In vitro and *in vivo* evaluation of the inhibition potential of risperidone toward clozapine biotransformation

Manoranjenni Chetty, Fabrizio d'Esposito, Wei V. Zhang, John Glen,¹
Glenys Dore,² Zvijezdana Stankovic,¹ Robert J. Edwards,³
Iqbal Ramzan & Michael Murray

Faculty of Pharmacy, University of Sydney, Sydney, ¹Macquarie Hospital, North Ryde and ²Drug and
Alcohol Service, Royal North Shore Hospital, St Leonards, NSW, Australia, and ³Imperial College of
Medicine, University of London, Hammersmith Hospital, London, UK

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The clozapine (CLZ)–risperidone (RISP) combination is effective in schizophrenic patients but may lead to elevated plasma CLZ concentrations and adverse effects.
- Reports of potential inhibitory effects of RISP on cytochrome P450 (CYP)-dependent CLZ oxidation have been inconsistent.
- It has been suggested that variations in expression of CLZ/RISP oxidases, such as CYPs 1A2, 2D6 and 3A4, may influence inhibitory interactions.

WHAT THIS STUDY ADDS

- Inhibition of CLZ biotransformation by RISP was assessed in microsomal fractions that expressed varying amounts of CYPs 1A2, 2D6 and 3A4.
- RISP did not inhibit the formation of major oxidized CLZ metabolites, regardless of the CYP expression profile; findings from an *in vivo* study in patients were consistent with this finding.
- Pharmacokinetic interactions due to the RISP–CLZ combination are unlikely to involve CYPs.

Correspondence

Dr Michael Murray, Faculty of Pharmacy,
University of Sydney, NSW 2006, Australia.
Tel: + 61 2 9351 2326
Fax: + 61 2 9351 4391
E-mail: michaelm@pharm.usyd.edu.au

Keywords

clozapine, cytochrome P450, drug
interaction, risperidone

Received

10 February 2009

Accepted

2 June 2009

AIMS

To study the impact of risperidone (RISP) on clozapine (CLZ) biotransformation *in vitro* in microsomal fractions containing varying expression of CYP oxidases and *in vivo* in patients.

METHODS

Human liver microsomes ($n = 11$) were assessed for expression of CYPs 1A2, 2D6 and 3A4, because these enzymes mediate RISP and CLZ oxidation. Inhibition of CLZ oxidation by RISP was assessed. Plasma CLZ elimination was estimated in patients with schizophrenia who received either CLZ alone or the CLZ–RISP combination ($n = 10$ per group).

RESULTS

(i) The CYP3A4 and CYP1A2 inhibitors ketoconazole and fluvoxamine inhibited CLZ oxidation to varying extents in individual microsomal fractions. (ii) RISP did not inhibit CLZ oxidation, regardless of variations in CYP expression. (iii) RISP co-administration did not impair CLZ clearance.

CONCLUSIONS

No evidence was found for CYP-mediated inhibitory or pharmacokinetic interactions between RISP and CLZ. Occasional literature reports of such interactions may involve other pathways that participate in CLZ disposition.

Introduction

Clozapine (CLZ) is an important antipsychotic agent, but monotherapy with the drug is often ineffective. The combination of CLZ with low-dose risperidone (RISP) has improved psychiatric ratings in some [1, 2], but not all studies [3, 4]. Reports of toxicity in CLZ–RISP-treated patients may be due to increased plasma CLZ concentrations, but the mechanism of potential pharmacokinetic interactions is unclear [5, 6].

Cytochromes P450 (CYPs) mediate CLZ and RISP oxidation in human liver. CLZ is *N*-demethylated to norCLZ by CYPs 1A2 and 3A4 [7–9] and to CLZ *N*-oxide by CYP3A4 [8–10]. RISP is oxidized by CYP2D6 and, to a lesser extent, CYP3A4 to its 9-hydroxy metabolite [11, 12]. Inhibition of CYP-dependent CLZ oxidation by RISP could account for increased plasma CLZ concentrations during concurrent therapy. However, inhibition of CYP1A2 or CYP2D6 is unlikely because of the selective roles of these enzymes in CLZ and RISP oxidation, respectively. Instead, there is potential overlap with CYP3A4, which contributes to the biotransformation of both drugs. In this study we tested the inhibition by RISP of CLZ oxidation *in vitro* in human liver microsomes in which CYPs 1A2, 3A4 and 2D6 were expressed to varying extents. CLZ clearance was also estimated in patients who received the drug alone or in combination with RISP. The principal finding was that RISP minimally influenced microsomal CLZ oxidation and RISP did not influence CLZ clearance in schizophrenic patients. Thus, pharmacokinetic interactions due to the CLZ–RISP combination are unlikely to be mediated by CYP inhibition.

Methods

Materials

CLZ, RISP, fluvoxamine (FLUV), ketoconazole (KETO), quinine (QUIN), norCLZ, CLZ *N*-oxide and biochemicals were from Sigma Aldrich (Castle Hill, NSW, Australia) or Roche Diagnostics (Castle Hill, NSW, Australia). High-performance liquid chromatography (HPLC)-grade solvents were from Rhone-Poulenc (Baulkham Hills, NSW, Australia) and analytical reagents were from Ajax (Sydney, NSW, Australia). Reagents for sodium dodecylsulphate (SDS)–polyacrylamide gel electrophoresis were from Bio-Rad (Richmond, CA, USA). Hyperfilm-MP, Hybond-N⁺ filters, and reagents for enhanced chemiluminescence (ECL) were from Amersham GE Healthcare (Rydalmere, NSW, Australia). The antiCYP peptide antibodies have been reported previously [13].

Liver donors and preparation of microsomal fractions

Studies in human liver microsomes were approved by ethics committees of the Western Sydney Area Health

Service and the University of Sydney, in accordance with the Declaration of Helsinki. Surplus tissue from liver donors and biopsies ($n = 11$) was obtained through the Queensland and Australian Liver Transplant Programs (Princess Alexandra Hospital, Brisbane, Queensland, and Royal Prince Alfred Hospital, Sydney, NSW, Australia, respectively). Tissue was perfused with cold Viaspan solution (DuPont, Wilmington, DE, USA) and frozen in liquid nitrogen. Information on recent drug intake was available for six of the donors and included adrenaline, ceftriaxone, desmopressin, dexamethasone, dopamine, flucloxacillin, imipenem, penicillin, rantidine, spironolactone and thyroxine; seven subjects were cigarette smokers. Hepatic microsomes were prepared by differential ultracentrifugation [14].

HPLC analysis of CLZ biotransformation

Incubations (37 °C) contained microsomal protein (0.2 mg) and CLZ (100 μM) in potassium phosphate buffer (0.1 M, pH 7.4; 250 μl); reactions were initiated with reduced nicotinamide adenine dinucleotide phosphate (1 mM) and terminated after 15 min with ice-cold formic acid (0.1%, 1 ml). Metabolite formation was linear under these conditions. Inhibitory effects of RISP, FLUV, KETO and QUIN on CLZ oxidation were determined in duplicate in microsomal fractions at two different inhibitor concentrations.

CLZ metabolites were isolated by solid-phase extraction on Oasis HLB cartridges (Waters Corp., Milford, MA, USA) and resolved on a Synergy Fusion-RP polar embedded C₁₈ column (250 × 4.6 mm, particle size 4 μm; Phenomenex Australia Pty Ltd, Pennant Hills, NSW, Australia) as described previously [9].

Other assays of CYP function in human hepatic microsomes

7-Ethoxyresorufin *O*-deethylation (EROD), testosterone 6β-hydroxylation, midazolam 1'-hydroxylation and dextromethorphan *O*-demethylation activities were estimated as described elsewhere [15–18]. Microsomal protein (15 μg) was electrophoresed on 7.5% polyacrylamide gels containing 5% 2-mercaptoethanol and 2% SDS [14]. After transfer to nitrocellulose, antiCYP peptide antibodies [13] were used for CYP quantification by ECL and densitometry (Bio-Rad).

Patient studies

The patient study was approved by the Human Research Ethics Committees of the Royal North Shore Hospital and the University of Sydney. Inpatients at Macquarie Hospital (North Ryde, NSW, Australia), aged between 20 and 60 years and diagnosed with schizophrenia or schizoaffective disorder, were recruited after written informed consent was obtained. Twenty patients were available with 10 assigned to either the CLZ (seven male, three female;

seven smokers) or CLZ–RISP (six male, four female; seven smokers) group. Concurrent prescription medications included amoxicillin/clavulanate, benztropine, finasteride, fluvastatin, levonorgestrel/ethinyl oestradiol, lithium carbonate, metformin, quetiapine, ranitidine, salbutamol, trihexyphenidyl and venlafaxine, which have not been associated with pharmacokinetic interactions with CLZ or RISP, and citalopram, paroxetine, sertraline and valproate, which may occasionally elicit pharmacokinetic interactions with the drugs. Patients were on their current therapy for at least 1 month and were matched within groups for age, weight, CLZ dose (250–750 mg day⁻¹), smoking habit and concurrent medication.

CLZ and norCLZ concentrations in human plasma

Blood was taken from the median cubital vein 12 h after the evening dose of CLZ and collected into lithium heparin tubes. Plasma was separated by centrifugation, frozen at –18 °C and transported to Hunter Area Pathology (Newcastle, NSW, Australia) where CLZ and norCLZ concentrations were determined [19]. The limit of quantification was 10 ng ml⁻¹ for CLZ and norCLZ and assays were linear over the range 10–2000 ng ml⁻¹. The coefficients of variation over 20 runs at 100 ng ml⁻¹ were 5.6% and 2.7%, respectively.

Estimation of CLZ clearance

Population pharmacokinetics software PKS (Abbotbase Version 1.1, Chicago, IL, USA) was used to estimate the plasma CLZ clearance in patients at steady-state. Literature pharmacokinetic parameters were V_d (volume of distribution) = 5.4 l kg⁻¹; CL (clearance) = 0.37 l h⁻¹ kg⁻¹; K_a (absorption rate constant) = 1 h⁻¹; F (bioavailability) = 0.7 [20, 21]. Individual plasma concentration data were fitted to a one-compartment first-order model by the Bayesian technique, which allows clearance estimation using sparse plasma concentration data from individual patients. Predictive power was established with a separate patient dataset; calculated vs. observed serum CLZ concentrations were well correlated ($r^2 = 0.97$).

Statistical analysis

Kolmogorov–Smirnov analysis indicated that the clinical data were not normally distributed. The Mann–Whitney U -test was used to compare CLZ clearance (CL_{CLZ}), total absolute (mg) and total relative (mg kg⁻¹) dose-corrected CLZ plasma concentrations ([CLZ] mg⁻¹ dose or [CLZ] mg⁻¹ kg⁻¹ dose), norCLZ concentration ([norCLZ]), and the norCLZ/CLZ serum concentration ratio between the two patient groups (SPSS, Version 14, SPSS Inc., Chicago, IL, USA; or Statview, version 4.5, SAS Institute, Cary, NC, USA). Linear regression was used to correlate relative CYP content and CYP activities in microsomes. Inhibition data

are presented as percentage of control (mean $\pm$ SD in each of the 11 available fractions).

Results and discussion

In vitro studies in hepatic microsomes

Median norCLZ formation in the hepatic microsomal fractions was 0.47 nmol mg⁻¹ protein min⁻¹ (range 0.13–1.01), whereas median CLZ N -oxide formation was 0.80 nmol mg⁻¹ protein min⁻¹ (range 0.24–1.80). Comparative data for other activities were: EROD (CYP1A2, median 13 pmol mg⁻¹ protein min⁻¹, range 7.3–49), testosterone 6 β -hydroxylation (CYP3A4, median 2.4 nmol mg⁻¹ protein min⁻¹, range 0.6–6.1), midazolam 1'-hydroxylation (CYP3A4, median 41 pmol mg⁻¹ protein min⁻¹, range 10–160) and dextromethorphan O -demethylation (CYP2D6, median 51 pmol mg⁻¹ protein min⁻¹, range 1.1–250). EROD, testosterone 6 β -hydroxylation and dextromethorphan O -demethylation activities in nine of the 11 livers were reported previously [9]. Relationships between CYP1A2, CYP3A4 and CYP2D6 expression and associated substrate oxidations were significant ($r = 0.60$ – 0.86 , $P < 0.01$ – 0.05 , $n = 11$). Microsomal norCLZ formation was correlated with CYP1A2-dependent EROD activity ($r = 0.83$, $P < 0.01$) and CLZ N -oxide formation was correlated with CYP3A4-dependent testosterone 6 β -hydroxylation ($r = 0.74$, $P < 0.01$).

CYPs 2D6 and 3A4 have been implicated in RISP biotransformation. We tested the capacity of RISP to inhibit CLZ biotransformation in microsomal fractions, which contained different levels of CYPs that have been implicated in CLZ and RISP oxidation (Figure 1a). However, RISP (1 or 50 μ M) did not inhibit norCLZ (97 ± 5 and $95 \pm 8\%$ of control, respectively) or CLZ N -oxide (87 ± 12 and $77 \pm 9\%$, respectively) formation (Figure 1b). In one liver RISP (1 and 50 μ M) decreased CLZ N -oxide, but not norCLZ, formation (to 55–60% of control). However, total RISP concentrations in patient plasma are lower than 0.2 μ M [22], so that the clinical significance appears low. In accord with previous findings, the CYP3A4 inhibitor KETO (0.2 and 2 μ M) decreased norCLZ formation (to $75 \pm 29\%$ and $60 \pm 23\%$ of control, respectively) and CLZ N -oxide formation (to $65 \pm 24\%$ and $50 \pm 25\%$ of control, respectively) [9, 10]. Similarly, the CYP1A2 inhibitor FLUV (1 and 10 μ M) decreased norCLZ formation ($78 \pm 26\%$ and $62 \pm 21\%$, respectively), whereas CLZ N -oxide formation was refractory ($92 \pm 16\%$ and $84 \pm 20\%$ of control, respectively). In contrast, the CYP2D6-selective inhibitor QUIN (0.5 and 5 μ M) was ineffective against CLZ biotransformation.

CYP3A4 participates in CLZ oxidation in at least a proportion of subjects [8–10]. In principle, dependence on CYP3A4 for CLZ clearance could render individuals susceptible to drug interactions with RISP if their CYP2D6 capacity is also compromised. Inhibition of CYP3A4 by RISP may

Figure 1

(a) Immunoblot analysis of CYP1A2, CYP3A4 and CYP2D6 expression in human hepatic microsomal fractions. (b) Box plots showing the variation in the inhibition of clozapine (CLZ) biotransformation to norCLZ and CLZ N-oxide by ketoconazole (KETO, 0.2 and 2 μM ; CYP3A4 inhibitor), fluvoxamine (FLUV, 1 and 10 μM ; CYP1A2 inhibitor), quinidine (QUIN, 0.5 and 5 μM ; CYP2D6 inhibitor) and risperidone (RISP, 1 and 50 μM). Inhibitors were tested in duplicate in each of the microsomal fractions (variation < 8% of the indicated mean values)

influence serum concentrations of CLZ [5]. In the present study, however, minimal inhibition of CLZ oxidation was observed in microsomal fractions that expressed CYP3A4, but in which CYPs 2D6 and/or 1A2 were low. Thus, there was little direct evidence for inhibitory interaction involving CYP3A4.

Plasma concentrations of CLZ and CLZ clearance in patients with schizophrenia

Combination therapy in the management of complex psychiatric disorders may offer therapeutic advantages, but increases the potential for pharmacokinetic interactions. RISP may promote CLZ accumulation in plasma [5, 23], and precipitate toxicity [24].

Twelve-hour CLZ plasma concentrations were variable in patients who received CLZ and the CLZ–RISP combination (range 155–523 ng ml^{-1} , median 295; and range 131–859 ng ml^{-1} , median 345, respectively; Table 1). Adjustment of CLZ concentrations for variations in total absolute (mg) dose (ng ml^{-1} per mg dose: range 0.31–1.49; median 0.49 in the CLZ group vs. range 0.24–2.28; median 0.70 in the CLZ–RISP group) or total relative (mg kg^{-1}) dose (ng ml^{-1} per mg kg^{-1} dose: range 24–135; median 46 in the CLZ group vs. range 19–171; median 57 in the CLZ–RISP group) did not alter these findings (differences not significant, Mann–Whitney *U*-test).

CLZ clearance did not differ between the groups (CLZ alone 0.245–0.720, median 0.299 $\text{l h}^{-1} \text{kg}^{-1}$ and CLZ–RISP

Table 1

CLZ pharmacokinetic parameters in patients

Patient	[CLZ] (ng ml^{-1})	[norCLZ] (ng ml^{-1})	[norCLZ]/[CLZ] ratio	CLZ ($\text{l h}^{-1} \text{kg}^{-1}$)
CLZ alone				
1	523	285	0.569	0.658
3	388	57	0.154	0.340
7	307	103	0.351	0.720
8	313	218	0.727	0.245
10	178	163	0.957	0.669
11	251	207	0.862	0.388
12	295	108	0.382	0.516
14	294	268	0.953	0.368
15	286	220	0.804	0.577
17	155	152	1.025	0.667
Median	295	185	0.766	0.299
Range	(155–523)	(57–285)	(0.154–1.025)	(0.245–0.720)
CLZ–RISP combination				
2	281	254	0.945	0.260
4	179	188	1.097	0.770
5	430	291	0.707	0.410
6	750	291	0.405	0.150
9	684	120	0.183	0.326
13	131	167	1.332	0.437
16	408	142	0.361	0.316
18	228	410	1.879	0.347
19	859	764	0.929	0.229
20	180	337	1.956	0.658
Median	345	273	0.937	0.337
Range	(131–859)	(120–764)	(0.183–1.956)	(0.150–0.770)

CLZ, clozapine; RISP, risperidone.

0.150–0.770, median 0.337 l h⁻¹ kg⁻¹; Table 1). Plasma norCLZ concentrations were also unchanged by concurrent RISP administration. In patient 19 plasma [norCLZ] was approximately threefold of the upper value in the CLZ-only group but plasma [CLZ] was also high and the [norCLZ]/[CLZ] ratio was within the normal range (0.93; Table 1).

The results of the present study suggest that CL_{CLZ}, [CLZ], [norCLZ] and the [norCLZ]/[CLZ] ratio in plasma were not significantly altered in patients who also received RISP. Thus, use of the CLZ–RISP combination appears to be safe in most patients and potential pharmacokinetic interactions in which RISP increases serum CLZ concentrations are unlikely to involve competition for CYP oxidation. Instead, it is possible that the function of alternate pathways that influence CLZ disposition may be affected by RISP.

Competing interests

None declared.

This study was supported by grants from the Australian National Health and Medical Research Council, The Rebecca Cooper Foundation and The University of Sydney Research and Development Fund. The authors are grateful to the nursing staff of Macquarie Hospital for their assistance with the clinical study.

REFERENCES

- Henderson DC, Goff DC. Risperidone as an adjunct to clozapine therapy in chronic schizophrenia. *J Clin Psychiatry* 1996; 57: 395–7.
- Morera AL, Baseiro P, Cano-Munoz JL. Risperidone and clozapine combination for the treatment of refractory schizophrenia. *Acta Psychiatr Scand* 1999; 99: 305–7.
- Honer WG, Thornton AE, Chen EY, Chan RC, Wong JO, Bergmann A, Falkai P, Pomarol-Clotet E, McKenna PJ, Stip E, Williams R, MacEwan GW, Wasan K, Procyshyn R. Clozapine alone versus clozapine and risperidone with refractory schizophrenia. *N Engl J Med* 2006; 354: 472–82.
- Josiassen RC, Joseph A, Kohegyi E, Stokes S, Dadvand M, Paing WW, Shaughnessy RA. Clozapine augmented with risperidone in the treatment of schizophrenia: a randomized, double-blind, placebo-controlled trial. *Am J Psychiatry* 2005; 162: 130–6.
- Tyson SC, Devane LC, Risch SC. Pharmacokinetic interaction between risperidone and clozapine. *Am J Psychiatry* 1995; 152: 1401–2.
- Kontaxakis VP, Havaki-Kontaxaki BJ, Stamouli SS, Christodoulou GN. Toxic interaction between risperidone and clozapine: a case report. *Prog Neuropsychopharmacol Biol Psychiatry* 2002; 26: 407–9.
- Bertilsson L, Carillo JA, Dahl ML, Llerena A, Alm C, Bondesson U, Lindstrom L, Rodriguez de la Rubia I, Ramos S, Benitez J. Clozapine disposition covaries with CYP1A2 activity determined by a caffeine test. *Br J Clin Pharmacol* 1994; 38: 471–3.
- Pirmohamed M, Williams D, Madden S, Templeton E, Park BK. Metabolism and bioactivation of clozapine by human liver *in vitro*. *J Pharmacol Exp Ther* 1995; 272: 984–90.
- Zhang WV, D'Esposito F, Edwards RJ, Ramzan I, Murray M. Interindividual variation in relative CYP1A2/3A4 phenotype influences susceptibility of clozapine oxidation to cytochrome P450-specific inhibition in human hepatic microsomes. *Drug Metab Dispos* 2008; 36: 2547–55.
- Eiermann B, Engel G, Johansson I, Zanger UM, Bertilsson L. The involvement of CYP1A2 and CYP3A4 in the metabolism of clozapine. *Br J Clin Pharmacol* 1997; 44: 439–46.
- Fang J, Bourin M, Baker GB. Metabolism of risperidone to 9-hydroxyrisperidone by human cytochromes P450 2D6 and 3A4. *Naunyn Schmiedeberg Arch Pharmacol* 1999; 359: 147–51.
- Bork JA, Rogers T, Wedlund PJ, de Leon J. A pilot study on risperidone metabolism: the role of cytochromes P450 2D6 and 3A. *J Clin Psychiatry* 1999; 60: 469–76.
- Edwards RJ, Adams DA, Watts PS, Davies DS, Boobis AR. Development of a comprehensive panel of antibodies against the major xenobiotic metabolising forms of cytochrome P450 in humans. *Biochem Pharmacol* 1998; 56: 377–87.
- Murray M, Zaluzny L, Farrell GC. Drug metabolism in cirrhosis: selective changes in cytochrome P-450 isozymes in the choline-deficient rat model. *Biochem Pharmacol* 1986; 35: 1817–24.
- Prough RA, Burke MD, Mayer RT. Direct fluorometric methods for measuring mixed function oxidase activity. *Methods Enzymol* 1978; 52: 372–7.
- Murray M. Metabolite intermediate complexation of microsomal cytochrome P450 2C11 in male rat liver by nortriptyline. *Mol Pharmacol* 1992; 42: 931–8.
- Kanazawa H, Okada A, Igarashi E, Higaki M, Miyabe T, Sano T, Nishimura R. Determination of midazolam and its metabolite as a probe for cytochrome P450 3A4 phenotype by liquid chromatography-mass spectrometry. *J Chromatogr A* 2004; 1031: 213–8.
- Vielnascher E, Spatzenegger M, Mayrhofer A, Klinger P, Jäger W. Metabolism of dextromethorphan in human liver microsomes: a rapid HPLC assay to monitor cytochrome P450 2D6 activity. *Pharmazie* 1996; 51: 586–8.
- Lovdahl MJ, Perry PJ, Miller DD. The assay of clozapine and N-desmethylclozapine in human plasma by high-performance liquid chromatography. *Ther Drug Monit* 1991; 13: 69–72.
- Guitton C, Kinowski JM, Gomeni R, Bressolle F. A kinetic model for the simultaneous fit of clozapine and norclozapine concentrations in chronic schizophrenic patients during long-term treatment. *Clin Drug Investig* 1998; 16: 35–43.

- 21** Doude van Troostwijk LJA, Koopmans RP, Vermeulen HDB, Guchelaar HJ. CYP1A2 activity is an important determinant of clozapine dosage in schizophrenic patients. *Eur J Pharm Sci* 2003; 20: 451–7.
- 22** Nesvåg R, Hendset M, Refsum H, Tanum L. Serum concentrations of risperidone and 9-OH risperidone following intramuscular injection of long-acting risperidone compared with oral risperidone medication. *Acta Psychiatr Scand* 2006; 114: 21–6.
- 23** Koren AR, Lieberman JA, Kronig M, Cooper TB. Cross-tapering clozapine and risperidone. *Am J Psychiatry* 1995; 152: 1690.
- 24** Godleski LS, Sernyak MJ. Agranulocytosis after addition of risperidone to clozapine treatment. *Am J Psychiatry* 1996; 153: 735–6.