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Bioequivalence Study of two Formulations Containing 30 mg of Aripiprazole in Healthy Human Volunteers

U. MANDAL, A. GHOSH, T.K. PAL* and T.K. CHATTARAJ†
Department of Pharmaceutical Technology, Bioequivalence Study Centre
Jadavpur University, Kolkata-700 032, India
E-mail: tkpal_12@yahoo.com; uttamkumarmandal@yahoo.co.in

Aripiprazole is a new anti-psychotic drug used for the management of Schizophrenia, a brain disorder. A simple and sensitive reversed phase high performance liquid chromatography coupled with UV detector set at 215 nm has been used to determine plasma concentration of aripiprazole in healthy male human volunteers. The method has been validated over a linear range of 20-400 ng/mL from plasma. The minimum quantifiable concentration (LOQ) obtained was 20 ng mL^{-1} (% CV < 10 %). The pharmacokinetic parameters (C_{max}, AUC_{0-t}, $AUC_{0-\infty}$) of this drug have been evaluated by the above method to compare the single oral dose (30 mg) bioavailability of aripiprazole with the reference formulation in 12 healthy male volunteers of two way, two periods cross over randomized study. Adverse effects leading to postural dizziness and blood pressure lowering have been observed in some of the volunteers. Although ECG reports confirm the non-significant physiological change. The pharmacokinetic parameters were C_{max} = 118.82 ± 20.76 ng mL^{-1} at t_{max} = 3.96 ± 0.69 h, AUC_{0-t} = 4962.67 ± 1189.54 ng h mL^{-1}, $AUC_{0-\infty}$ = 7266.75 ± 2146.78 ng h mL^{-1}, K_{el} = 0.0138 ± 0.0025 h^{-1} and $t_{½}$ = 51.69 ± 9.80 h.

Key Words: Aripiprazole, Bioequivalence, Pharmacokinetics, HPLC.

INTRODUCTION

Aripiprazole (7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl] butoxy]-3, 4-dihydro carbostyril) is a new anti-psychotic drug used for the management of schizophrenia, a brain disorder. It has potent partial agonist activity at dopamine D2 receptor[1], partial agonist activity at serotonin 5-HT_{1A} receptors[2] and antagonist activity at 5-HT_{2A} receptor[3]. The clinical trial[4] involving pharmacokinetic[5-9], efficacy and safety evaluation has been revealed that aripiprazole is an effective and well-tolerated drug in the management of schizophrenia. Development of a rapid sensitive and selective

†NRS Medical College and Hospital, Kolkata-700 014, India.

method for the determination of aripiprazole in human plasma is essential for understanding the pharmacokinetics of this drug when administered orally. The numbers of published methods for analysis are limited[10]. This paper describes a simple and selective HPLC method with UV-detection to analyze aripiprazole in human plasma. The aim and objective of the present study was to evaluate the pharmacokinetic parameter for bioequivalence study of tablet aripiprazole 30 mg (aripiprazole tablet from Psycho Remedies, Ludhiana, India) as test formulation and ARIPRA (containing aripiprazole 30 mg) from Ranbaxy Solus, New Delhi, India as reference formulation.

EXPERIMENTAL

Acetonitrile (ACN), HPLC grade water, methylene chloride, KH_2PO_4 and KOH. All the reagents were of HPLC grade.

Study population and study design: Adult, healthy, male human volunteers within 18-40 years were selected for the panel of volunteers recruited by CPU (Clinical Pharmacology Unit). The bioequivalence study of the test preparation was assessed utilizing a typical, two periods randomized, two-way complete cross over design in 12 healthy, male human volunteers. There were two dosing session with a wash out period of 15 d between them. All the volunteers are required to participate in two dosing sessions. In each dosing session, volunteers received either of test or reference preparation of tablet aripiprazole 30 mg as a single dose only on the study day as per the randomization code (Table-1) at a fixed time.

TABLE- 1
RANDOMIZATION CODE
(A = REFERENCE PREPARATION, B = TEST PREPARATION)

Subject No.	Period I	Period II	Subject No.	Period I	Period II
1	A	B	7	B	A
2	A	B	8	B	A
3	A	B	9	B	A
4	B	A	10	B	A
5	B	A	11	A	B
6	A	B	12	A	B

Approval from the Drugs Controller General of India (DCGI) as well as clearance from the Institutional, Ethical Committee (IEC) was received prior to undertaking this study. The whole study was conducted under the active guidance of clinical pharmacologists.

Blood collection, dietary control and adverse events: A total of 15 blood samples were collected from anticubital vein at 0 h (before adminis-

tration) 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 18.0, 24.0, 36.0 and 48.0 h in coded, centrifuge tubes containing EDTA. Blood samples were centrifuged immediately, the plasma separated into duplicate polypropylene tubes and stored frozen at -20 ºC.

A standard breakfast, lunch and dinner was served to subjects at 3, 6-8 and 14 h, respectively after drug ingestion. On the study day volunteers were permitted normal activities excluding strenuous exercise.

Abnormal symptoms/signs were monitored during the study period and for 1 week after the study period and if noticed their details were entered in the case report sheets and tabulated at the end of the study.

Stock solution: Stock solution of aripiprazole (1 mg mL^{-1}) were prepared by dissolving the drug in acetonitrile and stored at -20 ºC. Appropriate dilution of the stock solutions were prepared by diluting the stock solutions with mobile phase.

Calibration curves: For calibration curve six different concentrations (20, 50, 100, 150, 200 and 400 ng mL^{-1}) in plasma were prepared by adding required volume of working solution of analyte to blank plasma. Valdecoxib was taken as internal standard and its concentration in plasma was 200 ng mL^{-1}. The plasma sample was subjected to the sample preparation procedure and injected onto HPLC. Plasma calibration curve was prepared by taking area ratio of analyte to internal standard as Y-axis and concentration of analyte (ng mL^{-1}) as X-axis and a linear equation in the form $y = 0.00377x + 0.007772$ derived by least square regression analysis, where x is the spiked concentration and y is peak area ratio of aripiprazole to internal standard.

Chromatographic conditions:

Instrument:	Knauer HPLC, Germany
Column :	Hypersil BDS, C18, 150 × 4.6 mm, 5 µ particle size, stainless steel.
Mobile phase :	10 m mol phosphate buffer:acetonitrile 50:50 (v/v)
Flow rate :	1 mL min^{-1}
Wave length of detection:	215 nm
Injector:	Fixed loop rheodyne injector system filled with a 20 ml Rhd. Loop
Integrating software :	Eurochrom 2000

Extraction procedure: 1 mL of plasma spiked with aripiprazole raw drug was taken in a stoppered test tube. To this 10 µL of internal standard (valdecoxib in acetonitrile) was added and mixed well. This mixture was extracted with 8 mL of methylene chloride followed by shaking for 10 min and then centrifuged for three minutes at 3000 rpm. The organic layer was removed in a separate centrifuge tube with cap. The resulting organic layer

was evaporated to dryness in water bath in presence of nitrogen atmosphere at 40 ºC. The residue was reconstituted with 150 µL of dilute H_3PO_4 and the same was injected onto HPLC for chromatographic analysis.

Accuracy, precision and freeze thaw recovery: Within-run, between -run precision and accuracy as well as freeze thaw recovery was carried out as per the protocol described in earlier papers[11-13].

RESULTS AND DISCUSSION

Excellent linearity was observed between the peak area and drug concentration over the range 20-400 ng mL^{-1}. The lower limit of detection defined as three times the base noise was 10 ng mL^{-1} for this analytical method and the limit of quantitation was 20 ng mL^{-1} (n = 13, SD = 1.09). The sensitivity of the assay was sufficient for determination of aripiprazole in human plasma for a period of 1 to 48 h. Between-run and within-run accuracy was over 95 % and their % CV did not exceed 10 %. Freeze thaw recovery was found to be suitable for this study.

Fig. 1 shows the mean aripiprazole concentration *vs.* time profile after oral administration of single dose of 30 mg aripiprazole for both brands (standard and test).

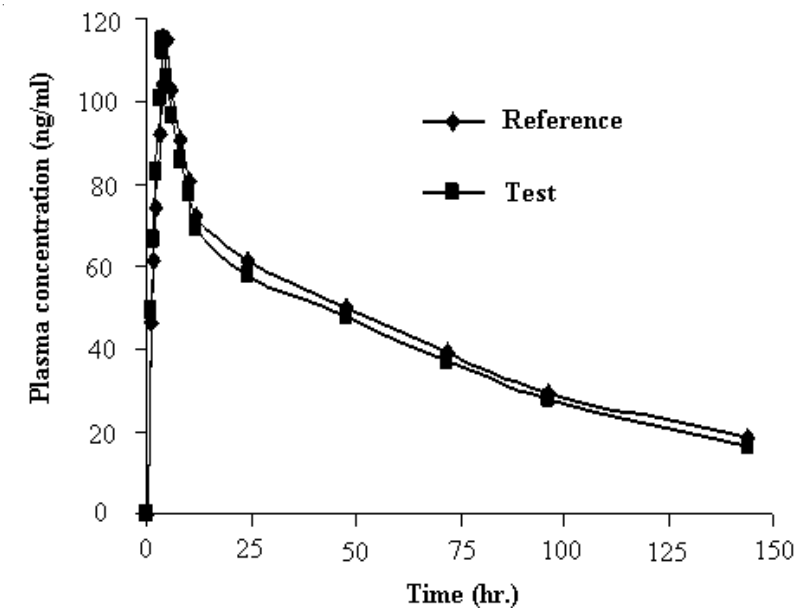

Fig. 1. Mean plasma concentration (ng/mL) *vs.* time (h) curve after oral administration of aripiprazole 30 mg tablet (test and reference) to 12 human volunteers

The mean pharmacokinetic parameters of all the 12 human volunteers are tabulated in Table-2.

TABLE-2
PHARMACOKINETIC PARAMETERS OF ARIPIPRAZOLE (MEAN ± SD) IN 12 HEALTHY VOLUNTEERS WITH TEST AND REFERENCE FORMULATION

Pharmacokinetic parameters	Reference	Test
$AUC_{0-\infty}$ (ng L mL^{-1})	7249.75 ± 1480.64	7266.75 ± 2146.78
AUC_{0-t} (ng L mL^{-1})	5049.37 ± 902.53	4962.67 ± 1189.54
C_{max} (ng mL^{-1})	119.35 ± 16.01	118.82 ± 20.76
t_{max} (h)	3.83 ± 0.54	3.96 ± 0.69
K_{el} (h^{-1})	0.0139 ± 0.0019	0.0138 ± 0.0025
$t_{½}$ (h)	50.58 ± 7.95	51.69 ± 9.80
Relative bioavailability (%)	100	98.28

It is evident from the table that all the pharmacokinetic parameters (C_{max}, t_{max}, AUC_{0-t}, $AUC_{0-\infty}$, K_{el} and $t_{½}$) are found to be most comparable with those of reference preparation. The 90 % confidence interval (CI) for C_{max}, AUC_{0-t} and $AUC_{0-\alpha}$ values of test and reference preparation (Table-3) were within the accepted limit of DCGI guidelines (0.8-1.2).

TABLE-3
90 % CONFIDENCE INTERVAL OF VARIOUS PHARMACOKINETIC PARAMETERS OF TEST AND REFERENCE FORMULATION

Pharmacokinetic parameters	Untransformed data	Transformed data
C_{max}	0.87-1.09	0.97-1.02
$AUC_{0-\alpha}$	0.86-1.14	0.98-1.01
AUC_{0-t}	0.85-1.11	0.98-1.01

On the basis of comparison of AUC_{0-t} for aripiprazole 30 mg after single dose administration, the relative bioavailability of test preparation of tablet aripiprazole 30 mg was 98.28 % to that of reference preparation and the test preparation was bioequivalent to reference preparation.

During the whole study, no serious adverse effects were observed, although postural dizziness and blood pressure lowering have been observed in some of the volunteers (2 out of 12 volunteers) but ECG reports confirm the non significant physiological change. Moreover, it is also not statistically significant.

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