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Research Article

Quantitative Levels of Aripiprazole and its Metabolites in Urine

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Abstract

A total of twenty subjects were enrolled in the study to find the lowest oral dose of drug in which the parent compound and/or one of the two major metabolites would be reliably detected in 80% of the urine samples. Five subjects received 5mg and fifteen received 2mg for 5 days of observed dosing with urine sampled on days 1, 4 and 5. OPC3373 was found in 93% of the samples compared to 50% for aripiprazole and 8% for dehydroaripiprazole. Aripiprazole serum samples on days 1 and 5 were similar indicating that steady state had been achieved. This information is consistent with the subjects being adherent to their prescribed dosing at study entry. In conclusion, urine levels of OPC3373 appear to be the most reliable marker for monitoring compliance with aripiprazole.

INTRODUCTION

Strict compliance to an antipsychotic medication regimen is vitally important in maximizing positive outcomes for individuals suffering from schizophrenia [1-3]. Unfortunately, adherence is usually poor due to the prevalence of adverse side effects. While adherence is poor across a wide variety of physical and psychiatric conditions [1,4-6], it is estimated that half of the patients with schizoaffective disorder and schizophrenia take less than 70% of their prescribed dose [7]. Although new generations of drugs are becoming increasingly available with broader efficacy and improved side-effect profiles, levels of adherence remain alarmingly low [1,5].

In a review of the literature [8], the most common method used to assess adherence was the report of the patient. In a follow-up study by Velligan [9] comparing patient self-report or physician assessment of compliance with more objective measures, it was shown that patients and physicians were not able to identify adherence accurately.

The goal of this study was to determine if, among known adherent individuals, a specific metabolite of a psychoactive drug could be consistently observed in the urine, which can serve as a marker of drug compliance. This could help to delineate the potential relationship between the oral drug exposure and the concentration of a reliable metabolite. The drug chosen for the study was aripiprazole, which is a second- generation antipsychotic and adjunctive treatment for depression that is highly prescribed in this country. The second goal was to observe if serum levels of aripiprazole were similar, when sampled five days apart during a period of observed dosing, in individuals who reportedly had taken the drug for a period of two weeks prior to their entry into the trial. Similar serum levels would confirm the reported adherence with the medication during the prior two-week period.

MATERIALS AND METHODS

A total of twenty subjects were enrolled in the study to find the lowest oral dose of drug in which the parent compound and/or one of the two major metabolites would be reliably detected in 80% of the urine samples. This study was conducted at the offices of Carolina Behavioral Care, PA in Durham, North Carolina and Hillsborough, North Carolina. All subjects met the eligibility criteria and each signed an informed consent form and completed all protocol assessments. Inclusion criteria included: at least 18 years of age, prescribed and judged to be likely compliant with a

stable once daily dose of aripiprazole for at least two weeks prior to study day 1. Subjects with known renal or kidney disease were excluded from the study. All the subjects had observed aripiprazole dosing for five consecutive days. Table 1 represents the baseline characteristics of the enrolled population. As can be seen, a relatively large proportion of subjects had a diagnosis of depression and had mild disease, both of which are consistent with their need for relatively low daily doses of aripiprazole. This drug is also indicated for the treatment of schizophrenia and bipolar disease.

Table 1: Baseline Characteristics of Subjects (N=20).

Variable	N (%)
Gender, N (%) Male Female	 6 (30%) 14 (70%)
Race, N (%) Black Caucasian	 9 (45%) 11 (55%)
Ethnicity, N (%) Hispanic Non Hispanic	 0 (0%) 20 (100%)
Marital Status, N (%) Single Married Separated Divorced Lives with parent Widowed	 4 (20%) 4 (20%) 7 (35%) 3 (15%) 2 (10%)
Education, N (%) Did Not Complete High School High School Graduate Some College College Graduate	 3 (15%) 6 (30%) 10 (50%) 1 (5%)
Employment, N (%) Unemployed Part Time Employed Full Time Employed Retired Disabled	 5 (25%) 4 (20%) 1 (5%) 2 (10%) 8 (40%)
Cigarette Smoking, N (%)	
Current Past Never	 12 (60%) 3 (15%) 5 (25%)
Body Mass Index (kg/m^2) Median Extremes	 29 19.2, 44.3
Psychiatric Diagnosis, N (%) Schizophrenia Bipolar Disorder Depression	 4 (20%) 5 (25%) 11 (55%)
Global Impression of Illness Severity, N (%) 1=Normal, not at all ill 2=Borderline mentally ill 3=Mildly ill 4=Moderately ill 5 or higher	 1 (5%) 3 (15%) 12 (60%) 4 (20%) 0

Table 2: Urine drug/metabolite testing on days 1, 4 and 5 on subjects on 5 mg/day (N=5).

	Aripiprazole	Dehydroaripiprazole	OPC3373
Study Day 1 N(%) Positive Mean ± SD Median Extremes	 4 (80%) 12.4 ±8.8 11.1 0 – 22.3	 2 (40%) 2.3 ± 3.2 0 0 – 6.5	 4 (80%) 375.7 ±493.1 175 0 – 1214
Study Day 4 N(%) Positive Mean ± SD Median Extremes	 2 (40%) 5.8 ± 8.0 0 0 – 16.3	 1 (20%) 1.2 ± 2.7 0 0 – 6.1	 4 (80%) 245.3 ± 16.5 228.2 0 – 571.4
Study Day 5 N(%) Positive Mean ± SD Median Extremes	 2 (40%) 6.1 ± 9.2 0 0 – 18.3	 1 (20%) 1.6 ± 3.7 0 0 – 8.2	 4 (80%) 216.8 ± 92.9 182.4 0 – 493.9

A total of 60 urine samples were obtained from the 20 subjects. Initially, 5 subjects were recruited who were taking a dose of 5 mg. If at least 4 out of 5 urines tested had at least one of the metabolites detected in each urine, then the next 5 subjects were to have been recruited at the next lowest available dose (which in this case was 2 mg) If fewer than 4 out of 5 urines tested did not have a detectable metabolite the next 5 subjects recruited were to have been at the next higher dose. This process was to have been continued until there were at least 15 subjects recruited with the same dose that had at least one of the 3 metabolites detected in 80% of the urines. In this case, the OPC3373 metabolite was detected in 4 out of 5 of the first subjects taking a dose of 5mg, and the next 15 subjects were recruited at 2mg.

Urine samples were analyzed to determine detection sensitivity for aripiprazole and the two major metabolites dehydroaripiprazole and OPC3373 using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPCL/MS/MS).

The urine samples were prepared by dilution with deionized water and methanolic internal standard solution. Samples were not hydrolyzed prior to analysis. Internal standards aripiprazole D8 and clozapine D4 and Waters Acquity UPC® BEH Phenyl, 2.1 x 50mm, 1.7um was the LC column used. The mobile phases utilized were: 2mM ammonium acetate with formic acid and methanol. Prepared samples were run on a Waters Acquity TQD® in positive electrospray ionization acquisition mode. Mass spectrometer temperatures were set as follows: source temperature of 160°C and desolvation temperature of 350°C. The desolvation gas flow was 800 L/Hr and cone gas flow was 10 L/Hr. The urine cut off levels are as follows: Aripiprazole 5 ng/ml, Dehydroaripiprazole 5 ng/ml, OPC 3373 25 ng/ml. Serum levels were measured using High Performance Liquid Chromatography /Tandem Mass

Spectrometry (LCMS/MS). The lower limit of detection of the serum testing was 20 ng/ml.

RESULTS AND DISCUSSION

The urine sample results for the five subjects on 5 mg aripiprazole per day are shown in Table 2. The parent compound aripiprazole was found in four of the five subjects on day 1 and in two of the subjects in days 4 and 5. An analysis of the actual urine levels shows no significant differences between days 1, 4 and 5. Dehydroaripiprazole was observed in two subjects on day 1 and in only one subject on days 4 and 5, which was far less frequent than the parent compound. As expected, the urine levels of this metabolite were quite low. Conversely, OPC3373 was consistently observed in four of the five subjects on all three days of testing. The urine levels ranged from 376 ± 493 ng/ml (SD) on day 1 to 217 ± 193 ng/ml on day 5. There were no significant differences observed between the levels on all three days. Included in the tables are the median and the extremes for the collected data. The wide range of the extreme values in conjunction with the fairly large standard deviations indicates the wide variability in the obtained data.

The results for the urine sample for the fifteen subjects on 2 mg aripiprazole per day are shown in Table 3. The parent compound aripiprazole was found in eight of the 15 subjects on day 1 and day 4; and in 7 subjects on day 5. There was no significant difference observed in the urine levels comparing all three days. On the other hand, dehydroaripiprazole was observed far less frequently. It was only observed on day 1 in one subject and the levels were less than 1 ng/ml. Conversely, OPC3373 was consistently observed in all fifteen subjects on days 1 and 4 and in 14 subjects on day 5. As can be seen, there were no significant differences in the urine levels in this metabolite on all three days. The median, extremes and standard deviation reflect the wide variability of the collected data.

Table 3: Urine drug/metabolite testing on days 1, 4, and 5 on subjects on 2 mg/day (N=15).

	Aripiprazole	Dehydroaripiprazole	OPC3373
Study Day 1			
N(%) Positive	8 (53%)	1 (7%)	15 (100%)
Mean ± SD	5.1 ± 5.7	0.5 ± 1.9	186.1 ±183.8
Median	5.8	0	122.2
Extremes	0 – 17.4	0 – 7.4	63.6 – 649.8
Study Day 4			
N (%) Positive	8 (53%)	0 (0%)	15 (100%)
Mean ± SD	5.0 ± 5.9	0 ± 0	248.6 ±195.1
Median	5.8	0	197.1
Extremes	0 – 18.3	0 - 0	55 – 820.5
Study Day 5			
N(%) Positive	7 (47%)	0 (0%)	14 (93%)
Mean ± SD	4.1 ± 4. 9	0 ± 0	194.9 ±235.2
Median	0	0	182.4
Extremes	0 – 12.8	0 - 0	0 – 493.9

Table 4: Change in Serum Aripiprazole Day 1 to Day 5 (5 Subjects).

Dose (Mg/ Day)	Day 1 Serum Aripiprazole (ng/ml)	Day 5 Serum Aripiprazole (ng/ml)	Difference Day 1 to Day 5 (ng/ml)	% Difference Day 1 to Day 5
5	76	78	2	2.6
5	55	49	-6	-11.5
5	36	49	13	30.6
5	51	37	-14	-31.8
5	27	22	-5	-20.4
2	49	55	6	11.5
2	48	Not obtained	N/A	N/A
2	Not detected	22	N/A	N/A
2	25	23	-2	-8.3
2	24	Not obtained	N/A	N/A
2	46	44	-2	-4.4
2	34	48	14	34.1
2	29	23	-6	-23.1
2	21	20	-1	-4.9
2	51	52	1	1.9
2	48	42	-6	-13.3
2	22	27	5	20.4
2	Not detected	22	N/A	N/A
2	24	25	1	4.1
2	**Not detected**	**39**	**N/A**	**N/A**

Serum aripiprazole levels were fairly constant from day 1 to day 5 as shown in Table 4. Serum aripiprazole was not obtained on study day 5 for 2 subjects. Serum levels of the drug were as likely to increase as to decrease over the five days of observed dosing. The percent change was small in the majority of subjects (<25% change in 12 of 15 subjects). In fact, in seven of the subjects, the levels on day 1 were greater than on day 5. While in eight of the subjects, the levels on day 1 were less than on day 5. Of interest, the parent drug/or metabolite was detected in the urine on day 1 for all three subjects with aripiprazole not detected in serum at day 1. As stated above, the level of detection of the serum testing was 20 ng/ml. The stability in serum levels from Day 1 to 5 is consistent with the subjects being adherent to their prescribed dosing at study entry as intended for the study.

In humans, aripiprazole is primarily converted in the liver to two major metabolites. It undergoes dehydrogenation to form dehydroaripiprazole, which is pharmacologically active. It is also converted through N-dealkylation to form the inactive compound OPC3373. These pathways involve both CYP2D6 and CYP3A4 enzymatic pathways. Less than 1% of aripiprazole is excreted unchanged in the urine. Blood levels of aripiprazole have been shown to be increased in individuals with hepatic impairment [10]. As expected, the blood levels of the dehydroaripiprazole derivative are reduced in liver toxicity [10]. Results of the same study revealed that the blood levels of aripiprazole were increased during renal impairment.

Ketoconazole as an inhibitor of CYP3A4 enhances aripiprazole serum concentrations. Likewise, quinidine an inhibitor of CYP2D6 also increases aripiprazole concentrations [11].

In the current study, aripiprazole was observed in 50% of the urine samples. The dehydroaripiprazole was only observed in 8% of the samples. Conversely, OPC3373 was observed in 93% of the urine samples. No difference was observed in the urine results for OPC3373 comparing days 1, 4 and 5. Thus, OPC3373 should be considered as the most reliable marker for monitoring compliance with drug administration. Importantly, these findings were observed among known adherent subjects. Serum levels of aripiprazole were monitored on day 1 and 5 of the study. Table 4 reveals that there were no differences observed comparing the serum levels on these two days. This information suggests that steady state had been achieved. Since the drug reaches steady state only after 14 days of constant administration it can be concluded that, in addition to the documented adherence during the observed five-day dosing period, the subjects had been consistently taking their medication for the time period prior to entering into the study.

CONCLUSION

In conclusion, urine levels of OPC3373 appear to be the most reliable marker for monitoring compliance with aripiprazole.

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Conflict of Interest

Gregory L. McIntyre is an employee of Ameritox Ltd, the organization that funded this research project.

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