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SERUM CONCENTRATION OF PYRAZINAMIDE SUSPENSION IN CHILDREN WITH TUBERCULOSIS: A THERAPEUTIC DRUG MONITORING

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ABSTRACT

Rationale. Therapeutic drug monitoring (TDM) is a process of adjusting drug dosages on the basis of serum drug concentrations for the purpose of optimizing drug therapy. This study introduces the use of TDM in the management of mycobacterial infections. Pyrazinamide (PZA) has been marketed as tablet in other countries. It is only in the Philippines wherein pyrazinamide is available both in tablet and suspension forms. No study has been done on pyrazinamide suspension use for the treatment of tuberculosis to this date.

Objectives. To examine the serum concentration of pyrazinamide suspension in children with tuberculosis

Design. Descriptive study

Methods. Thirty pediatric patients who were taking pyrazinamide suspension for at least 1 week as part of chemotherapy for tuberculosis were included in this study. Blood was taken prior to the dose then 2, 4, 8 hours after administration of PZA suspensionfor thefirst 4 patients. Specimens were submitted to the Pharmacology Department Laboratory of the University of the Philippines – College of Medicine and were analyzed using High Performance Liquid Chromatography technique. The samples from the first 4 patients were used to determine the time when the drug reaches its maximum concentration (Tmax). For subsequent patients, 2 determinations were taken at the time when the drug reaches its maximum concentration and trough level.

Results. At at a Tmax of 2 hours, the mean serum concentration of PZA suspension is at 34.6± 11.86 ug/ml. The mean serum trough level is 4.55± 4.63 ug/ml. There were no significant differences in serum concentration of PZA suspension among 3 brand names of PZA (p-value: 0.506).

Conclusion. Mean serum concentration of PZA suspension falls within the established therapeutic range for pyrazinamide. But 2 subjects failed to reach the therapeutic levels. No subject reached toxic levels

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Keywords. Pyrazinamide, antituberculosis, therapeutic drug monitoring, pharmacokinetics, tuberculosis

INTRODUCTION

In the past 4 years, tuberculosis ranks 6th in the leading causes of morbidity in the Philippines. It is also the 5th leading cause of mortality from 1989-1993. Children under 15 years of age represent 1.3 million cases per year and 450,000 deaths per year. Despite the widespread use of BCG vaccine and the availability of effective drugs, TB remains a major health problem. The eradication of TB has proven to be an elusive goal for clinicians and policy-makers. Failure of TB control is not a new phenomenon in our country. Several factors, including irrational antibiotic use, collapse of public health infrastructures, the HIV epidemic, war, famine, increasing inequality and poverty, and prohibitive cost of medicines, have all contributed to the increasing incidence of TB all over the world. In certain situations, drugs provide suboptimal serum concentrations and these are associated with worse treatment outcomes. Recurrence occurs in 2.4-5.5% of cases even when the patient receives directly observed treatment. It is important to maintain high standards of quality assurance, as low quality drugs often penetrate emerging markets, resulting in low cure rates for patients and increased resistance.

Definition of Terms

1. Therapeutic drug monitoring (TDM) – is the process of using serum drug concentrations to optimize drug therapy. TDM is useful when serum concentrations show a better correlation with the therapeutic effects or the incidence of adverse effects than does the size alone. TDM requires the accurate timing of doses and blood collection and the avoidance of assay interferences.

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2. Tmax – time when drug reaches maximum/peak serum concentration
3. Trough – serum concentration of the drug at time 0
4. Cmax - peak serum concentration

Review of literature

Pyrazinamide is one of the most frequently administered drugs for the treatment of TB. It was synthesized in the 1940s by Hall and Spoerri and formerly used only as salvage therapy. Convincing results of experimental studies have elevated it to a central role in tuberculosis chemotherapy as an essential addition to isoniazid and rifampicin which make it possible to shorten the treatment to 6 months. The major contribution of pyrazinamide is to increase the sterilizing power of an antituberculosis regimen, measured by prevention of relapse after initially successful therapy. Pyrazinamide is administered at 15-30 mg per kg with a maximum daily dose of 2 grams. When administered twice weekly, the dose of pyrazinamide is 50-70 mg/kg/dose with a maximum dose of 4 grams. A randomized, four-period, crossover study, a single oral dose of 30 mg/kg of pyrazinamide tablet in a fasting state results in a mean peak serum concentration of 53.4 ug/ml at 1.43 hours. [11] The mean serum concentration of pyrazinamide tablet was slightly increased by antacid and modestly decreased by food (p=0.0138). The optimum sampling time was 1 hour after the dose, which is the closest to Cmax. Similarly, a prospective study of C. Lacroix regarding pyrazinamide kinetics in 9 healthy subjects showed a rapid absorption in the fasting state (tmax </= 1 hour).[12] In Peloquin's two-way, randomized, crossover study of isoniazid, rifampicin, and pyrazinamide in 1997, the calculated steady-state range for pyrazinamide (dose of 20mg/kg) was 27.03 to 53.12 ug/ml.[16]

Current knowledge on PZA came from results described mostly in healthy volunteers. However, the pharmacokinetics of pyrazinamide in children with tuberculosis differ from that found in healthy volunteers. In the prospective, multiple-dose population pharmacokinetic study of Min Zhu has shown these differences.[17]

Pyrazinamide has been marketed as tablet (500 mg) in other countries. In the Philippines, it is available in tablet and suspension form. The suspension form (250mg/5 ml) is very unstable. It crystallizes when it is allowed to stand in room temperature and especially when it is refrigerated. Much of the studies published to this date used the tablet form of the drug. Bioavailability studies on PZA were based of the tablet preparation. Locally, according to the Bureau of Food and Drug Administrative Order 67 series of 1989, all drug manufacturers, traders and distributors are required to submit bioavailability tests on the products sought to be registered. At present, there are 5 existing brands of pyrazinamide in the market. The purpose of this study is to determine the serum level of pyrazinamide suspension in order to optimize drug therapy and verify that they achieve the therapeutic level. The National Jewish Center for Immunology and Respiratory Medicine proposed a therapeutic range for PZA at 2-hour which is at 20-60 ug/ml. Generally speaking, drug concentration at the site of drug action are difficult to determine and typically not obtainable. Therapeutic drug monitoring assumes that there is a better correlation between serum concentration and drug effects than between the dose prescribed and the drug effects.

Peloquin of the Infectious Disease Pharmacokinetics Laboratory in Denver suggested an approach to the use of antibiotic serum concentration.[15] It is clear that some drug concentration is required to achieve a therapeutic effect. The target ranges should be designed with a margin of safety with respect to their efficacy. Once a decision is made to use a given antibiotic, a goal should be set for the desired serum concentration. Monitoring antimycobacterial drug levels allows clinician to identify those patients who are not absorbing, metabolizing or eliminating their drugs normally.

OBJECTIVES

General objective

To examine the serum concentration of PZA suspension in children with tuberculosis

Specific objectives:

1. To present the demographic characteristics of subjects in terms of the following: age, sex, duration of therapy, organ of affectation
2. To compute the Tmax in 4 patients in order to determine the correct timing of peak level in subsequent patients
3. To compare the peak and trough of PZA suspension in children with tuberculosis with the established therapeutic range and duration of therapy
4. To compare the peak and trough levels of 3 most commonly used brands of PZA suspension with each other

METHODOLOGY

Study design. Descriptive study

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Study population

Thirty pediatric patients who were taking PZA suspension for at least 1 week as part of chemotherapy for tuberculosis were included in this study. The subjects were recruited from the TB registry of the INTROP section of the Department of Pediatrics and from admitted patients at Ward 11.

Inclusion criteria:

1. Thirty pediatrics patients diagnosed to have tuberculosis of any organ and are taking PZA suspension as part of antituberculosis therapy for at least 1 week
2. normal CBC, serum creatinine, AST, ALT, uric acid
3. informed consent from parent or guardian

Exclusion criteria:

1. poor compliance with the medications
2. abnormal CBC, serum creatinine, AST, ALT, uric acid
3. failure to give consent.
4. liver or renal insufficiency
5. adverse reactions to anti-TB medications

DATA COLLECTION

Last intake of pyrazinamide suspension of the thirty patients is 24 hours prior to the procedure. The investigator observed the patient take the pyrazinamide suspension and recorded the exact date and time. Three to five ml of blood was drawn from the subjects, who were at least on their 2nd week of pyrazinamide suspension therapy, via direct venipuncture. An indwelling IV catheter attached to a heplock was inserted in order to minimize punctures. Blood was taken prior to the dose then 2, 4, 8 hours after administration of pyrazinamide suspension. The specimens were placed in glass test tubes labeled with the patient's name, date and time of collection, and the drug to be assayed. It was then allowed to clot and then stored in an icebox at –20 degrees centigrade and was submitted to the Pharmacology Laboratory of the UP-College of Medicine. Blood specimens were centrifuged for 5 minutes. The plasma was analyzed using High Performance Liquid Chromatography technique. This method can measure the amount of pyrazinamide in the blood to as low as 0.1 ug/ml. Samples for the first four patients were used to determine Tmax. For subsequent patients, 2 determinations were taken at Tmax and trough.

Outcome measured.

Serum concentration of pyrazinamide suspension in children with tuberculosis

STATISTICAL ANALYSIS

Frequency of distribution included measures of central tendency for age, duration of treatment, serum concentrations at hour 0 and 2. Differences among subjects and groups were determined by analysis of variance (ANOVA) model. Paired samples were compared. Sample size calculations were based on an estimated mean difference of 20 and a standard deviation of the difference of 15. The test of equality of means was carried out at 0.017 level of significance (overall level of significance for pair wise comparisons of pyrazinamide brand names is 0.05). A sample size of 9 pairs per brand name gives a probability of 0.815 of rejecting the null hypothesis of equal means if the alternative holds.

RESULTS

The characteristics of subjects who participated in the study are described in Table 1 and 2. Thirty subjects (16 males; 14 females) were included. The mean age is 9.46 +/-4.65 (range: 1-18 years). The median duration of therapy is 9 days (range: 7-35 days). The subjects received 20-25 mg/kg/day of pyrazinamide suspension in keeping with the standard clinical practice of the institution. Twenty-six subjects have concomittant intake (intravenously and orally) of other drugs on the day of blood sampling such as oxacillin, cefepime, penicillin G, cefuroxime, amikacin, furosemide, acetazolamide, dexamethasone, prednisone, phenobarbital, propranolol, lanoxin, kalium durule, vit B complex, dibencozide and multivitamins. All of these drugs were assayed to check for interference in pyrazinamide level. Only acetazolamide was found to interfere with the assay of pyrazinamide. All patients received pyrazinamide in combination with other antituberculosis drugs including isoniazid, rifampicin, ethambutol and streptomycin. Among the study population, 40% has pulmonary tuberculosis. This was followed by tuberculous meningitis and Potts disease at 23% and 13%, respectively.

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Table 1. Mean age and duration of therapy of patient population

Variable	Mean	Median	Standard Deviation	Range
Age (years)	9.4667	10.5	4.6589	1.00-18.00
Duration of therapy (days)	12.1	9.0	8.1213	7.00-35.00

Table 2. Patient Population Demographics

Variable	N	Percentage
Male	16	53.3%
Female	14	46.6%
Organ of affectation		
Lungs	12	40%
CNS	7	23.3%
Bone	4	13.3%
Disseminated	3	10%
Heart	2	6.6%
Endobronchial	1	3.3%
Kidney	1	3.3%

Table 3 shows that Tmax was determined using the first four subjects. Based on the computation, Tmax of 2 hours was used for the subsequent subjects (mean of 2.98+/-1.1).

Table 3. Computed Tmax in first 4 patients

Patient	Hour 0	Hour 2	Hour 4	Hour 8	Tmax
#1	4.24	20.25	8.43	4.55	2.28
#2	0.26	7.14	10.55	8.26	1.98
#3	12.17	24.06	23.09	15.85	4.44
#4	9.23	28.62	20.37	13.32	3.22

Table 4. Suspention trough and Tmax serum concentration of pyrazinamide

Patient no.	Duration of therapy	Serum concentration (ug/ml) 0 hour (Through)	2 (Tmax)	4	8
ZINAPLEX® by Pediatrica					
3	16 days	9.23	28.62	20.37	13.32
13	7	4.76	25.98		
14	9	0**	58.64		
17	7	2.94	45.80		
22	10	0**	44.48		
23	7	0**	42.02		
24	10	0**	41.94		
25	8	0**	21.48		
28	7	8.02	38.62		
29	10	13.97	52.58		
ZCURE® by Natrapharm					
1	30 days	0.26*	7.14	10.55	8.26
2	14	4.24	20.25	8.43	4.55
4	9	12.17	24.06	23.09	15.85
8	11	0.88*	34.36		
9	9	4.19	27.05		
10	30	14.49	43.53		
18	9	0.85*	45.79		
19	7	2.64	47.42		
21	8	7.12	37.43		
20	8	0.93*	24.30		
PZA-CIBA® by Novartis					
6	35 days	0**	40.03		
7	7	0.52	32.20		
11	7	0**	16.55*		
12	30	4.9	18.38*		
15	7	3.28	22.35		
16	7	3.83	40.45		
26	9	9.28	38.81		
27	9	10.10	34.62		
28	8	11.62	44.95		
5	18	6.49	37.32		

*failed to reach therapeutic level (20-60ug/ml)
**trough levels <1ug/ml

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Table 5. Mean serum level of pyrazinamide suspension in population grouped into brand names

Zinaplex®	n	Mean ug/ml	Standard Deviation
Hour 0	0	3.8920	4.9944
Hour 2	0	40.0160	11.7294
Duration of therapy	0	9.1	2.7669
Zcure®			
Hour 0	0	4.7770	5.0055
Hour 2	0	31.1330	12.87226
Duration of therapy	0	13.5	8.9100
PZA-Ciba®			
Hour 0	10	5.0020	4.2789
Hour 2	10	32.6660	9.9970
Duration of therapy	10	13.7	10.5098

Repeated Measures Analysis of Variance was used to analyze the data. Hour (0 hour, 2 hour) was considered as within-subject effect; group (Zinaplex, Zcure, PZA-Ciba) as between–subject effect and duration as a covariate. A significant difference between 0 hour and 2 hours was noted (p-value: 0.000). However there were no significant differences in serum concentration of pyrazinamide suspension among three brand names of pyrazinamide (p-value: 0.506).

Table 6.

Within-subject effects	
Effect/Source	p-value
Hour	0.000*
Hour•duration	0.378
Hour•group	0.242
Between-subject effects	
Duration	0.550
Group	0.506

*Significant at the 0.05 level

DISCUSSION

Subjects were grouped according to brand names of pyrazinamide suspension. Ten subjects were included for each brand name. The serum concentration of pyrazinamide suspension and duration of therapy is reported in Table 4. In the zinaplex group, half of the subjects had a trough level of 0 ug/ml. On two occasions, PZA-Ciba also had the same serum trough level, while 4 out of 10 subjects belonging to the Zcure group reported a serum trough concentration of <1 ug/ml. Generally speaking, a low to absent serum drug level prior to the next dose indicates that the drug did not sustain the desired serum concentration necessary for it to exert bactericidal activity most probably because of its short half-life.

Pharmacodynamic parameters of efficacy can either be concentration-dependent or time-dependent. In concentration-dependent killing agents, the higher the drug concentration, the greater the extent of bactericidal activity (Cmax:MIC ratio). On the other hand, time-dependent agents kill bacteria only when the concentration at the site is higher than the minimum inhibitory concentration or MIC (time of the concentration above MIC). Thus, the extent of killing is dependent on the time of exposure. Drug concentration at the site of drug action or tissue sample is difficult to determine and that is the why serum drug concentration represent the next best alternative.

Previous studies have shown that the mean serum concentration of pyrazinamide tablet in healthy volunteers is at 5-7 ug/ml at the 23rd hour following administration of 1.5 gram dose of pyrazinamide tablet once daily (C. Peloquin, 1998). In addition, Min Zhu et al in 2002 determined the population parameter of pyrazinamide tablet in children and adults with tuberculosis. The absorption of pyrazinamide tablet in children was 32% slower than in adults. The median Cmax was 21.1 ug/ml, which was almost 50% lower than the adult value of 41.1 ug/ml. The volume of distribution was 71% lower in children and the half-life was 43% shorter for children. According to this data, children appeared to absorb pyrazinamide tablet more slowly but eliminated it more quickly than adults. Another study done in India showed that slow absorption was also found in 10 patients with tuberculosis, aged 6-12 years old after a single oral dose of pyrazinamide tablet. It is not clear if these differences were due to chance, formulation, brand name, race, concurrent TB infection, or a combination of these factors. Whether or not the trough level 0-1 ug/ml is clinically significant, a full-scale pharmacokinetics and pharmacodynamics on pyrazinamide suspension on Filipino children needs to be done.

Table 3 presents the computed Tmax from the first 4 subjects. There is a perceptible wide variability of time for drug to reach maximum concentration. Again, this could be due to several circumstances as mentioned earlier. Additional research is needed to explore how the derived parameters can be used to optimize antituberculous drug therapy.

As regards to the Cmax, 93% of subjects were able to achieve a serum concentration level which was within the expected therapeutic range (mean 34.6+/-11.86

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ug/ml). Two out of the 30 patient population reported a serum concentration of <20 ug/ml, both belonging to the PZA-Ciba group. Zinaplex group had the highest mean serum concentration of 40 +/- 11.72 ug/ml.

In terms of duration of therapy, the present study shows that there is no significant difference in serum concentration of pyrazinamide suspension as presented in Table 6.

The therapeutic range of pyrazinamide has been reported to be at 20-60 ug/ml. In our study, at Tmax of 2 hours, the mean serum concentration of pyrazinamide suspension is 34.6+/-11.86 ug/ml. Thus, pyrazinamide suspension provides sufficient serum drug level at the second hour to elicit the desired therapeutic response. The result of the present study justifies that the suspension form achieves the desired serum concentration at Tmax but this study poses many questions such as, what would be the role of race, formulation, brand name in the serum level of pyrazinamide; what is a better measure or parameter of efficacy, Cmax:MIC or time>MIC? In this context, the results of the present study might constitute a useful baseline or reference for future bioavailability studies on pyrazinamide suspension.

CONCLUSION

Serum concentration of pyrazinamide suspension in 30 pediatrics patients aged 1-18 years old, who are on this formulation for 7-35 days were analyzed in this study. Forty percent of the study population had pulmonary tuberculosis. The Tmax of 2 hours was used based on the computed value from the first four subjects. The mean serum trough level of pyrazinamide suspension is 4.557+/-4.63 ug/ml. Seven out of 30 subjects accounted for the lowest serum trough level of 0 ug/ml, 5 of whom belong to the zinaplex group. The mean serum concentration of pyrazinamide suspension at Tmax is within the therapeutic level for pyrazinamide and is notably being achieved also by the suspension form. However, there were two patients who failed to reach therapeutic levels and both of them belong to the PZA-Ciba group.

Zinaplex has the highest mean serum concentration at 2 hour followed by PZA-Ciba and Zcure. However, it also has the lowest mean serum trough level and has the most numbered of subjects (5) that recorded the lowest value of 0 ug/ml, but these differences did not reach statistical significance.

This study also demonstrates that duration of therapy does not significantly affect serum drug concentration. Neither the duration of therapy nor the brand name of the formulation statistically significantly affect the serum drug level of pyrazinamide suspension of Tmax.

RECOMMENDATION

A full-blown bioavailability study is needed to confirm the kinetics of pyrazinamide suspension particularly on Filipino children. The role of race, formulation, burden of TB disease on serum drug level is yet to be determined.

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