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INFLUENCE OF TOBACCO SMOKE ON THE PHARMACOKINETICS OF CITALOPRAM AND ITS ENANTIOMERS

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The purpose of this study was to evaluate the influence of tobacco smoke on the pharmacokinetics of citalopram (CIT) and desmethylcitalopram (DCIT) and its enantiomers on an animal model. High performance liquid chromatography (HPLC) with a diode array detector (DAD) was used for the identification and quantification of the studied compounds. The HPLC quantification of racemic mixtures of CIT was performed on a C18 column. The limits of detection (LOD) and quantification (LOQ) were: 7 and 10 ng/ml respectively. HPLC separation of citalopram enantiomers (S- and R-CIT) was performed on a Chirobiotic V column. The limits of detection (LOD) and quantification (LOQ) were: 6 and 15 ng/ml for R- and S-CIT respectively. The experiment was carried out on male Wistar rats. The rats were exposed to tobacco smoke for five days (6 hours per day). After the exposure, citalopram was administered in a dose of 10 mg/kg intragastrically. In the control group (non-exposed animals), citalopram was administered in the same way and at an equal dose. The blood of the animals was collected at nine time points. It was found that tobacco smoke exposure inhibits the biotransformation of citalopram. The half-life of the racemic mixture of citalopram after intragastric administration was increased by about 287%. Changes in the pharmacokinetic parameters of S-citalopram (active isomer) show a similar tendency to those of the racemic mixture. The pharmacokinetics of R-citalopram showed no statistically important differences after tobacco smoke exposure. Alterations in the pharmacological parameters of desmethylcitalopram presented an opposite trend to the parent drug. After exposure to tobacco smoke, the induction of metabolism of this compound was observed.

Key words: *citalopram, desmethylcitalopram, enantiomers, high performance liquid chromatography, pharmacokinetics, selective serotonin reuptake inhibitors, tobacco smoking*

INTRODUCTION

Depression is one of the most frequent disorders nowadays. The World Health Organization (WHO) reported that 5-10% of the general human population suffers from this disease. Pharmacotherapy is the most important treatment method of this disease. Recent studies on animals show that opiorphin, as a physiological dual inhibitor of both Zn-ectopeptidases, neutral endopeptidase and aminopeptidase N, potentiates the enkephalinergic pathway which then induces an antidepressive-like effect (1). However, at present, serotonin reuptake inhibitors are the first choice drugs in depression treatment. Citalopram belongs to one of the most commonly prescribed drugs in the pharmacotherapy of depression (2, 3). Citalopram is a selective serotonin reuptake inhibitor (SSRI) and, similar to other drugs from this group, CIT is a racemic mixture of S(+) and R(–) enantiomers. S-citalopram is a therapeutically active enantiomer. Baumann and co-workers reported that S-citalopram is approximately twice as potent as an inhibitor of serotonin reuptake in comparison to citalopram, and 100-fold more potent than the R-enantiomer (4). The S-citalopram enantiomer and its metabolites are metabolized faster than their antipodes. The S/R ratio in the plasma of patients after an oral dose of the drug (ratio 1:1) has been shown as 0.56 and 0.69 for CIT and DCIT, respectively (5).

Citalopram is metabolized to desmethylcitalopram by cytochromes CYP2C19 and CYP3A4, and to didesmethylcitalopram (DDCIT) by cytochrome CYP2D6 (6). An *in vitro* study suggested that citalopram is at least 4 times more potent than DCIT and 13 times more potent than DDCIT in the inhibition of serotonin reuptake, but Brosen and co-workers reported that these two metabolites are not active (7, 8). After a single dose of citalopram, as well as at steady state, the concentrations of metabolites are 30–50% of DCIT and 5–10% of DDCIT (4, 7).

Cigarette smoking is widely spread among depressive patients. Tobacco smoke contains more than 4200 chemical compounds (9, 10). Some tobacco smoke ingredients (*e.g.*, tobacco-specific N-nitrosamines, TSNA) are metabolized by cytochrome P-450, which is also involved in the metabolism of citalopram (11, 12). Carbon monoxide, heavy metals and reactive nitrogen species present in tobacco smoke can also influence enzyme activity. The cytochrome activity changes may affect the metabolism of citalopram (13). Thus it can be assumed that this influence can be different for particular enantiomers of this drug.

The aim of this study was to evaluate of tobacco smoke influence on the pharmacokinetics of citalopram enantiomers in animal model.

MATERIAL AND METHODS

Animals

Male Wistar rats with an average body weight of 225 g bred at the Department of Toxicology, University of Medical Sciences (Poznan, Poland), were housed in polycarbonate cages with hardwood chip bedding. A standard laboratory diet of Labofeed (Feeds and Concentrates Production Plant, Certificate of Quality System No 181/1/98, Kcynia, Poland) and water were available, with no limitations. Throughout the entire study period, a 12/12 h light/dark cycle was maintained. After 14 days of initial acclimatization, the rats were randomized and divided into two groups of 24 rats each. The protocol for this animal experiment was approved by the Local Ethics Commission for Animal Studies in Poznan (No.32/2008, June 20th 2008).

Group I was exposed to tobacco smoke in a dynamic toxicological chamber for 5 days (6 hours per day). The chamber is a glass rectangular cuboid of 308 dm^3 capacity, fitted with parallel tubing. There is a movable cover situated in the upper part and carbon monoxide detectors located in the lateral walls of the tank. Tobacco smoke is introduced into the chamber through a pipe, connected to perforated tubing. The air outlet vent is located on the opposite side. This tubing system provides an even and uniform distribution of smoke within the chamber (14). Tobacco smoke was generated from Polish cigarettes without a filter tip ("Poznanskie", 20 pieces per pack, Imperial Tobacco Poland S.A.). The CO concentration in the chamber reflected the smoke content in the inhaled air and was continuously monitored by a gas analyzer, Infralyt 1110/1210 (infrared measurement), to maintain 1,500 mg CO/m^3 of air. The level of oxygen was established at 20±0.5% of the air volume. The air in the chamber was changed 10 times per day. After exposure, citalopram (Lundbeck, Denmark) was administered by a gavage at a dose of 10 mg/kg body weight.

Group II (control - without exposure to tobacco smoke) - citalopram (Lundbeck, Denmark) was administered by a gavage at a dose of 10 mg/kg body weight. After administration of citalopram, the rats were anesthetized (a mixture of xylocaine 40 mg/kg and ketamine 5 mg/kg). Blood samples from the jugular vein were collected into tubes without an anticoagulant at eight time-points (0.33; 0.66; 1; 1.5; 2; 4; 8; 24 h) with three rats per point. The serum was subsequently separated by centrifugation for further analysis.

Citalopram and desmethylcitalopram measurement

For the determination and quantification of citalopram and its main metabolite (desmethylcitalopram), high performance liquid chromatography with a diode array detector was used. The analytes were isolated from the plasma by liquid-liquid extraction with the use of *n*-hexane and isoamyl alcohol mixture (98.5: 1.5 v/v). The HPLC separation of CIT and DCIT was performed on a C18 column (Spheri-5, 100×4.5 mm, 5 µm diameter), using a mixture of diluted solution of phosphoric (V) acid with an addition of 1% diethylamine at pH 2.36 and acetonitrile (40 : 60 (v/v)) as a mobile phase. The validation parameters are presented in *Table 1*.

Enantiomers of citalopram and desmethylcitalopram measurement

For the determination and quantification of enantiomers of citalopram (R-CIT and S-CIT) and its main metabolite (R-DCIT

Table 1. Validation parameters of determination of citalopram and desmethylcitalopram in plasma.

Parameter	Citalopram	Desmethylcitalopram
Linearity [ng/ml]	10–500	10–300
Slope of calibration curve, a	0.0052	0.0038
Intercept, b	-0.047	0.018
Correlation coefficient, R^2	0.9990	0.9984
LOD [ng/ml]	7	8
LOQ [ng/ml]	10	10
Precision of retention time, (n=5), RSD%	0.2	0.2
Intra-day precision at 75 ng/ml, (n=4), RSD%	12.4	10.3
Intra-day precision at 500 ng/ml (CIT) and at 300 ng/ml (DCIT), (n=4), RSD%	11.9	8.0
Inter-day precision at 75 ng/ml, (n=4), RSD%	10.7	15.4
Inter-day precision at 500 ng/ml (CIT) and at 300 ng/ml (DCIT), (n=4), RSD%	11.3	16.0
Accuracy at 75 ng/ml (n=4), relative error [%]	2.6	10.5
Accuracy AT 500 ng/ml (CIT) and at 300 ng/ml (DCIT), (n=4), relative error [%]	29.7	18.8
Efficiency of extraction at 75 ng/ml, (n=4)	89.25	73.43
Efficiency of extraction at 500 ng/ml (CIT) and at 300 ng/ml (DCIT), (n=4)	86.64	70.96

Table 2. Validation parameters of determination of R- and S-citalopram and R- and S-desmethylcitalopram in plasma.

Parametr	R–CIT	S–CIT	R–DCIT	S–DCIT
Linearity [ng/ml]	15–500	15–500	15–500	15–500
Slope of calibration curve, a	0.0400	0.0442	0.0345	0.0313
Intercept, b	-0.2713	0.1917	-0.2513	-0.4604
Correlation coefficient, R^2	0.9937	0.9913	0.9977	0.9919
LOD [ng/ml]	6	6	4	7
LOQ [ng/ml]	15	15	15	15
Precision of retention time, (n=5). RSD%	0.2	0.2	0.2	0.2
Intra-day precision at 25 ng/ml, (n=4). RSD%	2.2	4.7	2.2	3.7
Intra-day precision at 200 ng/ml, (n=4). RSD%	7.3	5.4	3.5	1.8
Inter-day precision at 25 ng/ml, (n=4). RSD%	8.9	21.0	4.3	7.9
Inter-day precision at 200 ng/ml. (n=4). RSD%	5.9	21.3	3.5	11.5
Accuracy at 25 ng/ml (n=4). relative error [%]	0.9	24.9	0.9	24.8
Accuracy at 200 ng/ml. (n=4). relative error [%]	20.7	11.7	15.2	3.9
Efficiency of extraction at 25 ng/ml. (n=4)	87.05	54.01	60.27	63.24
Efficiency of extraction at 200 ng/ml. (n=4)	82.31	73.21	63.04	66.37

and S-DCIT), HPLC-DAD was used. The separation of analytes from the plasma was achieved by liquid-liquid extraction with the use of an *n*-hexane and isoamyl alcohol mixture (98.5: 1.5 v/v). The HPLC separation of enantiomers of citalopram and desmethylcitalopram was performed with a modified method of Kosel and co-workers (15) on a Chirobiotic V column (250×4.5 mm and 5 μm diameter) purchased from Aldrich, Germany. Elution was performed with a mixture of methanol: acetic acid : triethylamine (99.9: 0.055: 0.060 v/v). The limits of detection and quantification are presented in *Table 2*.

Statistical and pharmacokinetic analysis

The pharmacokinetic analysis of citalopram, desmethylcitalopram and its enantiomers was carried out by the model-independent method (statistical moment analysis) using the SPLINE computer program.

The following parameters were calculated: the area under the plasma concentration time curve (AUC), the area under the first moment curve (AUMC), the mean resident time (MRT), the elimination rate constant (k), the biological half-life ($t_{0.5}$), the total clearance (Cl_s) and the volume of distribution (V_d).

The t-Student's test was used to assess the statistical significance of differences between the mean values of individual parameters in both the tobacco smoke exposed and non-exposed groups. In the calculations of probability distribution, a single track and 95% confidence interval were assumed (α=0.05).

RESULTS

The experiment was performed on two groups of Wistar rats. The first group was exposed to tobacco smoke for five days (6 hours per day). After exposure they were administered citalopram by a gavage at a dose of 10 mg/kg. The second (control) group of rats was given citalopram in the same way and dose, although it was not exposed to tobacco smoke. The time profile of citalopram and desmethylcitalopram concentration is presented in *Fig. 1*.

Changes in concentrations of citalopram enantiomers and desmethylcitalopram depending on the time elapsed from drug administration are presented in *Fig. 2* and *3*, respectively.

Between the two studied groups, particular parameters were compared - the area under the plasma concentration time curve (AUC), the area under the first moment curve (AUMC), the mean time of residence (MRT), the elimination rate constant (k), the half-life of the drug ($t_{0.5}$), the total clearance (Cl_s) and the volume of distribution (V_d). The results are summarized in *Table 3* and *4*.

DISCUSSION

In the present study, the influence of tobacco smoke on the pharmacokinetics of citalopram and desmethylcitalopram and its enantiomers has been investigated. In the animal model experiment, two groups of rats were formed. The first group was exposed to tobacco smoke for five days (6 hours per day). After the exposure, 10 mg/kg of citalopram was administered intragastrically. The second group (control) received citalopram in the same way and at an equal dose, although there was no exposure to tobacco smoke. Generally there are no significant differences between altering the concentrations of citalopram and its metabolite determined in the plasma of rats exposed to tobacco smoke and in the control group (*Fig. 1*). However, the slope of the concentration-time curve suggests that the elimination of the drug in the control group was more rapid.

As for the enantiomers of citalopram, it was observed that the S-isomer was eliminated much faster in the group exposed to tobacco smoke than in the control group (*Fig. 2B*). Faster metabolism and a higher first past effect associated with it

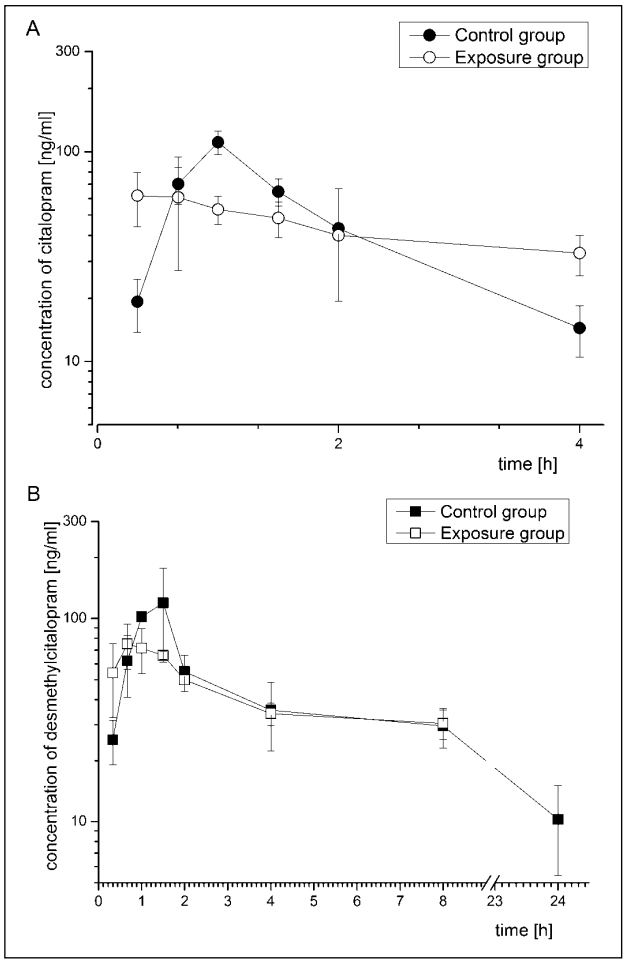

Fig. 1. Time profile of citalopram (A) and desmethylcitalopram (B) concentration in plasma of rats after intragastric administration of citalopram in dose 10 mg/kg.

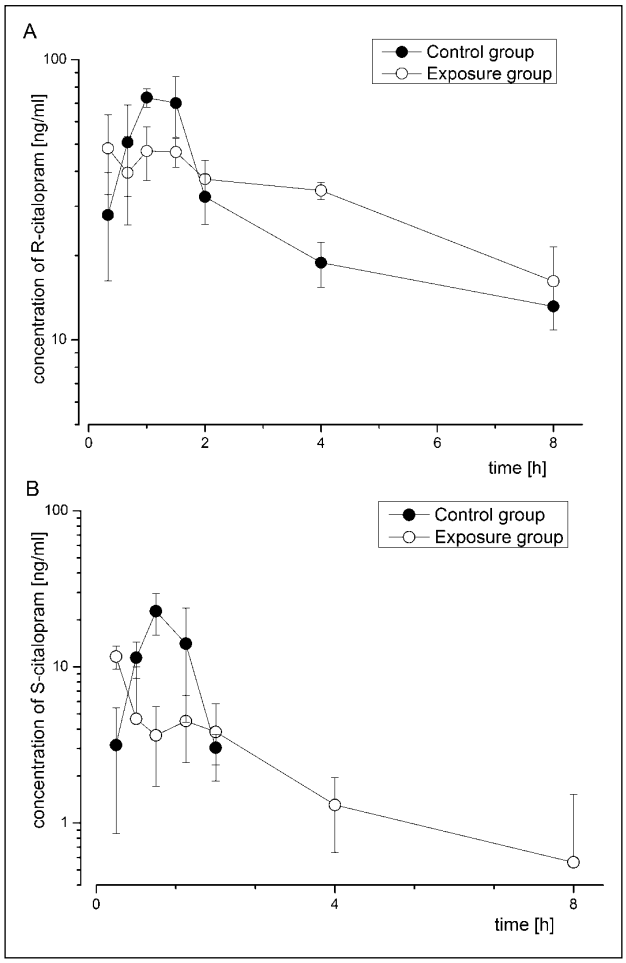

Fig. 2. Time profile of R-citalopram (A) and S-citalopram (B) concentration in plasma of rats after intragastric administration of citalopram in dose 10 mg/kg.

Table 3. Pharmacokinetic parameters of racemic mixture of citalopram and its enantiomers designated in the group of exposure to tobacco smoke and in the control group. Animals of both groups were given citalopram intragastrically at a dose 10 mg/kg.

Parameter		AUC [μg·h/l]	%	AUMC [μg·h^2/l]	%	Cls [l/h·kg]	%	Vd [l/kg]	%	MRT [h]	%	$t_{0.5}$ [h]	%
CIT	C	204.1±58.9	91	426.9±174.6	530	52.0±15.8	48	103.2±16.9	72	2.04±0.3	231	1.20±0.1	287
	E	390.5±102.1*		2689.5±957.0*		26.9±7.7*		177.8±28.4*		6.75±0.8*		4.64±0.8*	
R-CIT	C	267.0±38.7	33	1899.4±1163.0	17	19.0±2.3	24	134.6±77.2	35	7.12 ±4.2	14	3.09±0.2	30
	E	355.7±76.5		2231.3±824.9		14.5±3.3		86.6±3.9		6.12±1.2		4.02±0.9	
S-CIT	C	21.1±6.6	16	31.94±17.0	142	256.6±96.1	12	354.3±81.5	70	1.45±0.4	100	0.63±0.5	236
	E	24.6±9.8		77.5±57.4		226.2±87.2		603.7±94.3*		2.90±1.0*		2.12±0.9*	

C - control group; E - cxposure group; % - percentage change of parameter exposed group compared to the control group; * - statistically significant difference assuming significant level p equal to 95% (α=0.05).

resulted in a lower concentration of S-enantiomer (*Fig. 2B*) when compared to the racemic mixture (*Fig. 1A*) and a much lower one in comparison to R-enantiomer (*Fig. 2A*). A similar tendency was previously observed in the racemic mixture of citalopram, for which the change was not as significant as in the case of S-citalopram. This phenomenon can be explained by the lack of changes in the R-citalopram elimination rate (*Fig. 2A*).

The opposite tendency can be observed in the metabolite of citalopram. R-desmethylcitalopram showed no changes after tobacco exposure, while S-isomer persisted longer in the bodies of unexposed animals (*Fig. 3*).

Statistically significant changes were found for all of the pharmacokinetic parameters of citalopram racemic mixture between the group of exposed animals and the control group (*Table 3*). The half-life of the racemic mixture of citalopram, following intragastric application, increased by about 287%. As a consequence, the area under the citalopram concentration time curve (AUC) determined in the plasma samples of the exposed animals increased by almost 100%. Alterations in the biotransformation of citalopram may be the underlying cause of this situation. Citalopram is metabolized mainly by CYP2D6, CYP2C19 and CYP3A4 isoenzymes (5, 7). Additionally,

Table 4. Pharmacokinetic parameters of racemic mixture of desmethylcitalopram and its enantiomers designated in the group of exposure to tobacco smoke and in the control group. Animals of both groups were given citalopram intragastrically at a dose 10 mg/kg.

Parameter		AUC [μg·h/l]	%	AUMC [μg·h^2/l]	%	MRT [h]	%	$t_{0.5}$ [h]	%
DCIT	C	856.0±294.0	53	11589.2±4588.8	80	13.36±0.8	60	10.71±1.5	68
	E	399.9±159.1*		2323.3±1863.3*		5.25±2.1*		3.46±1.5*	
R-DCIT	C	260.1±25.2	48	2499.7±376.3	101	9.75 ±2.4	27	34.54±2.5	97
	E	386.0±126.2		5025.3±2596.4		12.40±2.9		8.9±2.3*	
S-DCIT	C	177.6±49.5	-	960.7±476.5	71	5.19±1.19	54	3.43±0.9	69
	E	115.4±20.0		276.9±80.7*		2.38±0.4*		1.06±0.6*	

C - control group; E - exposure group; % - percentage change of parameter exposed group compared to the control group; * - statistically significant difference assuming significant level p equal to 95% (α=0.05).

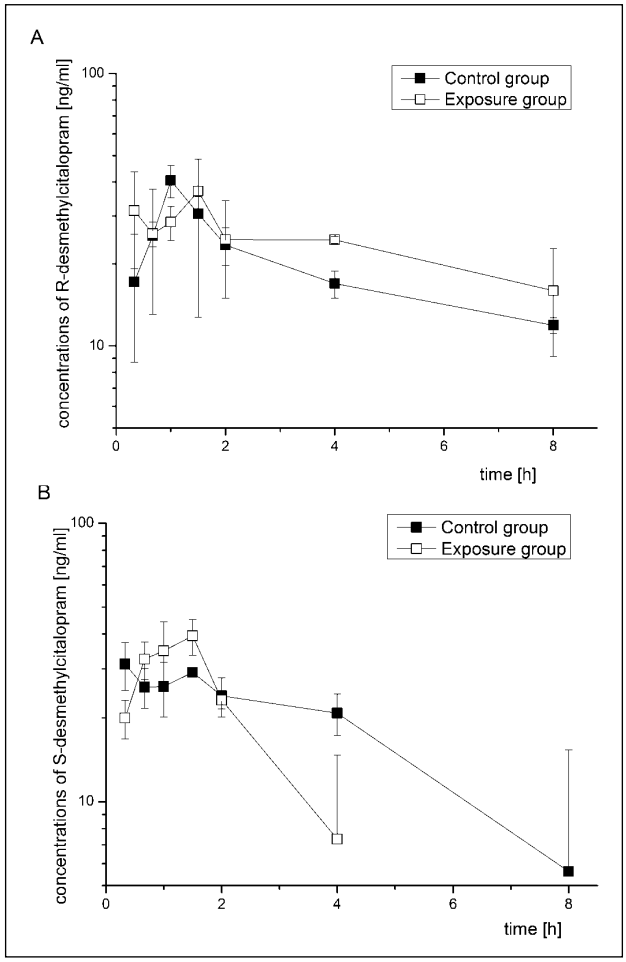

Fig. 3. Time profile of R-desmethylcitalopram (A) and S-desmethylcitalopram (B) concentration in plasma of rats after intragastric administration of citalopram in dose 10 mg/kg.

according to Kobayashi and co-workers, CYP3D4 is the most active factor in the N-demethylation of citalopram in the human liver (16), which is contrary to the result by Sindrup and co-workers, who suggested that the major isoensyme in this process is CYP2C19 (17).

Isoforms of enzymes CYP2D6 and CYP3A4 also participate in the biotransformation process of tobacco-specific N-nitrosamines (NAST) (11). During the simultaneous biotransformation of citalopram and NAST, competition for the active site of enzymes may occur between them. The result may slow down the biotransformation of the drug.

Changes in the pharmacokinetic parameters of S-citalopram (active isomer) show a similar tendency to those of the racemic mixture. Under the influence of tobacco smoke, the changes in the mean time of residence and half-time of S-citalopram were extended by about 100% and 236% respectively. There were no statistically important differences between these pharmacokinetic parameters for R-citalopram in the studied group.

Changes in the pharmacokinetic parameters of desmethylcitalopram represented a tendency opposite to the parent compound (*Table 4*). After exposure to tobacco smoke, the MRT and the biological half-life decreased substantially. Desmethylcitalopram is metabolized further to didesmethylcitalopram only by CYP2D6, which is also involved in the biotransformation of NAST. It is assumed that prolonged exposure of this cytochrome to these xenobiotics can lead to its induction and thus to an increase in the elimination of desmethylcitalopram.

For both S-desmethylcitalopram and the racemic mixture of the compound, inhibition of the biotransformation was observed, whereas the changes of R-desmethylcitalopram were of no statistical importance.

It can be concluded that the elimination rate of citalopram is decreased after tobacco smoke exposure due to inhibition of the S-enantiomer elimination, whereas the R-citalopram elimination rate remains constant.

In the case of the metabolite of citalopram, the changes observed were in the opposite direction to those described for the parent drug. It was determined that tobacco smoke exposure induces the biotransformation processes of the desmethylcitalopram racemic mixture. Additionally, a similar effect occurred under the influence of tobacco smoke in the S-enantiomer. The R-isomer, on the other hand, showed no statistically significant differences.

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Conflict of interests: Non declared

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