Iron concentration in breast milk normalised within one week of a single high-dose infusion of iron isomaltoside in randomised controlled trial

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ABSTRACT

Aim: We compared the iron concentration in breast milk after a single high dose of intravenous iron isomaltoside or daily oral iron for postpartum haemorrhage.

Methods: In this randomised controlled trial, the women were allocated a single dose of intravenous 1200 mg iron isomaltoside or oral iron at a mean daily dose of 70.5 mg. We included 65 women with sufficient breast milk three days after inclusion – 30 from the intravenous iron group and 35 from the oral iron group – and collected breast milk and maternal blood samples three days and one week after allocation.

Results: The mean (±SD) iron concentration in breast milk in the intravenous and oral groups was 0.72 ± 0.27 and 0.40 ± 0.18 mg/L at three days (p < 0.001) and 0.47 ± 0.17 and 0.44 ± 0.25 mg/L after one week (p = 0.64). Baseline samples were not available that soon after birth.

Conclusion: A single high dose of intravenous iron isomaltoside for postpartum haemorrhage led to a transient increase in the iron concentration in breast milk three days after treatment compared with oral iron. The difference disappeared one week after treatment, and mean iron concentrations were within the normal range in all samples.

INTRODUCTION

Breast milk contains iron, which is necessary for the normal growth and development of a newborn infant (1), but information is lacking on the regulation of iron in human mammary glands. Findings from animal studies suggest that when iron reaches the alveoli of the mammary tissue, it is transported through a divalent metal transporter in the basolateral membrane, where transferrin receptors are also likely to be involved in iron uptake (2). The regulation seems to occur after uptake of iron in the epithelial cells. Ferroportin is believed to transport iron through the apical membrane into secretory vesicles targeted for export into the lumen of the alveoli and into milk, and this process is believed to be one of the primary regulators of milk iron secretion (1,3).

There is minimal evidence of any association between breast milk iron concentration and maternal iron status. Studies have found that low maternal iron status has no effect on breast milk iron concentration, except when the mothers have severe anaemia (4–9). According to Quinn, this general independence of milk iron from maternal haemoglobin (Hb) and iron status supports the likelihood of an active, rather than passive, transfer of iron into maternal milk. However, milk iron concentration may be compromised in mothers with severe anaemia (10).

Iron concentration in human milk is relatively low (0.6–0.9 mg/L) in colostrum and declines further, to 0.2–0.3 mg/L, in mature milk (11–14). The major iron-binding protein in human milk is lactoferrin, which binds a significant proportion of iron. Specific receptors for lactoferrin have been reported in the small intestine in humans. The presence of such receptors may explain the high bioavailability of milk iron (7). Due to the much lower

Key notes

- We compared the breast milk of women with postpartum haemorrhage after 30 received one high dose of intravenous iron isomaltoside and 35 received daily oral iron.
- Intravenous iron isomaltoside led to a transient increase in the iron concentration in breast milk three days after treatment compared with oral iron.
- Any differences disappeared within a week of treatment, and the mean iron concentrations were within the normal range at all sampling times.
bioavailability of iron from infant formula compared with breast milk, infant formulas are fortified with higher iron concentrations (15–18). Current guidelines recommend an iron concentration in formula of between 2 of 12 mg/L (19,20). However, most formulas used in Europe have an iron content of between 4 and 8 mg/L (21).

Excess iron in infant nutrition poses two different hypothetical risks (10). The first risk is iron overload, whereby excess iron is absorbed by the immature intestine of the infant, which can potentially lead to accumulation in tissues and cause organ damage. The second potential risk is the unabsorbed excess iron in the intestines. Although the majority of iron will be lost in the stools, it might provide an essential nutrient for pathogenic iron-requiring bacteria in the intestines, thereby changing the bacterial composition of the gut flora and potentially causing neonatal infections (22–24).

The aim of this study was to measure the concentration of iron in breast milk after treatment with a single high-dose infusion of iron isomaltoside, compared with oral iron supplementation after postpartum haemorrhage (PPH).

PATIENTS AND METHODS

The study population comprised women included in a randomised, controlled, open-labelled, single-centre trial performed at the Department of Obstetrics, Rigshospitalet, University of Copenhagen, Denmark, from May 2, 2013, to 18 September, 2014 (25). The study was approved by the National Committee on Biomedical Research Ethics on April 12, 2013 (approval number H-4-2013-019), and by the Danish Medicines Agency (approval number EudraCT 2012-005782-12). Healthy women with a singleton delivery and PPH ≥700 and ≤1000 mL or PPH >1000 mL and Hb >6.5 g/dL within 48 hours of delivery were allocated to either a single high dose of intravenous (IV) iron (IV iron group) or current treatment practice with oral iron supplementation (oral iron group). The results on patient-reported outcome, as well as haematopoietic response, will be reported elsewhere.

The study population consisted of the fraction of the 196 participants included between February 2, 2014, and September 18, 2014, who had sufficient breast milk production three days after inclusion to provide a 3 mL sample of breast milk.

The randomisation was carried out using the eClinical OS interactive web response system (Merge Healthcare, North Carolina, USA). The randomisation was 1:1 and was stratified by bleeding volume of 700–1000 mL or >1000 mL.

The IV iron group received a single dose of 1200 mg Monofer iron isomaltoside (Pharmacosmos A/S, Holbaek, Denmark), which was diluted in 100 mL of 0.9% sodium chloride and infused intravenously for 15 minutes.

The oral iron group received current treatment practice, which involved a recommendation to either continue oral iron supplementation as they had performed during pregnancy or to take 100 mg oral iron one or two times daily for a variable time period. The individual intake of elemental oral iron, including the type of preparation, dose and treatment duration, was monitored throughout the study period.

We collected baseline maternal blood samples when the women were postpartum and then again prior to treatment. Maternal blood and milk samples were collected from participants with sufficient milk production three days (range: two to four days) and one week (range: six to eight days) after inclusion. The breast milk was collected using the Breast Express hand pump (Nuby Inc, Los Angeles, USA). The 3 mL milk samples were stored in a frozen condition (<−18°C) and sent for analysis to Interlab GmbH, Munich, Germany. The total iron concentration in breast milk was determined using the SpectrAA 220 Z atomic absorption spectroscopy (Varian Medical Systems Inc, California, USA). Blood samples were analysed for Hb, ferritin, transferrin, iron and transferrin saturation (TSAT).

Statistical analysis

The statistical analyses were performed using SAS software version 9.4 (SAS Institute, North Carolina, USA). We summarised demographics and baseline characteristics, as well as the haematological parameters, using descriptive statistics. Categorical data were summarised by treatment, using the number and percentages of participants. Continuous data were presented by the mean and standard deviation (SD). The iron concentration in breast milk is presented by the mean, SD, lower quartile, upper quartile, minimum and maximum. The mean values of iron concentration in breast milk were compared between treatment groups by a two-sided t-test. p values of <0.05 were considered significant.

RESULTS

During the study period, 65 women – 30 of 97 women from the IV iron group and 35 of 99 from the oral iron group – had sufficient milk production three days after inclusion. The baseline characteristics for both groups are presented in Table 1. There was no overall difference between the two groups. The mean (±SD) oral daily dose of elemental iron in the oral iron group was 45.3 ± 60.2 mg from baseline to day three and 89.5 ± 76.7 mg from day four to one week.

| Table 1 Demographics and baseline characteristics in the two treatment groups |
|----------------|-------------|-------------|
|                | IV iron     | Oral iron   |
| Full analysis set (n, %) | 30 (100.0) | 35 (100.0) |
| Age (years) | 32.5 (±4.6) | 32.2 (±4.0) |
| Prepregnancy weight (kg) | 65.8 (±11.5) | 65.5 (±8.5) |
| Postpartum haemorrhage (mL) | 1181.7 (±433.8) | 1237.1 (±478.1) |
| Primipara (n, %) | 18 (60.0) | 27 (77.1) |

Values are mean (SD) unless indicated otherwise. IV = Intravenous; n = Number of participants; SD = Standard deviation.
The mean iron concentration in breast milk three days after the intervention was significantly different between the two treatment groups: 0.72 mg/L in the IV iron group and 0.40 mg/L in the oral iron group (p < 0.001) (Table 2, Fig. 1). There were no statistically significant differences between the groups in mean iron concentration one week after the intervention: 0.47 mg/L in the IV iron group and 0.44 mg/L in the oral iron group (p = 0.64).

The mean Hb and TSAT at baseline indicated iron deficiency anaemia in both groups. The mean Hb increased in both groups from baseline to one week, with no between-group difference. In the IV iron group, there was a marked and sustained increase in ferritin, which reflected the high IV iron dose given to the women and the fact that their bodies’ iron stores were replenished. This increase was accompanied by a decrease in transferrin, indicating that sufficient iron was bound to transferrin and was therefore bioavailable. A transient marked increase in serum iron and TSAT was seen on day three, declining towards normal at one week, indicating sufficient iron bound to transferrin. In the oral iron group, ferritin remained unchanged from baseline to one week, indicating no significant body iron store repletion. This was accompanied by a rise in transferrin, unchanged serum iron and a TSAT below the lower normal limit, which indicated insufficient bioavailable iron (Table 3).

**DISCUSSION**

This was the first randomised study to compare the iron concentration in breast milk after a single high dose of IV iron and oral iron supplementation with concurrent collection of breast milk and maternal blood samples.

A single-dose intravenous infusion of 1200 mg iron isomaltoside led to a significant increase in the iron concentration in breast milk three days after treatment compared with oral iron supplementation. The increase was

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**Table 2** Summary and comparison of the iron concentration in breast milk (mg/L) three days and one week after intervention

|                | IV iron | Oral iron | Significance | p-value* |
|----------------|---------|-----------|--------------|----------|
| Day 3          |         |           |              |          |
| N              | 30      | 35        |              |          |
| Mean (SD)      | 0.72 (0.27) | 0.40 (0.18) | <0.001      |          |
| q25–q75        | 0.53–0.81 | 0.29–0.50   |              |          |
| Min–Max        | 0.33–1.64 | 0.08–0.99  |              |          |
| Week 1         |         |           |              |          |
| N              | 30      | 34        |              |          |
| Mean (SD)      | 0.47 (0.17) | 0.44 (0.25) | 0.64       |          |
| q25–q75        | 0.34–0.55 | 0.24–0.56  |              |          |
| Min–Max        | 0.18–1.00 | 0.08–1.15  |              |          |

N = Number of participants; SD = Standard deviation; q25 = 25% Quartile; q75 = 75% Quartile.

*pTwo-sided t-test for independent samples.

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**Table 3** Summary of haematology in the two treatment groups at baseline and three days and one week after intervention

|                | IV iron | Oral iron | Difference between treatment groups | p     |
|----------------|---------|-----------|------------------------------------|-------|
| Full analysis set (n, %) | 30 (100.0) | 35 (100.0) |                                  |       |
| Haemoglobin (g/dL) |         |           |                                   |       |
| Baseline          | 9.4 (1.3) | 9.5 (1.3) |                                   |       |
| Day 3             | 10.0 (1.3) | 9.7 (1.3) | 0.094                              |       |
| Week 1            | 11.0 (1.2) | 10.9 (1.2) | 0.400                              |       |
| Ferritin (ng/mL)  |         |           |                                   |       |
| Baseline          | 72 (52) | 66 (57)   |                                   |       |
| Day 3             | 1018 (341) | 63 (37)  | <0.001                             |       |
| Week 1            | 1013 (352) | 51 (23)  | <0.001                             |       |
| Iron (μg/dL)      |         |           |                                   |       |
| Baseline          | 41.3 (26.0) | 44.0 (26.9) |                                   |       |
| Day 3             | 190.9 (93.5) | 50.1 (15.9) | <0.001                             |       |
| Week 1            | 74.9 (29.6) | 50.4 (25.2) | 0.015                              |       |
| Transferrin (mg/dL) |         |           |                                   |       |
| Baseline          | 325.6 (58.5) | 306.4 (59.5) |                                   |       |
| Day 3             | 312.0 (52.3) | 324.5 (54.1) | 0.052                              |       |
| Week 1            | 298.1 (44.1) | 330.8 (45.6) | <0.001                             |       |
| Transferrin saturation (%) |         |           |                                   |       |
| Baseline          | 9.3 (5.5) | 10.3 (5.9) |                                   |       |
| Day 3             | 45.5 (22.3) | 11.3 (3.8) | <0.001                             |       |
| Week 1            | 18.5 (7.0) | 11.2 (5.8) | 0.004                              |       |

Values are expressed as mean (SD) unless indicated otherwise. IV = Intravenous; n = Number of participants; SD = Standard deviation.
transient and disappeared one week after the start of treatment. The mean levels of iron concentration in breast milk found in this study were all within what is considered the normal range of iron concentration in breast milk found in previous studies, ranging from 0.6 to 0.9 mg/L in colostrum and from 0.2 to 0.3 mg/L in mature milk (11–14).

Studies on the iron concentration in breast milk from mothers given iron supplementation have shown diverging results. In a comparative study by Zavaleta et al., anaemic lactating women were treated with oral iron supplementation and no association between treatment and iron levels in the breast milk was found (7). In a study by Breymann et al., lactating women with mild anaemia and iron deficiency (defined in the study as Hb 10–12 g/dL and TSAT <15%) were treated with either 100 mg IV iron sucrose or no iron treatment. Milk iron concentration was measured before treatment and for the following four days. No significant difference between the groups was found at any time point (26). By contrast, in a subsequent study by Breymann et al., lactating women were randomised to 1000 mg IV iron carboxymaltose or oral ferrous sulphate of 100 mg twice daily. The milk iron concentration increased from 0.50 mg/L at baseline to a maximum of 1.45 mg/L 24 hours after treatment with IV iron. The mean iron breast milk values were significantly higher in the IV iron group at 48 hours after treatment compared to the oral iron group (0.60 vs. 0.33 mg/L, respectively, p = 0.0052) (27).

In our study, the maternal blood samples showed significant differences between the treatment groups, with regard to the iron metabolism in maternal blood compared to the moderate variations in iron concentration in the breast milk. The IV iron treatment resulted in normalisation of the TSAT in the maternal blood and repleted the maternal iron stores with a mean ferritin level that was 16 times larger than in the oral iron group. The mean serum iron level and TSAT peaked on the third day after treatment with IV iron, with increases that were 3.8 and 4.0 times greater than the serum iron level and TSAT in the oral iron group, respectively. However, the mean iron concentration in breast milk only increased by a factor of 1.8 in the IV iron group compared with the oral iron group. These results supported the assumption that the mammary glands actively regulate iron uptake, even after one treatment with high-dose IV iron (2,7,26).

We can speculate about whether the transient elevation in iron concentration in colostrum after treatment with IV iron raises a safety concern or is beneficial for the infant. The risks associated with high iron concentrations, such as neonatal infection or iron overload, are considered irrelevant given that the mean iron concentration at the peak still remained within the normal range of iron in colostrum and given that the iron level in infant formula is 10-fold higher, even though the bioavailability is much lower (19,20).

In terms of benefit, we do not expect that the short transient elevation of the iron concentration in breast milk after treatment with a single high-dose iron infusion would be of any benefit for the infant’s iron status. However, our study shows benefits from IV iron treatment for mothers with postpartum haemorrhage followed by iron deficiency and anaemia, as their iron stores were repleted and iron transport normalised within a few days. This was in contrast to the oral iron group mothers, who remained iron deficient.

This study had certain limitations. First, did not have any measurements of the iron concentration in breast milk before treatment. The women were included within 48 hours of delivery, before the onset of the postpartum lactogenesis, which meant that we were unable to collect the baseline breast milk samples of 3 mL required for analysis. The maternal blood measurements at baseline did not differ between the two groups and we would expect the same for the iron concentration in breast milk. Second, we might have missed a higher peak of iron concentration in breast milk before or after the collection of milk samples three days after inclusion and treatment, but as the milk volume before full lactogenesis is small, it would have had little influence on the iron level in the infant. Several factors influence the iron concentration in breast milk, including the diurnal variation and the difference in the composition of colostrum and mature milk. These factors were not considered in this study.

CONCLUSION
Treatment with single high-dose intravenous iron isomaltoside for postpartum haemorrhage led to a transient increase in the iron concentration in breast milk three days after treatment compared with oral iron. The difference disappeared one week after treatment. However, mean iron concentrations in breast milk were within the normal range at all sampling times. The transient peak calls for further investigation of biochemical outcomes, including the free iron component in breast milk.

ACKNOWLEDGEMENTS
We would like to thank Jens K Slott Jensen, MSc, who provided statistical support and the project midwives Trine Markussen Larsen, Katrine Høybye and Sara Winther.

CONFLICT OF INTERESTS
Charlotte Holm is an industrial PhD student engaged in a collaborative research project between Pharmacosmos A/S, manufacturers of the Monofer iron isomaltoside used in the study and the University of Copenhagen. Lars Lykke Thomsen and industrial PhD student Veronika Markova are employed by Pharmacosmos A/S. The other authors have no conflict of interests to declare.

FUNDING
The study is sponsored and funded by Pharmacosmos A/S, the University of Copenhagen and the Innovation Fund Denmark.
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