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SAFETY ASSESSMENT OF EFAVIRENZ AFTER A SINGLE–DOSE BIOEQUIVALENCE STUDY: A TREND TO CORRELATE CENTRAL NERVOUS SYSTEM EFFECT AND PLASMA CONCENTRATION

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ABSTRACT

The objective of the current study was to examine safety and pharmacokinetic data obtained from a bioequivalence study of two brands of efavirenz carried out in healthy volunteers in order to assess the relationship between concentrations and appearance of adverse events. Drowsiness was reported in almost all the subjects 1 or 2 hours post dosing and generally the onset of this adverse event was 30 or 40 minutes before high efavirenz plasma concentrations, evidencing a lag time between venous plasma concentration and effect. As many studies reported, arterial drug concentration is higher than the respective venous concentration during drug inputs so adverse events experienced during drug input would correlate with arterial drug concentration rather than the respective venous concentration.

Keywords: Efavirenz, central nervous system, adverse events, venous concentration, arterial concentration

INTRODUCTION

Efavirenz (EFV) is a non-nucleoside reverse transcriptase (RT) inhibitor used as a first- line agent in the treatment of human immunodeficiency virus type 1 (HIV-1) infection in adult and pediatric patients.[1] It binds directly and reversibly to the catalytic site of the RT. The RT of HIV-type 2 and human cellular DNA polymerase are not inhibited by EFV. Its efficacy is well documented when it is used in combination with either a protease inhibitor or/and with nucleoside reverse transcriptase inhibitors.[2-4]

EFV is strongly bound to plasma protein mainly albumin (99%) and is extensively metabolized by the cytochrome P450 enzymes, primarily CYP3A4 and CYP2B6.[1] The metabolism of EFV is an autoinducible enzymatic process. [5] The long terminal half-life after a single oral dose ranges from 52 to 76 hours and after multiple doses decreases to 40 -55 hours.

Major concerns in treatment with highly active antiretroviral therapy are the occurrence of adverse events that could result in failure of compliance and thus development of viral resistance.

In a large clinical trial [3], more than 50 % of the patients treated with EFV-based regimens experienced central nervous system (CNS) effects.These symptoms typically occur within the first week of treatment and usually resolve within 4 weeks of treatment. Symptoms include confusion, dizziness, insomnia, somnolence, impaired concentration. Also, a mild-to-moderate rash (15-30%) can develop within two weeks after starting EFV and does not require discontinuation. The rash usually disappears within two weeks.

Some data [6,7] indicate that there might be a relationship between high plasma EFV concentrations and appearance of CNS effects whereas some authors did not find such relationship.[8,9] Fumaz et al.[10] failed to demonstrate an association between CNS toxicity and EFV plasma concentrations in patients receiving long-term therapy with this drug. Commonly, venous plasma drug concentrations are used in pharmacokinetic and pharmacokinetic/pharmacodynamic studies, but

many investigations [11-13] have been carried out explaining the different relation between venous and arterial blood concentrations and the correlation with the effect. Arterial drug concentration is higher than the respective venous concentration during drug inputs where the opposite phenomenon is observed during the elimination process. In these studies venous maximum serum concentration (C_{MAX}) and the time to reach it (T_{MAX}) did not coincide with the corresponding arterial values and a much higher arterial C_{MAX} and a shorter arterial T_{MAX} were found.

Time of day has to be regarded as an additional variable influencing the kinetics of a drug. Time-dependent changes in kinetics may proceed from circadian variations at absorption, distribution, metabolism and elimination processes. Thus, circadian variations in gastric acid secretion and pH, motility, gastric emptying time, gastrointestinal, hepatic and renal blood flow, drug protein binding, among others may play a role in such kinetic variations. A higher sympathetic tone in the morning in comparison to the evening-period leads to a higher cardiac output. This could result in a greater absorption rate in the morning and thus a greater difference between arterial and venous plasma concentrations. Besides, this higher sympathetic tone deviates a higher blood flow fraction to the extra-splanchnic-renal regions during the morning [14], and so, higher brain/plasma concentration ratios can be obtained, increasing even more than predicted the CNS drug effects.

A study conducted in 24 healthy adult males, comparing the pharmacokinetics of 600 mg of EFV at steady state when administered daily in the morning versus at bedtime, showed an exposure to the drug slightly but significantly higher after the evening dose. [15] According to the results obtained by our group with carbamazepine [16], an anticonvulsant drug with similar pharmacokinetic characteristics to EFV, there would be a higher extravascular EFV concentration during the day (lowering plasma concentration) than at night (increasing plasma levels). This fact would result in more adverse effects during the day despite lower plasma concentrations.

Food may alter the bioavailability of orally administered drug. EFV is lipophilic, and enhanced absorption with fat meals is expected. As reported in the literature, a high-fat meal increased area under the plasma concentration–time curve (AUC) and maximum plasma concentration (C_{MAX}) of a daily morning administration of EFV by 28% and 79%, respectively.[17] For this reason, the manufacturer recommends administration of EFV without food since elevated efavirenz concentrations may lead to an increased frequency of adverse events.

So administration of EFV in the morning or in the evening or under fed or fasting conditions will impact on its concentrations and thus on the efficacy and tolerability of the drug.

The objective of the current study was to examine safety and pharmacokinetic data obtained from a bioequivalence study of two brands of EFV carried out in healthy volunteers in our Bioavailability and Bioequivalence Centre for Medicine Evaluation in order to assess the relationship between EFV concentrations and the occurrence of adverse events (AEs).

MATERIALS AND METHODS

Study design: A total of 16 adult Caucasian subjects (8 men, 8 women) were recruited for the study.

Laboratory values and electrocardiograms for all subjects had to be within normal range. Negative test for HIV, hepatitis B and C viruses were also required. Female subjects were required to have a negative pregnancy test at screening and agree to use a highly effective contraception method while on study treatment and for a month after the last dose of EFV.

The clinical trial was performed according to a randomized, two-treatment, two-period, two-sequence, single dose, crossover design with a wash-out period of 28 days.

During each period, each subject received one film-coated tablet (600 mg) of either the REFERENCE product or the TEST product in the evening. The study was performed in the Bioavailability and Bioequivalence Centre for Medicine Evaluation, situated in ''Dr. Juan J. Crottogini'' Hospital (Montevideo, Uruguay).

Volunteers came to the Centre the first day of each week at 6:00 p.m., with at least a four-hour fasting period. Subjects received dinner at 7 p.m. and at 21:00 p.m. the TEST or REFERENCE product was administered with 200 mL of water. The same standard meals were administered in both study periods. Subjects remained in the Centre the first 24 hours and were required to return to the Centre at specific times for plasma collection at 48, 72 and 96 hours post-dose administration.

The study protocol was approved by the Institutional Ethics Review Committee of the Faculty of Chemistry – *Universidad de la República*, Uruguay. Written consent was obtained from all subjects prior their entry into the study.

Sample collection and analytical methodology for drug determination: Blood samples were collected prior to dosing (0 hour) and at 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 12 – 16 – 20 – 24 – 48 – 72 and 96 hours following dose administration from the antecubital

vein through cannulation and placed in heparinized tubes. Plasma was separated by centrifugation and stored at -25 °C until analysis.

Plasma EFV concentrations were measured by a high performance liquid chromatography method developed and validated at the Centre. Full methodological validation was carried out according to FDA guidance for bioanalytical method validation. [18] By this method, 50 microliters of internal standard solution (lopinavir, 0.074 mg/mL in methanol) were added to 1.0 mL of plasma. Plasma was alcalinized by adding 1 mL of sodium carbonate 0.50 M. The extraction of analytes was performed by adding 2 mL of ethyl acetate and 2 mL of hexane then vortexed for 1 minute. After centrifugation, the supernatant was separated and dried under nitrogen stream at 40°C. Dry residue was dissolved with 100 µL of mobile phase and 20 µL injected into a Dionex Ultimate 3000 series chromatograph. A Phenomenex®Luna C18 (5µm, 150 mm x 4.6 mm) column was used as a reversed stationary phase. The mobile phase was a mixture of disodium phosphate 50 mM pH 5.9/acetonitrile/methanol (40:40:20) pumped with a flow rate of 1.5 mL/min. The column compartment was kept at 40°C and the wavelength detection was 205 nm. Under these conditions, the retention times of analytes were 8.0 and 12.0 for EFV and lopinavir respectively.

The HPLC method was linear between 50 (the lower limit of quantification, LLOQ) and 5000 ng/mL. Inter and intraday coefficients of variation (CVs) and accuracy of method were below 15%.

Pharmacokinetic analysis: The maximum EFV blood concentration ($\mathbf{C_{MAX}}$) and the time-to-peak ($\mathbf{T_{MAX}}$) were computed for each volunteer from experimental data. The area under the plasma concentration–time curve from zero to infinite (AUCinf) was calculated using the trapezoidal rule until the last quantifiable concentration ($\mathbf{C_{LAST}}$): $AUC_{0\text{-}T}$, and extrapolated to infinitive adding the term $\mathbf{C_{LAST}}/\beta$, being β the first order elimination rate constant calculated from the slope of the log-linear concentration-time regression of data collected 96 h post-dose. $\mathbf{C_{MAX}}$/AUCinf ratio was calculated for each volunteer as an estimator of the absorption rate. [19]

Safety assessment: Physical examination, psychiatric evaluation, hematology, platelets count, serum chemistry (fasting glucose, liver functional and enzymogram analysis, creatinine, urea, uric acid, potassium, sodium), urinalysis, were performed before enrollment as it was mentioned above and at study termination for safety purposes.

For female, serum pregnancy test was performed at screening and on urine samples previous to each dosing period. An abbreviated physical examination was carried out on the evening before drug administration. Vital signs (systolic and diastolic blood pressure in supine position and heart rate) were recorded during immediately before and after drug administration. Safety and tolerability assessments included the monitoring and recording of all adverse events, and any concomitant medications used to treat adverse events.

RESULTS AND DISCUSSION

Fourteen subjects (8 women, 6 men) completed both periods of the study. One of the subjects did not appear the day of the study and another subject (volunteer 11) experienced rash after completing the first period, so treatment was discontinued. Subject characteristics are summarized in table 1.

Figure 1 shows mean plasma concentration - time curves after administration of 600 mg of TEST and REFERENCE products of EFV. As it can be observed, REFERENCE product exhibits a higher C_{MAX}. Plasma pharmacokinetic parameters for EFV in women and men are summarized in table 2.

From pharmacokinetic analysis, TEST and REFERENCE products resulted biopharmaceutical equivalent regarding AUC and the rate of absorption (C_{MAX}/AUC, T_{MAX}). Considering the sex of individuals, the TEST product showed lower maximum exposure than the REFERENCE in men.

From a pharmacodynamic point of view, EFV was well tolerated by all subjects. Drowsiness as a mild adverse event (grade 1 on the World Health Organization scale) was reported in almost all the subjects 1 or 2 hours post dosing. After those hours, the event resolved alone without treatment. EFV concentrations in male and female after TEST and REFERENCE administration are shown in tables 3 and 4 respectively. Adverse events are painted in orange. Volunteer 11 was computed in the first period of the study.

As it can be observed, the AE started one or two hours after drug administration, women presented this AE with the two formulations, except for subject 6 who only experienced drowsiness after TEST administration; men experienced more AEs after REFERENCE intake; time duration of the effect was more prolonged in women and generally the onset of the adverse event was 30 or 40 minutes before high EFV concentrations were observed.

TEST product was better tolerated than REFERENCE in men whereas women showed similar response to both formulations. The higher C_{MAX} registered in women for TEST and

REFERENCE products (table 2) could explain the more frequent observation of AEs, giving evidence of a concentration-dependent mechanism. In this study, C_{MAX} higher than 3000 ng/mL were associated with CNS adverse events.

The onset of adverse events preceded a high plasma EFV concentration, evidencing a lag time between venous plasma concentration and effect. As it was explained in the introduction, this could be the consequence of a higher arterial EFV concentration during drug absorption, concentration that closely correlates with the concentration in the neuronal sites when drugs with high lipophilia, and then fast transference across the membranes, as is the case of EFV, are considered. This could be related with EFV toxicity in brain and with the non-correlation observed by some authors between venous plasma concentrations and adverse events.

The shorter arterial T_{MAX} for EFV would comply with the rapid onset of the adverse event and the higher arterial C_{MAX} for EFV would be the concentration that really correlates with the effect. Figure 2 illustrates what would happen with arterial and venous concentrations after a single-dose extravascular administration of EFV. Time of onset of adverse events would correspond to the time the arterial C_{MAX} should be attained.

The administration of EFV with food could increase the frequency of adverse events due to an increase of plasma concentrations. However, all the studies mentioned in the literature were carried out after a morning administration, and as it was mentioned in the introduction section, a more elevated cardiac output in the morning in relation to the evening hours could worsen the appearance of side effects.

Although AEs are usually transient and decrease over time as previously reported [20], avoidance of drug toxicity should be an objective in order to accomplish patient compliance. According to some authors [21], a dose-escalating regimen may provide a better tolerance profile without evidence of decreased antiviral activity in the short term.

CONCLUSIONS

Adverse events experienced during drug input would correlate with arterial drug concentration rather than the respective venous concentration. In order to diminish efavirenz-related CNS, the drug should be taken in the evening separated from food intake. Stepwise dose escalation of efavirenz over 2 weeks is advisable as it reduces the incidence and intensity of such events while maintaining efficacy.

Table 1. Subject demographic characteristics [a]

	Total	Men	Women
Subjects	14	6	8
Age (years)	28 (19-49)	28 (20-46)	28 (19-49)
Weight (kg)	75.3 (56.0-109.5)	90.5 (74.0-109.5)	63.9 (56.0-70.0)
Height (cm)	170.1 (160.0-184.0)	176.6 (173.0-184.0)	165.2 (160.0-173.5)
BMI (kg/m^2)	26.4 (22.0-38.0)	29.6(25.0-38.0)	24.0 (22.0-27.0)
CT	0	----	0

[a] Expressed as average (range) when appropriate. BMI: body-mass index; CT: contraceptive therapy

Table 2. Pharmacokinetic parameters for EFV obtained after 600 mg of TEST or REFERENCE dose administration in 14 healthy subjects

Pharmacokinetic parameters	MEN (TEST)	WOMEN (TEST)
$\beta(h^{-1})$	0.0111 (±0.0070)	0.0085 (±0.0028)
C_{MAX} (ng/mL)	2984 (±527)	4535 (±729)
T_{MAX} (h)	3.33 (±0.65)	2.38 (±0.63)
Medians [Q25; Q75]	3.5 [2.5; 4]	2 [2; 3]
$AUC_{[0-T]}$ (ng.h/mL)	67858 (±23084)	83828 (±11639)
AUC_{inf} (ng.h/mL)	130562 (±55466)	158546 (±42303)
C_{MAX}/AUC_{inf} (1/h)	0.0273 (±0.0101)	0.0317 (±0.0079)
	MEN (REFERENCE)	**WOMEN (REFERENCE)**
β (h^{-1})	0.0106 (±0.0051)	0.0077 (±0.0023)
C_{MAX} (ng/mL)	4208 (±690)	4452 (±1072)
T_{MAX} (h)	2.5 (±0.67)	2.75 (±0.61)
Medians [Q25; Q75]	2 [2; 3,5]	2.5 [2; 3]
$AUC_{[0-T]}$ (ng.h/mL)	71911 (±21720)	79607 (±9381)
AUC_{inf} (ng.h/mL)	138537(±65067)	177656 (±75955)
C_{MAX}/AUC_{inf} (1/h)	0.0376 (±0.0132)	0.0319 (±0.0102)

Table 3. EFV concentrations in ng/mL after TEST administration in female subjects (1-8) and male subjects (9-15) for each time post dosing. Concentrations where adverse events occurred are painted in orange.

Time (h) post dosing	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8
1	3674	3112	638,7	281,0	4621	650,6	6507	747,3
2	3879	3961	4390	2981	4878	3070	3106	5402
3	3829	4389	3131	3368	4707	2626	2943	4066
4	2012	2649	3342	2568	2740	3464	2208	2972
5	1368	2039	2594	1545	2378	1940	1644	1553
6	1279	1200	1803	1472	1892	1707	1440	1251
7	1517	1110	1722	1241	1823	1234	1111	1108
8	931,5	1776	1350	1094	2152	1110	1085	1021
12	1040	1555	856,3	992,3	1906	1060	1108	970,8
16	796	1620	777,1	972,5	1293	1273	817,4	820,8
20	753,4	1174	801,9	922,9	1658	1165	783,3	654,7
24	797,7	1300,0	558,5	845,5	1169	840,4	706,5	679,2
48	640,7	555,7	468,4	844,9	1027	981,4	678,0	517,4
72	716,4	463,4	413,0	633,1	913,2	740,0	709,7	402,2
96	331,8	382,1	543,2	413,1	597,4	803,7	406,7	404,7

Time (h) post dosing	Subject 9	Subject 10	Subject 11	Subject 12	Subject 13	Subject 14	Subject 15
1	72,1	2982	58,0	1200	261	0	615
2	1330	3011	2012	3149	1128	1907	3242
3	2653	3459	3354	2441	2152	1788	3367
4	2559	2337	3335	2050	2867	1945	3829
5	1437	1789	2704	1566	1747	1490	3355
6	1250	2138	2024	1466	1145	1076	1257
7	1038	1485	2098	1434	1418	923,3	2435
8	884,4	1238	1999	1302	795,5	907,9	2346
12	758,1	1171	1824	1202	553,4	614,0	2496
16	708,8	865,6	1440	1126	516,2	535,1	1814
20	638,6	1019	1294	1027	409,0	497,8	1912
24	634,5	820,1	1138	908	401,0	504,6	1403
48	475,3	645	718,6	432,4	378,6	411,6	903,4
72	433,3	462	483,1	232	404,6	329,4	1035
96	314,5	402,5	406,0	127,4	337,9	300,4	635,5

Table 4. EFV concentrations in ng/mL after REFERENCE administration in female subjects (1-8) and male subjects (9-10, 12-15) for each time post dosing. Subject 11 was excluded. Subjects experienced adverse events in the concentrations painted in orange.

Time (h) post dosing	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8
1	0	278,6	131,6	2062,0	1897	177,9	569,0	1210
2	1290	5255	3502	5090	6078	1037	6335	3800
3	3201	4603	3844	4140	4868	1533	3049	3970
4	2954	3592	3970	2493	2814	1714	2492	2359
5	2003	2146	2765	1441	1959	1589	1185	1452
6	1473	2218	1652	1950	1654	1325	1004	1140
7	1741	1921	1618	1146	1444	1268	986,4	1199
8	1061	1497	1174	1143	1534	1127	965,2	1057
12	974,7	1212	943,1	1119	1203	1527	784,8	924
16	900,0	1009	837,3	968,2	1184	1022	737,2	942,6
20	837,6	1478	759,1	990,4	1070	1049	727,7	668,1
24	772,1	904,5	697,0	874,7	1089	854,1	731,7	667,3
48	672,6	454,4	608,2	662,1	867,6	1110	584,5	712,6
72	451,8	599,1	499,0	622,8	891,0	721,4	408,4	382,9
96	396,3	434,4	464,5	446,1	773,3	859,1	473,7	379,1

Time (h) post dosing	Subject 9	Subject 10	Subject 12	Subject 13	Subject 14	Subject 15
1	1714	0	190,1	109,2	192,7	1602
2	3978	547,9	4488	1424	4350	5683
3	2741	3383	2721	3334	2562	4315
4	1759	3286	1838	3365	1524	3690
5	1325	2548	1627	1736	907,9	3056
6	2414	1920	1295	1334	839,6	2949
7	1126	1610	1821	992,8	983,5	2390
8	990,5	1508	1553	880,7	737,5	2198
12	857,9	991,9	1567	633,8	573,9	2024
16	772,3	872,3	1061	565,9	564,4	1786
20	720,6	804,3	981,4	504,9	655,1	1689
24	610,1	773,8	883,5	429,6	475,4	1329
48	512,5	625,0	546,1	405,3	501,1	1073
72	431,0	484,9	393,0	545,5	342,0	833,7
96	367,1	337,5	176,0	364,9	287,4	798,0

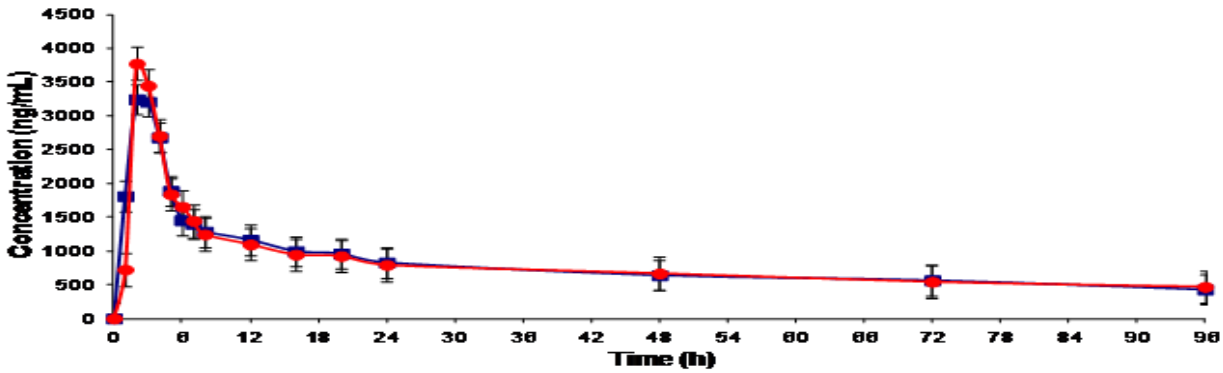

Figure 1. Mean plasma EFV concentration - time curves after administration of 600 mg of TEST (blue curve) and REFERENCE (red curve)

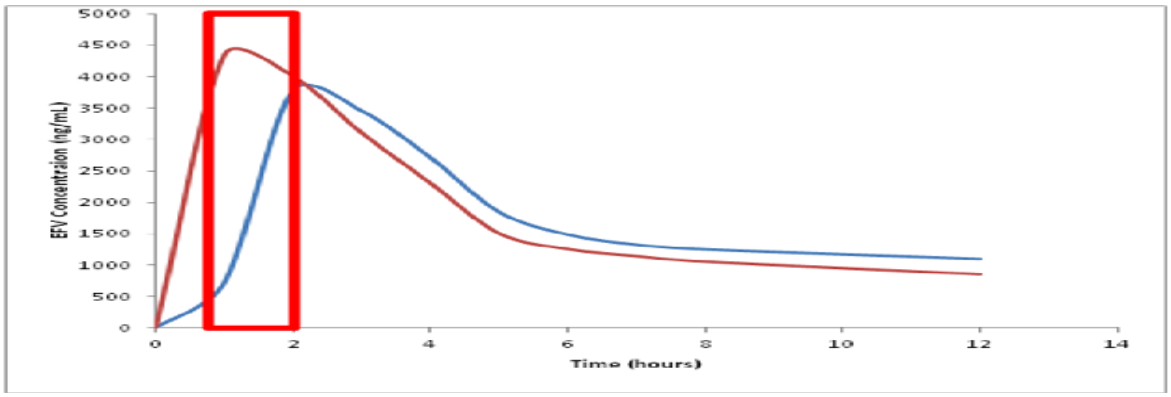

Figure 2. Illustration of arterial (red curve) and venous (blue curve) concentrations after an extravascular administration of EFV in a single dose. Time of onset of adverse events corresponds to the time the arterial C_{MAX} should be attained (red rectangle).

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