Excretion of low molecular weight heparin in human milk

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Aims The excretion of low molecular weight heparin (LMWH) in breast milk was investigated in 15 lactating mothers after Caesarean section.

Methods Blood and milk samples were collected before and 3–4 h after once daily routine subcutaneous injection of 2500 IU dalteparin. Anti-Xa activity was measured by an assay utilizing prolonged clotting times in plasma or breast milk as an index of LMWH activity.

Results Plasma anti-Xa activities ranged from 0.074 to 0.308 IU ml^{-1} of plasma. Anti-Xa activities in breast milk ranged from <0.005–0.037 IU ml^{-1} of milk. This is equivalent to a milk/plasma ratio of <0.025–0.224.

Conclusions Therefore, it appears highly unlikely that puerperal thromboprophylaxis with LMWH has any clinically relevant effect on the nursing infant.

Keywords: breast milk, low molecular weight heparin, puerperium

Introduction

Low molecular weight heparin (LMWH) is widely used for thromboprophylaxis during pregnancy and puerperium. Numerous animal studies and clinical trials have shown that LMWH, like conventional unfractionated heparin, does not cross the placenta [1, 2] and provides safe, effective prophylaxis of thromboembolic complications [3, 4], with no evidence of teratogenicity [5]. Moreover, LMWH offers major clinical advantages over unfractionated heparin, a fact accounting for its increasing use in obstetric practice in recent years. Postpartum, LMWH is used primarily following Caesarean section. To our knowledge, the extent to which LMWH is excreted into breast milk, if at all, has yet to be established.

Methods

The trial protocol was approved by the ethics committee of the University of Zurich, and informed consent was obtained from all patients. Blood and milk samples were collected in 15 patients at University Women's Hospital, Zurich, before and 3–4 h after once daily routine subcutaneous injections of 2500 IU dalteparin (Low Liquemin®, Roche Pharma (Switzerland) Ltd, Reinach, Switzerland) on days 4–8 following Caesarean section. The milk and plasma samples were analysed individually. Pregnancies and deliveries had been uncomplicated, and lactation had previously been established in all patients. Mid-expression milk samples (5 ml) from one breast were obtained from each patient by manual breast massage immediately prior to and 3–4 h after dalteparin injections and filled into polystyrene tubes. Blood was withdrawn at the same time using Vacutainer tubes containing 0.5 ml of 0.1 mol l^{-1} sodium citrate for anti-Xa activity measurement and EDTA-containing tubes for haemograms. Plasma was immediately separated by centrifugation at 2000 *g* for 20 min at 4° C and then filled into polystyrene tubes. Milk and blood samples were both stored at −20° C.

Anti-Xa activity is an accepted parameter for monitoring responses to LMWH. In plasma and other watery solutions anti-Xa activity is normally determined photometrically by measuring proteolysis of a specific chromogenic substrate. Such assays cannot be used on an optically turbid suspension like breast milk, however. For this reason, an assay was developed utilizing prolonged clotting times in plasma or breast milk as an index of LMWH activity. For each test, 0.1 ml of sample and 0.1 ml of anti-Xa solution (Heptest, Haemachem Inc., Saint Louis, MO, USA) were incubated for 120 s at 37° C in a Schnittger and Gross–type coagulometer (on loan from

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Received 16 March 2001, accepted 16 August 2001.

DADE Diagnostics, Düdingen, Switzerland). The reaction was then started immediately using 0.1 ml of Recalmix (Heptest, Haemachem Inc., St Louis, MO, USA). The coagulometer measures clotting times in seconds.

A standard calibration curve was constructed for each patient to determine plasma LMWH concentrations. Plasma and milk samples before s.c. injection of LMWH were used for the standard calibration and standard curve determination for each patient (Table 1a, b). Curves were generated by adding LMWH (Low Liquemin®, Roche Pharma (Switzerland) Ltd, Reinach, Switzerland) in three concentrations within the expected range (0.1, 0.2 and 0.3 IU ml^{-1}) to plasma at time $t=0$ and measuring the clotting times. Plasma LMWH concentrations at $t=4$ h were obtained by comparing the clotting times for these samples with the curves.

To determine LMWH levels in breast milk, samples were diluted with two parts Human Control Plasma Citrated Normal (Roche Diagnostic Systems, Basel, Switzerland). As the samples showed a marked tendency to clot spontaneously at 37° C, each sample was prepared separately and then assayed immediately. To plot calibration curves, freshly prepared mixtures of breast milk and control plasma were spiked at $t=0$ with four different concentrations of LMWH within the expected range (0.0, 0.005, 0.01 and 0.02 IU ml^{-1}) and clotting times were determined. LMWH levels in breast milk at $t=4$ h were obtained by comparing the clotting times for these samples with the curves.

Table 1 Example for a standard curve ($\Delta t=0$ h) and results ($\Delta t=4$ h) in patient plasma (a) and milk (b).

(a) Plasma standard curve

(Δt = 0 h) Heparin concentration (IU ml^{-1})	*Clotting time (s)*	*Mean (s)*
0.10	42.6; 41.0; 39.0; 40.6	40.8
0.15	54.6; 54.6; 52.7; 54.1	54.0
0.20	62.7; 62.5; 62.1; 62.1	62.4
0.25	70.6; 72.5; 71.1; 71.1	71.3

Plasma result

(Δt = 4 h) Clotting time (s)	*Mean (s)*	*Heparin activity (IU ml^{-1})*
61.1; 64.1; 62.1; 62.1	62.4	0.201

(b) Milk standard curve

(Δt = 0 h) Heparin concentration (IU ml^{-1})	*Clotting time (s)*	*Mean (s)*
0	22.9; 23.0; 22.1; 22.6	22.65
0.005	23.0; 24.1; 23.1; 23.6	23.45
0.01	24.8; 25.1; 24.1; 24.1	24 52
0.02	24.9; 25.6; 24.6; 24.6	24.93

Milk result

(Δt = 4 h) Clotting time (s)	*Mean (s)*	*Heparin activity (IU ml^{-1})*
23.9; 23.6; 22.6; 23.1	23.3	0.012

Results

Mean lactation time at sample collection was 5.3 days. The mean duration of thromboprophylaxis at a daily dose of 2500 IU subcutaneous dalteparin was 5.7 days. Treatment compliance and tolerability were good in all 15 patients; no adverse events were seen. Haemograms including haematocrit, haemoglobin and platelet and white cell counts were in the normal range for all patients. Table 2 gives an overview of anti-Xa activities in plasma and milk and milk/plasma ratios 3–4 h after s.c. injection of 2500 IU of dalteparin. Plasma anti-Xa activities ranged from 0.074 to 0.308 IU ml^{-1} of plasma. This corresponds to the expected therapeutic range for once daily dosing of 2500 IU dalteparin by subcutaneous injection. In breast milk, activities ranged from <0.005–0.037 IU ml^{-1} of milk. This is equivalent to a milk/plasma ratio of <0.025–0.224.

Table 2 Anti-Xa activity in plasma and breast milk.

Patient number	*IU ml^{-1} plasma*⋆	*IU ml^{-1} breast milk*⋆	*Milk/plasma ratio*
01	0.191	0.017	0.089
02‡	0.308	0.006	0.02
03	0.201	0.012	0.06
04	0.087	0.017	0.195
05	0.260	0.027	0.104
06	0.264	0.012	0.046
07	0.192	$<0.005^{+}$	<0.026
08	0.198	$<0.005^{+}$	<0.025
09	0.103	0.017	0.165
10	0.180	$<0.005^{+}$	<0.028
11	0.165	0.037	0.224
12	0.254	0.021	0.083
13	0.175	0.028	0.16
14	0.074	$<0.005^{+}$	<0.068
15	0.163	0.007	0.043

⋆Anti-Xa activity determined using a clotting time assay developed and performed by P. Lang, Quality Control & Assurance, F. Hoffmann-La Roche, Basel, Switzerland.

+Limit of quantification was set at the lowest point on the standard calibration curve plotted for each sample ($t=0$).

‡The recovery rate for a 0.045 IU ml^{-1} spike of low molecular weight heparin added to the plasma of this patient ($t=0$) was 88.9%.

Discussion

Unfractionated heparin remains the standard drug for thromboprophylaxis during pregnancy and puerperium. With a mean molecular weight of 12 000–14 000 Da, heparin does not pass into breast milk [6]. LMWH, in contrast, has a mean molecular weight of 4000–6000 Da. Based on what is currently known about drug excretion into breast milk, the probability of a drug's passing from blood to milk increases the lower its molecular weight, regardless of such characteristics as degree of ionization, pH and lipid solubility. According to a case report by Harenberg *et al.* [7], no anti-Xa activity was detected in breast milk following anticoagulation with a dose of 5000 IU of LMWH. In the present trial, excretion of LMWH into breast milk was seen in at least 11 of 15 patients. No quantitative correlation was noted between anti-Xa activities in plasma and milk.

Anti-Xa activities were fairly widely dispersed at baseline. Although milk samples were processed immediately in an effort to minimize spontaneous clotting, such clotting may nevertheless have contributed to the poor correlation between activities in plasma and milk and may tend to under- or overestimate the heparin activity. It should also be noted that the concentration ranges were very low for the clotting assay used in this trial, and therefore a certain degree of measurement error is likely – another factor which may help explain the lack of correlation between plasma and milk.

Dryjski *et al.* [8] observed no measurable plasma activity in patients given LMWH orally at a dose of 5000 anti-Xa units. In a study in rats, antithrombotic activity was seen following oral doses of roughly 5000–10 000 IU kg^{-1} of body weight [9]. In man this would be equivalent to a total dose of roughly 600 000 anti-Xa units. Assuming newborns have an average milk intake of 250 ml on the fifth day of life, and based on a maximum measured activity of 0.037 IU ml^{-1} of milk, oral exposure does not exceed 9 IU 24 h^{-1} equivalent to 5.4% of the maternal dose kg^{-1} bodyweight.

Milk samples at days 4–8 represent immature milk which is different from mature milk. In respect to this limitation it is not clear whether these results fully represent the excretion into mature milk. Drug measurements in infant plasma would supply the most precise index of exposure. No blood samples were taken from infants, however, as the amount of blood required seemed unacceptably large. Based on the present data and the very low bioavailability of heparin ingested orally, it appears highly unlikely that puerperal thromboprophylaxis with LMWH has any clinically relevant effect on the nursing infant.

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