

Excretion of clofazimine in human milk in leprosy patients

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Summary Clofazimine is an important and effective constituent of multi drug therapy for leprosy. A study has been conducted to determine the distribution of clofazimine in maternal milk so that the safety of breast-feeding during maternal ingestion of the drug can be ascertained. Eight female leprosy patients (LL/BL) on clofazimine, 50 mg daily or 100 mg on alternate days for 1–18 months, (mean 5.0 ± 1.81 months; median 3.25 months) and in the early lactating phase were studied. Blood samples and milk specimens were collected 4–6 hr after the last daily dose. Clofazimine was assayed in the milk and plasma samples by HPTLC. Mean plasma and milk clofazimine levels were 0.9 ± 0.03 $\mu\text{g/ml}$ and 1.33 ± 0.09 $\mu\text{g/ml}$ respectively. The ratio of milk to plasma drug concentration ranged from 1.0 to 1.7 with a mean of 1.48 ± 0.08 . The amount of drug ingested by the infants was 0.199 ± 0.013 mg/kg/day which represented $22.1 \pm 1.9\%$ of the maternal dose.

Introduction

Clofazimine, 3-(p-chloroanilino)-10-(p-chlorophenyl)-2, 10-dihydro-2-(isopropylimino) phenazine, is an effective antileprosy drug. It is a part of the multi drug therapy (MDT) for leprosy and is given at a dose of 50 mg daily or 100 mg on every other day.¹ It is also useful in the management of erythema nodosum leprosum at higher doses, usually 200–300 mg daily.² Slow absorption, relatively much slower distribution and longer retention in selective tissues are peculiar features of clofazimine metabolism.³ The plasma concentration of the drug is dose related, although there does not appear to be a linear relationship between doses and plasma concentrations. A steady state is expected to be attained with oral administration of clofazimine 50 mg daily for more than 30 days.⁴

There are isolated reports on pigmentation of the infants born to leprosy patients on clofazimine therapy^{5,6} and the mammary excretion of the drug in experimental animals.⁷ Recently there has been a report on excretion of significant amounts of clofazimine in the breast milk of a woman in Nepal.⁸ Since the safety of drug ingestion via maternal milk by suckling infants is of considerable importance, the present study was carried out to quantitate the mammary excretion of clofazimine in patients and determine milk to plasma concentration ratio as well as the approximate levels of the drug ingested by suckling infants.

Materials and methods

Eight female leprosy patients (LL/BL) who were in the early lactation period of upto 4 months formed the subjects of the study. They were aged 19–35 years with the mean age of 28 ± 2.17 (SE) years. As a part of MDT (comprising of rifampicin 600 mg once a month, dapsone 100 mg daily and clofazimine 50 mg daily or 100 mg on alternate days) all patients had taken clofazimine for 1–18 months with a mean of 5.0 ± 1.81 months. One patient had received the drug 100 mg daily while 2 patients received 100 mg on alternate days and remaining patients were on 50 mg daily. Milk specimens and blood samples were collected 4–6 hr after administering the day's dose with breakfast.

Plasma was separated and both milk and plasma samples were either processed for clofazimine assay immediately or stored at -20°C till assay. Clofazimine was assayed by the method of Lanyi & Dubois⁹ based on high performance thin layer chromatography (HPTLC) coupled with densitometric scanning. Briefly 1–3 ml of plasma/milk were acidified with 2 ml of acetate buffer (1M, pH 5.0) and extracted with 6 ml of toluene for 15 min. After brief centrifugation, the organic layer was removed and evaporated to dryness under a stream of nitrogen at 40°C . The residue was redissolved in $100 \mu\text{l}$ of methanol and applied on to HPTLC silica gel 60 Plate (Merck) predeveloped with chloroform : methanol (1 : 1 v/v) prior to use. The plates spotted with clofazimine extracts and standards were developed in toluene–acetic acid–water (50 : 50 : 4 v/v). *In situ* quantitation of the TLC spots were performed with a Shimadzu (Japan) TLC Scanner at 550 nm. Two samples containing aliquot of clofazimine extracts were also run on high performance liquid chromatography (HPLC) Shim pack C8 column using a mobile phase of 0.0425 M Phosphoric acid in 81% methanol (pH 2.4) at a flow rate of 1.5 ml/min and quantitated at 285 nm.¹⁰

The ratio of milk to plasma clofazimine concentrations was calculated and the approximate amount of the drug ingestion by suckling infants was calculated assuming that an infant aged 2–3 months and weighing 2.5 kg would consume breast milk at a rate of 150 ml/kg bodyweight/day on average.¹¹ The percentage of last maternal dose, in mg/kg, ingested by the infants was also determined.

Results

The plasma clofazimine levels ranged between 0.8 and $1.0 \mu\text{g/ml}$ with mean value of $0.9 \pm 0.03 \mu\text{g/ml}$ while the milk drug levels ranged from 0.8 to $1.7 \mu\text{g/ml}$ (1.33 ± 0.09). The ratio of milk drug concentration to plasma drug concentration (M/P) ranged from 1.0 to 1.7, (mean 1.48 ± 0.08) 4–6 hr after the last daily dose. The quantities of clofazimine ingested daily by suckling infants ranged from 0.120 mg/kg/day to 0.255 mg/kg/day (0.199 ± 0.013) which, when expressed as a percentage of the last maternal dose in mg/kg, represented 13.5 to 30% (22.11 ± 1.90) (Table 1).

Discussion

Clofazimine is a highly lipophilic drug. The mechanisms of its absorption, distribution and retention are far from clear. Leprosy patients receiving the drug at 100 mg thrice weekly or 100 mg daily for varying periods have shown plasma drug levels of 0.5 or $0.7 \mu\text{g/ml}$

respectively.¹² A single oral dose of 200 mg results in a mean plasma level of 0.4 µg/g.⁴ An average plasma concentration of 1.15 µg/ml has been reported in leprosy patients receiving a daily dose of 300 mg.¹³ The patients included in the present study were on clofazimine 50 mg daily or 100 mg alternate days for 1–18 months. Since a daily continuous daily administration of 50 mg of the drug for 30 days is likely to result in a 'steady-state level',⁴ plasma drug concentrations of 0.8–1.0 µg/ml presented by the patients in the present study might represent the steady-state levels and at this stage one could look for an equilibrium between plasma and milk compartments.

The distribution of clofazimine is relatively slower than its absorption. Clofazimine and related phenazines have been reported to show very selective tissue distribution. A significant proportion of the drug is found in adipose tissues and in cells of the reticuloendothelial system, where they can be seen concentrated in phagosome type inclusions and ultimately as crystals of pure drug.¹⁴ Presumably clofazimine after absorption circulates bound to plasma proteins and is engulfed in this form by the cells of the reticuloendothelial system, where it remains after digestion of the proteins.⁷ Clofazimine and its metabolites are excreted in urine in very small amounts constituting 1.6% of the daily dose (assuming 70% drug absorption).^{15,16} The low excretion rate is probably due to retention of the drug in the body for a long time. Based on an observation that infants born to mothers who had received the drug during pregnancy were deeply pigmented at birth, it has been reported that clofazimine crosses the placenta.⁵ However, no teratogenic effect has been reported with clofazimine.⁶

Decisions about the safety of breast-feeding during maternal ingestion of the drug require knowledge of the amount of the drug which might be present in the milk. There are only scanty reports on the excretion of dapsone, rifampicin and clofazimine in breast milk. Lactating mothers on 100–150 mg dapsone daily were found to have serum levels of 1.62 µl/ml with milk levels of 1.09 µg/ml resulting in milk : plasma (M/P) ratio of 0.7. The breast-fed infants had serum dapsone levels of 0.493 µg/ml.¹⁷ Experimental studies in female mice, rats, guinea-pigs and rabbits have shown transmission of significant amounts of clofazimine to the offspring via mother's milk. In a TLC–NMR Spectroscopic analysis of milk from a Nepali woman, a considerable amount of clofazimine was found to be released from the body with the mother's milk.⁸ Our study has included a larger number of lactating patients on varying lengths of chemotherapy and clofazimine has been quantitated in both plasma and single milk sample from all 8 patients.

Since the total quantity of milk during a period of 24 hr or part of it could not be collected the studies had to be limited to single samples. The drug ingestion by the suckling infants was calculated assuming that the volume of milk consumed by the infant aged 2–3 months is about 150 ml/kg/day.¹¹ The values for the suckling infants of 8 patients ranged from 0.120 mg/kg/day to 0.255 mg/kg/day with a mean of 0.199 ± 0.013 mg/kg/day. The highest level of drug ingestion by the infant of subject No. 1 would then represent 30% of last daily dose (mg/kg) by the subject who had taken 26.6 g of the drug cumulatively.

The accumulated total dose of clofazimine does not seem to proportionately influence the excretion of the drug in milk as shown by the figures of subject No. 5 versus subject No. 1 (Table 1). Even when the total intake by subject No. 5 was only 1.5 g, the amount of the drug excreted into milk was almost the same as in the case of subject No. 1 who had taken about 20 times more of the drug cumulatively. This indicates that the clofazimine mobilized from the fixed dose within the cells of the tissues into extra vascular space and subsequently into milk, may not be important in view of slow elimination phase for the drug. The extent of drug ingestion has, therefore, been expressed as a percentage of the last maternal daily dose in

Table 1. Milk and plasma levels of clofazimine in lactating female leprosy patients on administration of the drug orally for 1–18 months

Subject	Length of treatment (months)	Total intake (g)	Clofazimine levels 4–6 hr after last oral dose		M/P ratio	Drug ingested by infants (mg/kg/day)	% maternal doses‡
			Plasma (µg/ml)	Milk (µg/ml)			
1	18	26.6	1.0	1.7	1.7	0.255	30.0
2	6	17.6	0.9	1.5	1.7	0.225	13.5
3	4	6.5	0.8	1.3	1.6	0.195	23.4
4	4	6.0	1.0	1.4	1.4	0.210	25.2
5	1	1.5	0.8	0.8	1.0	0.120	14.4
6†	2	3.0	1.0	1.4	1.4	0.210	25.2
7	2	2.7	0.8	1.1	1.4	0.165	20.0
8†	3	4.3	0.9	1.4	1.6	0.210	25.2
Mean ± SE	5.0 ± 1.81	8.53 ± 2.93	0.9 ± 0.03	1.33 ± 0.09	1.48 ± 0.08	0.199 ± 0.013	22.11 ± 1.90
	Median	3.25					

* On clofazimine 100 mg daily.

† On clofazimine 100 mg on alternate days.

‡ Maternal doses as mg/kg.

mg/kg. The experimental data in animals have shown that the rate of drug passage between the mammary gland and plasma is more rapid for drugs that are highly lipid soluble and unionized.¹⁸ The high lipid solubility and moderately low molecular weight of clofazimine might favour rapid passage between mammary gland and plasma of lactating mothers.

It may be concluded that in spite of clofazimine in breast milk in significant quantities and the infant ingestion of about 0.225 mg/kg/day discontinuation of clofazimine use need not be recommended to lactating mothers till further studies with toxicity data are available.

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