VOLUME-8, ISSUE-7, JULY-2019 • PRINT ISSN No. 2277 - 8160

Original Research Paper

Pulmonary Medicine

VARIATIONS IN PLASMA LEVELS OF RIFAMPICIN AND PYRAZINAMIDE WITH PRE AND POST MEAL ADMINISTRATION OF THRICE WEEKLY REGIME OF RNTCP, AFTER TWO MONTHS OF THERAPY

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ABSTRACT

52 patients of New Smear Positive Pulmonary Tuberculosis were given 3 times a week RNTCP regime under observation. On completion of 2 months after taking predose pharmacokinetic sample, the study drugs were administered, in half of the patients before and in rest, after meal. Post dose samples were withdrawn at 2, 4, 6, and 10 hours after drug administration. Analysis of samples was done using HPLC.

Mean ± SD of C_{max} of Rifampicin was 6.42 ± 3.01 µg /ml, adjusted T_{max} was 4.45 hours. In pre-meal samples mean ± SD of C_{max} levels of Rifampicin were 7.01 ± 2.24 µg/ml, adjusted T_{max} was 3.38 hours. In post-meal samples, mean ± SD of C_{max} of Rifampicin was 5.93 ± 3.51µg /ml, adjusted T_{max} was 5.26 hours.

Mean ± SD of Pyrazinamide C_{max} levels was 49.23 ± 17.32 µg /ml, adjusted T_{max} was 3.79 hours. In pre-meal samples, mean ± SD of C_{max} levels of Pyrazinamide was 51.91 ± 16.69µg /ml, adjusted T_{max} was 3.36 hours. In post-meal blood samples, mean ± SD of C_{max} levels of Pyrazinamide was 46.55 ± 17.98 µg/ml, adjusted T_{max} was 4.21 hours.

KEYWORDS : Plasma levels; antitubercular: rifampicin; pyrazinamide

1. INTRODUCTION

Exposure of the organism to variable and sub-therapeutic concentrations of antitubercular drugs leads to acquired resistance which subsequently leads to increased primary drug resistance. Studies have demonstrated that plasma levels of first line antitubercular drugs are variable and they differ from place to place, population to population, with duration of therapy and with presence of other diseases etc. This increased interindividual and interoccasional variability may affect the efficacy of the drugs by increasing the likelihood of sub therapeutic concentrations.[1-5] This potentially complicated by Rifampicin's well reported induction of the activity of Cytochrome p450 enzymes could lead to delayed or incomplete response to treatment and an increase in the risk of the emergence of drug resistance.[6-8]

Pharmacokinetic data supplied by the manufacturer is generated from a small number of healthy subjects administered single drug in standard conditions which is difficult to reproduce in practice. We need to have therapeutic drug level monitoring at local levels to ensure that the plasma concentrations of these drugs is maintained within therapeutic limits irrespective of all variables so as to increase the success rate of the therapy and to decrease the incidence of drug resistance.

3. OBJECTIVES

The study aims to ascertain the variability in plasma concentrations of first line antitubercular drugs with thrice weekly regime of RNTCP during the course of therapy with pre and post-meal administration of drugs in the population of Kangra valley.

4. MATERIAL AND METHODS:

The study was conducted in Dr. Rajendra Prasad Government Medical College and Hospital Tanda, District Kangra (HP). The protocol was reviewed and approved by Institutional Ethical Committee before the recruitment of subjects started.

Diagnosis of the patients was done based on history, clinical examination, chest x-ray and sputum microscopy. After inclusion of the patients in the study, history of drug and nutritional supplement intake in the previous one week was taken. Detailed physical examination, screening for peripheral neuropathy and laboratory investigations (LFT, RFT, Routine Haematology, Blood glucose and HIV) were done. Pregnant women, nursing mothers, subjects with liver dysfunction and renal insufficiency, HIV positive subjects and subjects with any surgical or medical condition which could significantly alter the absorption, distribution, metabolism or excretion of the study drug were excluded from the study.

METHOD

On completion of two months of drug treatment with first line antitubercular drugs, all patients were divided in to two groups for Pre and post-meal administration of drugs. Patients were allotted these groups by systemic randomization. Subjects were admitted night before drug administration and were given only tap water from 21:00 PM.

In the morning (Approximately 6:00 AM) a Predose Pharmacokinetic sample was taken, the study drugs (600 mg of Isoniazid, 450mg of Rifampicin, 1200mg of Ethambutol and 1500mg of Pyrazinamide) supplied through RNTCP were administered at 7:00 AM with 200 ml of water and swallowed in sitting posture without chewing the drug. In group 1 dosing was done empty stomach and in group 2 dosing was done just after a meal comprising of pulses, vegetable, rice and chapati. Subjects were not allowed to exert physically.

Post dose samples were withdrawn at 2, 4, 6, and 10 hours after drug administration. Date and time of the sample collection were recorded and food was given 5 and 12 hours after dosing.

All the patients underwent detailed physical examination, screening for peripheral neuropathy and routine laboratory investigations (LFT, RFT, Routine Haematology and Blood glucose) which were done at the time of inclusion of the patient in the study, if suggested by the clinician.

Sample Processing

Collected serum samples were kept in liquid nitrogen. On the day of processing, serum samples were taken out from nitrogen container and thawed at room temperature. One ml of serum was mixed with one ml of acetonitrile to allow denaturation of proteins. The sample was centrifuged at 3000 rpm for 10 minutes. Equal volume of supernatant was mixed with equal volume of acetonitrile. The sample was centrifuged

again for 20 minutes for precipitation of any remaining protein. 20 μL of this supernatant was injected in HPLC system manually.

Analysis

Analysis of Isoniazid, Pyrazinamide and Rifampicin was done simultaneously using external control. Mobile phase was acetonitrile and phosphate buffer 50:50 (v/v). pH of phosphate buffer was 6.8 and flow rate was 1 ml/minute. Run time was 12 minutes. Peaks of INH, Pyrazinamide and Rifampicin were obtained at 2.6, 2.9 and 6.2 minutes respectively.

Calculation of pharmacokinetic parameters

The non-compartmental pharmacokinetic analysis method was employed to determine the pharmacokinetic parameter of the drugs. C_{max} and T_{max} were obtained directly from the observed data. AUC_{0-10} was calculated by the linear trapezoidal method.

5. RESULTS

A total of 52 patients with new smear positive tuberculosis were enrolled in the study. 37 (71.15%) patients were males and 15 (28.85%) were females with mean age ± SD being 44.8 ± 15.53 years. Mean weight ± SD of the patients was 45.52 ± 8.57 and mean body mass index (BMI) ± SD was 17.69 ± 3.41. Samples were collected on first day and at the end of two months. The results of first day samples are published in the past.[9]

Plasma levels

Out of 52 study subjects enrolled in the study, 39 turned up at the end of 2 months of their treatment and 217 samples were collected from them.

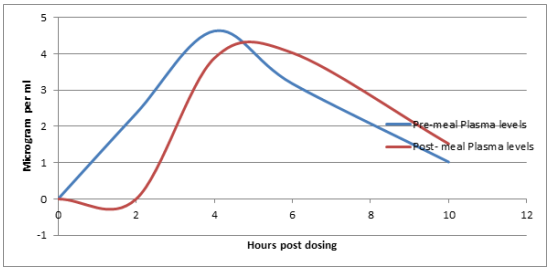
On completion of 2 months of antitbercular regimen, Mean±SD of C_{max} of Rifampicin was 6.42± 3.01 µg /ml; Range of C_{max} levels was 1.28- 14.43µg /ml and Median was 5.73 µg /ml (N=35. Mean ± SD of $AUC_{0-10}$was 31.30±18.78 µghr/ml; Range was 0- 73.11µghr/ml and Median 29.71µghr/ml (N=32). Adjusted $T_{max}$was 4.45 hours.

(Table 1)

Table 1: Plasma levels of rifampicin at the end of 2 months of treatment

N	Time post dosing	Mean ± 1SD (µg/ml)	Range (µg/ml)	Median (µg/ml)	N (Undetected levels)
39	2 hours	2.51 ± 3.76	0-14.43	0.50	19
39	4 hours	4.38 ± 2.73	0-9.52	4.41	4
37	6 hours	4.07 ± 2.71	0-13.38	3.52	4
33	10 hours	2.13 ± 2.15	0-7.25	1.38	4

Figure 1: Median plasma levels of Rifampicin at 2 months



Pre-meal

In pre-meal samples collected at the end of completion of 2 months of treatment Mean±SD of Cmax levels of Rifampicin were 7.01± 2.24 µg/ml; Range was 3.47- 11.43µg /ml and Median was 7.21µg/ml(N=16). Mean±SD of AUC0-10of plasma levels was 32.37± 20.67 µghr/ml; Range of AUC0-10 was 0- 73.11µghr/ml and Median AUC0-10 was 35.65 (N=17). Adjusted T max was 3.38 hours. (Figure 1)

Post-meal

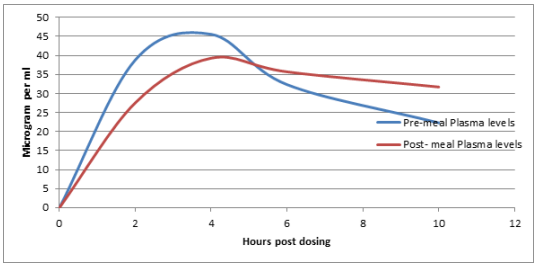
In post-meal samples,Mean±SD of Cmax of Rifampicin was 5.93±3.51µg /ml; Range was 1.28- 14.43µg /mland Median was 4.77µg /ml (N=19). Mean±SD AUC0-10 was 30.09± 17.02µghr/ml; Range 6.57- 62.21µghr/ml and Median was 23.49µghr/ml(N=15). Adjusted Tmax was 5.26 hours. (Figure 1)

Table 2: Plasma levels of pyrazinamide at the end of 2 months of treatment

N	Time post dosing	Mean ± 1SD (µg/ml)	Range (µg/ml)	Median (µg/ml)	N (Undetectable levels)
39	2 hours	33.77 ± 21.45	0-73.41	33.98	4
39	4 hours	42.92 ± 17.75	0-87.14	39.44	1
37	6 hours	36.33 ± 17.47	0-71.36	35.45	1
33	10 hours	27.80 ± 15.53	0-62.69	24.67	1

In the samples collected on completion of 2 months of treatment Mean±SD of Pyrazinamide C_{max} levels was 49.23±17.32 µg /ml; Range was 15.88-87.14µg /ml and Median was 47.79 µg /ml(N=38). Mean±SD of AUC_{0-10} was 323.17±136.86µghr/ml; Range of AUC_{0-10} was 0-609.68 µghr/ml and Median was 298.34 µghr/ml (N=32). Adjusted $T_{max}$was 3.79 hours. **(Table 2)**

Figure 2: Median plasma levels of Pyrazinamide at 2 months



Pre-meal

At 2 months, in pre-meal samples, Mean±SD of C_{max} levels of Pyrazinamide was 51.91±16.69µg /ml; Range was 23.03-76.05µg /ml and Median was 57.35 µg /ml (N=19). Mean±SD of $AUC_{0-10}$of Pyrazinamide plasma levels was 346.47±128.17µghr/ml; Range of AUC_{0-10} of Pyrazinamide plasma levels was 132.94- 580.53µghr/ml and Median was 323.89µghr/ml (N=16). Adjusted $T_{max}$was 3.36 hours. **(Figure 2)**

Post-meal

In post-meal blood samples collected on completion of 2 months of treatment with antitubercular regimen, Mean±SD of C_{max} levels of Pyrazinamide was 46.55±17.98 µg/ml; Range was 15.88-87.14µg /ml and Median of plasma levels was 43.66 µg /ml (N=19). Mean±SD of AUC_{0-10} of plasma levels was 319.86±125.62 µghr/ml; Range was 68.68- 609.68 µghr/ml and Median was 308.83 µghr/ml (N=15). Adjusted T_{max} was 4.21 hours. **(Figure 2)**

6. DISCUSSION

The ideal concentrations required for effective therapy with antitubercular drugs are only partially known. Precise targets for peak serum concentrations relative to the minimal inhibitory concentration (Cmax: MIC), or time above MIC, are not available from human studies. So the target of the therapy is to achieve the concentrations achieved in healthy volunteers under controlled, phase I study conditions. It is well known that the standard doses are generally effective so these concentrations also should be effective.[10]

The plasma levels of rifampicin reported in healthy volunteers are 8-24 µg/ml with T_{max} at 2 hours with 600 mg daily regime.[10] In patients of this study, the levels reported range from 1.28 to 14.43 µg/ml. Higher doses of rifampicin has been found

associated with improved early bactericidal activity and better treatment results.[11,12] The drug's activity has been found to be dependent on concentration and related to the AUC/MIC ratio. So more C_{max}/MIC will be related to more antibacterial activity.[13] Suitable dose adjustments by repeated drug level measurement can help in dealing with the problem by providing required therapeutic concentration and thus partly avoiding problem of drug resistance.

A dose of 25 mg/ kg/ day of pyrazinamide The plasma levels of pyrazinamide generates 20-50 μg/ml plasma levels and that of 50 mg/kg biweekly regime, 40-100 μg/ml with T_{max} at 1-2 hours in healthy volunteers.[10] In patients various studies report 29.9-84.4 mcg/ml.[14-16] The Cmax levels of pyrazinamide in our study vary widely from 15.88-87.14μg /ml. One of the reasons for this wide variability is difference in body weight that generated a wide variability of drug dose per kg body weight.

Pre and post-meal variability in plasma levels

Food is found to affect the bioavailability of antitubercular drugs. Antitubercular drugs should preferably be administered empty stomach.[10] In our study, there was a difference of 1.08 μg/ml in the mean and 2.44 μg/ml in the median levels of rifampicin though the difference in the AUC_{0-10} was only 1.28 μ ghr/ml. T_{max} was almost 1hr 50 minutes more in post-meal samples.

The levels of pyrazinamide were more in post meal samples, the difference in mean, median and AUC of pre and post-meal levels being 5.36 μg/ml, 13.69μg/ml and 26.61μghr/ml respectively. Tmax was 51 minutes more in post-meal samples.

8. CONCLUSION

It is evident from our study that there is a wide variability in plasma levels of rifampicin and pyrazinamide. Serum drug levels of all the patients of first line antitubercular drugs should be done at various stages of therapy. Patients with very low or very high serum levels should be segregated for dose modification to achieve target therapeutic range.

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