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Exposure to thiopurine drugs through breast milk is low based on metabolite concentrations in mother-infant pairs

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Aims
To determine infant exposure to 6-thioguanine and 6-methylmercaptopurine nucleotides (6-TGN and 6-MMPN, respectively) during maternal use of azathioprine in breastfeeding.

Methods
Mother-infant pairs provided blood for determination of 6-TGN and 6-MMPN concentrations, and *TPMT* genotype.

Results
Four women taking azathioprine 1.2–2.1 mg kg^{-1} day^{-1} and their infants were studied. All had the wild-type *TPMT* genotype. Maternal 6-TGN and 6-MMPN concentrations ranged from 234 to 291 and 284 to 1178 pmol per 8×10^8 red blood cells, respectively, and were consistent with those associated with improved therapeutic outcomes. Neither 6-TGN nor 6-MMPN was detected in any of the infants, despite a sensitive assay.

Conclusions
The data suggest that azathioprine may be 'safe' during breastfeeding in patients with the wild-type *TPMT* genotype (~90% of caucasian patients) taking 'normal' doses.

Breastfeeding offers substantial nutritional, sociological and economic benefits and is the best form of nourishment for infants in the first 6 months of life [1]. Human milk also provides health advantages, including protecting the offspring from immune-mediated diseases in later life, such as inflammatory bowel disease [2]. There are few reasons to avoid breastfeeding, although maternal pharmacotherapy is a frequent cause for concern. However, whereas it is clear that virtually all drugs transfer into milk to some extent [3], the risk for most suckling infants is likely to be small. Immunosuppressants are often considered to be an exception because of their low therapeutic index and a concern that long-term exposure to these drugs in milk may result in immunosuppression and carcinogenesis in the infant. Although this suggestion is unsubstantiated, it often leads to the recommendation that women requiring immunosuppressant drugs should avoid breastfeeding.

Azathioprine and its metabolite, 6-mercaptopurine (6-MP), are two immunosuppressants used extensively in

premenopausal women for a diverse range of conditions. Their use in pregnancy is increasing since the maternal and fetal consequences of inadequately treated disease often outweigh the adverse effects associated with the drug. Overall, azathioprine and 6-MP appear to pose low teratogenic risk, although they may increase the chances of perinatal complications such as myelosuppression [4]. Currently, the published data on outcomes with thiopurine use in breastfeeding are inadequate to discern safety. A search of Medline (1966 to October 2005) and Embase (1988 to October 2005) using the terms 'azathioprine', '6-mercaptopurine', '6-thioguanine', 'human milk', 'lactation' and 'breastfeeding' revealed 11 cases of solid organ transplant recipients electing to take azathioprine during pregnancy and breastfeeding [5–8]. Whereas no adverse effects in the offspring were apparent, this is only partially reassuring and concentrations of active drug were not measured.

Azathioprine itself is not expected to transfer appreciably into milk, due to rapid conversion to 6-MP. One report documents 6-MP concentrations in two lactating renal transplant recipients taking azathioprine [8], with one of the babies estimated to ingest <1% of the maternal dose (as azathioprine equivalents), corrected for weight [3]. Infant exposure is likely to be further decreased as a result of the low oral availability of 6-MP (~0.50) [9]. The small number of subjects studied and the fact that 6-MP is active only after conversion to 6-thioguanine nucleotides (6-TGN) limit the conclusions that can be drawn regarding the exposure of babies to thiopurine drugs through breast milk. As 6-TGN are present intracellularly, determination of their concentration in breast milk is unlikely to be helpful in the therapeutic setting.

The aim of this study was to determine the concentrations of 6-TGN in the blood of exposed infants and to compare these with maternal concentrations. Thiopurine methyltransferase (*TPMT*) genotype was also determined in the mothers and infants, as mutations within this gene may lead to decreased TPMT enzyme activity and significant elevations in the cytotoxic 6-TGN metabolites. Erythrocyte TPMT enzyme activity was also determined in the mothers. The concentrations of 6-methylmercaptopurine nucleotides (6-MMPN), other metabolites that are potentially hepatotoxic, were also determined.

Methods

Subjects and protocol

Mothers who were eligible for inclusion had made the decision to take azathioprine during breastfeeding independently of this study and were on a stable dose for at least 4 weeks. The mothers had to be at least 3 months postpartum if azathioprine had been taken during pregnancy to ensure that metabolite concentrations in the infant were a consequence of breastfeeding rather than of *in utero* exposure.

One to two weeks prior to the study, the mothers provided a blood sample (10 ml EDTA) for the determination of 6-TGN and 6-MMPN concentrations to confirm compliance with medication prior to sampling from the infant. On the study day, a blood sample (2–5 ml EDTA) was drawn from the infant for determination of 6-TGN and 6-MMPN concentrations and *TPMT* genotype. Maternal blood (25 ml EDTA) was collected within 2 h of infant sampling for the determination of metabolite concentrations, TPMT activity and genotype.

Approval was obtained from the Canterbury Ethics Committee, Christchurch, New Zealand. Informed written consent was obtained from the mothers for their own participation and for the involvement of their babies.

Determination of 6-TGN and 6-MMPN concentrations

The concentrations of 6-TGN and 6-MMPN in red blood cells (RBC) were determined using a previously described high-performance liquid chromatography method [10]. The limit of quantification for both compounds was 30 pmol per 8×10^8 RBCs. The intra- and inter-day coefficients of variation (CV) were <10% for both assays, at concentrations of 60 600 and 2400 (6-TGN) and 150, 600, 3000 and 12 000 (6-MMPN) pmol/8×10^8 RBCs/0.1 mL.

Determination of TPMT genotype and phenotype

Genomic DNA was obtained from 2 to 5 ml of peripheral blood using a guanidine isothiocyanate-based extraction method [11]. Each patient was genotyped for the common poor metabolizer alleles, *TPMT*2* (238G→C), *TPMT*3A* (460G→A, 719A→G) and *TPMT*3C* (719A→G) using a previously described multiplex allele-specific polymerase chain reaction [12]. The robustness of this assay was increased by decreasing the annealing temperature to 63 °C, increasing elongation to 90 s and replacing nonhot start *Taq* DNA polymerase (Roche Molecular Biochemicals, Indianapolis, IN, USA) with Platinum®*Taq* DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA).

A radiochemical method was used to determine TPMT activity, as described previously [13]. The assay can determine enzyme activity from 0–30 IU/mL (CV 10%), enabling classification into 'normal' (9.3–17.6 IU/mL), reduced (<9.3) and deficient (near 0 IU/mL) activity.

Results

Four women (63–76 kg, 29–35 years old, all self-defined as New Zealand Europeans) taking azathioprine for the prevention of renal transplant rejection (subject 1), Crohn's disease (subjects 2 and 3) or autoimmune hepatitis (subject 4) were recruited. These subjects had taken azathioprine throughout pregnancy and were stabilized on a dose of 1.2–2.1 mg kg^{-1} day^{-1}. Three of the four male offspring (5.7–7.2 kg; 3–3.5 months old) were exclusively breastfed, whereas the other (baby of subject 2) had 'formula' but only rarely. The infants were regarded as healthy, although one had chronic conjunctivitis (subject 2) and another had vesico-ureteric reflux (subject 3). As a crude guide to infant progress, growth charts [14] were used to assess body weight and all four infants were within the 50th to 95th percentiles.

The initial blood sample from the mothers confirmed adequate drug exposure, with concentrations of 6-TGN ranging from 228 to 295 pmol per 8×10^8 RBC (local range 235-450 pmol per 8×10^8 RBC). These values are close to or within the range associated with efficacy in inflammatory bowel disease (>235 pmol per 8×10^8 RBC) [15] and a decreased risk of myelotoxicity (<500 pmol per 8×10^8 RBC) [16]. 6-MMPN concentrations were <922 pmol per 8×10^8 RBC, which is substantially below the concentration (5700 pmol per 8×10^8 RBC) associated with hepatotoxicity [17].

In the study proper, maternal and infant blood samples were taken at variable times post dose (Table 1), which is reasonable based on the long half-life of 5 days of 6-TGN in those with 'normal' TPMT activity [18]. All of the mothers and their infants had a wild-type *TPMT* genotype (*TPMT*1/*1*) and the mothers had 'normal' TPMT enzyme activity (11.1–14.4 IU ml^{-1} RBC; local range 9.3–17.6). Maternal concentrations of 6-TGN and 6-MMPN on the study day ranged from 234 to 291 and 284 to 1178 pmol per 8×10^8 RBC, respectively, consistent with concentrations associated with therapeutic outcomes [15, 16]. In contrast, neither 6-TGN nor 6-MMPN could be detected in the exposed infants.

Discussion

The principle finding of this study was that infant exposure to azathioprine metabolites through milk was undetectable in these subjects who had a *TPMT*1/*1* genotype. This suggests that many mothers requiring azathioprine (or a comparable dose of 6-MP) could breastfeed with safety. This reassurance is assisted by the high sensitivity of the assays, which detect concentrations at around 10% of 'therapeutic' concentrations. However, it is possible that mothers taking higher doses or those with decreased TPMT activity may transfer more drug to their baby. For these reasons, and given the small number of subjects included in the present study, the decision to recommend breastfeeding must not be taken lightly, although the consistency of our findings is reassuring.

Table 1
Results for the individual mother-infant pairs

	Subject 1		Subject 2		Subject 3		Subject 4	
	Mother	Infant	Mother	Infant	Mother	Infant	Mother	Infant
Azathioprine dose (mg kg^{-1} day^{-1})	1.3	–	1.2	–	1.5	–	2.1	–
Concurrent drugs, dose per day	Tacrolimus 4 mg Diltiazem 180 mg Atenolol 100 mg Frusemide 20 mg	–	Mesalazine 1 g Prednisone 5 mg	–	Mesalazine 4 g	–	–	–
Time of sample (h postdose)	1.6	3.7	5.8	5.5	10.6	10.4	6.5	6.3
TPMT activity (IU ml^{-1} RBC)*	11.1	–	14.4	–	13.5	–	12.9	–
6-TGN (pmol per 8×10^8 RBC)	253	< 30	234	< 30	291	< 30	263	< 30
6-MMPN (pmol per 8×10^8 RBC)	310	< 30	284	< 30	290	< 30	1178	< 30

**All subjects had a TPMT *1/ *1 genotype TPMT, Thiopurine methyltransferase; 6-TGN, 6-thioguanine nucleotides; 6-MMPN, 6-methylmercaptopurine nucleotides.*

The methodology used in this preliminary study is unusual because azathioprine acts via intracellular metabolites. Robust studies of drugs and lactation focus on measuring average concentrations of drug in milk over a dose interval so as to enable calculation of infant dose via milk [19]. It is often considered to be a 'bonus' if blood can be sampled for the measurement of drug concentrations in the exposed infants. For azathioprine and 6-MP, the determination of drug concentrations in milk may not yield particularly helpful information as the compound most likely to be present (6-MP) is inactive. As the active 6-TGN reside intracellularly, they are unlikely to be quantified readily in milk, suggesting that the best option for confirming safety is to measure the metabolites in the suckling infants with a sensitive assay. The women were studied at least 3 months postpartum to ensure that if metabolites were detected in the infants, these were the result of drug exposure in breast milk rather than *in utero*. However, the older age of the infants also means that care should be taken when extrapolating these findings to younger or premature infants who may have a decreased ability to clear the drug.

In summary, these results suggest that breastfeeding during azathioprine treatment may be safe in mothers with 'normal' TPMT enzyme activity and blood concentrations of 6-TGN that are within the range associated with efficacy (>235 pmol per 8×10^8 RBC in inflammatory bowel disease) and reduced myelotoxicity (<500 pmol per 8×10^8 RBC). This work provides additional information to that which is already published [5-8]. It also suggests that sampling the infant for determination of concentrations of 6-TGN could prove helpful clinically, with undetectable metabolite concentrations indicating negligible or low exposure.

Conflict of interest

None to declare.

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