hippocampus, cerebellum, and the brainstem.[18,35] The level of the CYP enzymes, which is ~0.1–2% of that in the liver, is too low to significantly affect the overall pharmacokinetics of drugs in the body.[36] However, it is possible that psychotropic drugs could undergo a local cerebral metabolism that could have some pharmacological consequences.[37] The presence of CYP2D in the brain implies that the genetic variability in the liver may also be present in the brain, and consequently that the metabolism of drugs in the brain may show interindividual variation.[18,38] However, it is difficult to predict the consequences of CYP2D genetic variability in the CNS, but it may imply differences in the therapeutic drug response.[38]

In addition to the CYP system, the role of monoamine oxidase (MAO) enzymes in the metabolism of citalopram in rat and human brain microsomes has been investigated.[39] In that study, citalopram as well as dm-citalopram and ddm-citalopram were stereoselectively metabolized by MAO to a propionic acid derivative of citalopram. Kosel and co-workers[39] concluded that the biotransformation of citalopram in the rat and human brain occurs mainly through MAO and not, as in the liver, through the CYP enzymes. However, if the brain MAO activity differs between SD and DA rats is not known as of today. This is also the case for the P-glycoprotein (P-gp) activity. Uhr and Grauer[40] reported that citalopram is

a substrate of P-gp at the blood-brain barrier in the abcb1ab knockout mice model. In their study,[40] the brain concentrations of citalopram in the wild-type mice were less than 1/3 of the concentrations in the knockout mice, suggesting that citalopram is actively exported out of the brain by the P-gp transport mechanism. Unfortunately, no data are available on the enantiomers of citalopram and its metabolites. A challenging speculation might be that the uneven stereoselective disposition of citalopram in the brain of SD and DA rats could possibly be explained in part by a strain difference in P-gp activity. However, further evidence to corroborate this speculative suggestion is warranted.

In conclusion, the results from this study demonstrate clear differences in the steady-state pharmacokinetics of citalopram in female DA rats when compared with female SD rats. These results indicate that the CYP2D enzymes have a significant impact on the stereoselective metabolism of citalopram and its metabolites in these rat strains. Such differences in drug metabolism may cause significant differences not only in the results of pharmacokinetics of the drug but also in pharmacodynamics, especially when the metabolites of the test compound might have pharmacological and/or toxicological effects. In addition, if the drug is a racemic mixture also the distribution of the enantiomers could differ depending on the strain of the animal, which is evidenced by the results from this study.

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