Investigation of dabigatran secretion into breast milk: *implications for oral thromboprophylaxis in post-partum women*

Running title: Dabigatran secretion into break milk

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Pregnancy and the post-partum period are recognised risk factors for venous thrombo-embolism (VTE), one of the most important causes of maternal mortality [1]. Sub-cutaneous low molecular weight heparin (LMWH) is the current recommendation for post-partum VTE prophylaxis [1] but an effective and safe oral agent would have advantages over LMWH. Direct oral anticoagulants (DOACs) have been demonstrated to be effective alternatives to LMWH and warfarin for VTE prophylaxis after hip and knee replacement surgery, as well as stroke prophylaxis in patients with atrial fibrillation [2]. Dabigatran etexilate is a pro-drug of the active agent dabigatran. The latter is minimally absorbed from the gastro-intestinal tract [3][3] and its physicochemical properties indicate that its transfer into breast milk is likely to be limited. Consequently, dabigatran etexilate could be an oral alternative to LMWH for VTE prophylaxis in the post-partum period. In this proof of concept study, we investigated dabigatran secretion into breast milk following oral administration to non-breast feeding women. We then estimated the systemic exposure in the neonate and investigated the potential effect on neonatal (cord blood) coagulation indices.

This was a single-centre, open-label study which had approval from the National Research Ethics Service-Committee North East, Newcastle & North Tyneside (ref: 15/NE/0331). The study was registered with the ISRCTN Registry under the name ‘Dabigatran Presence in Breast Milk (DALMATION)’ (trial registration no: ISRCTN87845776). Women were approached to take part in the study 2-7 days after delivery and only if they had made an informed decision not to breastfeed their baby. Women were included if they were aged ≥18 years, had a vaginal birth and did not require pharmacological thromboprophylaxis. Women were excluded if they had a known contra-indications to dabigatran, an increased risk of bleeding, abnormal renal (serum creatinine >90 μmol/L) or hepatic function (serum ALT ≥ 40 IU/L), on-

going treatment with anti-fungal drugs, an artificial heart valve, history of heart attack, irregular heartbeat, or use of other investigational study drug(s) within 30 days. Following the administration of a single oral dose of dabigatran etexilate (220mg), venous blood (10 ml) and breast milk (15 ml maximum) samples were collected from 2 women at t = 0 (pre-dabigatran dose), 1, 2, 3, 5, 7, and 10 h post-dose. Venous blood samples were collected into citrated tubes and plasma separated. All samples were stored at -80 °C until analysed in triplicates. Dabigatran concentrations in breast milk and plasma were measured using HPLC-MS technique. The lower limit of detection of the assay was 20 pg/ml in plasma and 75 pg/ml in breast milk. The inter- and intra-day coefficient of variation of the assay for the measurement of dabigatran at 0.03 ng/μl were in plasma: 4.8% and 7.0% and breast milk: 8.4% and 18.9%, respectively, and at 2 ng/μl were in plasma: 2.8% and 6.3% and breast milk: 0.4% and 3.4%, respectively. Milk to plasma (M/P) ratio was calculated from the area under the milk and plasma concentration – time curves (AUC) estimated using the trapezoidal rule. The absolute infant dose (had the woman been breast feeding) was calculated from the average dabigatran concentration in breast milk (AUC0-t/t) and an average (range) milk intake in the first 24 hours after birth of 13 (3-32) g/kg, increasing to 98 (50-163) g/kg and 155 (110-196) g/kg on days 3 and 5, respectively [4]. The estimated plasma dabigatran concentration in the individual babies was calculated on the assumption of (a) 100% drug oral bioavailability, (b) dabigatran being distributed entirely within plasma, (c) each baby weighed 3.5 kg at birth (d) each baby had a blood volume of 80 ml/kg and (e) for estimated values for days 3 & 5 there is no residual dabigatran from the previous day's milk intake. To measure the effect of dabigatran on neonatal coagulation indices, blood was collected from the umbilical vein into citrated tubes following birth at term (≥ 37 weeks gestation; n = 10) or pre-term (< 37 weeks

gestation; n = 7). Plasma was separated and stored at -80^{o}C until analysis. Dabigatran (Cayman Chemical, Michigan USA) was solubilised in HCl 1M (Sigma-Aldrich, Missouri USA), then diluted in distilled H_2O to 100µg/ml and aliquoted for storage at -20^{o}C. Solubilised dabigatran was further diluted in Tris-NaCl-BSA buffer (0.05M Tris, 0.15M NaCl and 1% BSA at pH 7.4; Hyphen Biomed, Neuville sur Oise, France), to a concentration of 15µg/ml. Using serial dilutions, four working solutions were prepared to achieve a final plasma dabigatran concentration ranging from $5x10^{-5}$ng/ml to 150ng/ml. Plasma samples were incubated with dabigatran (0, $5x10^{-5}$, 0.05, 0.5, 5, 50 and 150 ng/ml) and coagulation assays performed using the IL TOP ACL 700 CTS analyzer (Instrumentation Laboratory Company, Bedford, MA, USA). Thrombin time (TT), fibrinogen Clauss (Fib-C) and direct thrombin inhibitor assay (DTI) were measured using respective HemosIL® reagents (Instrumentation Laboratory Company, Bedford, MA, USA). Bovine thrombin reagent was diluted with 5ml buffer at a ratio of 1:5 with distilled H_2O. Fibrinogen based on the Clauss method was measured at a dilution of 1:10. Clotting factor II activity in plasma was measured with standard clotting assays based on PT (Recombiplastin 2G®) and factor II (FIIa) immunodepleted plasma (HemosIL®).

The milk and plasma dabigatran concentration-time profiles for the 2 participants are shown in **Figure 1**. Dabigatran was detectable in plasma 1h after dosing and peak concentrations of 204.6 ng/ml in subject 1 and 414.9 ng/ml in subject 2 were reached between 2-3 hours post-dose. Dabigatran was detected in breast milk 2-3h post-dose and peak dabigatran concentrations of 8.0 ng/ml and 53.0 ng/ml (4% and 12% of their respective peak plasma concentration) were detected at 5-7 hours post-dose. The pharmacokinetic characteristics of dabigatran in plasma and breast milk are summarised in **Table S1**. The breast milk AUC versus time was 0.016 µg/ml.h for

subject 1 ($AUC_{0\text{-}5h}$) and 0.17 µg/ml.h ($AUC_{0\text{-}10h}$) for subject 2. The estimated doses of dabigatran ingested by the suckling baby are shown in **Table S2**. The estimated doses expressed as equivalent of maternal dose on days 1, 3 and 5 are shown in **Table S3**. The estimated plasma concentrations for the suckling baby are shown in **Table S4**. Dabigatran at ≤5.0 ng/ml final plasma concentration had no significant effect on neonatal TT, FIIa, or plasma Clauss-fibrinogen concentration compared to the corresponding baseline values (**Figure S1**). The average coefficient of variation for replicate analysis of samples were as follows; TT5 (10.5%); DTI (16.4%, with values at 5ng/ml mainly contributing to variability); fib-C (8.8%); FIIa (5.7%).

DOAC use has not been extended to thromboprophylaxis in pregnancy and the post-partum period because of concerns about fetal and neonatal consequences. Dabigatran is a zwitter ion and is not absorbed from the gastrointestinal tract. The less hydrophilic pro-drug, dabigatran etexilate, was designed to overcome this obstacle and produce a more membrane permeable drug with favourable pharmacokinetic properties. With this modification, the absolute bioavailability of dabigatran following oral administration of dabigatran etexilate is 6.5% [5]. Following absorption, dabigatran etexilate is rapidly converted to dabigatran by plasma esterases. The physicochemical properties of dabigatran base indicate that its transfer across the mammary gland epithelium into breast milk is likely to be limited. In this study we investigated whether dabigatran is secreted into breast milk following oral ingestion of a standard oral dose of dabigatran etexilate in post-partum women. The plasma dabigatran concentration profiles in the two study subjects are consistent with the previously published data [5] and transfer of dabigatran from plasma into breast milk is delayed. The variability in milk/plasma (M/P) concentration ratio at individual time points illustrates the importance of using AUC method for

calculating M/P so that time-related variations can be minimised. The M/P AUC ratio was 0.02 and 0.1 for subject 1 and subject 2 respectively. Drugs with an extremely low M/P values (e.g. 0.1) are likely to be safe during breastfeeding (excluding those drugs contraindicated because of extreme toxicity) [6]. We were able to estimate the dose of dabigatran ingested by the neonate through breastfeeding during the first 5 days after birth and the resulting plasma concentrations. Our data indicate that the estimated maximum plasma dabigatran concentrations in the neonate are 100,000 times below the concentrations that will have a significant effect on coagulation indices. The results of this study indicate that dabigatran etexilate is a suitable candidate for further investigation as an oral agent for thromboprophylaxis in post-partum women. The strengths of this study include the use of a highly sensitive HPLC-MS assay to measure low level of dabigatran in breast milk over 7-10 hours. We were able to generate breast milk dabigatran concentration – time curves and calculate AUC. We examined the potential effects of low concentrations of dabigatran on coagulation indices in both term and pre-term neonates. The inclusion of only 2 women is an important limitation and further studies with a larger sample size are required to establish inter-individual variability in dabigatran secretion into breast milk. In addition, the secretion of dabigatran into breast milk is delayed and future studies should extend breast milk sampling time to 24 hours, simulating scenarios where the woman does not breast feed overnight.

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Authorship

Contribution: P.A., and F.K. designed the study. P.A., L.M., and J.A. conducted the trial. A.T., F.S. performed MS-HPLC analyses. E.K. and P.M. were responsible for the hematological assays. J.A. led focus-group discussions and monitored the trial. P.A. and F.K. analysed and interpreted data. All authors contributed to the manuscript.

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Figure legend

Figure 1. Concentration-time profile for dabigatran plasma and breast milk in two subjects after a single oral dose of dabigatran etexilate 220 mg. Data are mean ± sem for triplicate samples.

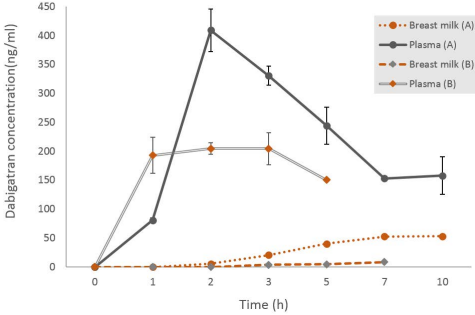
Dabigatran concentration(ng/ml)
450
400
350
300
250
200
150
100
50
0
0
1
2
3
5
7
10
Time (h)
Breast milk (A)
Plasma (A)
Breast milk (B)
Plasma (B)

Figure S1: Figure S1. Effect of dabigatran on neonatal coagulation indices. Cord blood was obtained from n = 10 placentas (full term) and n = 7 placentas (Pre-term). Analysis limited to a dabigatran concentration of 5 ng/ml in pre-term samples because of insufficient volume of plasma. For pre-term data in graph (a), n = 5 at 0 and 5 x 10^{-5} ng/ml, n = 3 at 0.05 ng/ml, n = 2 at 0.5 ng/ml and n = 1 at 5 ng/ml.

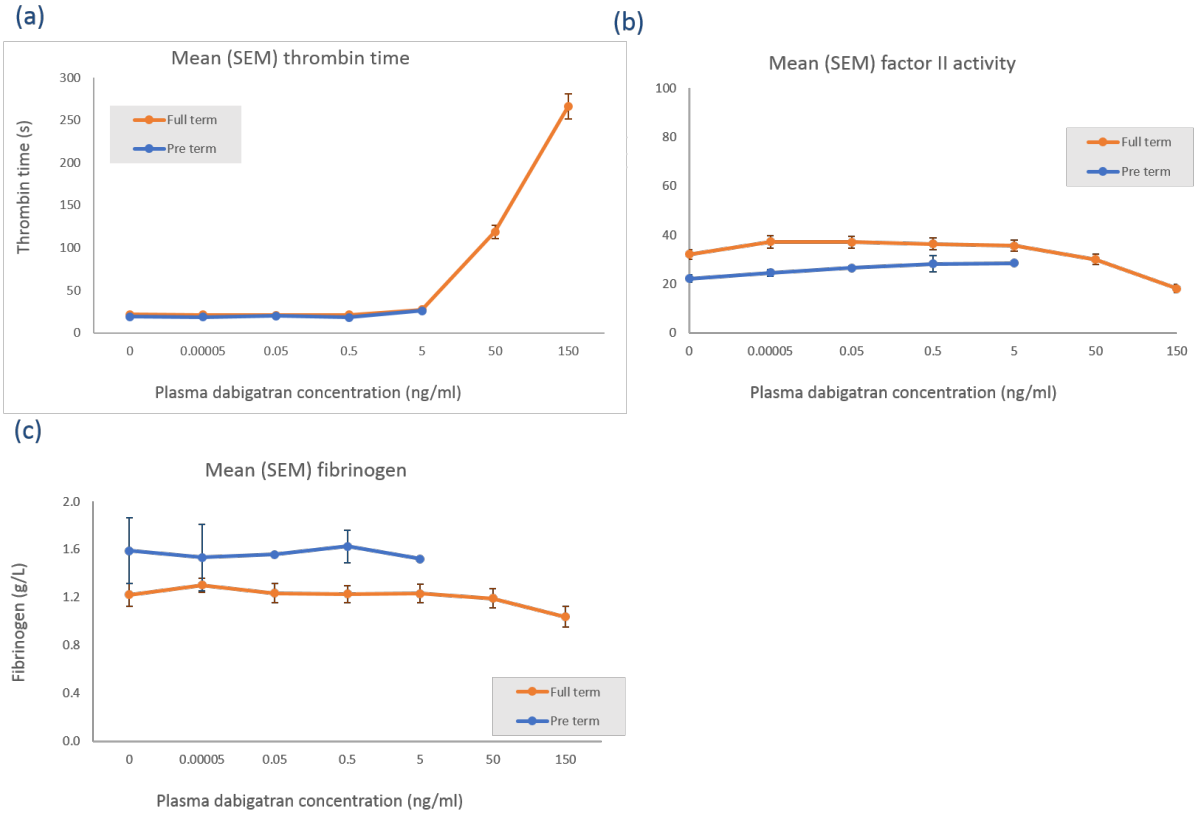

Table S1. Dabigatran pharmacokinetics in plasma and breast milk in the two study subjects

Subject	**1**			**2**		
	Plasma	Breast milk	M/P	Plasma	Breast milk	M/P
Time to dabigatran detection (h)	1	3		1	2	
Cmax (ng/ml)	204.6	8.2	0.04	414.9	52.6	0.12
Tmax (h)	2	7		3	7	
AUC 0-t (ng/ml.h)	752.7	16	0.02	1523	171	0.1

Table S2. Estimated dose of dabigatran (mg/kg) ingested by baby through breastfeeding by study subjects on days 1, 3, and 5 following birth

	Subject 1 **Dose (range) mg/kg**	**Subject 2** **Dose (range) mg/kg**
Day1	29.7×10^{-6} (6.8×10^{-6} – 73.1×10^{-6})	2.2×10^{-4} (5.1×10^{-5} – 5.5×10^{-4})
Day3	2.2×10^{-4} ($1.1.7 \times 10^{-4}$ – 3×10^{-4})	1.7×10^{-3} (8.6×10^{-4} – 2.8×10^{-3})
Day5	3.5×10^{-4} (2.5×10^{-4} – 4.5×10^{-4})	2.7×10^{-3} (1.9×10^{-3} – 3.4×10^{-3})

Table S3. Estimated equivalent dose that a baby is exposed to following breastfeeding expressed as percentage of maternal dose

	Subject 1	Subject 2
Day1	9×10^{-4} (2×10^{-4} – 21×10^{-34}) %	6×10^{-3} (1×10^{-3} –1×10^{-2}) %
Day3	7×10^{-3} (3×10^{-3} – 11×10^{-3}) %	4×10^{-2} (2×10^{-2} – 8×10^{-2}) %
Day5	1×10^{-2} (7×10^{-3} – 13×10^{-3}) %	7×10^{-2} (5×10^{-2} – 9×10^{-2}) %

Table S4. Estimated plasma dabigatran concentration (ng/ml) in baby with an average weight of 3.5 kg after breastfeeding

	Subject 1	Subject 2
Day1	37×10^{-10} (8×10^{-10} – 87×10^{-10})	27×10^{-7} (6.2×10^{-7} – 67×10^{-7})
Day3	28×10^{-7} (15×10^{-7} – 46×10^{-7})	20×10^{-6} (11×10^{-8} – 36×10^{-6})
Day5	43×10^{-7} (31×10^{-7} – 56×10^{-7})	32×10^{-6} (23×10^{-6} – 41×10^{-6})