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Monitoring of methylergometrine in human breast milk by solid-phase extraction and high-performance liquid chromatography with fluorimetric detection

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Received August 25, 2011, accepted September 30, 2011

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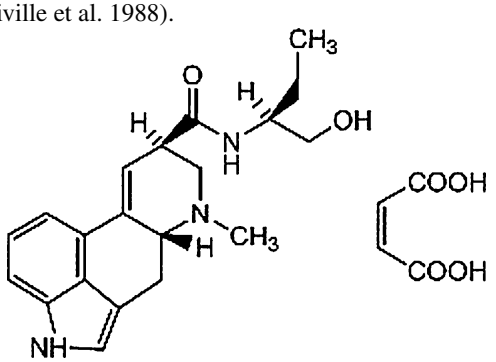
Pharmazie 67: 482–484 (2012) *doi: 10.1691/ph.2012.1127*

A high-performance liquid chromatographic assay has been developed for the detection and quantification of the conventional postnatal uterotonic drug, methylergometrine, in human breast milk using a C-18 reversed-phase column by isocratic elution. The analytical method consisted of sample clean-up by solid-phase extraction, and the fluorescence detection required only 8.5 min per sample for separation and quantitation. This assay gave intra- and inter-assay coefficients of variation of less than 7.9% and 7.7%, respectively, and the detection limit was approximately 50 pg/ml. This method was applied for drug level monitoring in the breast milk of patients given methylergometrine.

1. Introduction

Breast milk is the optimum food for infant nutrition. In addition to the psychological benefit that breast-feeding provides to the mother, Japanese women are increasingly attempting to breast feed because of the numerous health benefits offered by breast milk. However, drugs can be excreted by the mother into the breast milk and, because metabolic activity is low in infants compared with adults, this can have a number of negative effects on children consuming this milk. The final dose encountered by an infant must be determined with respect to both the milk secretion rate and the disposition of the drug in the infant (Itoh and Lee 2003; Hale 2004; McNamara and Abbassi 2004).

Methylergometrine belongs to the group of oxytocic drugs that enhance uterine motility. Prophylactic use of drugs such as ergometrine, methylergometrine and oxytocin in the third stage of labor reduces the risk of post partum hemorrhage (PPH) and the need for further oxytocic therapy in the puerperium (Van Dongen et al. 1991). The use of oxytocics in the post partum period is advocated for the prevention and management of PPH (Prendiville et al. 1988).



Methylergometrine maleate

Several chromatographic methods have been published for the determination of ergot alkaloids in pharmaceutical preparations and in urine or plasma (Sondack 1978; Edlund 1981; Tokunaga et al. 1983; Smith and Molinaro 1988; De Groot et al. 1993b; De Groot et al. 1995; Marigny et al. 2006). In contrast to these analyses, there are few articles describing analytical methods for the determination of methylergometrine in human breast milk. A radioimmunoassay using lysergic acid antiserum (Erkkola et al. 1978; Mantyla and Kanto 1981), and a high-performance liquid chromatographic (HPLC) method using tandem mass spectrometry (Vogel et al. 2004), have been reported. The latter method involves sample clean-up by liquid-liquid extraction, and subsequent application to HPLC, but no fundamental data such as validation parameters (e.g. sensitivity) of this method were described.

In this paper, we describe a selective HPLC method using fluorimetric detection with solid-phase extraction for the monitoring of methylergometrine in human breast milk from patients in the puerperium.

2. Investigations, results and discussion

Analyses of drugs in breast milk have been impeded by the high protein and lipid concentrations of the milk matrix. We investigated an efficient extraction method for the measurement of methylergometrine in human breast milk. The extraction method presented here is rapid, efficient and has sufficient specificity to eliminate interference peaks in human breast milk. Previous attempts to analyze methylergometrine in human breast milk by HPLC used liquid-liquid extraction (Vogel et al. 2004), but this is inefficient because it requires periodic flushing with an organic solvent to prevent deterioration of the chromatography column.

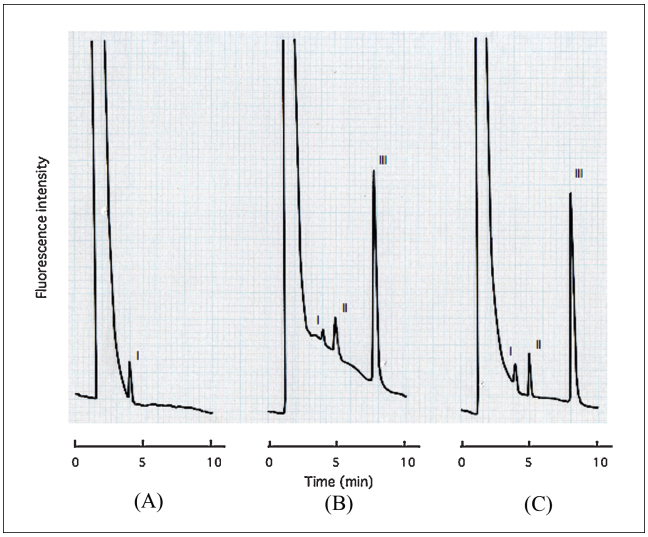


Fig. 1: HPLC chromatograms for (A) unmodified human breast milk, (B) human breast milk spiked with 0.5 ng/ml methylergometrine maleate and the internal standard, lisuride maleate (100 ng/ml), and (C) a human breast milk sample obtained 2 h after the oral administration of 0.125 mg methylergometrine maleate. Conditions are described in the Experimental section. Peaks: I, unknown compound; II, methylergometrine maleate; III, lisuride maleate

Our HPLC-fluorimetric detection system for the measurement of methylergometrine in human breast milk was found to be very selective and reliable. The calibration curve for methylergometrine showed good linearity below 25 ng/ml, and was defined by the equation:

$$y = (\text{slope} \times x) + (\text{intercept})$$

where slope = 19.540 ± 4.099 and intercept = 0.111 ± 0.023. The coefficient of determination (r^2) was 0.9997. The slope and y-intercept values represent the mean of five different curves determined on the day of assay.

The limit of detection (LOD) and the lower limit of quantitation (LLOQ) were found to be 50 and 80 pg/ml, respectively, for methylergometrine in our analytical system. Data regarding recovery and precision for methylergometrine spiked into human breast milk are shown in the Table. The recovery (mean ± S.D., n = 5) at five different concentrations was between 93.5 and 103.0%. The intra- and inter-assay precision, expressed as relative standard deviation (R.S.D.), was no more than 7.9% and 7.7%, respectively, and varied with drug concentration. This statistical validation indicates that the assay is acceptable for practical use.

Figure 1 shows a typical chromatographic pattern for the separation of methylergometrine maleate and the internal standard, lisuride hydrogen maleate, in a human breast milk sample obtained 2 h after the oral administration of methylergometrine maleate (0.125 mg). The retention times for methylergometrine maleate and lisuride hydrogen maleate were 4.8 and 7.8 min, respectively. Both peaks were well separated from other detectable components in human breast milk.

Figure 2 shows time-dependent changes in methylergometrine concentration in human breast milk after a single oral dosing with 0.125 mg. Methylergometrine levels rose steadily, peaked at 0.437 ± 0.110 ng/ml at 2 h and then declined to 0.086 ng/ml at 5 h. The levels of methylergometrine in human breast milk were only marginally lower than those of a previous report that levels measured using radioimmunoassay (Erkkola et al. 1978) and are in close agreement with those in the literature (Vogel et al. 2004).

The prophylactic use of oxytocic drugs such as methylergometrine is generally advocated to prevent maternal death

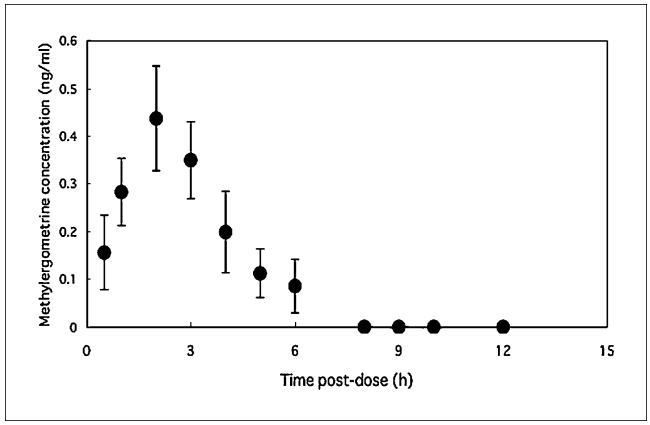


Fig. 2: Time course of mean methylergometrine maleate concentration in human breast milk in 12 subjects after a single 0.125 mg oral dose (mean ± S.E.M., n = 12)

from PPH. Problems are encountered when these prophylactic drugs are administered intravenously, which can cause severe hypertensive and cardiovascular accidents (De Groot et al. 1993a). Furthermore, convulsions, respiratory depression and apnea have occurred upon accidental administration in newborn infants (Donatini et al. 1993; Baum et al. 1996). From these observations, a precise assay for the determination of methylergometrine concentrations in human breast milk is required to aid the risk-benefit assessment of oxytocic therapy in *postpartum* women who are breastfeeding. The selective and accurate assay described in this paper for measuring methylergometrine levels in human breast milk may be useful for this purpose.

3. Experimental

3.1. Chemicals

Methylergometrine maleate salt was purchased from Sigma (St. Louis, MO, USA). R-(+)-lisuride hydrogen maleate was obtained from MP Biomedicals (Irvine, CA, USA). Oasis MCX cartridges were from Waters (Milford, MA, USA). Acetonitrile was of HPLC grade (Wako, Tokyo, Japan). All other chemicals and solvents were of analytical reagent grade.

3.2. Stock solutions

Stock solutions of methylergometrine maleate salt and R-(+)-lisuride hydrogen maleate were prepared in water at a concentration of 1 mg/ml. After preparation, stock solutions were stored at −20 °C and were stable at least for four weeks. Working standard solutions were prepared by dilution of stock solutions before use and were stable for at least 3 days at 4 °C.

3.3. Subjects

The participants in this study were twelve women who had been treated with oral methylergometrine, 0.125 mg, three times daily (Metenarin tablets, Teikoku Zouki) for 5 days because of incomplete *postpartum* uterine involution. Body weight, height, age and haemoglobin level of the patients were recorded. Breast milk samples were collected from all patients, each of whom gave informed consent for the clinical drug monitoring. This study was approved by the Experimental Research Involving Human Subjects committee of the Saiseikai Yokohamashi Nanbu Hospital (Yokohama, Japan). Standardized milk sample collection (about 3 ml into polypropylene tubes) was performed with self-collection. A baseline milk sample was taken 1–3 h after a typical Japanese breakfast, immediately after suckling the baby at the opposite breast. The allocated drug was then administered and further milk samples at the same breast were taken 0.5, 1, 2, 3, 4, 5, 6, 8, 9, 10 and 12 h afterwards. Each milk sample was stored at −80 °C.

3.4. Sample preparation

Milk aliquots (2 ml) were pipetted into polypropylene tubes and stored at −80 °C until analysis. An aliquot (1 ml) of breast milk was spiked with 100 ng/ml R-(+)-lisuride hydrogen maleate as an internal standard, and briefly vortex-mixed. The human breast milk samples were diluted with 5 ml of McIlvaine buffer containing 5% EDTA (pH 4.1) (MacNeil et al.

Table: Statistical validation for the determination of methylergometrine in human breast milk

Spiked concentration (ng/ml)	Intraassay (n = 5) measured concentration mean ± S.D. (ng/ml)	R.S.D. (%)	Recovery (%)	Interassay (n = 5) measured concentration mean ± S.D. (ng/ml)	R.S.D. (%)	Recovery (%)
0.1	0.103 ± 0.008	7.9	102.6	0.099 ± 0.008	7.7	98.9
0.2	0.190 ± 0.008	4.1	95.1	0.189 ± 0.013	6.9	94.4
0.5	0.495 ± 0.036	7.3	98.9	0.490 ± 0.024	5.0	97.9
1.0	0.995 ± 0.074	7.4	99.5	0.935 ± 0.070	7.5	93.5
5.0	5.065 ± 0.285	5.6	101.3	5.153 ± 0.344	6.7	103.0

1996) and the resultant solutions were briefly mixed. After centrifugation (8000 × g, 10 min, 4 °C), the supernatant was loaded onto a solid-phase extraction cartridge. Solid phase extraction was performed using OASIS MCX cartridges (3 cm^3/60 mg). These cartridges were conditioned with 1 ml of methanol and then equilibrated with 1 ml of water. Each specimen was applied to the cartridge and passed through the bed at a flow rate of 1 ml/min. Cartridges were sequentially washed with 4 ml of 0.1 M hydrochloric acid and 4 ml of methanol. Analytes were eluted with 2 ml of methanol containing 5% ammonium hydroxide and the clear eluent obtained was evaporated *in vacuo* using an AES1010 speed-vac concentrator (Savant, Holbrook, NY, USA). The resulting residue was dissolved into 500 µl of water, and an aliquot of this solution was subjected to HPLC analysis.

3.5. Chromatographic conditions

Analysis of methylergometrine in human breast milk was conducted using a Japan Spectroscopic HPLC system consisting of an 880-PU pump, an 821-FP spectrofluorometer, an 860-CO column oven, an 880–50 degasser, an 880–02 gradient unit, an 802-SC system controller, and an 807-IT integrator. The system was operated at room temperature at a flow rate of 1.0 ml/min employing a Finepak SIL C18S (particle size: 5 µm) reversed-phase column (150 mm × 4.6 mm i.d.) (Jasco) fitted with an Inertsil ODS-2 guard gel (10 mm × 4.0 mm i.d.; particle size: 5 µm) (GL Sciences). The mobile phase consisted of 10 mM sodium acetate buffer (pH 4.0):acetonitrile (20:80, v/v). Fluorescence was monitored with excitation at 323 nm and emission at 430 nm.

3.6. Validation parameters

Before applying an analytical method, it is necessary to validate it according to the guidelines set out by the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH 2003; ICH 2005) and USP 30 (USP 2007). Validation was performed using a standard curve of 5–8 points with reproducible linear or non-linear responses and statistical fits. The LOD, defined as the lowest concentration of analyte that can be detected with a signal-to-noise ratio greater than 3:1, was established by serial injection of human breast milk spiked with different concentrations of methylergometrine. The LLOQ was defined as the lowest concentration of analyte at which the percentage deviation from the nominal concentration (accuracy) and R.S.D. (precision) are less than 20%, and were determined from the linearity tests. Recovery was assessed by spiking known amounts of methylergometrine into the human breast milk. The intra- and inter-assay precision and accuracy were evaluated to ensure reproducibility before using the assay to measure human samples. The intra-assay precision was assessed by analyzing five samples at five concentrations (0.1, 0.2, 0.5, 1.0 and 5.0 ng/ml) in a single day. The inter-assay precision was assessed using the same method on five different days.

Acknowledgments: We are very grateful to Dr. Akira Tanaka for his valuable and helpful advice and for manuscript preparation.

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