THE 1-(2,3-DICHLOROPHENYL)-PIPERAZINE-TO-ARIPIPRAZOLE RATIO AT STEADY STATE IN RATS AND SCHIZOPHRENIC PATIENTS

Milena Colovic[a], Giuseppe Campiani[b], Stefania Butini[b], Alberto Parabiaghi[a] and Silvio Caccia[a]

[a]*Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy*
[b]*Dipartimento Farmaco Chimico Tecnologico and European Research Centre for Drug Discovery and Development, Universita` di Siena, Siena, Italy*

Summary

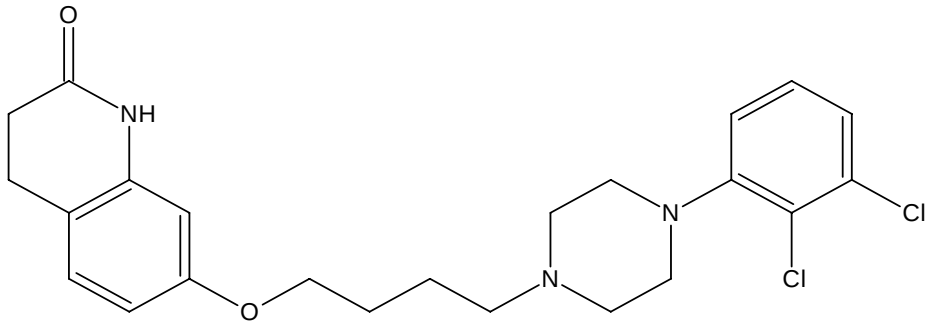
Aripiprazole metabolism includes N-dealkylation to 1-(2,3-dichlorophenyl)-piperazine which was present as a minor metabolite in serum of eight schizophrenic outpatients taking 5-15 mg/day. As phenylpiperazines concentrate in the brain, causing serotonin receptor-related effects, the authors examined the compounds' concentrations in brain of rats given 10 mg/kg aripiprazole dihydrochloride orally, approximately every half-life for five half-lives, and evaluated the metabolite-to-parent drug ratio at steady state. The metabolite had higher brain uptake (about 26) than aripiprazole (about 5) and therefore the metabolite-to-parent drug ratio was higher in brain (0.43 ± 0.07, on molar basis) than in blood (0.08 ± 0.01). However, assuming that the compounds concentrate in human brain to the same extent as in the rat, one can conclude that at the site of action the metabolite represents only about 6% of aripiprazole. It is therefore conceivable that the metabolite contributes to the central effects of aripiprazole for rats, but this is unlikely for patients taking therapeutic doses.

Key Words: Aripiprazole; 1-(2,3-dichlorophenyl)-piperazine; brain-to-blood distribution; metabolite-to-parent drug ratio

With a pharmacological profile that differs from currently marketed antipsychotics for the treatment of schizophrenia and schizoaffective disorders, aripiprazole is a first-in-class drug. It causes partial agonism at dopamine D2 receptors, whereas all other effective antipsychotics act as pure antagonists [1]. Like other atypical agents it shows strong serotonin $(5\text{-HT})_{2A}$ antagonism but is similar to only some of them [2] in also having partial agonistic activity at the 5-HT_{1A} receptor [3]. On the basis of animal studies it has been suggested that aripiprazole stabilizes both the dopaminergic and serotoninergic systems, an action that may improve the positive and negative symptoms of schizophrenia [4].

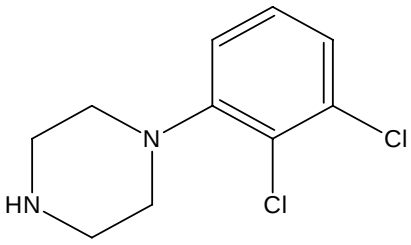
Structurally too aripiprazole, a quinolinone piperazine derivative, differs from other antipsychotics. However, perospirone, ziprasidone and their related compound tiospirone also have an arylpiperazine moiety in their chemical structure, namely a diclorophenylpiperazine in the new antipsychotic and benzisothiazolepiperazine in its older derivatives.

Like for arylpiperazines in general, cytochrome P450 (CYP)3A4-mediated N-dealkylation of the aliphatic chain attached to the piperazine nitrogen occurs for all these antipsychotics [5, 6]. For aripiprazole it results in the formation of 1-(2,3-dichlorophenyl)-piperazine (2,3-ClPP) (Fig. 1) which reaches the systemic circulation after oral aripiprazole, although at much lower concentrations than the parent drug and its major metabolite dehydroaripiprazole in healthy subjects [7].



ARIPIPRAZOLE

CYP3A4



1-(2,3-DICLOROPHENYL)-PIPERAZINE

Figure 1. The biotransformation of aripiprazole to 1-(2,3-dichlorophenyl)-piperazine in man

Little is known about the pharmacological properties of 2,3-ClPP. In binding studies it has lower affinity than aripiprazole for D2 and D3 receptors, supporting pharmacokinetic findings that this metabolite presumably does not contribute to the final outcome [7]. However, as phenylpiperazines such as the well known 1-(2-chlorophenyl)-piperazine (mClPP) cause a variety of serotonin (5-HT) receptor-related neurochemical, neuroendocrine and behavioural effects [8, 9] the metabolite 2,3-ClPP might have similar actions in sensitive species.

Since mClPP and 1-aryl-piperazines in general concentrate markedly in brain tissue [10] the 2,3-ClPP-to-aripiprazole concentration ratio in the central nervous system may differ from that expected from blood measurements. Therefore the contribution of 2,3-ClPP to the activity of aripiprazole remains to be clearly established.

In this study we measured the concentrations of 2,3-ClPP and its parent drug in serum of schizophrenic patients taking therapeutic doses of aripiprazole, to preliminarily evaluate the steady-state concentrations of 2,3-ClPP relative to its parent drug. The concentrations of aripiprazole and 2,3-ClPP were also evaluated in serum and brain of rats given repeated doses of aripiprazole to find out to what extent the two compounds concentrate in brain tissue, compared to serum, under steady-state conditions.

Methods

Subjects

Seven females and one male with a diagnosis of schizophrenia of any subtype according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), taking aripiprazole, participated in this study. Their age ranged from 36–44 years and their weights from 57–96 kg. There was one smoker, and one of the females was taking contraceptive steroids. All were physically healthy outpatients. These data and those regarding the dosing regimen and the interval between the last dose and the sampling time were provided by the clinicians. The study conformed to the Declaration of Helsinki. Consent was obtained as per Institutional Review Board instructions before collection of the specimens.

Five patients took their daily aripiprazole dose at night and three in the morning. Serum was obtained by cubital venipuncture about 11 h after the evening dose or 1.5 h after the morning dose. This was done at the clinical centres when patients attended for routine blood tests. The blood was allowed to clot at room temperature for 30–60 minutes in the collecting tube, and then the samples were centrifuged at 2500 *g* for 10 minutes. The serum was transferred to a new test tube and stored at -20°C.

Animals and drug treatment

Male Sprague-Dawley CD-COBS rats (Charles River, Italy) weighing about 200 g were used. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl. 40, 18 Febbraio 1992, Circolare No. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJL 358, 1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

Rats were given five oral doses of 10 mg/kg of aripiprazole dihydrochloride (8.6 mg/kg free base) at 1.5 h intervals. The drug was dissolved in water and administered in a volume of 10 mL/kg. The animals were killed by decapitation under deep anesthesia 2 h after the last dose. Serum and brain samples were stored at -20°C.

Because pure aripiprazole was not available at that time for these studies it was synthesized at Dipartimento Farmaco Chimico Tecnologico of University of Siena. The key intermediate, 7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one, was obtained by cyclization of 3-chloro-*N*-(3-methoxyphenyl)propanamide, in turn obtained after reaction between *m*-methoxyaniline and 3-chloropropionyl chloride. Then the lactone was O-alkylated to give 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1*H*)-one as previously described [11]. This was used as the alkylating agent for commercially available 2,3-ClPP to obtain aripiprazole, from which the water-soluble dihydrochloride salt was obtained by a standard procedure. The structure of all compounds was assessed by spectroscopic analysis (NMR and mass). The chemical-physical data of aripiprazole (results not shown) were identical to those reported by Oshiro et al. [11]. Briefly:

3-Chloro-N-(3-methoxyphenyl)propanamide. To a cold solution of *m*-methoxyaniline (8.12 mmol) in dichloromethane (20 mL) and pyridine (8.12 mmol), a solution of 3-chloropropionyl chloride (8.93 mmol) in dichloromethane (20 mL) was added. The reaction mixture was stirred at 25°C overnight. Then water and 4N HCl were added and the organic phase was washed with a saturated solution of $NaHCO_3$. The crude brown solid was recrystallized from ethyl acetate/hexane to give 3-chloro-*N*-(3-methoxyphenyl)propanamide as a white crystalline solid, (yield 96.5%).

7-Hydroxy-3,4-dihydroquinolin-2(1H)-one. In a sealed tube NaCl (11.9 mmol), KCl (9.32 mmol) and $AlCl_3$ (38.3 mmol) were added to 3-chloro-*N*-(3-methoxyphenyl)propanamide (4.6 mmol); the mixture was heated at 170 °C for 1h. The mixture was then cooled to 0 °C and 40 mL of H_2O were added. The aqueous phase was extracted with CH_2Cl_2 and Et_2O, the collected organic layers were dried, filtered and evaporated. The crude was purified by flash chromatography (Et_2O) to provide a white solid in 66% yield.

7-(4-Bromobutoxy)-3,4-dihydroquinolin-2(1H)-one. To a solution of the lactone (2.9 mmol) in 10 mL of DMF, Ce_2CO_3 (3.5 mmol) and 1,4-dibromobutane (8.7 mmol) were added. The mixture was stirred for 12 h at 65 °C. Then the solvent was removed and 25 mL of H_2O were added. The aqueous phase was extracted with CH_2Cl_2 and the collected organic layers were dried, filtered and evaporated. The crude was purified by flash chromatography (Et_2O/*n*-hexane, 15:1) to provide a white amorphous solid in 36% yield.

*7-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butoxy)-3,4-dihydroquinolin-2(1H)-one***.** To a solution of 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1H)-one (1.03 mmol) in 15 mL of acetonitrile, NaI (1.5 mmol) was added and the mixture was refluxed for 30 min. 1-(2,3-dichlorophenyl)piperazine hydrochloride (1.1 mmol) and TEA (3.1 mmol) were added and the mixture was refluxed for 3h. Then the solvent was removed, water was added and the aqueous phase was extracted with $CHCl_3$. The collected organic layers were dried, filtered and evaporated. The crude was purified by flash chromatography (CH_2Cl_2/MeOH, 25:1) to provide a white solid in 65% yield.

Chemical analysis

Aripiprazole and 2,3-ClPP were quantified by liquid chromatography tandem mass spectrometry after solid-phase extraction (1 mL/30 mg Oasis^{TM} HLB cartridges, pre-wetted with 1 mL of CH_3OH and 1 mL of H_2O). Before extraction the samples (0.2 mL) were brought to 1 mL with 5.16 M H_3PO_4 and the internal standard (N-[4-[4-(2,3-diclorophenyl)piperazine-1-yl]butyl-3-metho- xy]benzamide) was added. The cartridges were washed with 1 mL of 2% NH_4OH in water, containing 2% of CH_3OH and 1 mL of 2% CH_3COOH, and the compounds were eluted with 2 mL of CH_3OH, and dried under the stream of nitrogen. Brain samples were homogenized in CH_3CN/ 0.1N HCl(10:90, v/v) and 0.2 mL of the homogenate was processed as plasma.

The reconstituted residues were chromatographed on an X Bridge MSC18 column 3.5 m at 30°C. The mobile phase was (A) CH_3CN/5mM NH_4HCO_3 (90: 10 v/v) and (B) CH_3CN/5mM NH_4HCO_3 (10: 90 v/v) delivered in gradient conditions at 0.3 mL/min. The eluent was introduced through the ESI source of a triple-quadrupole mass spectrometer, with capillary voltage set at 3.45 kV. Cone voltage and collision energy were set respectively at 50 V and 18 eV for 2.3-ClPP and 100 V and 30 eV for aripiprazole. The principal transition ions 231>188 and 448>285 were used for quantification of 2.3-ClPP

and aripiprazole, respectively.
Calibration standards ranged from 0.5-250 ng/mL and 5-2500 ng/g and 1.25-500 ng/mL and 12.5-5000 ng/g for 2.3-ClPP and aripiprazole respectively in serum and brain. Mean coefficients of variation for quality samples were 8.8-11.3% for aripiprazole and 3.1-14.1 % for 2,3-ClPP in rat brain, and 2.1-8.4 for aripiprazole and 2.2-11.3 for 2,3-ClPP in serum regardless of the species.

Results

Serum samples from patients taking aripiprazole once daily showed steady-state aripiprazole concentrations from 0.15 nmol/mL (5 mg; one patient) to 0.85 nmol/mL (15 mg; three patients) about 1.5 or 11 h after the last dose (Table 1). 2,3-ClPP concentrations ranged from 0.002 to 0.013 nmol/mL, yielding mean metabolite-to-parent drug ratios between 0.01 (5 mg) and 0.02 (15 mg, in the morning).

Table 1

Steady-state serum concentrations of aripiprazole and 1-(2,3-dichlorophenyl)-piperazine in schizophrenic patients

Dose (mg)	Concentrations (nmol/mL)		
	Aripiprazole	2,3-ClPP	Metabolite-to-parentdrug ratio
5 (1)[a]	0.15	0.002	0.01
10 (2)[a]	0.46 - 0.51	0.002 - 0.004	0.004 - 0.008
(2)[b]	0.28 - 0.89	0.005 - 0.005	0.02 - 0.006
15 (2)[a]	0.53 - 1.08	0.006 - 0.011	0.01 - 0.01
(1)[b]	0.97	0.021	0.02

Patients (the number is shown in parenthesis) took their daily dose of aripiprazole in the evening[a] or in the morning[b].

Serum and brain levels of aripiprazole and 2,3-ClPP resulting from five oral doses of 10 mg/kg at 1.5 h intervals in rats are listed in Table 2. In brain, the concentrations of the two compounds were higher than in serum, about five times for the parent drug but 26 times for 2,3-ClPP. Therefore the metabolite-to-parent drug ratio averaged 0.08 in serum but approached 0.43 in brain.

Table 2
Steady-state serum and brain concentrations of aripiprazole and 1-(2,3-dichlorophenyl)-piperazine in rats

	Concentrations (nmol/mL or g ± SD)		
Tissue	Aripiprazole	2,3-ClPP	Metabolite-to-parent drug ratio
Serum	1.26 ± 0.47	0.10 ± 0.03	0.08 ± 0.01
Brain	5.82 ± 1.88	2.54 ± 1.13	0.43 ± 0.07
Brain-to-serum ratio	4.7 ± 0.6	26.0 ± 7.7	

Rats were given five oral doses of 10 mg/kg aripiprazole hydrochloride at 1.5 h intervals and were killed two hours after the last dose for parent drug and metabolite quantification in serum and brain. Each value is the mean (with SD) of six rats.

Discussion

This study confirmed that 2,3-ClPP is a minor metabolite of aripiprazole in blood and examined the concentrations of the metabolite and its parent drug in rat brain and serum to evaluate their brain-to blood-distribution ratio and their ratio at the site of action at steady state. Previous studies showed that in healthy subjects aripiprazole steady-state concentrations are attained within 14 days of dosing and are linearly related to the dose up to 30 mg/day [12]. In rats, however, the drug showed nonlinear pharmacokinetics with oral bioavailability more than dose-proportional above 10 mg/kg [13]. Therefore, in this study the brain uptake and metabolite-to-parent drug ratio were evaluated after 8.6 mg/kg (free base), given orally to mimic the route of aripiprazole therapy. This dose was given approximately every half-life (1-2 h) [7, 13] for about five half-lives to achieve steady-state concentrations. This is because the drug elimination half-life in rat is much shorter than in man (about 60 h in CYP2D6 normal metabolizers) and the usual once-daily therapeutic regimen was clearly inadequate to reach a steady-state concentration in the rat body. Weigmann et al. [14]used a similar study design to extrapolate clinical conditions of clozapine therapy to rats.

Using this treatment schedule the mean aripiprazole concentrations were close to those in the schizophrenic patients after the recommended initial and target doses, from 238 to 323 µg/L after 10-15 mg/day (0.55 to 0.72 nmol/mL). These serum levels substantially agree with those found in male schizophrenic patients by Kirschbaum et al. [15] who reported through serum concentrations from 105 to 549 µg/L after 10-30 mg once daily. Broadly similar concentrations were also reported for mean plasma maximum concentrations in healthy males, 163 to 452 µg/L after the same doses[12], possibly because aripiprazole has a very long half-life so there is little fluctuation in plasma concentrations over the dosing interval. Interindividual variability was comparably large in all studies. It possibly reflects the high variability in the expression and activity of the CYP enzymes active in the primary routes of aripiprazole metabolism, i.e CYP2D6 and CYP3A4 for dehydrogenation and hydroxylation and CYP3A4 for the N-dealkylation pathway [1, 7].

The 2,3-ClPP concentrations in rat serum averaged 8% of the drug concentrations at approximately the time of aripiprazole maximum concentrations [13].

The metabolite concentrations were lower and more variable in patients, with a metabolic ratio of less than 0.02 regardless of the dose and sampling time. Again, this agrees with early pharmacokinetic studies where 2,3-ClPP was quantifiable in only some (healthy) subjects at a few time points after 5 mg oral aripiprazole. However, urinary excretion of total p-hydroxy-2,3-ClPP (unchanged metabolite and its sulfate and glucuronide conjugates) amounted to about 30% of the cleared radioactivity, indicating that 2,3-ClPP is extensively biotransformed before excretion.[7] p-Hydroxylation before conjugation and excretion is also the primary route of metabolism of other 1-aryl-piperazines [16], including mClPP [17] and 1-(2-pyrimidinyl)-piperazine [18]. In man this oxidative route is mediated by CYP2D6 [16 - 19] which certainly contributes to the interindividual differences in the ratios of the 1-aryl-piperazine metabolites to their several parent drugs.
While in rat serum the concentrations of 2,3-ClPP were much lower than those of aripiprazole, its brain concentrations amounted to about 40% of the parent drug concentrations. This is because 2,3-ClPP penetrates the blood-brain barrier better than aripiprazole. In view of the wide spectrum of pharmacological actions [9], some of which have been attributed to their interaction with 5-HT sites at which phenylpiperazine derivatives such as mClPP may act as agonists or partial agonists at some subtypes and antagonists at others [8, 20, 21], the present results highlight the need for appropriate pharmacological studies of 2,3-ClPP in the rat and other animal models to clarify its role in the centrally-mediated effects of aripiprazole. However, it is unlikely that the metabolite plays a role for the clinical outcome: assuming that the brain-to-blood distribution ratios of aripiprazole and 2,3-ClPP in man are proportionally similar to those in the rat one can conclude that the mean metabolite concentrations at the site of action approaches about 6% of the parent drug concentrations, ranging from 2% to 12%.
In conclusion, this study indicates that 2,3-ClPP concentrates in the brain more than aripiprazole, confirming that for 1-aryl-piperazines total blood concentrations may be poor indicators of the metabolite-to-parent drug ratio at the site of action. However, while it is conceivable that 2,3-ClPP contributes to the central effects of aripiprazole for the rat, the metabolite's effects should be of minor importance in patients given the usual therapeutic doses.

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