
ISONIAZID AND ACETYLISONIAZID KINETICS IN SERUM AND URINE IN PULMONARY PRIMARY COMPLEX WITH INTERMITTENT REGIMEN

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ABSTRACT

Twenty patients, 1 through 13 years of age from Pediatric Tuberculosis Clinic of All India Institute of Medical Sciences, New Delhi, suffering from pulmonary primary complex (PPC) were investigated for serum and urine concentrations of isoniazid (INH) and acetylisoniazid (AcINH). Patients were put on an intermittent regimen - 2HR, 4H₂R₂, INH (H) was given in a dose of 10 mg/kg/day for first 2 months (the daily dose phase), followed by 20 mg/kg/dose in biweekly phase of regimen for rest of the 4 months, whereas, rifampicin (R) was given as 12 mg/kg in both daily as well, as biweekly phases. In the biweekly phase of regimen, after 7 days of biweekly administration of drugs, INH and AcINH concentrations were estimated by IPLC at 0,1,3,5 and 7 hours in serum, and at 0-3, 3-6, 6-12 and 12-24 hour-intervals of drug administration in urine. Peak concentrations of INH and AcINH (Mean \pm SD) were 2.6 ± 1.8 and 5.5 ± 2.6 μ g/ml in serum (C_{max}), and 5.7 ± 4.8 and 21.5 ± 12.1 mg in urine, respectively. Time to achieve C_{max} (T_{max}), for INH and AcINH were 1 and 5 hours respectively while time of peak concentration in urine for INH was 3-6 hours and for AcINH 6-12 hours. The half-life (T_{1/2}) of INH was 4.5 hours and area under serum-concentration time-curve (AUC_{0-7h})

Isoniazid (INH) has dominated as the vital drug in the chemotherapy of tuberculosis despite the availability of newer anti-tubercular drugs. It is well absorbed orally and reaches serum concentrations which far exceed its minimum inhibitory concentration (MIC) for most strains of *Mycobacterium tuberculosis* (1). After oral administration, peak serum concentrations of INH ranging from 6 to 20 μ g/ml are achieved within 1-2 hours in children(2,3)- *M. tuberculosis* is inhibited by a serum INH concentration of 0.05 μ g/ml which is much below

was 20.7 μ g/ml/h (mean values). In biweekly phase (4HJ₂J of regimen, just before administration of next dose, 0 hour (or 72 hours) concentration of INH was estimated at 0.47 ± 0.3 μ g/ml.

The frequency and dose of a drug depend on the duration for which a specific dose can maintain levels above minimum inhibitory concentration (MIC). In the present study which is first of its kind in children, 0 hour (or 72 hours) concentration of INH was 10-20 times of its MIC. Logically, then the desired serum and tissue concentrations vis-a-vis clinical response may be expected to be achieved with a much lower dosage with all its advantages. We suggest undertaking pharmacokinetic studies with lower dosage of INH in relation to achieving desired clinical response in children on intermittent regimen.

Keywords: Isoniazid, Acetylisoniazid, Rifampicin, Pharmacokinetics, Tuberculosis,

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the peak serum concentrations. Seth *et al.*(1) and Beotra *et al.*(4) have also stated that serum concentrations are frequently 50-100 times of the MIC for susceptible tubercle bacilli when oral doses of 10 to 20 mg/kg/day are used.

INH along with rifampicin can be given as intermittent therapy without compromising on treatment efficacy. This cuts down the cost of the therapy which is the main concern for poor drug compliance. The World Health Organization (WHO) recommends that in twice weekly intermittent regimen, the dosage of INH should be kept in the range of 12-15 mg/kg body weight instead of 20 mg/kg, both in adults and children(5). Unfortunately, these dosage recommendations are based on clinical impressions rather than inferences drawn from well designed pharmacokinetic studies. No published data regarding pharmacokinetics of INH in intermittent regimen in tuberculous children is available in medical literature. Hence, the present study, which is first of its kind in Indian children as well, was designed to investigate serum and excretion kinetics of INH and acetylisoniazid (AcINH)—an active metabolite of INH—in pulmonary primary complex in the biweekly phase of the intermittent regimen.

Material and Methods

Twenty patients, 8 females and 20 males, with established diagnosis of pulmonary primary complex (PPC) in the age group of 1-13 years, attending the Pediatric Tuberculosis Clinic of All India Institute of Medical Sciences, New Delhi, were admitted to the study. They had no renal or hepatic functional impairment, had not taken any antitubercular therapy previously and were on no other concomitant drugs during the study period. These patients were put

on supervised isoniazid (H) and rifampicin (R)—containing two-drug intermittent regimen 2HR, 4H₂R₂. Rifampicin was administered in the dosage of 12mg/kg/day in the daily as well as biweekly phase. INH was given in a dose of 10 mg/kg/day for the first two months in the daily-dose phase (2HR), followed by 20 mg/kg/dose in biweekly phase (4H₂R₂) of the regimen for rest of the four months. In the biweekly phase after 7 days of biweekly administration of drugs, concentrations of INH and AcINH were estimated by High Performance Liquid Chromatography (HPLC)(6) at 0, 1, 3, 5 and 7 hours in serum and at 0-3, 3-6, 6-12 and 12-24 hour-intervals in urine. The samples were deproteinized by precipitating with acetone. The drug was then extracted from the filtrate by butanol/chloroform and injected. A Waters Millipore (USA) Chromatographic System (HPLC) equipped with 501 Pump, 7125 Rheodyne injector and 481 LC UV detector and Micro-Bondapak C₁₈ Column was used for the purpose. The detection was recorded on 745 data module. Each intermittent dosage was administered by calling the patients to the clinic for supervision. For sampling, the patients were admitted in the short stay unit of our pediatric ward. For ethical reasons, each child was bled only twice and informed consent was duly obtained.

Since urine samples were difficult to collect in the younger children, urine from patients above 3 years of age (preferably males) was collected for the purpose of study. Some serum samples taken at 1 hour interval were lost due to refrigeration error. Hence, the number of readings at that point of time have been unfortunately less. Also, since estimation of AcINH concentrations can only be done upto 48 hours after col-

lection of sample as compared to INH concentrations estimation of which can be done in upto 3 months, there is difference in the number of samples in which INH and AcTNH were estimated (Table I).

The pharmacokinetic parameters calculated were-maximum (peak) serum concentration (C_{max}), time to achieve maximum (peak) serum concentration (T_{max}) and half-life of elimination ($T_{1/2}$). The area under the serum concentration-time-curve (AUC) was also calculated in the interval of 0-7 hours according to the trapezoidal rule(7).

Results

Serum INH and AcINH Concentrations

INH and AcINH concentrations (Mean \pm SD) in serum are shown in Table I & Fig. 1. Time to achieve peak serum concentration (T_{max}) of INH was estimated at 1 hour and the mean value of peak serum concentration (C_{max}) attained was 2.6 ± 1.8

TABLE I- Serum Isoniazid (INH) and Acetylisoniazid (AcINH) Concentrations by HPLC at Different Time Intervals in Intermittent Regimen (2HR, 4H₂R₂) in Pulmonary Primary Complex.

Time interval (hrs)	Serum concentration (mg/ml) (Mean \pm SD)	
	INH	AcINH
0	0.47 \pm 0.3 (12)	2.2 \pm 1.8 (14)
1	2.6 \pm 1.8 (9)	4.0 \pm 2.4 (4)
3	1.9 \pm 1.3 (17)	5.4 \pm 2.4 (11)
5	1.69 \pm 1.3 (20)	5.5 \pm 2.6 (14)
7	0.96 \pm 0.4 (13)	4.8 \pm 3.3 (13)

Figures in parentheses are number of samples.

μ g/ml, whereas T_{max} and C_{max} for AcINH were 5 hours and 5.5 ± 2.6 μ g/ml, respectively. The half-life ($T_{1/2}$) of INH was 4.5 hours and AUC was 20.78 μ g/ml/h (mean values) (Table II).

Urine INH and AcINH Concentrations

INH and AcTNH concentrations (Mean \pm SD) are given in Table III & Fig. 2. Mean peak concentration of INH was observed as 5.71 ± 4.82 mg in 3-6 hour and that for AcINH as 21.5 ± 12.1 mg in 6-12 hour urine samples after oral intake of INH.

Discussion

The frequency and dose of the drug depend on the duration for which a specific dose can maintain concentrations above its MIC. From the experiments it has been concluded that INH damages the resting tubercle bacilli irreversibly, provided the bacilli are exposed to the drug for a long time. The necessary concentration, which has been shown to be corresponding to the MIC of INH to *M. tuberculosis*, i.e., 0.05 μ g/ml, can be achieved in the tubercle lesions in man. The MIC of intracellularly located tubercle bacilli is practically the same

TABLE II- Isoniazid Kinetics in Serum in Intermittent Regimen (2HR, 4H₂R₂) in Pulmonary Primary Complex.

C_{max} (μ g/ml)	C_{max}^* (h)	$T_{1/2}^*$ (h)	AUC 0-7hr* (μ g/ml/h)
2.6	1.0	4.5	20.78

* = Mean values,

C_{max} = Maximum serum concentration,

T_{max} = Time to attain C_{max}

$T_{1/2}$ = Serum half-life,

AUC = Area under the serum concentration-time-curve.

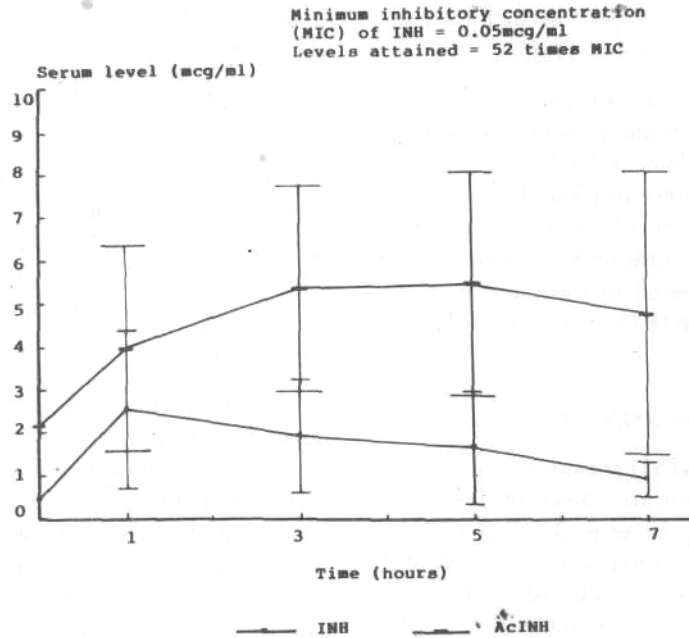


Fig. 1. Serum isoniazid (INH) and acetylisoniazid (AcINH) concentrations in intermittent regimen (2HR, 4H₂R₂) in pulmonary primary complex (Mean ± SD).

TABLE III— Urine Isoniazid (INH) and Acetylisoniazid (AcINH) Concentrations by HPLC in Intermittent Regimen (2HR, 4H₂R₂) in Pulmonary Primary Complex

Time interval (hours)	No. of Samples	Urine concentration (mg) (Mean ± SD)	
		INH	AcINH
0 - 3	9	1.82 ± 1.29	6.7 ± 6.5
3 - 6	9	5.71 ± 4.82	15.7 ± 9.7
6 - 12	9	5.21 ± 6.41	21.5 ± 12.1
12 - 24	9	21.9 ± 1.92	10.2 ± 6.6

as for extracellular bacilli. With a slide-culture technique, it has been shown that the MIC of INH kills 98% of the bacterial populations and that a concentration of 4

µg/ml is no more effective than 1 µg/ml. A remarkable reduction in viable counts of tubercle bacilli has been established at low INH concentrations of 0.1 -0.2 /µg/ml which

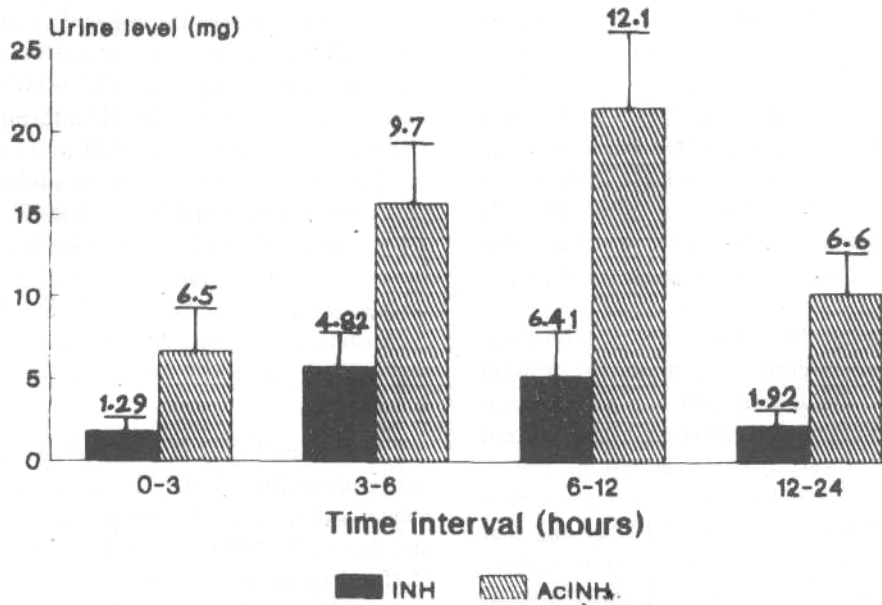


Fig. 2. Urine isoniazid (INH) and acetyloniazid (AcINH) concentrations in intermittent regimen (2HR,4H₂R₂) in pulmonary primary complex (Mean \pm SD).

is due largely to the killing effect of the drug on multiplying bacilli and not to the host's defence mechanism acting on bacilli held in stasis by the drug(8). The experimental studies firmly establish that concentrations above 1 μ g/ml have no higher killing activity on continuous exposure and that INH is bactericidal for tubercle bacilli (both intra- and extracellular) at its MIC of 0.05 μ g/ml. Its bactericidal activity depends on the multiplication rate of the bacilli.

The bactericidal effect is growth-dependent and above a certain optimal concentration (1-2 μ g/ml), it is concentration-independent. Hobby and Lenert(9) have shown that killing kinetics of proliferating tubercle bacilli in INH-containing media slows down the more the generation time of the bacilli increases after the phase of

exponential growth has been passed. It could thus be expected that a time-limit exposure will be sufficient to produce a delay before growth restarts after removal of the drug which has been termed as "lag-period" and this has indeed been observed by Dickinson and Mitchison(10). They showed in experiments that an exposure of the H37Rv strain of *M. tuberculosis* to 1 μ g/ml of INH in a liquid medium for 24 hours produced a growth delay of 2.5 days to 6.9 days, and exposure for 96 hours a delay of 6.2 days to 19 days. Exposure period for 2 hours to 12 hours did not cause any retardation of the lag phase. It is this delay in the resumption of multiplication that forms the basis for intermittent treatment with INH.

Pulsed exposure to INH *in-vitro* has been studied by Gangadharam *et al.*(11), Dickin-

son and Mitchison(10), and Awaness and Mitchison(12), and they showed that an exposure of INH to *M. tuberculosis* for a few hours to concentrations attainable in man is sufficient to produce a bactericidal effect and a marked delay in the resumption of multiplication, atleast if the exposures are repeated several times. In some experiments even a single exposure was infact sufficient to elicit these phenomena.

Keeping these microbiological observations and inferences in mind, the present study provides an insight into the pharmacokinetic aspect of INH as a constituent drug of intermittent regimen in Indian children. The peak serum INH concentration of $2.6 \pm 1.8 \mu\text{g/ml}$ at 1 hour and that for AcINH of $5.5 \pm 2.6 \mu\text{g/ml}$ at 5 hours in the biweekly phase of the intermittent regimen was observed in the study. The concentration of INH is an average of 52 times and a maximum of 88 times of its MIC when administered orally in the dose of 20 mg/kg/dose. The study further shows that the concentration at 7 hours is $0.96 \pm 0.4 \mu\text{g/ml}$ which is 19 times of its MIC. Even at 0 (or 72) hours (3 days), *i.e.*, just before the administration of next due dose of INH, the concentration is sustained at $0.47 \pm 0.3 \mu\text{g/ml}$ which is 9 times higher than its MIC. It is thus clear that an oral dose of 20 mg/kg of INH in biweekly phase of therapy is able to sustain a concentration which is much above its MIC value. Hence, the bactericidal concentration upto $1 \mu\text{g/ml}$ in a child with PPC, where the number of tubercle bacilli is relatively small compared to the severe forms of tuberculosis or to that in adults, can be achieved even with much lower doses without compromising with the efficacy. Further, in diseases like tuberculous meningitis where risk of drug-induced hepatotoxicity is much higher, use

of INH in lower doses in intermittent regimens with therapeutic concentrations in CSF, will not only prove to be as efficacious but also more acceptable. The WHO recommends that in twice weekly intermittent regimen, lower dosage of INH as 12 to 15 mg/kg should be given both in adults and children(5). The usual belief that rifampicin is responsible for hepatotoxicity is a myth because the liver enzyme induction produced by rifampicin will metabolise INH. Accordingly, it will produce the more toxic metabolite, an acylating agent, which is hepatotoxic.

Excretion of INH in urine is fairly good and comparatively early as its maximum concentration is seen between 3 and 6 hours and that of AcINH between 6 and 12 hours of the drug intake. It suggests that in patients with normal renal functions, INH does not have the tendency to accumulate in serum and therefore can be given safely in prescribed doses irrespective of the status of acetylation phenotype (rapid or slow) of the patients in 17Hgeneral.

Based on present study, it is suggested that pharmacokinetic studies be done using INH in lower doses *vis-a-vis* the desired clinical response in intermittent regimens in children. More such studies should be undertaken in children for INH in continuous regimens and also for rifampicin and pyrazinamide in relation to their therapeutic dosages and clinical efficacies as these are more expensive drugs. In developing countries where cost of therapy has a direct bearing on drug-compliance and hence the clinical efficacy, the use of antitubercular drugs in lower but therapeutically effective doses will considerably reduce the cost-burden of therapy on patients. A marked improvement in compliance can then be anticipated.

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