LACK OF CYP3A4 INHIBITION BY GRAPEFRUIT JUICE AND KETOCONAZOLE UPON CLOZAPINE ADMINISTRATION *IN VIVO*

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SUMMARY

The drug-food and drug-drug interaction between grapefruit juice (GFJ) and ketoconazole (KETO) was evaluated in schizophrenic patients given a single dose of clozapine (CLZ). CLZ is metabolized primarily by CYP isozymes 3A4 and 1A2 to two principal metabolites, desmethylclozapine (DCLZ) and clozapine *N*-oxide (CNO). GFJ and KETO are well known potent CYP 3A4 inhibitors in the gastrointestinal tract and hepatic isozymes, respectively. Twenty-one schizophrenic patients participated in the co-administration of CLZ 50 mg and GFJ. After a one-week washout, five patients were given double the GFJ (HGFJ) dose for 7 consecutive days. In another group of five patients, ketoconazole (KETO) 400 mg was given for 7 consecutive days. At the end of the 7-day period for both groups, CLZ was co-

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administered with the HGFJ and KETO groups. CLZ, DCLZ and CNO were assayed by HPLC. GFJ, HGJF and ketoconazole failed to significantly change CLZ disposition. Metabolites DCLZ and CNO concentrations remained unchanged during the study. The only exception was decreased C_{max} in DCLZ and CNO concentrations. These results indicate that CYP 3A4 inhibition may not be clinically significant compared to CYP 1A2, as previous studies show a dramatic increase in CLZ plasma concentrations with fluvoxamine (CYP 1A2 inhibitor). The reasons for the lack of drug-food and drug-drug interactions with CLZ and CYP 3A4 inhibitors can be explained by the higher K_i values for gastrointestinal and hepatic CYP 3A4 isozymes.

KEY WORDS

clozapine, grapefruit juice, ketoconazole, drug interactions

INTRODUCTION

Clozapine (CLZ) is an atypical antipsychotic medication used mainly for treatment-resistant schizophrenic patients /1,2/. Unlike other typical antipsychotics, CLZ does not produce significant extrapyramidal side effects (EPS). However, CLZ produces agranulocytosis in about 1-2% of treated patients. Therefore, CLZ use is restricted to refractory patients who are also compliant with the required complete blood count monitoring.

CLZ metabolism has been extensively evaluated in animal and human models /3/. CLZ is converted into two principal metabolites - desmethylclozapine (DCLZ) and clozapine *N*-oxide (CNO) /2/. Numerous other metabolites have been identified in humans; however, these metabolites are in much smaller quantities than DCLZ and CNO and are not measured in clinical studies /4,5/.

Plasma concentrations of DCLZ and CNO exhibit wide interpatient variability. Between 10-75% and 10-30% of a CLZ dose is converted to DCLZ and CNO, respectively. Conversion to these principal metabolites and other CLZ metabolites has been thoroughly investigated in *in vitro* and *in vivo* models /2/. Metabolism to these metabolites comprises a variety of cytochrome P450 isozymes: CYP

1A2, 3A4, 2C9, 2D6, and 2C19 /6-10/. Investigations have suggested that the main CYP isozymes involved in the conversion to DCLZ are CYP 3A4 and 1A2, whereas only CYP 3A4 is involved in the formation of CNO /11/. In addition to CYP isozymes, other enzymes systems such as the flavin monooxygenases (FMOs) have been shown to metabolize CLZ /12/.

A variety of drug-drug interactions have been reported with CLZ /13-15/, but only a few of these drug-drug interactions have been extensively evaluated. Compounds that stimulate CYP 1A2 activity (e.g. cigarette smoking) reduce plasma CLZ concentrations, whereas potent CYP 1A2 inhibitors (e.g. fluvoxamine) dramatically elevate amounts in the body. Fluvoxamine was reported to significantly elevate plasma CLZ concentrations while slightly lower (but statistically not significant) plasma DCLZ and CNO concentrations were found /5/.

Erythromycin is a macrolide antibiotic and a known CYP 3A4 inhibitor, as reported in two CLZ-treated patients who experienced toxic reactions that included seizures, slurred speech, disorientation and urinary incontinence /16,17/. CLZ plasma concentrations were not measured prior to CLZ treatment, and only one report measured steady-state CLZ levels when restarting the drug after a temporary cessation /17/. CLZ plasma concentration during toxicity was 1,150 µg/l (600 mg dose) when erythromycin was added, compared to 385 µg/l without the antibiotic at the same drug dose.

The interaction between erythromycin and CLZ was evaluated in 12 healthy male volunteers /18/. Addition of erythromycin did not alter any pharmacokinetic parameter of CLZ or its metabolites, DCLZ and CNO. These findings suggest that clinically relevant erythromycin dosages do not inhibit CLZ metabolism, and possibly the CYP 3A4 pathway is of lesser importance than CYP 1A2. Itraconazole is a widely used oral antimycotic and is a potent hepatic CYP 3A4 inhibitor /19/. Itraconazole was shown not to affect steady-state CLZ and DCLZ concentrations in seven schizophrenic patients, supporting the hypothesis that CYP 3A4 may be of minor importance in CLZ metabolism /19/. Ketoconazole is a more potent CYP 3A4 inhibitor than itraconazole and erythromycin. *In vitro* models have clearly demonstrated that ketoconazole inhibits CLZ metabolism /6-9/. Therefore, to clearly demonstrate *in vivo* that drug interactions occur with CYP 3A4, ketoconazole co-administration with CLZ is needed.

Grapefruit juice (GFJ) is well known to produce significant gastrointestinal CYP 3A4 inhibition. Many different drugs are substrates of CYP 3A4 and have been shown to be altered in their pharmacokinetic profiles when co-administered with GFJ /20,21/. A small study with nine patients did not report changes in steady-state CLZ and DCLZ plasma concentrations /22/. The aims of this study were to evaluate *in vivo* interactions with gastrointestinal and hepatic CYP 3A4 inhibitors, GFJ and ketoconazole (KETO), upon CLZ administration in schizophrenic patients.

MATERIALS AND METHODS

Subjects

Twenty-one stable non-smoking male schizophrenic patients (mean age 28.2 ± 8.5 years, mean weight 67.6 ± 9.4 kg) gave informed consent to participate in this study. The study was approved by the facility's Institutional Review Board prior to commencement. Each patient met the DSM-IV criteria for schizophrenia, had not been previously treated with CLZ, and had not consumed beverages containing caffeine, alcohol, or grapefruit juice for at least one month prior to the study. Patients had also not taken any known CYP 1A2 or CYP 3A4 inducers or inhibitors or other routinely prescribed medications for a minimum of two weeks. Patients were washed out from their routine antipsychotic medications one week prior to CLZ administration. Only lorazepam 2 mg "as needed" every 2-4 h was allowed during the study, and not within 24 hours of CLZ administration. If patients clinically deteriorated as reported by their family members or by clinician evaluation, patients were discontinued from study participation and placed on antipsychotic treatment. All patients were evaluated to be physically healthy by physical examination, medical history, and routine hematological, biochemical and urinalysis tests.

Study design

This study was conducted with single clozapine 50 mg (2 x 25 mg tablets) dose. All 21 schizophrenic patients participated; each patient received clozapine 50 mg at 08.00 h and was randomly assigned to

take the drug with 250 ml water or 250 ml regular strength grapefruit juice (Ocean Spray Cranberries, Inc. MA, USA). Venous blood samples (5 ml) were collected in heparinized tubes and obtained prior to the dosage administration and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24, 36, and 48 h after drug administration. After a two-week interval, the patients crossed over to take clozapine plus water or grapefruit juice (GFJ), dependent upon to which group they had been previously assigned. Blood samples were obtained at the same time periods as in the first study. Blood samples were centrifuged at 3000 rpm for 15 min and the separated plasma frozen at -20°C until assay.

After a one-week washout period, five patients participated in a repeat study in which each patient received 500 ml grapefruit juice (Ocean Spray Cranberries, Inc. MA, USA) for 7 consecutive days. On the 7^{th} day, clozapine 50 mg was co-administered with the higher dose of grapefruit juice (HGFJ). Venous blood samples (5 ml) were collected at the same time points and then separated plasma frozen as stated above.

In another group of five patients, after a one-week washout period from regular strength GFJ, each patient received 400 mg ketoconazole for 7 consecutive days. On the 7^{th} day, clozapine 50 mg was co-administered with the ketoconazole. Venous blood samples (5 ml) were collected at the same time points and then separated plasma frozen as stated above.

Laboratory procedure and data analysis

CLZ, DCLZ, and CNO were assayed by high performance liquid chromatography (HPLC) with ultraviolet detection. The intra-assay and interassay coefficients of variation were 8.0-14.7% at 50 ng/ml for CLZ and its metabolites. The lower limits of detection were 1 ng/ml for CLZ and 2 ng/ml for DCLZ and CNO /23/. All samples were assayed in duplicate.

The pharmacokinetic parameters for CLZ and its metabolites were determined by a model-independent method with non-linear least-square regression analysis (WinNONLIN). The area under the plasma concentration time curve (AUC) up to 48 h was calculated according to the trapezoidal method and extrapolated to infinity by dividing the last concentration measured (C48) by the elimination rate constant (K_{el}). Oral clearance (CL/F) was determined by dividing the oral dose (D) by the AUC. The elimination half-life was determined according

to ln2 divided by K_{el}. Values for maximal concentration (C_{max}), time to maximal concentration (T_{max}), and plasma concentrations 12 and 24 hours after drug administration (C12 and C24) were determined by visual inspection of the data for each patient. Student's paired two-tailed t-test was used to compare the pharmacokinetic parameters of CLZ and its metabolites before and after the addition of GFJ in the first section of the study. A two-way analysis of variance (ANOVA) with repeated measures was used to compare the parameters of CLZ and its metabolites when the HGFJ and the ketoconazole were co-administered. Statistical significance was defined as $p < 0.05$.

RESULTS

Twenty-one schizophrenic patients were administered CLZ and CLZ plus GFJ in a cross-over study. Adverse side effects were not observed in any patient during CLZ administration with or without the lower dose of GFJ. A wide inter-patient variability in CLZ disposition was observed. Addition of GFJ did not result in any significant changes in CLZ plasma concentrations at any time point (see Fig. 1). A summary of the pharmacokinetic parameters of CLZ and its two metabolites prior to and with the addition of GFJ in the 21 patients is presented in Table 1. No significant change in the disposition of CLZ or its metabolites or any pharmacokinetic parameter was found upon GFJ co-administration.

A smaller number of patients (n = 5) agreed to participate in the HGFJ and ketoconazole sections of the study. When the dose of GFJ was increased and administered for a longer time period of 7 consecutive days, no significant differences were found in CLZ, DCLZ and CNO disposition (Table 2). Plasma CLZ and CNO concentrations were analyzed in all five patients. However, DCLZ concentrations could be only quantified in four patients; DCLZ plasma concentrations were undetectable for one patient due to unknown reasons, most likely due to assay problems that day. A large inter-patient variability was found for CLZ and its metabolite disposition. HGJF did not significantly alter any pharmacokinetic parameter of CLZ, DCLZ and CNO. The AUC for CLZ in the HGFJ group was 71% of that when taken with water. Correspondingly, CLZ clearance was about 25% greater in the HGFJ group than the water or GFJ groups. CNO results showed a decrease in AUC by about 45% and the

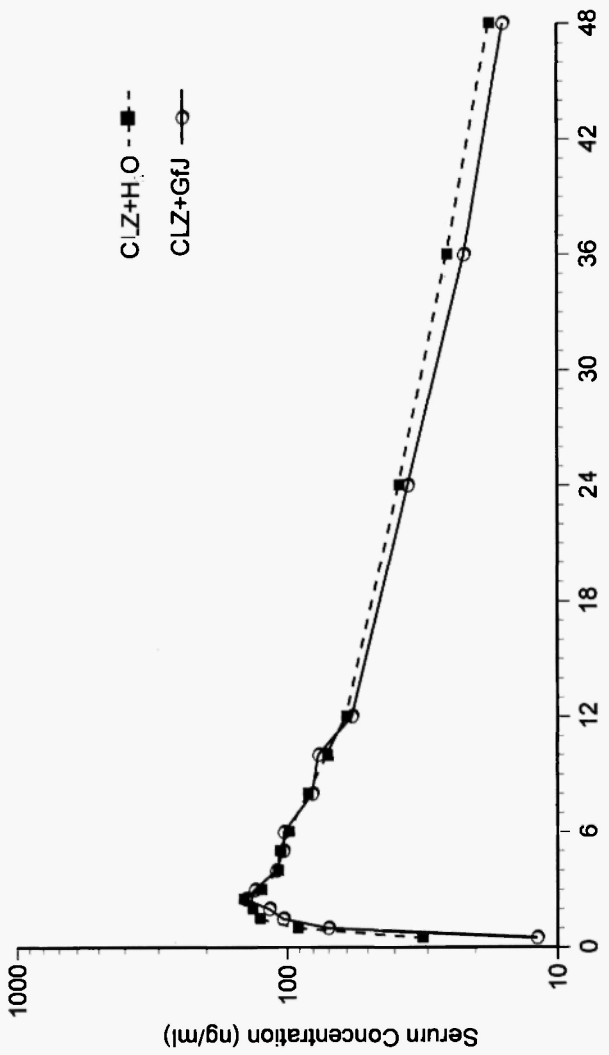

Fig. 1: Plasma levels of clozapine (CLZ) in 21 schizophrenic patients co-administered with water (H_2O, filled squares) or grapefruit juice (GfJ, open circles).

TABLE 1

Summary of the pharmacokinetic parameters (mean ± SD) of clozapine (CLZ) and its metabolites after co-administration with water or grapefruit juice (GFJ) in schizophrenic patients (n = 21)

	CLZ + water	CLZ + GFJ		CLZ + water	CLZ+ GFJ.
CLZ			**Clozapine *N*-oxide (CNO)**		
AUC (ng/ml • h)	2384.2 ± 1278.9	2198.5 ± 1019.6	**AUC** (ng/ml • h)	348.6 ±107.9	334.4 ± 105.6
T_{max} (h)	2.5 ± 1.3	2.6 ± 0.9	T_{max} (h)	2.3 ± 0.8	2.3 ± 0.6
C_{max} (ng/ml)	175.3 ± 91.9	166.5 ± 81.0	C_{max} (ng/ml)	45.2 ± 21.1	42.5 ± 20.9
CL/F (l/h)	25.9 ± 20.0	25.2 ± 18.1	**Desmethylclozapine (DCLZ)**		
K_{el}	0.049 ± 0.023	0.051 ± 0.025	**AUC** (ng/ml • h)	1087.5 ± 444.5	1120.6 ± 454.1
$t_{½}$ (h)	17.2 ± 8.2	20.2 ± 20.3	T_{max} (h)	3.1 ± 2.5	3.7 ± 3.1
			C_{max} (ng/ml)	43.9 ± 11.4	47.4 ± 14.7

AUC = area under the plasma concentration time curve; T_{max} = time to maximal plasma concentration; C_{max} = maximal plasma concentration; CL/F = oral clearance; K_{el} = elimination rate constant; $t_{½}$ = elimination half-life.

TABLE 2

Summary of pharmacokinetic parameters (mean ± SD) of clozapine (CLZ) and its metabolites after co-administration with water, grapefruit juice (GFJ) and double quantity GFJ (HGFJ) in schizophrenic patients (n = 5)

	CLZ + water	CLZ + GFJ	CLZ + HGFJ
CLZ			
AUC (ng/ml • h)	2205.5 ± 746.9	2284.4 ± 713.4	1568.4 ± 691.5
T_{max} (h)	2.2 ± 0.8	4.3 ± 4.4	1.6 ± 0.8
C_{max} (ng/ml)	199.8 ± 98.8	207.3 ± 77.9	126.5 ± 44.7
CL/F (l/h)	20.7 ± 9.2	18.8 ± 8.8	27.5 ± 16.4
K_{el}	0.043 ± 0.022	0.044 ± 0.020	0.040 ± 0.020
$t_{½}$ (h)	19.8 ± 10.2	19.7 ± 11.1	27.9 ± 20.4
Clozapine *N*-oxide (CNO)			
AUC (ng/ml • h)	328.3 ± 53.6	354.4 ± 109.0	175.9 ± 56.6
T_{max} (h)	2.4 ± 1.0	2.6 ± 0.6	2.0 ± 0.7
C_{max} (ng/ml)	55.0 ± 31.9	49.7 ± 32.6	24.1 ± 11.3**
Desmethylclozapine (DCLZ)*			
AUC (ng/ml • h)	1092.1 ± 546.3	1091.9 ± 426.9	787.4 ± 535.3
T_{max} (h)	2.8 ± 1.0	3.3 ± 0.5	2.5 ± 0.5
C_{max} (ng/ml)	50.1 ± 20.4	48.3 ± 22.7	29.7 ± 13.5

See Table 1 for abbreviations.
* n = 4 for DCLZ; ** $p < 0.05$.

only significant finding was a lower C_{max}. The AUC CNO/CLZ ratio was slightly lower with HGFJ, but not statistically significant ($F = 3.284$, $p = 0.153$). Lower DCLZ AUC by about 28% in the HGFJ group compared to the other two groups was also found, but was not statistically significant ($F = 0.707$, $p = 0.428$).

In the study section with KETO, CLZ, DCLZ and CNO were detected; their disposition with water, low dose GFJ and KETO co-administration is presented in Table 3. Similar to the previous findings, a broad variability in CLZ pharmacokinetics was found. GFJ did not significantly alter any pharmacokinetic parameter of CLZ,

TABLE 3

Summary of pharmacokinetic parameters (mean ± SD) of clozapine (CLZ) and its metabolites after co-administration with water, grapefruit juice and ketoconazole (KETO) in schizophrenic patients (n = 5)

	CLZ + water	CLZ + GFJ	CLZ + KETO
CLZ			
AUC (ng/ml • h)	2810.3 ± 1581.9	3148.7 ± 1795.0	1989.6 ± 1756.6
T_{max} (h)	2.4 ± 0.5	2.7 ± 1.9	3.4 ± 1.5
C_{max} (ng/ml)	203.5 ± 101.9	240.8 ± 120.4	110.2 ± 16.0*
CL/F (L/h)	19.1 ± 10.5	17.7 ± 10.8	21.0 ± 13.4
K_{el}	0.046 ± 0.011	0.046 ± 0.019	0.031 ± 0.016
$t_{½}$ (h)	15.8 ± 4.8	19.4 ± 13.5	28.7 ± 16.5
Clozapine *N*-oxide (CNO)			
AUC (ng/ml • h)	403.9 ± 108.8	401.4 ± 147.1	208.1 ± 83.0*
T_{max} (h)	2.5 ± 0.6	2.2 ± 0.5	3.0 ± 0.8
C_{max} (ng/ml)	33.7 ±10.9	39.2 ± 16.2	17.1 ± 6.3*
Desmethylclozapine (DCLZ)			
AUC (ng/ml • h)	1261.3 ± 371.6	1445.6 ±382.9	840.6 ± 299.4
T_{max} (h)	4.0 ± 2.6	4.5 ± 4.4	2.5 ± 0.5
C_{max} (ng/ml)	52.1 ±14.1	54.7 ± 9.7	28.6 ± 13.1*

See Table 1 for abbreviations.
*p <0.05.

DCLZ and CNO. Interestingly, co-administration of KETO resulted in a 29% decrease in CLZ AUC, but this change was found not to be significant (F = 1.958, p = 0.199). CLZ clearance, shown in Figure 2, showed no significant changes between water, GFJ and KETO. One patient actually had a slight increase in CLZ clearance from 35.5 l/kg to 43.5 l/kg. The CLZ elimination half-life was found to be longer with KETO co-administration, but this change was also found not to be significant (F = 0.353, p = 0.568). Significant decreases in CNO AUC (by about 48%, F = 19.121, p = 0.002) and C_{max} (by about 49%, F = 12.215, p = 0.008) were found with KETO. Although CNO AUC decreased, the change in CNO/CLZ AUC ratio did not change significantly (F= 2.373, p = 0.161). A strong statistical trend in DCLZ AUC decrease (by about 33%, F = 3.853, p = 0.051) was found, and

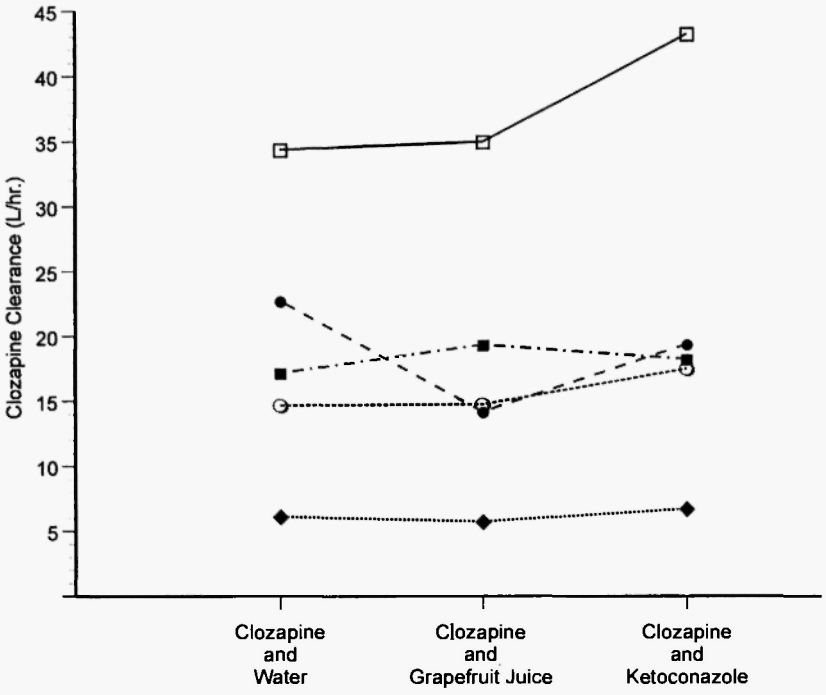

Fig. 2: Individual clozapine clearance in five schizophrenic patients when co-administered with water, grapefruit juice or ketoconazole.

only C_{max} (by about 45%, F = 7.716, p = 0.024) was found to be significant with KETO. Similar to CNO, DCLZ AUC decreased but the DCLZ/CLZ AUC ratio did not change significantly (F = 1.116, p = 0.321).

DISCUSSION

Our results indicate that the CYP3A4 inhibitors GFJ and KETO did not significantly alter the disposition of CLZ or its metabolites, DCLZ and CNO. This potential drug-drug and drug-food interaction was evaluated with CLZ due to earlier reports with a small number of patients or volunteers.

In our study with regular strength GFJ, the disposition of CLZ and its metabolites did not change, as shown in Table 1. When taken with water (active control group), CLZ disposition in this study resembled the previous results of other CLZ pharmacokinetic studies /24-26/. Further, the overall plasma level time course displayed in Figure 1 also demonstrates the lack of effect when GFJ was co-administered with CLZ. Even though some studies previously reported that a single GFJ dose alters the pharmacokinetics of many drugs that are CYP 3A4 substrates /20,21/, this effect was not found with CLZ. Our findings with a larger number of patients with GFJ confirm the previous results of Vandel *et al.* /22/ in whose study CLZ and DCLZ steady-state plasma concentrations remained unchanged when GFJ was given with stable CLZ dosages for at least three months in nine patients (actual daily doses were not included in the article).

Similar to the previous findings with GFJ, although a much smaller number of patients participated, HGFJ also did not significantly increase CLZ plasma concentrations (see Table 2). Interestingly, a decrease in CLZ AUC was found, with ensuing reductions in DCLZ and CNO AUCs. This occurrence may be explained by the wide inter-patient variability and the smaller number of study patients. An interesting observation from Table 2 is that in the HGFJ group, CLZ clearance actually increased by about 25%. Although inter-patient variability is the most likely explanation for this finding, one might speculate that another potential drug interaction mechanism may be involved. Besides GFJ's inhibitory actions upon CYP 3A4, GFJ has also been shown to stimulate the P-glycoprotein drug transport system /27/. Drugs that are P-glycoprotein substrates can show decreased drug

absorption through the gastrointestinal tract, as this active drug transport system promotes drug movement from the intracellular compartment back into the intestinal lumen. Some medications that are CYP 3A4 substrates also appear to be P-glycoprotein substrates, and when given with GFJ would result in decreased drug amounts in the body or increased drug clearance. The decreased drug amounts reflected in CLZ AUC could lead to decreased metabolite concentrations. This occurrence was reported with itraconazole, when drug concentrations decreased with GFJ co-administration /28/. An additional study with a larger number of patients could increase our understanding of the potential interaction with HGFJ.

In our study, KETO also failed to produce significant changes in CLZ disposition, as shown in Table 3. Both DCLZ and CNO AUCs decreased slightly, but this was only statistically significant for CNO ($p = 0.002$). These findings can also be explained by the large inter-patient variability of CLZ metabolism, and the smaller number of patients. CLZ clearance did not show any significant change when GFJ and KETO were compared to water (see Fig. 2). Only one patient had a marginal elevation in CLZ clearance with KETO, which is most likely related to intra-patient variability. The remaining four patients had negligible alterations in CLZ clearance when given with either GFJ or KETO.

These findings support the results of previous studies showing a lack of interaction between CLZ and erythromycin or itraconazole /18,19/. Unfortunately, KETO plasma concentrations were not measured in our study, but the dose used in this study was consistent with the dose used in other studies to produce significant interactions /29/. The results in this study with KETO are similar to the previous study with itraconazole in which three of seven subjects showed a decrease in CLZ serum concentrations after itraconazole co-administration /19/. The lack of interaction between CLZ and KETO *in vivo* considerably differs from the *in vitro* results that reported significant inhibition by KETO. The explanation for these inconsistent findings can be related to the K_i properties of CYP isozymes. It is well known that fluvoxamine is a potent CYP 1A2 inhibitor and produces a significant *in vitro* and *in vivo* drug-drug interaction with CLZ /5,30-32/. Besides CYP 1A2 inhibition, *in vitro* studies showed that fluvoxamine also inhibited other isozymes, CYP 2C19, 3A4 and 2D6 /33,34/. However, the K_i of fluvoxamine inhibitory action upon CLZ

metabolism was about 575 times greater for CYP 1A2 than CYP 3A4 (0.041 μM vs 23.5 μM) /34/. This much higher potency for CYP 1A2 than CYP 3A4 can account for the lack of interaction with specific CYP 3A4 inhibitors with CLZ. The effects of multiple isozymes, including the FMO system /12/, cannot be ruled out and is difficult to evaluate independently.

This study has the limitations of a single dose drug-drug and drug-food interaction evaluation. Single dose drug administration may not reflect changes in drug disposition under steady-state or clinical conditions. Significant interactions that are dose- or time-dependent would not be found in a single dose study. Another potential possibility is that the metabolic inhibitors chosen, GFJ and ketoconazole, were unspecific for the CYP isozymes involved, or that the multiple isozymes involved in CLZ's metabolism obscured these findings.

Drug-drug interactions involving CYP 3A4 are becoming increasingly important in clinical psychopharmacology /35/. However, careful clinical research must be completed prior to providing clinicians with information regarding potential CLZ interactions based upon a few patient reports or with only *in vitro* data /36/. Clinicians must always monitor patients individually, and pay attention both to the wide inter-patient variability in CLZ disposition and the variability in known and potential drug-drug and drug-food interactions.

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