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Citalopram enantiomers in plasma and cerebrospinal fluid of *ABCB1* genotyped depressive patients and clinical response: A pilot study

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ABSTRACT

The antidepressant activity of citalopram (R,S-CIT) is mainly due to its (S)-enantiomer (S-CIT). P-glycoprotein (P-gp), encoded by the *ABCB1* gene, is a membrane transport protein which regulates the efflux of many drugs. Polymorphisms in the *ABCB1* gene may have an impact on the expression and function of P-gp, thereby influencing the response to treatment with antidepressants, which are substrates of this protein. The influence of *ABCB1* polymorphism on the disposition of R,S-CIT in plasma and cerebrospinal fluid (CSF) was examined under steady-state conditions in 15 patients with major depression treated with 40 mg/d R,S-CIT for 4 weeks. In contrast to the *ABCB1 C3435T* polymorphism, only the *ABCB1 G2677T* polymorphism significantly influences R,S-CIT plasma and CSF concentrations (46 ± 11 ng/ml versus 69 ± 20 ng/ml for *TT* versus *GT/GG* in plasma, $p = 0.027$; 24 ± 5 ng/ml versus 32 ± 9 ng/ml for *TT* versus *GT/GG* in CSF, $p = 0.05$). On the other hand, no significant influence of *G2677T* polymorphism was found on the plasma and CSF (S)/(R) ratio, suggesting a lack of stereoselectivity in the activity of this transporter. The *2677 GG/GT* genotype was associated with a better treatment response ($p = 0.001$) compared with *2677TT* genotype. Furthermore, higher R,S-CIT plasma and CSF concentrations were observed in treatment responders. This study is the first to demonstrate that a P-gp polymorphism significantly influences plasma and CSF concentrations of R,S-CIT in depressive patients, therefore possibly influencing the activity of this antidepressant. These findings should be replicated in future studies with larger groups of patients. Because of the small number of subjects in the present study, future studies with larger groups of patients, also with different ethnicities.

1. Introduction

The selective serotonin reuptake inhibitor (SSRI) citalopram (R,S-CIT) is a widely prescribed antidepressant drug [1,2]. The pharmacologic effect of the racemate with regard to its serotonin reuptake inhibiting properties in rat synaptosomes resides mainly in (S)-CIT (S-CIT) and to some extent in the metabolite (S)-desmethylcitalopram, while the corresponding (R)-enantiomers have no therapeutic effect and may actually interfere with the therapeutic effect of S-CIT [3,4]. Escitalopram (S-CIT) has also been introduced as an antidepressant drug [5,6].

P-glycoprotein (P-gp) is a member of the ATP-binding cassette superfamily of membrane transport proteins, responsible for the efflux of many drugs. It represents a major component of the blood–brain [7] and the intestinal barrier [8], and it contributes to renal and biliary elimination of drugs [9,10]. P-gp can limit the penetration into and retention within the brain and thus modulate effectiveness and central nervous system toxicity of numerous compounds [11]. While there is evidence that paroxetine and venlafaxine are substrates of P-gp [12], the results are contradictory for citalopram [13,14]. However, in *abcb1ab* double knockout mice, a higher cerebellum to plasma ratio of R,S-CIT than in wild type mice was demonstrated after a 10-day subcutaneous administration of R,S-CIT. This suggests that R,S-CIT is a substrate of murine P-gp [15].

In humans, P-gp is encoded by the *ABCB1* gene, for which several single-nucleotide polymorphisms (SNPs) have been described. Contradictory results have been reported on the possible effect of some *ABCB1* SNPs (e.g. *3435C > T* and *2677G > T*) on the expression of P-gp and on the kinetics of its substrates [16]. As polymorphisms in the *ABCB1* gene reportedly could predict the response to antidepressant treatment in depressed patients receiving drugs that have

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been identified as substrates of this protein (i.e R,S-CIT, paroxetine, amitryptiline, and venlafaxine) [15], it was hypothesized that P-gp regulates intracerebral concentrations at the blood–brain barrier and, by extension, affects the clinical response of CNS-targeting drugs [15]. However, to our knowledge, no study has yet directly measured the intracerebral concentrations of psychotropic drugs and determined whether they could be influenced by polymorphism of the *ABCB1* gene.

Relationships between the clinical outcome and plasma and cerebrospinal fluid (CSF) concentrations of S-CIT [17], and the effect of R,S-CIT on the hypothalamic–pituitary–adrenal axis [18] in patients with major depression treated with R,S-CIT were recently reported. In some of these patients [17,18], we examined in the present study the influence of the *ABCB1 C3435T* and *G2677T* polymorphisms on the steady-state concentrations of R,S-CIT, S-CIT and R-CIT in plasma and CSF and we examined the hypothesis whether these two genetic polymorphisms of the *ABCB1* gene could influence the response to a R,S-CIT based antidepressant treatment.

2. Patients and methods

2.1. Study population

Fifteen patients with a major depressive episode (19–55 years old (mean ± SD: 36.2 ± 8.3 years)) including seven females were enrolled. Mean (SD) heights, weights and body mass index (BMI) of the patients were 165 (5.2) cm, 70 (6.8) kg, 25.7 (1.48) kg/m^2, respectively. Written informed consent was obtained after explanation of the purpose and design of the study. The protocol was approved by the Ethics Committee of the Philipps-University of Marburg, Germany, according to the 1975 Declaration of Helsinki. All patients received active treatment with R,S-CIT, starting with 20 mg once daily, and from day 5 onwards 40 mg daily at breakfast. The study was carried out in the Klinikum Fulda gAG, Department of Psychiatry and Psychotherapy, Fulda, Germany. It is a substudy from an investigation described earlier in detail [17–19].

2.2. Blood and cerebrospinal fluid (CSF) collection and assays

Blood samples were obtained at 8:00 a.m., after a 4 week R,S-CIT treatment, before any medication. CSF samples were obtained by lumbar puncture from 15 patients after the 4 week R,S-CIT treatment. The lumbar puncture was performed in the lateral decubitus position between 8:00 a.m. and 9:00 a.m. after overnight bed rest and fasting. Heparinised plasma and CSF samples were submitted to liquid–liquid extraction before R,S-CIT, S- and R-CIT were determined by chiral HPLC using a fluorescence detector set at 240 nm and 296 nm for excitation and emission, respectively. The limit of quantification was 5 ng/ml for each enantiomer. Intra- and interassay coefficients of variation were 2.9–8.6% [17,20].

2.3. ABCB1 genotyping

Genomic DNA was extracted from EDTA blood samples with the FlexiGene DNA Kit (Qiagen, Hombrechtikon, Switzerland). *ABCB1 2677G>T* and *3435C>T* SNPs were detected by real-time polymerase chain reaction (PCR) with the use of 5′-nuclease allelic discrimination assays (ABI PRISM 7000 Sequence Detection System, Applied Biosystems, Rotkreuz, Switzerland) with primers and probes obtained from Applied Biosystems as previously described [21].

2.4. Data analysis

Statistical analyses were carried out by use of SPSS for Windows, version 16 (SPSS, Chicago, IL, USA). We used standard descriptive statistics to describe population characteristics and outcome data. For P-gp expression analysis, mean values were compared between groups using the Mann–Whitney *U*-test. Hardy–Weinberg equilibrium was assessed by *Monte Carlo Markov Chain* (1,000,000 steps and 100,000 dememorisation steps) [22]. *Mantel–Haenszel* test was applied to allow for combining information from tables stratified for a confounding variable as gender. The level of significance was set at 0.05.

3. Results

3.1. Clinical data and treatment response

The mean ± SD (range) HAM-D$_{21}$ scores at baseline were 25.1 ± 3.0 (21–30) in the 15 depressed patients. After 4 weeks of treatment with 40 mg R,S-CIT, they decreased to 14.5 ± 4.4 (9–25).

Seven patients (47%) were considered as responders, and eight patients (53%) were non-responders. Response was defined as a ≥50% decrease in the HAM-D score after 4 week treatment with R,S-CIT. The two groups were comparable in sex distribution (*Fisher's exact test*: $p = 0.38$) but they did not differ with regard to age, duration of illness, number of previous episodes, or HAM-D$_{21}$ scores at pre-treatment baseline (*t-tests*: *p*-values between 0.49 and 0.63).

3.2. R,S-CIT concentrations in plasma and CSF

After 28 days of R,S-CIT treatment, plasma concentrations of R,S-CIT and its enantiomers were (mean ± SD; $n = 15$): R,S-CIT: 61.5 ± 20.4 ng/ml; S-CIT: 19.2 ± 8.9 ng/ml; R-CIT: 42.3 ± 12.2 ng/ml. The corresponding figures for their concentrations in CSF were: R,S-CIT: 29.6 ± 8.7 ng/ml; S-CIT: 9.6 ± 3.9 ng/ml and R-CIT: 20.1 ± 5.5 ng/ml.

3.3. ABCB1 genotypes frequency distributions

The frequencies of the *ABCB1* genotypes in this group of patients ($n = 15$) were 20.0% for *CC*, 60.0% for *CT*, and 20% for *TT* (*C3435T*); for *G2677T* they were 26.7% for *GG*, 40.0% for *GT*, and 33.3% for

Table 1
Plasma and cerobrospinal fluid (CSF) steady-state concentrations (mean ± SD) of citalopram (R,S-CIT) (ng/ml) and its enantiomers in patients with major depression genotyped for *ABCB1 G2677T* polymorphism and treated with 40 mg/day R,S-CIT for 28 days.

Genotype	Patients (*n*)	Plasma				CSF				CSF/Plasma		
		R,S-CIT	S-CIT	R-CIT	S/R-CIT	R,S-CIT	S-CIT	R-CIT	S/R-CIT	R,S-CIT	S-CIT	R-CIT
G2677T												
GG/GT	10	68.9 ± 20.2	21.9 ± 9.1	47.1 ± 11.8	45%	32.4 ± 9.1	10.4 ± 3.8	22.0 ± 5.7	47%	48%	50%	47%
TT	5	46.4 ± 10.6	13.8 ± 6.1	32.6 ± 5.9	42%	24.1 ± 4.6	7.9 ± 2.8	16.2 ± 2.5	48%	52%	58%	50%
p-values												
GG/GT versus TT		0.027	0.043	0.020	0.244	0.050	0.066	0.043	0.579	0.348	0.178	0.805