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Acute Pharmacokinetics of First Line Anti-tuberculosis Drugs in Patients with Pulmonary Tuberculosis and in Patients with Pulmonary Tuberculosis Co-infected with HIV

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Abstract: Background: The aim of this study was to compare the pharmacokinetics of antituberculosis drugs in patients with pulmonary tuberculosis (PTB) and in patients with PTB and HIV during the first 24 h of treatment. Methods: Designed a case-control study, it compares the pharmacokinetics of first line antituberculous drugs, in HIV-positive (cases) and HIV-negative (control) patients both presenting with pulmonary tuberculosis. Blood samples were collected before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 8, 12 and 24 h after administration of drugs. Drugs plasma levels were tested using HPLC assays. Results: Fourteen HIV positive (7 males and 7 females) and 17 HIV negative (9 males and 8 females) enrolled. Rifafour, a combination tablet including rifampicin, isoniazid, pyrazinamide and ethambutol was used in HIV positive patients, CD4 counts were significantly lower, renal function mildly decreased in 85% patients and moderately decreased in 7% patients. Liver function was normal in both groups. None of these patients was on other drug therapy. In the HIV positive group isoniazid $T_{1/2}$ and AUC were decreased and Cl increased whereas Tmax and Cmax were unchanged. Pyrazinamide Tmax and Cmax were significantly decreased in HIV positive patients and no significant changes were noticed in the $T_{1/2}$, AUC and CL. Conclusion: The study suggest that ethambutol, pyrazinamide and rifampicin pharmacokinetics was not affected by HIV infection and that isoniazid disposition is affected by HIV.

Key words: Pharmacokinetics, rifampicin, isoniazid, ethambutol, pyrazinamide, HIV infection.

1. Introduction

Tuberculosis (TB), in sub-Saharan Africa, remains a leading cause of mortality in HIV infected patients and is the leading cause of death in South Africa where the life expectancy is only 49 years of age. The country has the highest incidence of HIV/AIDS world wide with an estimated 5.7 million people infected in 2009 [1, 2]. Co-infection of HIV/AIDS patients with TB is a substantial health problem. The cure rate in 2008 of new smear-positive patients with TB in South Africa was 64%. Therapeutic response is influenced by the method of administration with Directly Observed Treatment (DOT) estimated to have averted 8 million deaths related to TB [3]. Appropriate management of TB in South Africa is particular important because of the high incidence of HIV, a known risk factor for development of TB, and the high rates of MDR-TB with over 6,000 cases expected to be treated in South Africa in 2010.

Drug treatment of tuberculosis consists of 2 months of isoniazid, refiampin, pyrazinamide and ethambutol followed by 4 months of rifampin and isoniazid [4].

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Therapeutic response is related to serum concentrations of drugs received with poor outcomes associated with low pyrazinamide and isoniazid concentrations [5, 6]. Lower treatment outcomes are seen in patients co-infected with HIV [7], which may explain why the cure rates in South Africa are low.

There have been a few studies that have investigated the influence of AIDS on the absorption of drugs used to treat tuberculosis. Some authors concluded that drug malabsorption may contribute to the emergence of acquired drug resistance [7–10]; and they have advocated for routine therapeutic drug monitoring of antituberculous drugs in HIV-infected patients, particularly those with advanced HIV disease [7–10]. Other studies found that the call for screening of antimycobacterial drug levels in HIV-infected patients with pulmonary tuberculosis is still premature since no impairment in the bioavailability of antituberculosis drugs was found [11, 12].

The aim of this study was to describe the pharmacokinetics of the first line anti-tuberculosis (TB) drugs during the first 24 hours after drug administration in patents infected with pulmonary tuberculosis (PTB) and compare the pharmacokinetic parameters obtained in patients co-infected with PTB and HIV.

2. Patients and Methods

2.1 Study Design, Subjects, Inclusion and Exclusion Criteria

Designed a case-control study, the study compares the pharmacokinetics of first line antituberculous drugs, in HIV-positive (cases) and HIV-negative (control) patients both presenting with pulmonary tuberculosis. The study involved male and female patients without previous history of TB, 18 to 60 years old, with sputum microbiology test positive for Mycobacterium tuberculosis (MTB) which was sensitive to rifampicin (RIF), isoniazid (INH), ethambutol (ETH) and pyrazinamide (PZA). Patients were recruited from their home area TB clinics where they had just been tested for HIV after counseling. After informed and written consent to join the study, patients were admitted to the Karl Bremer Hospital in Cape Town (South Africa), in the evening before the start of TB treatment. They were excluded from the study on request by the patient, if they were critically ill and in case of previous exposure to anti-TB drugs. Other exclusion criteria included the presence of chronic disease such as diabetes mellitus, hypertension and cardiac failure, as determined by physical examination.

2.2 Drugs Used

For the initial phase of treatment (2 months) Rifafour® e-200 tablets were used. Each tablet contains rifampicin (RIF) 120 mg, isoniazid (INH) 60 mg, pyrazinamide (PZA) 300 mg and ethambutol (ETH) 200 mg. Patients weighing 30–50 kg body weight (BW) received 4 tablets Rifafour® e-200 daily dose. Those with a body weight (BW) > 50 kg received 5tablets Rifafour® e-200 daily dose. For the continuation phase of treatment Rifinah ® – 150 and Rifinah ® – 300 were used. Each Rifinah ® – 150 tablet contains RIF 150 mg and INH 100 mg. Each Rifinah ® – 300 tablet contains RIF 300 mg and INH 150 mg. Patients weighing less than 50 kg received 3 tablets of Rifinah ® – 150 and those with a BW ≥ 50 kg received 2 tablets of Rifinah ® – 300. Tablets were taken in front of a nurse every day, five days a week (Monday to Friday). Each patient was also given pyridoxine 25 mg daily in order to prevent peripheral neuropathy due to INH. All tablets were supplied by Aventis Pharma (Pty) Ltd, 2 Bond Street, Midrand 1685, South Africa. The acetylation status of our patients was unknown.

2.3 Collection of Blood Samples

The following morning, after an 8-hour overnight fast, blood samples were collected for base line tests including liver function tests (LFTs), renal function tests (RFTs), full blood count (FBC), CD4 counts and plasma concentrations of the anti-TB drugs. Rifafour was administered to patients for the first time using the dosages recommended by the Department of Health.

Then blood samples were collected for TB drugs plasma levels determination at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 8, 12 and 24 hours after drug administration. Patients were served the same breakfast 1 hour after the beginning of the treatment. They were also offered the same meal at lunch and super time. The following morning, the 24 hours blood sample for TB drug plasma levels determination was collected before breakfast and administration of the following morning anti-TB drugs. Blood samples for determination of TB drugs plasma levels were collected in a heparinized vacutainer tube from an intravenous catheter fixed on a forearm vein of the patient prior to dosing and centrifuged immediately, with the plasma stored in a minus 20˚C freezer for no more than 12 hours. Thereafter, it was stored in a minus 80˚C deep freezer until analysis.

2.4 Laboratory Tests

The following laboratory tests were conducted at the National Laboratory Services

(NHLS) Green Point Lab (Cape Town–South Africa) for each patient: liver function tests (LFTs), renal function tests (RFTs), full blood counts (FBC), sputum microscopy after Auromine or Ziehl-Neelsen stains. If mycobacteria were observed, susceptibility tests were determined forall first — line anti TB agents mentioned above HIV test (Elisa) was done at the University of Cape Town (UCT) Virology Department for each patient. Plasma levels were performed using high performance liquid chromatography (HPLC) assay methods. The lower limit of quantification for all four drugs was 0.05 µg/ml. The linear range for isoniazid and rifampicin was 0.05–30 µg/ml and for pyrazinamide it was 0.05–100 µg/ml. All four drugs were measured from the same assay run.

2.5 Determination of Pharmacokinetic Parameters

After determination of rifampicin, isoniazid, pyrazinamide and ethambutol plasma concentrations, the plasma concentration-time profile for each drug by patient was plotted using Graph Pad Prism program. Drugs pharmacokinetic parameters were calculated based on the non-compartmental analysis (NCA) as follows:

2.5.1 Maximum Concentration and the Time to Reach Maximum Concentration

The maximum concentration (Cmax) and the time to reach the maximum concentration (T_{max}) were obtained directly from the plasma concentration-time profile.

2.5.2 Half-life

The half-life (T½) was calculated using the formula: $T½ = 0.639/K_e$ and K_e was determined from the terminal slope of the ln-concentration vs time curve.

2.5.3 The Area under the Plasma Concentration-Time Curve

The area under the plasma concentration-time curve from zero to 24 hours (AUC_{0-24}) was calculated by the trapezoidal method using Graph Pad Prism software.

2.5.4 The Total Body Clearance

The apparent total body clearance (Cl_{tot}) was calculated using the following formula: Cl_{tot} = Dose/ AUC_{0-24}. Low Cmax values were defined using the following method reported by Jordan W. Tapero et al. [7]: isoniazid, < 3 µg/mL (300 mg dose); rifampicin, <8 µg.mL (weight –adjusted dose, 450 or 600 mg); pyrazinamide, <35 µg/mL (median dose, 35 mk/kg); and ethambutol, <2 µg/mL (median dose, 21 mk/kg). Very low Cmax values were defined as follows: isoniazid, <2 µg/mL (300 mg dose) or <3 µg/mL (400 mg dose); rifampicin, <4 µg/mL; pyrazinamide, <20 µg/mL; and ethambutol, <1 µg/mL. Delayed absorption was defined as a Tmax >3 h.

2.5.5 Ethical and Statistical Considerations

The study was conducted according to the declaration of Helsinki and ICH guidelines. The protocol was approved by the ethics committee of the University of the Western Cape. Permission to conduct the study was granted by the medical superintendent of Karl Bremer Hospital. The information obtained during the study was treated as confidential.

For each of 4 drugs responses for those who are HIV Positive were compared to those who were negative on each of the 9 parameters. Some of the parameters are related, so test results are not independent. Nevertheless there are many tests being done so a more stringent level of significance was used (a p value of < 0.01 rather than the customary 0.05 was required for significance). In many cases the distribution of the measurements was skewed and decidedly non-normal. Consequently groups were compared using the Wilcoxon Rank Sum test.

3. Results

Thirty one (31) patients were involved in the study with 14 (7 males and 7 females) patients determined to be HIV positive (cases) and seventeen patients (9 males and 8 females) HIV negative (control) (Table 1). All of them were infected with Mycobacterium tuberculosis (MTB) sensitive to anti-TB drugs used. During the intensive phase (first 2 months) rifafour, a combination tablet including rifampicin 120 mg, isoniazid 60 mg, pyrazinamide 300 mg and ethambutol 200 mg, was used at the dose of 4 tablets daily five days a week (Monday to Friday) for patients under 50 kg body weight and 5 tablets daily for patients weighing 50 kg and more.

The median body weight was 54 (range 51–60) kg in HIV-positive patients (cases) and 56 (range 51–65) kg in HIV-negative patients (control).

Table 1 Demographics, renal and hepatic function data.

Characteristics	HIV (+) patients (Nb:14)	HIV (-) patients (Nb:17)
Gender	7 males, 7 females	9 males, 8 females
Age : average (range)	29.46 (18.5–55)	26.79 (18–36)
CD4 counts: average (range)	133.14 (2–332)	1015.18 (674–1458)
GFR: range (ml/min)	30–59 (12 patients) 60 – 89 (1 patient) >90 (1 patient)	> 90: normal
ALT (normal values: 5-40)	12 – 35	6 – 25
AST (normal values: 5-40)	17 – 39	8 – 30
GGT (normal values: 0 – 60)	5 – 55	7 – 35

As indicated in Table 1, the age distribution is similar in both groups. The mean age is 33.06±10.12 in HIV negative group (control) and 35.14±12.05 in HIV positive group (cases).

Out of 14 HIV-positive patients, 9 were stage 3 (WHO staging system) and 5 were stage 4 (WHO staging system). The mean CD4 cells was 356.38 and the mean period of HIV infection was 5.9 yrs.

The CD4 counts were significantly lower in the HIV positive patients (cases) than in the HIV negative patients (control). In the HIV control group, the renal function was normal. However, in cases group, the renal function was mildly decreased in 85.71% of patients, moderately decreased in 7.14% of patients and normal in 7.14% of patients. The hepatic function tests were normal in both groups. None of these patients was on other drug therapy.

Tables 2a, 2b, 2c and 2d provide the descriptive statistics for the pharmacokinetic parameters for the test drugs in HIV positive and HIV negative patients. In general the presence of HIV did not impact the disposition of ethambutol, pyrazinamide and rifampicin. The one exception was isoniazid which showed a modest effect of HIV on its disposition. A statistical reduction in T_{max} in HIV positive patients was observed and borderline changes in half-life, AUC_{0-24} and Cl. It appears that the presence of HIV may modestly decrease AUC and half-life which is consistent with either an observed increase in clearance or reduction in bioavailability.

4. Discussion and Conclusion

The current study evaluated the disposition of

Table 2a Pharmacokinetic parameters in HIV negative and HIV positive patients.

Ethambutol (1000 mg-dose)	HIV-negative	HIV-positive	Statistical significance
Subject Number	17	14	
T_{max} (h)	2.4 ± 0.32	2.4 ± 0.32	NS
C_{max} (µg/ml)	2.8 ± 0.65	2.8± 0.77	NS
$T_{½}$ (h)	4.5 ± 0.61	4.4± 0.46	NS
AUC_{0-24}(µg/h.ml)	11.5 ± 1.5	11.6± 2.9	NS
Cl (L/h)	88.7 ±11.4	90.5± 20.9	NS

Table 2b Isoniazid pharmacokinetic parameters in HIV negative and HIV positive patients.

Isoniazid (300 mg-dose)	HIV-negative	HIV-positive	Statistical significance
Subject Number	17	14	
T_{max} (hrs)	2.5±0.12	2.1±0.21	<0.001
C_{max} (µg/mL)	2.1±0.26	2.2±0.37	NS
$T_{½}$ (hrs)	4.8±3.2	3.5±2.6	0.0299
AUC_{0-24} (µg/h.ml)	15.4±9.5	9.6±3.3	0.0365
Cl (L/hr)	25.4±11.2	34.3±9.8	0.0365

Table 2c Pyrazinamide pharmacokinetic parameters in HIV negative and HIV positive patients.

Pyrazinamid (1500 mg dose)	HIV-negative	HIV-positive	Statistical significance
Subject number	17	14	
T_{max} (hrs)	2.5±0.26	2.4±0.43	0.084
C_{max} (µg/ml)	61.7±8.6	57.2± 9.8	0.124
$T_{½}$ (hrs)	8.8±2.3	9.7±2.6	NS
AUC_{0-24} (µg/h.ml)	698.9±237.4	730.2±194.6	NS
Cl (L/hr)	2.4±0.98	2.2±0.59	NS

Table 2d Rifampicin pharmacokinetic parameters in HIV negative and HIV positive patients.

Rifampicin (600 mg-dose)	HIV negative	HIV positive	Statistical significance
Subject Number	17	14	
T_{max} (hrs)	2.8±0.36	2.7±0.32	NS
C_{max} (µg/ml)	13.5±2.5	13.7±1.2	NS
$T_{½}$ (hrs)	6.0±2.9	6.5±2.7	NS
AUC_{0-24} (µg/h.ml)	95.3±34.8	113.5±49.9	NS
Cl (L/hr)	7.1±2.6	6.2±2.5	NS

ethambutol, pyrazinamide, isoniazid and rifampcin in patients with active TB who were either HIV-positive or HIV-negative. The data suggests that isoniazid disposition is affected by HIV with the T_{max} and $AUC_{0-\infty}$ being lower in patients co-infected with HIV. The presence of HIV had no effect in this study on the disposition of ethambutol, pyrazinamide or rifampicin. Several studies have evaluated the influence of HIV on the pharmacokinetics of first line anti-TB drugs. The demographic parameters (number, gender balance, mean age and mean CD4 lymphocytes count) of patients involved in this study are similar to those of patients who participated in the previous studies [12, 15–18]. Ethambutol C_{max}, T_{max} and half life found in this study are similar to those reported in previous studies and there is no significant difference between HIV-negative and HIV-positive patients [12, 15–18]. The presence of HIV does not influence the disposition of ethambutol. Isoniazid pharmacoki- netic parameters found in this study and those reported previously [12, 15–18] are quite variable. This could be explained by the differences between studies in the incidence of rapid and slow acetylators in the population. However, for each study, there is no significant difference between pharmacokinetic values in HIV-negative and HIV-positive patients. Our study does show a modest 37% reduction in AUC in HIV-positive patients. This result is in variance with other studies and does indicate the need for additional studies. The AUC that we report in HIV-negative patients is very comparable to that reported in normal health volunteers that are slow acetylators [19]. Whereas the AUC value we reported in HIV-positive patients is between that reported for rapid and slow acetylators of INH. The differences that we report in INH AUC as a function of HIV status could very well reflect differences in the number of patients who are rapid acetylators between these groups.

In this study, pyrazinamide and rifampicin Tmax, Cmax, $T_{1/2}$ and AUC values are higher than in studies previously conducted [12, 15–18]. However, in each individual study, there is generally no significant difference between HIV-negative and HIV-positive patients. The most recent study from Botswana [6] reported lower than expected concentrations of pyrazinamide and showed that these were related to treatment outcomes. This study, however, showed only very modest influences ($p < 0.04$) of HIV infection on the disposition pyrazinamide and rifampin. These results are not that much different that this study in South Africa.

In HIV positive patients, renal function was mildly decreased in 85.71% of patients and moderately decreased in 7.14% of patients. This however did not influence the pharmacokinetics of isoniazid, ethambutol, pyrazinamide and rifampicin.

In this study ethambutol and rifampicin pharmacokinetics were not affected by HIV infection. In the HIV positive group isoniazid $T_{1/2}$ and AUC were decreased and Cl increased whereas T_{max} and C_{max} were unchanged. Pyrazinamide, Tmax and Cmax were significantly decreased in HIV positive patients and no significant changes were notices in the $T_{1/2}$, AUC and CL.

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