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Short communication

Azathioprine and breastfeeding—is it safe?

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Traditionally, women receiving azathioprine have been discouraged from breastfeeding because of theoretical potential risks of neonatal bone marrow suppression, susceptibility to infection, and pancreatitis. The aims of this study were to measure the concentration of 6-mercaptopurine (6-MP) in breast milk of mothers receiving azathioprine and in the blood of their babies and to investigate any immunosuppressive effects on the babies. Women receiving azathioprine, who after appropriate counselling wished to breastfeed their babies, were approached for inclusion in the study. Breast milk samples were obtained from recruited women, and 6-MP levels were measured in each breast milk sample. Haemoglobin level, white cell and platelet counts, and 6-MP and 6-thioguanine nucleotides (6-TGN) levels were measured in the respective neonatal blood samples. Clinical signs of immunosuppression in the neonates were noted. Thirty-one breast milk samples were collected from ten women. Low concentrations of 6-MP (1.2 and 7.6 nanograms/ml, compared with therapeutic immunosuppressant level of 50 nanograms/ml in serum) were detected in two breast milk samples obtained from one woman. 6-MP was not detected in any of the other 29 samples. 6-MP and 6-TGN were undetectable in the neonatal blood. There were no clinical or haematological signs of immunosuppression in any of the ten neonates. We conclude that breastfeeding should not be withheld in infants of mothers receiving azathioprine.

Keywords 6-Mercaptopurine, azathioprine, breastfeeding.

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Introduction

Azathioprine has been used as an immunosuppressant in pregnancy for many years. On absorption, azathioprine is rapidly converted to 6-mercaptopurine (6-MP), which then follows the route of normal purine salvage within the cell to form 6-thioguanine nucleotides (6-TGN). Azathioprine and 6-MP cross the placenta in humans, but only very low concentrations have been detected in the fetus.[1] No teratogenic effects on the fetus have been described,[2] although concern regarding neonatal leucopenia and thrombocytopenia correlating with maternal leucocyte counts has been an issue.[3] Traditionally, breastfeeding in these women has been discouraged because of the theoretical potential risks of bone marrow suppression, susceptibility to infection, and pancreatitis in the neonate. A study by Coulam *et al.*,[4] involving two women (one breastfeeding and another non-breastfeeding) predicted that the concentration of 6-MP present in breast milk (peak levels 3–18 nanograms/ml) would expose the infant to far less drug than the 50 nanograms/ml in serum required for a therapeutic immunosuppressive effect.[4] The peak concentration of 6-MP in the breast milk was found at 2 and 8 hours after ingestion of azathioprine. In another study of two babies breastfed by mothers taking 75 and 100 mg azathioprine daily, respectively, the infants had normal blood cell counts, no increase in infections and above average growth rate (6-MP levels in the milk were not determined).[5] Similar observations were made by Khare *et al.*[6] On the basis of these studies, some clinicians advocate breastfeeding for women who are receiving azathioprine. The World Health Organization recommends against azathioprine in breastfeeding women due to its immunosuppressive effect.[7] The general advantages of breastfeeding, including protection against chest infections and gastroenteritis, are well known, and these protective effects are even more beneficial for premature babies. Women with connective tissue diseases, inflammatory bowel diseases and renal transplants are more likely to be receiving azathioprine and are at increased risk of premature delivery, so the advantages of breastfeeding in this group may outweigh the theoretical risks. Many such women delivered in our unit are keen to breastfeed.

The aims of this study were to measure the concentration of 6-MP in breast milk of mothers receiving azathioprine, to

measure the concentrations of 6-MP and 6-TGN in the red blood cells (RBC) of their neonates and to investigate any immunosuppressive effects on their babies.

This study was approved by St Thomas Hospital Research Ethics Committee.

Materials and methods

Women were recruited from the joint Obstetric–Renal and Lupus clinics of Guy's and St Thomas hospitals during the period June 2003 to February 2005. Those women who, after appropriate counselling, wished to breastfeed their babies while receiving azathioprine (75–150 mg in a single daily dose) were approached for inclusion in the study. Women were requested to provide breast milk samples on days 3–4, days 7–10 and day 28 after delivery. Two millilitres of expressed breast milk was collected before taking azathioprine and thereafter during each breastfeed for 12–18 hours. 6-MP levels were measured in each breast milk sample.

Blood samples were obtained from the neonates on days 1–6 of life if other tests were indicated clinically, days 7–10 (at the time of the Guthrie test) and day 28. Haemoglobin concentration, white cell and platelet counts, and 6-MP and 6-TGNs concentrations were measured. A neonatologist assessed each neonate for any clinical signs of immunosuppression at the time of venepuncture. All women were asked to inform one of the investigators about any illness in their baby (e.g. diarrhoea, chest infection).

Thiopurine methyltransferase (TPMT) activity was not known in the women prior to initiation of azathioprine. All women are, however, likely to have normal TPMT activity as they have tolerated standard doses of azathioprine without suffering adverse events. TPMT deficiency is associated with decreased methylation of 6-MP, high levels of TGNs and an increased risk of drug toxicity.[8]

Analysis of 6-MP

TGN levels in the neonatal blood (RBC) were measured as the hydrolysed bases 6-MP and 6-thioguanine as previously reported.[9] The assay of 6-MP in the breast milk sample was performed by the following method.

Trichloroacetic acid precipitation and ether extraction (trichloroacetic acid method)

Nineteen microlitres of a fresh 1.1M solution of dithiothreitol was added to 300 μl of breast milk, and the solution was briefly vortexed to mix. Three hundred microlitres of 10% trichloroacetic acid (TCA) was added to precipitate protein, and the solution was vortexed and centrifuged at 12 000 *g* for 3 minutes. The supernatant was transferred to a clean microfuge tube and extracted four times with water-saturated ether to remove TCA and fats. One hundred microlitres was injected onto a C18 (ODS) column (Phenomenex Hypersil 3u 150 × 4.6 mm, Macclesfield, UK) over 35 minutes and eluted with a gradient running from 100% buffer A (40 mM ammonium acetate, pH 5.0) to 30% buffer B (methanol: acetonitrile: tetrahydrofuran, 80:10:10) over 25 minutes, then back to initial conditions. Chromatograms were monitored at wavelengths of 324 nm for 6-MP (elution 10.6 minutes). A 1 mM standard of 6-MP was prepared in 0.1N NaOH. To estimate recovery, standards were diluted 1:31 into breast milk (final concentration 32258 nM) and processed as described above. Recovery of standards for 6-MP by using the TCA method was 91%.

Results

During the study period, 12 women were recruited but two were withdrawn, as they decided not to breastfeed their baby. The demographic and clinical data are shown in Table 1. In seven women, indication for azathioprine therapy was systemic lupus erythematosus, renal transplant was the indication in two cases, and Crohn's disease in one. Thirty-one breast milk samples were collected from ten women (one to six samples each). The day of sample collection varied from days 3 to 28 following delivery, and the timings of collections were before or 3–18 hours after the azathioprine intake. Three samples were collected just before azathioprine intake, nine samples within 3 hours, seven samples between 3 and 6 hours, eight samples between 6 and 12 hours and one sample after 18 hours of azathioprine intake. The timings of breast milk collection in relation to azathioprine intake were not recorded in three samples. 6-MP was only detected in case 5 (6-MP levels in breast milk were 1.2 and 7.6 nanograms/ml at 3 and 6 hours after ingestion of azathioprine, respectively, on day 28). 6-MP was not detected in any of the other 29 samples.

As shown in Table 1, 6-MP and 6-TGNs levels and full blood counts were measured in seven neonates since mothers withheld consent in three cases (cases 8, 9, and 10). 6-MP and 6-TGN were undetectable in neonatal blood. One baby had a borderline low neutrophil count (0.9). There were no clinical signs of immunosuppression in any of the ten neonates, three of whom were preterm.

Discussion

Azathioprine is an antimetabolite drug that is used as an immunosuppressant in many clinical conditions affecting women of childbearing age including renal transplantation, connective tissue disease, and inflammatory bowel disease. It is absorbed from the adult gastrointestinal tract and converted in the liver to 6-MP, which is the active metabolite. Most of the beneficial and adverse effects of azathioprine are due to 6-MP. The plasma half-life of azathioprine is 10 minutes. Due to the short half-life, it is not possible to measure azathioprine in the blood or breast milk nor is it

Table 1. Clinical data and blood results of all cases

Case	Indication for azathioprine therapy	Dose of azathioprine (mg/day)	Other medications	Gestational age at delivery (weeks)	Birth weight (gm)	Neonatal age at the time of blood sampling	6-MP and TGN levels (nanograms/ml)	White cell counts ($\times 10^9$/l)	Neutrophil counts ($\times 10^9$/l)
1	SLE	100	Prednisolone	38	3515	Day 8	0 and 0	10.7	3.5
2	SLE	100		32	1940	Day 1		4.6	2.3
						Day 2		5.7	1.2
						Day 10	0 and 0	5.9	1.1
3	Renal transplant	100	Tacrolimus	34	1790	Day 7	0 and 0	9.6	5.2
4	SLE	100	Prednisolone, hydroxychloroquine	37	3140	Day 1		26.4	14
						Day 9	0 and 0	9.9	0.9
5	SLE	100	Prednisolone	39	2750	Day 28	0 and 0	8.3	1.7
6	SLE	100	Prednisolone, hydroxychloroquine	37	3140	Day 20	0 and 0	25.5	20.4
7	SLE, lupus nephritis	100	Prednisolone, hydroxychloroquine	33	1940	Day 2		20.6	14.8
						Day 8	0 and 0	15.5	5.4
8	SLE, lupus nephritis	100	Prednisolone	39	2750	No blood sample			
9	Renal transplant	75	Tacrolimus	39	3630	No blood sample			
10	Crohn's disease	150	Prednisolone	37	3170	No blood sample			

SLE, systemic lupus erythematosus.

necessary because all of its effects are through 6-MP. Ding and Benet[10] have shown that a woman on therapeutic doses of azathioprine had a peak concentration of 6-MP in the serum within an hour of azathioprine intake, and 6-MP levels were detected for up to 7 hours of ingestion of the drug. In the present study, the concentration of 6-MP in the breast milk at various time intervals after ingestion of azathioprine was undetectable in all but detectable in two (6%) of the samples. However, given that 6-MP may only be detectable in the serum for 7 hours after ingestion of azathioprine[10] and only 16 breast milk samples were collected within 6 hours of ingestion, this would be a more appropriate denominator giving a percentage of 2/16 (12.5%). Where 6-MP was present in breast milk, the levels were well below the dose required to achieve the therapeutic immunosuppressant level of 50 nanograms/ml in the serum. Reassuringly, 6-MP levels were undetectable in the infant of subject 5, whose breast milk contained detectable 6-MP but only blood from day 28 was available. It would have been reassuring to confirm that there were undetectable or subtherapeutic levels of 6-MP in her infant after it received two doses of 6-MP within 6 hours from the breast milk with detectable levels.

The babies were followed for up to 28 days. They did not show any signs of immunosuppression nor 6-MP and 6-TGN were detectable in their RBCs.

Gardiner *et al.*[11] measured levels of 6-TGN and 6-methylmercaptopurine nucleotides (6-MMPN; metabolites of azathioprine) in the RBCs of four breastfed neonates, whose mothers were taking azathioprine. They found that neither 6-TGN nor 6-MMPN was detected in any of the neonates despite sensitive assay.

Although the number of mother–infant pairs in our series was small, this study is the largest published series. The absence of active metabolites of azathioprine in the neonatal blood suggests that the conventional advice to women with normal TPMT activity receiving azathioprine to avoid breastfeeding may be inappropriate. The effect of a heterozygous or homozygous genotype in the neonate on thiopurine metabolite levels is not known. The concern is whether a TPMT homozygous-deficient infant unable to inactivate very small amounts of 6-MP absorbed from breast milk will be exposed to significant blood levels of the drug. Measuring the TPMT phenotype in the red cells of infants born to a carrier woman will identify infants completely deficient in TPMT. In these rare cases, breastfeeding may be inappropriate.

6-MP was uncommonly present in breast milk. It is possible that multiple breastfeeds where 6-MP is present at 2–4 hourly intervals shortly after maternal ingestion of azathioprine may lead to cumulative significant levels of 6-MP in the neonate. However, there is no evidence from this small study of immunosuppression in the neonates.

Conclusions

From this prospective observational cohort study, we conclude that breastfeeding should not routinely be withheld in infants of mothers receiving azathioprine as the immediate benefits may outweigh the risks. Indeed, given the increased susceptibility of many of the babies because of prematurity, there should be individual discussion with women about benefits and theoretical risks of breastfeeding while taking azathioprine. There are no data on the effect of carrier

TPMT activity on metabolite levels in breast milk or the effects of a heterozygous or homozygous genotype in the breastfed neonate, and careful counselling of such women is important.

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