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Azathioprine treatment during lactation

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SUMMARY

Background
Thiopurines are widely used to maintain remission in inflammatory bowel disease. Treatment during pregnancy is generally recommended to improve the chance of a normal birth outcome, but advice concerning breastfeeding is conflicting.

Aim
To estimate the exposure of breastfed infants to 6-mercaptopurine, as a metabolite of azathioprine, from maternal milk.

Methods
Eight lactating women with inflammatory bowel disease receiving maintenance therapy with azathioprine 75–200 mg daily were studied. Milk and plasma samples were obtained 30 and 60 min after drug administration and hourly for the following 5 h.

Results
The variation in the bioavailability of the drug was reflected in a wide range of peak plasma values of 6-mercaptopurine within the first 3 h. A similar curve, but with an hour's delay and at significantly lower concentrations varying from 2–50 μg/L, was seen in maternal milk. After 6 h an average of 10% of the peak values were measured.

Conclusions
The major part of 6-mercaptopurine in breast milk is excreted within the first 4 h after drug intake. On the basis of maximum concentration measured, the infant ingests mercaptopurine of <0.008 mg/kg bodyweight/24 h. The findings confirm that breastfeeding during treatment with azathioprine seems safe and should be recommended, considering the extensive beneficial effects.

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INTRODUCTION

Treatment with the thiopurines: azathioprine (Aza) and mercaptopurine (MP) is widely used to maintain remission in inflammatory bowel disease, particularly in patients who are steroid-dependent.[1] The most recently published evidence-based recommendations also include prevention of post-operative recurrence and fistulizing Crohn's disease.[2, 3] Aza and MP are increasingly used during pregnancy as avoidance of recurrent disease is thought to outweigh the risk of side effects in the foetus.

Many women are advised not to breastfeed on maintenance medication because of concerns of immunosuppression in the infant, but few data are available to support this recommendation and no consensus exists in the literature.[4–6]

The metabolism of Aza is complex and involves extra- as well as intracellular metabolism. The bioavailability of Aza varies between 16% and 70%.[7] Aza is a prodrug which is rapidly converted to 6-MP by a non-enzymatic reaction in the liver. The plasma half-life of 6-MP is short, in general, <2 h.

Further 6-MP metabolism involves three enzymatic pathways: xanthin-oxidase in the gut and liver converts Aza and 6-MP into the metabolically inactive 6-thiouric acid (6-TU), which is excreted in the urine. Thiopurine S-methyltransferase (TPMT) methylates 6-MP and some of its metabolites and of these, the methyl-thioinosine monophosphate is a strong inhibitor of purine *de novo* synthesis. Finally, hypoxanthine–guanine–phosphoribosyl transferase converts 6-MP into 6-thioguaninenucleotides (6-TGN).The concentration of 6-TGN in red blood cells reflects the therapeutic effect as well as outlines possible toxic effects.[8] In one study of four mothers and infants, maternal concentrations of the intracellular 6-TGN and some of its metabolites were within normal therapeutic ranges and no values above detection limits were found in the infants.[4]

Data on long-term outcomes of breastfeeding are sparse; only few data on concentrations in breast milk of 6-MP in a low number of women are available[4, 9, 10] and these measurements were performed infrequently.

In this study, we explored the exposure of the infants to 6-MP in maternal milk, as 6-MP is the most important substance for further metabolism also in the infant.

MATERIALS AND METHODS

Subjects and protocol

Eight lactating women with inflammatory bowel disease, who, independently of the study, had chosen to breastfeed during Aza treatment, were included. All had given birth to healthy infants 1.5–7 months prior to the investigation and all but one had been treated with Aza during their pregnancy.

Four women had Crohn's disease and four had ulcerative colitis. Demographic and clinical details are given in Table 1. Seven were in remission, while one had mild active disease treated with prednisolone. The Aza treatment was initiated 1–6 years prior to the pregnancies in the remaining seven participants. The daily dose ranged from 75 to 200 mg (0.75–2 mg/kg bodyweight), given once daily. Four of the women were on concomitant 5-ASA treatment (Table 1). All had unremarkable biochemistry and haematology including normal liver function tests. All were breastfeeding five to eight times a day. The infants did not receive other nutritional supplements.

On the day of the investigation, trough plasma values of 6-MP were measured 24 h after last medication intake. The participants ingested their usual dose of Aza with breakfast and after 30 and 60 min respectively, milk- and plasma samples were obtained. Hourly plasma- and milk concentrations of 6-MP were then measured over the following 5 h.

Maternal TPMT phenotypes and genotypes were also determined.

Table 1. Clinical data and dose of azathioprine in eight breastfeeding mothers with inflammatory bowel disease

Case	Disease	Age (years)	Weight (kg)	Azathioprine dose (mg/day)	Duration of azathioprine treatment (years)
1	CD	31	70	100	6
2	CD	29	100	200	5
3	CD	30	75	150	4
4	CD	28	50	100	3
5	UC	29	75	75	7
6	UC	29	80	150	4
7	UC	40	67	100	0.3
8	UC	28	78	150	7

CD, Crohn's disease; UC, ulcerative colitis.

The study was approved by the local ethics committee and the women gave their written informed consent according to the Helsinki declaration.

Determination of 6-MP and 6-TU and TPMT

Dithiotreitol 0.43 μmol/mL was added to the milk and blood samples at the time of sampling. The samples were immediately frozen at −80 °C. The metabolites were analysed as previously described.[11, 12]

Thiopurine S-methyltransferase genotypes were determined by enzymatic cleavage of purified DNA.

The genotype of each individual was explored by means of polymerase chain reaction and restriction enzyme based assays for analyses of the G460A and A719G point mutations.

The TPMT phenotype was analysed by a modified RIA method.

RESULTS

The TPMT phenotypes all showed normal activity and the genotypes showed no mutation.

The maternal plasma values of 6-MP are shown in Figure 1. As expected, the large variation in systemic absorption was reflected by large interindividual values in 6-MP concentrations; however, peak values were observed within 3 h after intake in all study subjects.

The breast milk concentrations of 6-MP are shown in Figure 2. A similar but delayed and less pronounced increase in breast milk values is seen.

Based on the excretion in the milk, an estimate of the infants' exposure to 6-MP can be made. If the highest MP concentration measured in any of the milk samples (50 μg/L) is multiplied by the amount of milk of about 150 mL/daily/kg bodyweight[13] fed to the infant during 24 h, the infant will receive 0.0075 mg/kg bodyweight. This is considered 'worst case scenario', as the exposure in most cases will be <10% of the maximum concentration, according to the concentrations found in the remaining samples.

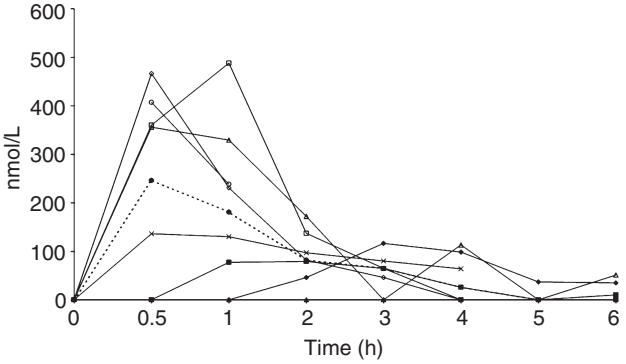

Figure 1. 6-mercaptopurine concentrations in maternal plasma.

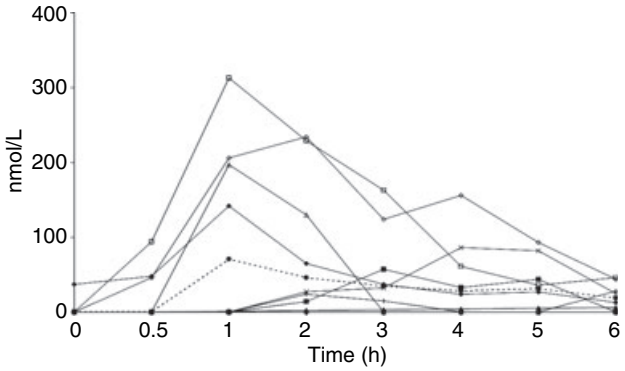

Figure 2. 6-mercaptopurine concentrations in maternal milk.

DISCUSSION

The extent of infant exposure to immunosuppressants in maternal milk is important to assess the risks and benefits of breast feeding. The absorption and metabolism of the thiopurines is highly variable, the half life of 6-MP is below 2 h and single measurements of milk concentrations are too simplified to predict the overall exposure of the drug to the infant. It is therefore crucial that frequency of measurements in maternal milk reflects the pharmacokinetics of the drug to ensure valid exposure of the drug to the infant.[15] In this study, the concentrations of 6-MP in plasma and breast milk were measured 30 and 60 min after drug intake, followed at hourly intervals to ensure that no peak values of the substance were missed. During the study interval, the concentrations decreased in all subjects, which indicate that the interval of observation is sufficient to estimate the infants' exposure. The plasma concentrations of 6-MP showed peak values within one hour in accordance with the findings by Ding and Benet,[16] but at very different levels, reflecting the large variation in bioavailability. The concentration of 6-MP in maternal milk showed peak values within the first 4 h after drug intake, also displaying a wide variation, from 2 to 50 μg/L. The maximum concentration

is higher than reported in any other published data and occurred in a woman, who received azathioprine 200 mg daily. In a study by Sau,[10] 31 milk samples were obtained from ten lactating women on daily doses Aza of 75 mg (n = 1); 100 mg (n = 8) and 150 mg (n = 1). Between 1 and 6 samples were drawn from each patient within 0–18 h after drug intake. Detectable concentrations were found in just one patient on Aza 100 mg of 1.2 and 7.6 μg/L at 3 and 6 h respectively. This finding emphasizes the importance of frequent measurements at regular intervals as performed in this study to establish the infant's actual exposure to MP. When multiplying the maximum concentration of 300 nmol/L with the amount of daily milk ingestion of 150 mL/kg bodyweight and calculated molecular weight of 158.2 g,[17] the infant receives <0.008 mg/kg bodyweight daily. Compared with the maternal exposition of 1–2.5 mg/kg bodyweight daily, the infant dose amounts to <1% of the maternal dose. All values decreased at the latest after 5 h and no subsequent values above 20 μg/L were measured.

The therapeutic effects as well as possible side effects such as myelosuppression and hepatic involvement are considered related to the intracellular concentrations of the metabolites 6-TGN and 6-MPMM respectively. The formation is among other enzymes determined by the TPMT activity. All participants had normal TPMT activity, and no measurement of 6-TGN and 6-MPMM in red blood cells was made in either study subjects or in infants. Gardiner investigated the concentrations in four infants and found no measurable concentrations in the infants, despite a sensitive method.[4] When the infant ingests 6-MP perorally, an absorption of 5–37% of the dose may be expected assuming the absorption in infants is similar to that in adults.[17] If the infant has a rare very low TPMT activity (one in 300),[18] possible myelosuppression cannot be excluded and special attention should be paid in case even higher doses than 200 mg are administered. However, the exposure can be reduced in all infants if the mother uses a breast pump to discard the first portion of milk produced after medicine intake instead of nursing the baby.

Four of the lactating women were also treated with 5-ASA containing drugs and the combined treatment with thiopurines has shown to lead to an increase in 6-TGN levels. The clinical importance of this interaction is unclear.[19, 20]

In summary, we have shown that the major part of 6-MP is excreted in breast milk within the first 4 h after drug intake and the estimated maximum exposure of drug to the infant is <0.008 mg 6-MP/kg bodyweight/day, representing <1% of the maternal dose. The present findings confirm that breastfeeding during treatment with Aza generally seems safe and should be recommended considering the otherwise beneficial effects.

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