

# Transfer of isoniazid from circulation to breast milk in lactating women on chronic therapy for tuberculosis

Neera Singh,<sup>1</sup> Anil Golani,<sup>1</sup> Zarine Patel<sup>2</sup> & Anurupa Maitra<sup>1</sup>

<sup>1</sup>Molecular Endocrinology Laboratory and <sup>2</sup>Genetics Research Centre, National Institute for Research in Reproductive Health, ICMR, Jehangir Merwanji Street, Parel, Mumbai, India

## WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Isoniazid is the most widely used first line antituberculosis drug.
- It is considered safe during lactation, but limited data are available on the transfer of isoniazid from circulation to milk in lactating women, which can provide an assessment of extent of exposure to the nursling.

## WHAT THIS STUDY ADDS

- The study documents the transfer pattern and milk to plasma (M : P) ratio of isoniazid at a steady state.
- Peak plasma and milk concentrations of isoniazid were reached within 1 h and the projected exposure of the drug to the infant is much lower than the prophylactic dose, supporting its safety during breast feeding.

## AIM

To determine milk to plasma (M : P) ratios and infant dose (absolute and relative) for isoniazid in lactating women on antituberculosis therapy.

## METHODS

Concentrations of isoniazid in plasma and milk were measured in exclusively breast feeding women taking 300 mg day<sup>-1</sup> as treatment for tuberculosis.

## RESULTS

Peak plasma and milk concentrations of isoniazid were observed at 1 h. A mean M : P<sub>AUC</sub> value of 0.89 (95% CI 0.7, 1.1) was calculated for isoniazid from seven women over 24 h. The mean absolute infant dose was estimated to be 89.9 µg kg day<sup>-1</sup> (95% CI 65.6, 114) and the relative infant dose was 1.2% of the weight adjusted maternal dose.

## CONCLUSIONS

The mean relative dose of isoniazid (1.2%) transmitted to the infant via breast milk is below the 10% notional level of concern. These data suggest that isoniazid therapy is safe during breastfeeding.

## Correspondence

Dr Anurupa Maitra,  
Molecular Endocrinology Laboratory,  
National Institute for Research in  
Reproductive Health, ICMR, Jehangir  
Merwanji Street, Parel, Mumbai –400 012,  
India.  
Tel.: + 91 22 2419 2009;  
Fax: + 91 22 2413 9412;  
E-mail: anurupamaitra@yahoo.co.in

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## Introduction

Breastfeeding offers innumerable advantages to a growing infant by meeting all its nutritional and immunological requirements. However, breastfeeding is contraindicated in several cases when the mother is on chronic drug therapy and the safety of the drug in lactation is not established. Ill health of nursing mothers requiring medical intervention is a common feature in developing countries like India. It is now well established that most of the drugs present in the maternal circulation can be transferred to breast milk and subsequently to the feeding infant [1, 2].

Thus infants become unintended recipients of maternal drugs. The possible effects of these medications on breast-fed infants are therefore of great concern and so it becomes important to know the extent of transfer of maternal drugs into breast milk, in order to assess the safety of breastfeeding. There is information available from previous studies on the apparent effects of many maternal drugs and chemicals on the nursling [3]. However the possible long-term effects of these drugs, particularly those that are chronically administered (for example tuberculosis and leprosy) on the breastfeeding infant are not clear. Although most of these drugs are transferred to milk in

minute quantities, rarely reaching toxic concentrations, even they can be 'pharmacologically active' [4].

In India, tuberculosis is still the first infectious cause of death among females in the reproductive age group, necessitating chronic long-term therapy even during critical phases like pregnancy and lactation [5]. Thus in India, with a high prevalence of tuberculosis (especially in people from lower socio-economic background due to poor living conditions); antituberculosis drugs are among those most commonly administered during breastfeeding. There are limited data available on the extent of transfer of antituberculosis drugs in milk and the subsequent exposure to the infants (American Academy of Pediatrics categorizes them as compatible with breastfeeding). Previous studies on transfer of isoniazid into human milk have reported that the peak concentration of isoniazid was achieved at 2 and 3 h after intake of a single oral dose of 200 and 300 mg, respectively [6–8]. The peak concentration of isoniazid varied from 2.1 to 10.6  $\mu\text{g ml}^{-1}$  in the above studies. Further, Snider & Powell have suggested that only a small fraction of the antituberculosis drugs appears in breast milk [9].

Isoniazid, a pyridine derivative of nictoinamide, is the most widely prescribed first line drug for the treatment of tuberculosis. It is mainly metabolized in the liver by N-acetyltransferase 2. It is also prescribed to infants as prophylactic therapy. The drug is known to be relatively non-toxic, but its potential for interference with nucleic acid metabolism and hepatotoxicity in infants has been documented [10, 11]. Toddywalla *et al.* have reported that isoniazid is capable of suppressing hepatic drug metabolizing activity (HDME) in nurslings of mothers on therapy [12]. It is therefore important to assess the extent of exposure of these infants to the drug through breast milk, so as to avoid over-exposure and its adverse effects. The present study has therefore been undertaken with the objective to assess the pharmacokinetic pattern of isoniazid in mother's circulation and its transfer to breast milk and also to determine the milk to plasma ratio of the drug.

## Methods

### Study protocol

The study design was approved by the Institutional Ethics Committee for conduct of clinical studies. Written informed consent was obtained from all the participants.

### Patients

Nine lactating women, mean age 23 years (range 18–28 years), mean body weight 40 kg (range 35–45 kg) diagnosed with tuberculosis were enrolled for the study. The women were on combination therapy (isoniazid 300 mg, rifampicin 450 mg and ethambutol 800 mg) and the standard single dose of 300 mg isoniazid was administered daily. Therapy with isoniazid had commenced a mean of 133 days (range 34–298 days) prior to the study day and all

the participants were considered to be at steady state at the time of study. All the women were of similar nutritional and socio-economic status.

### Sample collection

A maternal blood sample (5 ml) was collected in EDTA vacutainers by venipuncture at 0, 1, 2, 3 and 4 h after ingestion of the drug in the morning. Plasma was separated and stored at  $-20^{\circ}\text{C}$  until analysis for the estimation of drug concentrations by reverse phase HPLC. At approximately the same time interval, breast milk samples (3–5 ml) were collected manually in sterilized tubes and stored at  $-20^{\circ}\text{C}$  until analysis. Out of nine only seven patients agreed to give five time point sample collections while two cases agreed to three time point collections.

### Materials

Pure isonicotinic acid hydrazide (isoniazid) was a kind gift from Lupin Laboratories Ltd. (Mumbai). Cinnamaldehyde was purchased from Sigma-Aldrich. All other solvents and reagents were of analytical or HPLC grade. Millipore filters of 0.45 microns were used for filtration of the milk and plasma samples.

### High performance liquid chromatography (HPLC)

Estimation of isoniazid in plasma and milk was carried out using a reverse phase HPLC method as described previously by Sadeg *et al.* [13]. The plasma and milk standard curves were linear over the range (0.25–10  $\mu\text{g ml}^{-1}$ ) with a correlation coefficient of 0.997. The limit of detection of isoniazid for both plasma and milk samples was 0.02  $\mu\text{g ml}^{-1}$ . The intraday coefficients of variation for the plasma assay ranged from 4 to 10% at 0.5  $\mu\text{g ml}^{-1}$  and 2–5% at 4  $\mu\text{g ml}^{-1}$ . The interday coefficients of variation for the plasma assay ranged from 4 to 9% at 0.5  $\mu\text{g ml}^{-1}$  and 2.6–4% at 4  $\mu\text{g ml}^{-1}$ . For the milk assay intraday CVs ranged from 1 to 10% at 0.5  $\mu\text{g ml}^{-1}$  and 0–1% at 4  $\mu\text{g ml}^{-1}$ . The interday CVs for milk ranged from 3.5 to 11% at 0.5  $\mu\text{g ml}^{-1}$  and 1–8% at 4  $\mu\text{g ml}^{-1}$ . A Dionex HPLC system with UV detector, version 6.40 SP1 comprising of Acclaim-R-120 silica based C18 column (150  $\times$  4.6 mm) attached with a guard column (35  $\times$  15 mm) was used for the analysis.

### Data analysis

Pharmacokinetic parameters were estimated using a one-compartmental approach. The terminal elimination rate constant ( $\lambda_z$ ) was determined by log-linear regression analysis of the isoniazid concentration vs. time curve using 1–4 h data points. Using the terminal elimination rate constant, the concentration of isoniazid in milk and plasma was theoretically determined for all the seven patients at each hour from 5 to 24 h. The area under the concentration–time profiles (AUC (0, 24 h)) was calculated

using the trapezoidal rule for both plasma and milk. Data have been summarized as mean (95% CI or range) as appropriate.

### Calculation of M/P ratio and infant dose

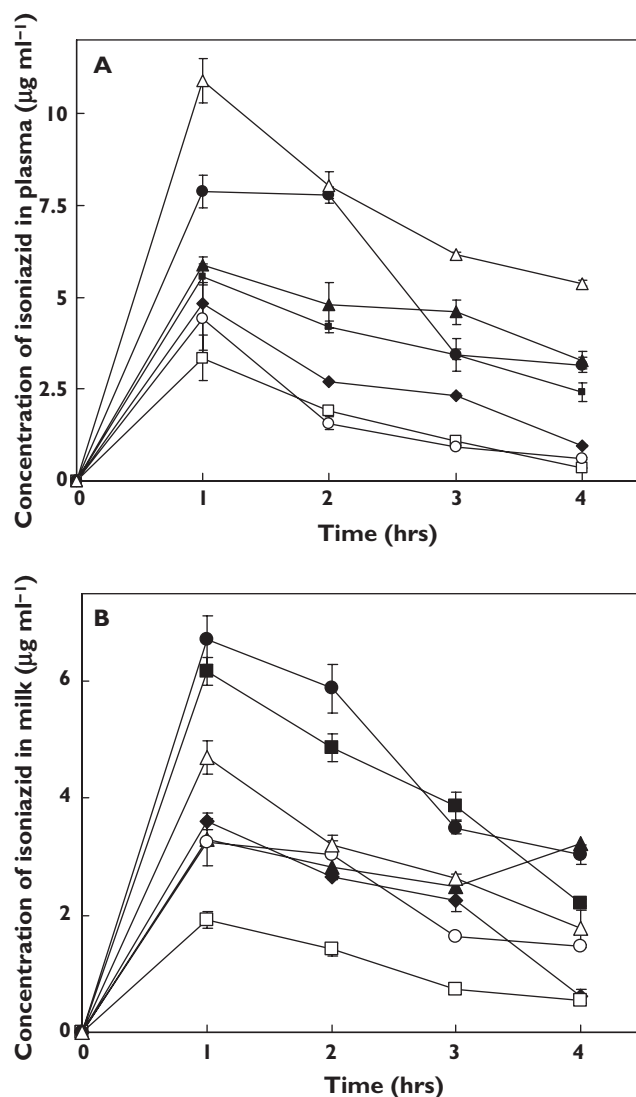
M:P ratios were calculated using AUC data. To calculate the absolute infant dose of isoniazid, an average infant milk intake of  $0.15 \text{ l kg}^{-1} \text{ day}^{-1}$  [14] and an oral bioavailability of 100% were assumed. The average milk concentration (AUC (0, 24 h)/24 h) was multiplied by average milk intake to give a weight-adjusted estimate of daily infant dose. The relative infant dose was then expressed as a percentage of the maternal weight-normalized dose.

## Results

Nine exclusively breast feeding women on chronic antituberculosis therapy were enrolled for this study. Blood and milk samples were collected at five time points (0, 1, 2, 3, and 4 h) in seven patients and at three time points (0, 1 and 3 h) in two patients. The data analysis was done on the basis of seven patients. Plasma and milk concentration-time profiles for isoniazid in all the seven patients are shown in Figure 1. The peak plasma concentration of isoniazid was achieved at 1 h in all cases and decreased sharply thereafter. The concentration of isoniazid in plasma at 4 h was significantly different from the isoniazid concentration at 1 h ( $P < 0.001$ ). In milk, the isoniazid concentrations were highest at 1 h and a similar trend was observed as in plasma suggesting that this transfer of isoniazid from plasma to milk is concentration dependent. The peak concentrations of isoniazid in plasma ( $3\text{--}10 \mu\text{g ml}^{-1}$ ) and milk ( $2\text{--}6.7 \mu\text{g ml}^{-1}$ ) were found to be highly variable in all the patients. The AUC was extrapolated to 24 h using a terminal elimination phase equation. Isoniazid concentration in milk and plasma and the M:P ratio calculated by AUC are summarized in Table 1. The mean AUC(0, 24 h) of isoniazid in plasma and milk in all the seven patients was  $18.4 \mu\text{g ml}^{-1} 24 \text{ h}^{-1}$  (95% CI 11.2, 25.6) and  $14.4 \mu\text{g ml}^{-1} 24 \text{ h}^{-1}$  (95% CI (10.5, 18.3), respectively. The mean M:P<sub>AUC</sub> ratios for all these patients were calculated to be 0.89 (95% CI 0.7, 1.1). Assuming the milk consumption of an infant was  $0.15 \text{ l kg}^{-1} \text{ day}^{-1}$ , the likely exposure of the drug to the infant was calculated to be  $89.9$  (95% CI 65.6, 114)  $\mu\text{g kg}^{-1} \text{ day}^{-1}$  which corresponds to 1.2% (95% CI 0.9, 1.5) of the weight normalized maternal dose (Table 1). In the two patients where we had only three time point collections, the M:P ratio was calculated to be  $0.8 \pm 0.1$  and  $0.8 \pm 0.04$ , respectively.

## Discussion

Our study shows that the peak concentrations of isoniazid in both plasma and milk were achieved at 1 h after the



**Figure 1**

Plasma (A) and milk (B) concentration-time profiles for isoniazid in seven patients {P1 (◆), P2 (■), P3 (▲), P4 (□), P5 (○), P6 (●) and P7 (Δ)} over 4 h from drug administration at steady state. Isoniazid concentrations were measured by HPLC as described in methods section. All data are presented as mean  $\pm$  SEM

drug administration. These results indicate that isoniazid is quickly absorbed and is transferred to milk in a concentration dependent manner. We have observed a high variation of isoniazid peak concentrations in both plasma ( $3.35\text{--}10.9 \mu\text{g ml}^{-1}$ ) and milk ( $1.9\text{--}6.7 \mu\text{g ml}^{-1}$ ) in spite of the similar maternal dose received by all the patients. The variation in peak isoniazid concentrations observed in our patients may be due to individual differences in their drug metabolizing capacity. A previous study by Lass & Bunger has reported the peak milk concentration of isoniazid at 2 h ( $1.7\text{--}2.3 \mu\text{g ml}^{-1}$ ) in six women after a single oral isoniazid dose of 200 mg [6]. However 1 h data were not available in this study. In another study by Ricci & Copaitich,

**Table 1**

AUC for plasma and milk, M : P ratios and infant dose for isoniazid

Volunteers	Milk (AUC) ( $\mu\text{g ml}^{-1} 24 \text{ h}^{-1}$ )	Plasma (AUC) ( $\mu\text{g ml}^{-1} 24 \text{ h}^{-1}$ )	M : P (AUC) ratio	Absolute infant dose ( $\mu\text{g kg}^{-1} \text{ day}^{-1}$ )	Relative infant dose as a %
P1	10.6	11.9	0.89	66	0.88
P2	19.4	18.4	1.05	121	1.62
P3	14.8	22.3	0.66	92	1.23
P4	6.8	7.9	0.86	42	0.57
P5	12.1	8.5	1.42	76	1.01
P6	22.2	24.9	0.89	139	1.85
P7	14.7	34.6	0.43	92	1.23
Mean	14.4	18.4	0.89	89.9	1.2
95% CI	(10.5, 18.3)	(11.2, 25.6)	(0.7, 1.1)	(65.6, 114)	(0.9, 1.5)

the peak milk concentrations were reported at 3 h ( $5.4\text{--}5.5 \mu\text{g ml}^{-1}$ ) after a single oral isoniazid dose of 300 mg in three women [7]. In another single case study, peak milk concentration ( $16.6 \mu\text{g ml}^{-1}$ ) was reported after 3 h [8].

The mean M : P ratio of isoniazid calculated by using AUC data was 0.89 (95% CI 0.7, 1.1) suggesting that isoniazid has a modest potential to penetrate into breast milk. We have estimated that an infant would receive an absolute dose of about  $89.9 \mu\text{g kg}^{-1} \text{ day}^{-1}$  (95% CI 65.6, 114). The calculated relative infant dose of isoniazid was 1.2% of the weight normalized maternal dose which is well below the notional 10% level of concern [15]. Our estimated relative infant dose can be compared with the suggested values of 0.3–1% [LactMed (Drug and lactation database, CASRN: 54-85-3)] and 0.75–2.3% [International Programme on Chemical Safety INCHEM (PIM 288, CAS no. 54-85-3)] available in the literature. This amount of drug transferred to breast milk may not be directly toxic for the infant since it is much lower than the isoniazid prescribed to the infant as a prophylactic dose ( $10 \text{ mg kg}^{-1} \text{ day}^{-1}$ ). However the low rate of transfer should not be ignored because drugs can be 'pharmacologically active' even at the lower concentration and particularly when the mother is on long-term chronic drug therapy for 9–12 months and infants are also on prophylactic therapy with isoniazid. It has already been established that infant hepatic drug metabolizing capacity is immature and in the case of N-acetyl transferase 2 (NAT2) the enzyme metabolizing isoniazid, this enzyme is not fully mature until the age of 4 years [16]. Therefore it becomes very important to monitor critically these breast-fed infants for any short-term and long-term adverse effects when the mother is on isoniazid therapy [5]. The previous finding by Toddywalla *et al.* [12] also suggests that isoniazid is able to suppress infant hepatic metabolizing capacity. Thus, while studying the presence of drugs in milk, not only should the drug characteristics and maternal factors be considered, but also the infant's capability to metabolize the drugs should not be ignored.

The study would have been more valuable with the estimation of actual isoniazid concentrations in infant's

plasma and the collection of their well being data, but this could not be done due to ethical constraints and lack of mother's consent. Nevertheless it is evident from our study that the level of exposure of the infant through breast milk can be minimized by counseling mothers for appropriate breastfeeding intervals so as to circumvent the peak time of exposure. These data suggest that isoniazid therapy is safe during breastfeeding. However each case should be considered as an individual risk/benefit analysis.

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