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The impact of the *CYP2D6* polymorphism on haloperidol pharmacokinetics and on the outcome of haloperidol treatment

Objectives: **The genetically polymorphic enzyme cytochrome P450 (CYP) 2D6 contributes to the biotransformation of the antipsychotic drug haloperidol. The impact of the polymorphism on haloperidol pharmacokinetics, adverse events, and efficacy was prospectively evaluated under naturalistic conditions in 172 unselected psychiatric inpatients with acute psychotic symptoms.**

Methods: **Serum trough levels of haloperidol and reduced haloperidol of patients receiving clinically adjusted doses were analyzed on days 3, 14, and 28 after hospital admission. Adverse events such as extrapyramidal symptoms were assessed by standardized rating scales. Efficacy was documented by recording the change in positive and negative schizophrenic symptoms. These parameters were correlated with the *CYP2D6* genotype determined by polymerase chain reaction analysis for alleles **1* to **15* and **17*.**

Results: **The serum concentrations showed wide interindividual variation. Reduced haloperidol trough levels and haloperidol total clearance correlated significantly with the number of active *CYP2D6* genes. In addition, body weight and smoking had significant effects on haloperidol kinetics, whereas age, gender, and comedication showed only slight effects. The ratings for pseudoparkinsonism were significantly higher in poor metabolizers of substrates of CYP2D6. On the other hand, there was a trend toward lower therapeutic efficacy with increasing number of active *CYP2D6* genes.**

Conclusions: **Treatment with haloperidol should be avoided in extremely slow and extremely rapid metabolizers of CYP2D6 substrates. Both genotyping and blood concentration measurement explained only a fraction of the adverse events; about 20 patients would have to be genotyped to achieve a significant benefit in 1 patient. It is interesting that genotyping was at least as good a predictor of adverse events as the measured drug concentrations. (Clin Pharmacol Ther 2002;72:438-52.)**

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The dopamine D_2-receptor antagonist haloperidol is prescribed as a high-potency antipsychotic drug for the treatment of acute and chronic schizophrenia and other psychiatric disorders worldwide. Its high efficacy is compromised by serious extrapyramidal adverse reactions (acute dystonia, pseudoparkinsonism, akathisia, and tardive dyskinesia), which occur with a high incidence.[1-3] The therapeutic serum concentration of haloperidol ranges from 5 to 17 μg/L according to several mostly small clinical trials,[4-14] but the lower limit, in particular is not well defined.

A linear correlation was observed between haloperidol dose and its serum concentration.[15] It was shown that dosages of haloperidol higher than 10 mg/d are usually not of additional benefit in the treatment of acute schizophrenia.[16] However, interindividual variations in haloperidol pharmacokinetics are considerable.[17] Haloperidol is extensively metabolized in the liver, and only 1% of the administered dose is excreted

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unchanged in the urine.[18] Hepatic biotransformation includes glucuronidation (about 50%-60% in vivo) and reduction and back-oxidation (23%), as well as *N*-dealkylation and pyridinium metabolite formation (about 20%-30%).[19-22] The glucuronide metabolite is inactive, whereas reduced haloperidol has significant pharmacologic effects (eg, high-affinity binding to sigma-type opioid receptor–binding sites and to dopamine D_2 and D_3 receptors).[23] The concentrations of haloperidol and reduced haloperidol appeared to correlate with clinical response and adverse effects.[24-27]

The formation of reduced haloperidol is mediated by a carbonyl reductase.[28] Reduced haloperidol is partially back-oxidized to haloperidol. In vitro, this reaction is mediated by cytochrome P450 (CYP) 3A4[29-31]; only one group of authors has reported a contribution of CYP2D6.[32] The oxidation of haloperidol to a pyridinium metabolite and oxidative *N*-dealkylation also appeared to be mediated mainly by CYP3A4,[31,33-35] but CYP2D6 appeared to be involved as well.[31]

Several studies have investigated the contribution of CYP2D6 to haloperidol metabolism in healthy volunteers or patients. Although in vitro data suggest that CYP2D6 might play only a minor role in haloperidol biotransformation in human beings, all studies except one (which included only one poor metabolizer of CYP2D6)[36] have found an effect of CYP2D6 activity on haloperidol pharmacokinetics. Poor metabolizers according to CYP2D6 showed higher haloperidol serum concentrations, lower haloperidol clearance, and higher concentrations of reduced haloperidol than extensive metabolizers.[37-42] These in vivo studies were either clinical trials that were conducted under standardized conditions in selected groups of inpatients[36,38,40,43] or single-dose studies with healthy volunteers.[37,42] One observational study examined the influence of *CYP2D6* genotype, smoking, and concomitant drug use in a naturalistic clinical setting,[39] in which the total sample size was 92 participants. The reason for the discrepancy between the in vitro and in vivo data with regard to the role of the polymorphic *CYP2D6* in haloperidol pharmacokinetics is not yet clear.

Higher reduced haloperidol and haloperidol levels in blood are associated with a higher risk of adverse drug reactions. Reduced haloperidol levels were even more strongly correlated with extrapyramidal side effects and poorer clinical outcome than those of haloperidol.[25,27]

In a retrospective analysis, the poor metabolizer phenotype of CYP2D6 was found to be significantly overrepresented in patients with adverse effects.[44] This finding is corroborated by several reports on the higher incidence and severity of adverse drug effects in poor metabolizers of CYP2D6.[44-46] We conducted a prospective naturalistic study, in which the impact of CYP2D6 on haloperidol pharmacokinetics, adverse events, and efficacy was tested in an unselected sample of 172 consecutively included inpatients. The aim was to determine the extent to which the *CYP2D6* genotype influences the risk of adverse drug reactions, therapeutic outcome, and interindividual pharmacokinetic variability under naturalistic clinical conditions. Furthermore, these results might serve as an empiric basis for future genotype-based optimization of antipsychotic drug treatment.

METHODS

Study design

This was a prospective, observational, multicenter study conducted at 5 psychiatric departments in Berlin, Germany. Patients were included within 3 days after hospital admission.

Patients

The study was approved by the ethics committees at the University Medical Center Benjamin Franklin and the University Medical Center Charité, Humboldt University, Berlin, Germany. One hundred seventy-five German psychiatric inpatients (34% women and 66% men), treated with haloperidol because of psychotic symptoms, were included. In accordance with the study protocol, patients were informed about the study and the genotyping for polymorphic drug-metabolizing enzymes, and consent was obtained from each patient. The mean age was 39 years. Most patients were diagnosed as having an acute exacerbation of schizophrenia (n = 114) or schizoaffective psychosis (n = 40) according to the criteria of the *International Statistical Classification of Diseases, 10th Revision*. Brief psychotic disorder occurred in 10 patients, mania requiring antipsychotic medication in 7 patients, substance-related psychotic disorders in 2 patients, and borderline personality disorder, as well as delusional disorder, in 1 patient. Patients with organic mental disorders, as diagnosed by means of clinical history, computed tomography, or nuclear magnetic resonance imaging, were excluded.

All medication was documented from hospital admission until discharge or, in cases of prolonged hospitalization, for at least 28 days after admission. On average, patients took 4 medications during the study period. During the 3-day period before sample taking, 32% of the haloperidol-treated patients received other antipsychotic comedication simultaneously. The antipsychotic agents most frequently administered in addi-

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tion to haloperidol were chlorprothixene (9%), perazine (6%), and promethazine (5%), all of which have low potency. In addition, 70% of the patients received benzodiazepines, 58% anticholinergics, and 34% other types of hypnotic drugs. Comedications were classified as CYP-interacting drugs and noninteracting drugs. CYP-interacting drugs for haloperidol were substances inhibiting CYP2D6.[47] Because CYP3A4 plays a role in haloperidol metabolism, we also evaluated the effect of CYP3A4 inhibitory drugs (eg, ranitidine and levonorgestrel) and CYP3A4 inducers (eg, carbamazepine).

The course of illness was documented by the global clinical assessment scale and by the positive and negative symptoms scale (PANSS)[48] on days 3, 14, and 28. The instruments for the assessment of adverse effects included the extrapyramidal symptom (EPS) scale,[49] the abnormal involuntary movement scale,[50] the Barnes akathisia rating scale,[51] a questionnaire asking for all of the typical adverse events of antipsychotic agents, and the Hamilton depression rating scale for the assessment of depressive symptoms (which gives only the undifferentiated sum of depressive symptoms occurring as side effects of haloperidol treatment in addition to possible depressive symptoms resulting from the psychotic disease plus depressive symptoms resulting from possibly existing depressive comorbidity). For genotyping, 9 mL of blood was taken from each patient, and haloperidol serum concentrations were determined at days 3, 14, and 28 or close to these dates. Psychiatric rating scales were assessed by psychiatrists who had no knowledge about the genotype data and serum concentration data when they performed the clinical examination and data documentation (blinded assessment). To improve interrater comparability, regular monitoring sessions in 6-week intervals took place in which all clinical investigators of the study monitored 1 patient. These sessions were followed by discussions of the possible discrepancies in the scores obtained by different monitors. Furthermore, the persons who performed the genotyping and serum concentration analyses were blinded to the clinical data, and clinical monitors, at the time of their documentation, had no knowledge about the plasma concentrations or genotypes.

HPLC quantification of haloperidol and reduced haloperidol in serum

Standards of haloperidol and reduced haloperidol were obtained from Biotrend (Cologne, Germany). Serum was separated from whole blood and stored at −20°C. Six calibration standards with concentrations from 0 to 20 μg/L and 2 controls with 1.25 μg/L and 7.5 μg/L were used. Haloperidol and reduced haloperidol were quantified by HPLC with electrochemical detection as described by Walter et al.[52] The lower limit of quantification was 0.31 μg/L for haloperidol and reduced haloperidol. The limit of detection was 40 pg for haloperidol and 50 pg for reduced haloperidol. The interassay coefficient of variation was 12.8% at 1.25 μg/L and 5.6% at 7.5 μg/L. Each patient sample was measured twice, and the mean was used for further calculations.

CYP2D6 genotyping

Leukocytes were isolated after erythrocyte lysis, and deoxyribonucleic acid (DNA) was extracted in phenol-chloroform.[53] DNA samples were processed by polymerase chain reaction for detection of the *CYP2D6* alleles **1* to **15* and **17*, as well as for gene duplication of alleles **1* and **2*. The methods of *CYP2D6* genotyping have been described previously.[54] As shown in Table I, the *CYP2D6* genotypes were classified into groups with no active gene (poor metabolizers), 1 active gene (intermediate metabolizers), 2 active genes (extensive metabolizers), or more than 2 active genes (ultrafast metabolizers), that is, individuals with gene duplications of allele **1* or **2*. Genotyping was performed in 172 patients; the blood samples of 3 subjects were lost before DNA extraction. After we had noted a surprisingly low frequency of genotypically identified poor metabolizers, 60% of all genotyping analyses were performed completely in duplicate by different investigators in a blinded fashion. No discrepancies with respect to the classification into the poor metabolizer or extensive metabolizer groups were found.

Data evaluation

Serum trough concentrations of haloperidol and the administered dosages of haloperidol were used to estimate the concentration-time curves by means of the Bayesian approach.[55] In brief, WinNonlin, version 1.5 (Pharsight Corporation, Mountain View, Calif), was used for the modeling of all individual concentration data on the basis of the complete individual dosing history. Concentrations after oral dosage and intramuscular dosage of the depot form of the drug were modeled according to a first-order absorption and 1-compartment disposition model. A population bioavailability of 60% for oral dosages, a bioavailability of 100% for intramuscular and intravenous application, a total systemic clearance of 11.9 $mL \cdot min^{-1} \cdot kg^{-1}$ (standard deviation [SD], 2.9 $mL \cdot min^{-1} \cdot kg^{-1}$), and a volume of distribution of 18 L/kg (SD, 7 L/kg) were applied,[56] whereas absorption constants after oral dos-

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age of haloperidol and intramuscular dosage of haloperidol decanoate were fixed at 1.5 h^{-1} and 0.006 h^{-1}, respectively. By use of this Bayesian approach, the estimation of the individual pharmacokinetic parameters was stabilized through use of population data from the literature. It should be considered, however, that the variability resulting from the *CYP2D6* genotype might be slightly underestimated because the Bayesian approach as applied here may direct parameter estimation toward one common population mean not differentiated by genotype. Fig 1 shows the approximated concentration-time curves of 2 patients to illustrate how the individual dose schemes of each patient were considered in the pharmacokinetic analysis. In Fig 1, *A*, the patient received haloperidol first orally and then intravenously; in Fig 1, *B*, haloperidol was administered intramuscularly and orally. Fig 1, *B*, also illustrates how the combined oral and intramuscular medication results in high serum concentrations; this combined treatment was applied in 20% of the patients included in this study.

Statistics

Subgroups were formed according to the number of active *CYP2D6* genes: 0 (poor metabolizers), 1 (intermediate metabolizers), 2 (extensive metabolizers), and more than 2 (ultrafast metabolizers). Nonparametric tests were used to evaluate differences between these groups (Kruskal-Wallis test or, as a test for trend, the Jonckheere-Terpstra test with a predefined trend according to the number of active *CYP2D6* genes). All tests were performed with SPSS, version 10 (SPSS Inc, Chicago, Ill).

Comedication was assessed as CYP2D6-inhibitory, CYP3A4-inhibitory, and CYP3A4-inducing. Inhibitory drugs were given a score of 1, inducing drugs were given a score of −1, and substances without interaction were given a score of 0. If more than one comedication was administered, the scores were summed. Clearance was a dependent variable, and age, gender, weight, smoking habits, *CYP2D6* genotype, and comedication factors were independent variables. The influence of factors such as age, weight, gender, smoking habits, and comedication on haloperidol clearance was tested with multiple linear regression analysis.

RESULTS

Among the 175 patients included, 5 (2.9%) were CYP2D6 ultrafast metabolizers (>2 active alleles), 106 (60.6%) were extensive metabolizers with 2 active genes, 56 (32.0%) were intermediate metabolizers with 1 active gene, and only 5 (2.9%) were identified by genotype as poor metabolizers (ie, subjects without any CYP2D6 activity for genetic reasons). A list of the allele combinations found is presented in Table I (no blood samples for genotyping were available from 3 patients).

Table I. *CYP2D6* genotypes of patients

*Metabolic phenotype**	*Total No. of patients*	*CYP2D6 genotypes†*	*No. of patients*
Ultrafast	5 (3%)	*2x2/1*	3
		2x2/2	2
Extensive	106 (61%)	*2x2/4*	1
		1/1	23
		1/2	49
		2/2	20
		1/10	1
		1/17	1
		1/9	2
		1x2/3	1
		2/10	5
		9/10	1
		9/9	1
		2/9	1
Intermediate	56 (32%)	*2/3*	4
		2/4	13
		2/5	3
		2/7	1
		1/3	7
		1/4	18
		1/5	3
		1/6	2
		3/9	1
		4/10	3
		4/9	1
Poor	5 (3%)	*4/4*	3
		4/5	1
		4/6	1

*Prediction of metabolic activity of CYP2D6 according to genotype. Poor metabolizer, zero active genes; intermediate metabolizer, 1 active gene; extensive metabolizer, 2 active genes; ultrafast metabolizer, 3 or more active genes.
†According to the international nomenclature.[69]

The mean haloperidol serum trough concentration was 8.4 μg/L (SD, 6.7 μg/L). The broad variability in serum concentrations is only partially explained by the different doses administered. The coefficient of determination (r^2) of dose was 0.31 ($P < .001$ for correlation) for haloperidol serum concentration and 0.10 ($P < .001$) for reduced haloperidol serum concentration, when correlated with the dose administered within 22 hours before the blood sample was drawn. The frequency distributions and the correlations with the previously given doses are illustrated in Fig 2.

The group mean of haloperidol concentrations for each CYP2D6 metabolizer group was calculated from the mean haloperidol concentrations for every

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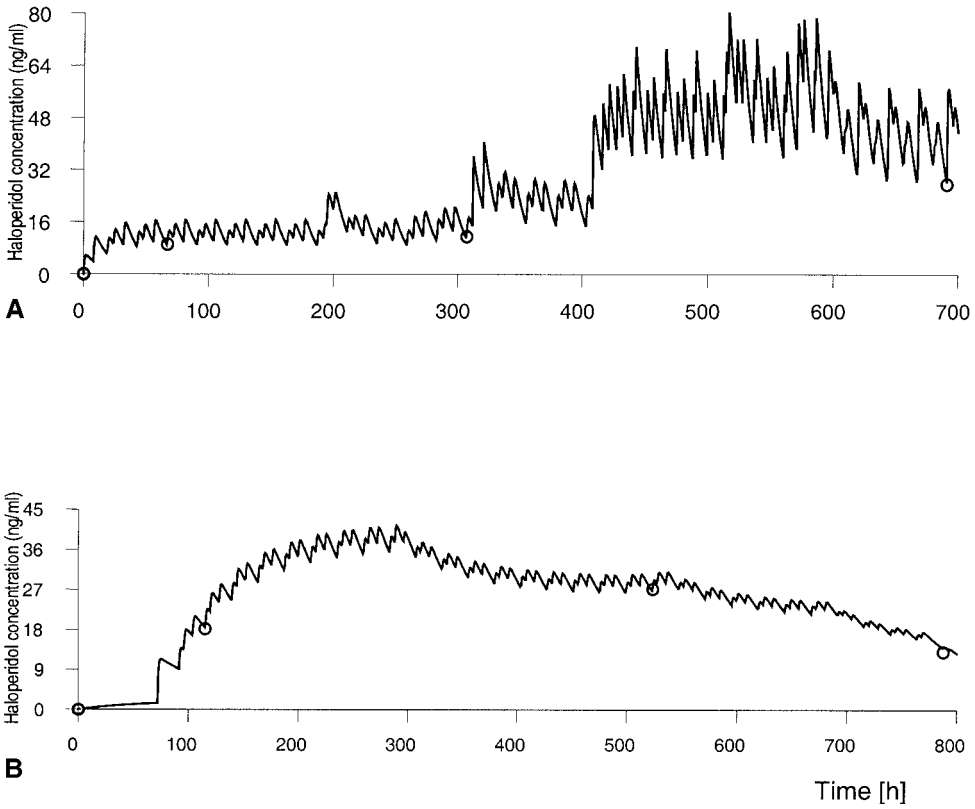

Fig 1. Illustration of typical concentration-time courses of haloperidol in 2 patients who received different application forms of haloperidol. **A,** Haloperidol serum concentration in a patient who received multiple intravenous and oral doses. **B,** Haloperidol concentration in a patient who received haloperidol intramuscularly and orally. The haloperidol concentrations measured are depicted by *circles*.

haloperidol-treated patient. A trend toward higher haloperidol serum concentrations was detected in individuals with low CYP2D6 activity or without CYP2D6 activity (Table II). This trend was statistically significant ($P = .05$, Jonckheere-Terpstra trend test) when serum concentrations were normalized for the total dose administered within the 24-hour period before blood samples were taken. The total haloperidol clearance was calculated by taking each individual dose into account, and this parameter depended significantly on the number of active alleles of *CYP2D6* ($P = .034$). As illustrated in Fig 3, individuals with more than 2 active alleles, classified as ultrafast metabolizers, had the highest total haloperidol clearance (mean, 57.3 L/h), which was 60% higher than total clearance in the poor metabolizer group (mean, 34.7 L/h).

Genotypically identified poor metabolizers also showed significantly higher serum levels of reduced haloperidol ($P = .004$, Jonckheere-Terpstra trend test) (Table II). The CYP2D6 effect on reduced haloperidol levels is illustrated in Fig 4; the effect was even more pronounced for the ratio of reduced haloperidol to haloperidol ($P = .002$).

Multivariate analysis revealed that body weight, smoking, and number of active *CYP2D6* alleles were the most important determinants of haloperidol clearance, whereas the comedication exhibited only moderate effects (Table III). For CYP3A4 inhibitors and inducers, correlations were not statistically significant.

Of the patients, 63% were smokers and 37% were nonsmokers. The mean haloperidol clearance was only marginally higher in smokers than in nonsmokers (49.7 L/h versus 44.3 L/h). The correlation between the number of cigarettes smoked per day and haloperidol serum concentrations was not statistically significant, but the corresponding correlation with reduced haloperidol was statistically significant ($P = .02$, Spearman rank correlation analysis).

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