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CASE REPORT

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Transfer of Everolimus into Colostrum of a Kidney Transplant Mother

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Background: Transplanted women are increasingly expressing their desire to breast-feed. Due to the unknown effects that might occur in newborns of everolimus-treated mothers, it is now recommended to inhibit lactation. This report discusses the assessment of everolimus levels in maternal, umbilical, and neonatal blood, and colostrum of a kidney transplant mother.

Case Report: A 28-year-old white primipara after second kidney transplant, treated with everolimus, conceived unintentionally. Due to the high risk of recurrence of primary disease, the immunosuppressive treatment remained unchanged. At 37 weeks of gestation, due to mild preeclampsia, the woman was qualified for induction of labor and vaginally delivered a healthy infant. The highest concentration of everolimus in the colostrum was observed 4 h after drug administration and was 0.066 ng/ml. The estimated maximal dose of everolimus in colostrum was 0.38% of the mother's dose.

Conclusions: Breast-feeding in transplanted women treated with everolimus seems possible, particularly in mothers who are willing to breast-feed, especially in the first days after labor, when levels of immunoglobulins in colostrum are high and the concentrations of everolimus are low.

MeSH Keywords: **Colostrum • Kidney Transplantation • TOR Serine-Threonine Kinases**

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Background

Milk, produced in proper amounts by a healthy mother, satisfies the nutritional needs of the neonate, ensuring its normal development until the first year of life. Breast-feeding not only entails health and protective benefits for the mother and baby, but it also affects the later taste preferences of the child. According to the recommendations of various societies, exclusive breast-feeding is recommended up to 6 months.

Current recommendations indicate that not all drugs are excreted into breast milk in clinically irrelevant concentrations and that detection of their presence does not always entail adverse effects in the infant [1]. In clinical practice there are few indications of maternal pharmacotherapy that breast-feeding must be temporarily or permanently discontinued. However, this situation does not apply to transplanted mothers, because they need to receive immunosuppressive medications to prevent a graft rejection.

Available data indicate that in many cases post-transplant women may choose to safely breast-feed their children. Currently, there is an increasing number (up to 35%) of organ donors who decide to breast-feed their infants during the immunosuppressive treatment [2]. In addition, children of transplanted mothers can achieve even greater breast-feeding benefits because of the increased risk of preterm birth. However, data are lacking on the risk of immunosuppressive use by breast-feeding mothers after organ transplants.

Everolimus, which is a mammalian target of rapamycin inhibitor (mTOR), reduces cell proliferation, angiogenesis, and glucose uptake. Currently, there is a lack of studies on the use of everolimus in pregnant women. However, there are a few animal reports indicating the possibility of adverse fetal outcomes following the use of mTOR inhibitors during pregnancy [3]. Thus, the potential risk for humans remains unknown. The U.S. Food and Drug Administration classifies mTOR inhibitors as category C, which comprises drugs that can only be used in pregnant women if the potential benefit to the mother outweighs the potential risk to the fetus. Therefore, it is recommended that women of childbearing age be advised to use effective birth control methods while receiving everolimus and up to 8 weeks after stopping this therapy.

There are limited data on the excretion of everolimus into rat milk at a concentration higher than that found in rat maternal blood. Due to the unknown effects on newborns after exposure to everolimus in breast milk, it is recommended that breast-feeding should be avoided when the mother receives this immunosuppressant.

This report presents analysis of the levels of everolimus measured in maternal, umbilical, and neonatal blood, as well as in colostrum collected by the kidney transplant mother who received everolimus throughout pregnancy and after delivery.

Case Report

A white 28-year-old primipara was admitted to the hospital at 38 weeks of gestation due to increasing proteinuria and deterioration of blood pressure control. In the interview, we found that at the age of 18 years, the woman was diagnosed with nephrotic syndrome in the course of the focal segmental glomerulonephritis and two years later the first kidney transplant was performed. At the age of 26 years, she underwent a second kidney transplantation due to recurrence of the underlying disease. Twenty-three months later, she conceived unintentionally. At that time, she received immunosuppressive treatment based on: azathioprine (125 mg/day), methylprednisolone (12 mg/day), and everolimus (0.5 mg/day). Due to the high risk of recurrence of primary disease, it was decided to continue the current immunosuppressive treatment. The drug concentrations and maternal graft function were monitored, without the need for modification of the immunosuppressive therapy in the course of pregnancy. The routine fetal ultrasound examination did not detect the presence of fetal anomalies or fetal growth restriction. At the 37th week of gestation, due to proteinuria of 1.5 g/24 h and moderate hypertension, she was admitted to the hospital and qualified for preinduction and subsequent induction of labor. She vaginally delivered a healthy female infant in good general condition, with normal Apgar score and a weight of 2600 grams.

After birth, the level of everolimus in the maternal, umbilical venous, and neonatal blood was measured (Table 1). The mother agreed to sustain lactation and to estimate the everolimus concentration in the colostrum. Each sample of colostrum had a volume of 2 milliliters and was stored in sterile tubes. The colostrum samples were collected before the next dose of everolimus, followed by 2, 4, 6, 8, and 12 h after drug administration. The result was multiplied by the amount of milk that the breast-fed newborn would ingest on the day of sample collection. The amount of ingested everolimus for a newborn was calculated in ng/24h and then recalculated by the dose, based on kilograms of bodyweight per 24 h. After all samples were collected, lactation was inhibited by bromocriptine.

The highest concentration of everolimus in colostrum measured, on the second postpartum day, was observed 4 h after drug administration and was to 0.066 ng/ml. The estimated infant dose of the everolimus per kilogram, based on the highest everolimus concentration which would be secreted with the colostrum, was estimated to be 4.224 ng/kg/24 h and was 0.38% of the mother's dose. The concentration of everolimus in umbilical vein blood was higher than in the neonatal blood.

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Table 1. Concentrations of everolimus in blood and colostrum.

Tested sample	Everolimus concentration (ng/ml)
Maternal blood after delivery	1.1
Umbilical venous blood	1.0
Neonatal blood after delivery	0.559
Colostrum on the second postpartum day	
Before drug administration	0.033
2 hours after drug administration	0.049
4 hours after drug administration	0.066
6 hours after drug administration	0.045
8 hours after drug administration	0.051
12 hours after drug administration	0.045

The highest level of bilirubin was noted in the sixth day of life and was 220.6 µmol/l.

Methodology

Chemicals: The chemicals used included the following: LC-MS grade methanol, HPLC grade methanol, HPLC grade acetonitrile, methyl-tert-butyl ether and formic acid (J.T. Baker), zinc sulfate monohydrate (Sigma-Aldrich, St. Louis, MO), and analytical grade ammonium acetate (POCH, Gliwice, Poland), Everolimus, and Everolimus$^{13}C_2D_4$ (Toronto Research Chemicals, Inc., North York, Canada). Ultra-pure water was obtained from a water purification system (Mili-Q, Millipore, Milford, MA, USA). A commercial kit *Chromsystems MassCheck® Immunosuppressants whole blood control* was obtained from Chromsystems Instruments & Chemicals GmbH (Munich, Germany).

Sample extraction procedure was as follows. A breast milk sample was prepared by vortexing 1.5 ml of milk with 500 µl of 2% aqueous zinc sulfate solution containing internal standard (5ng/ml) and 300 µl of acetonitrile. We used 3 ml of methyl-tert-butyl ether to extract everolimus. Samples were vortexed for 10 min and centrifugated for 10 min at 3000 rpm. The organic layer was transferred into a clean test tube and evaporated under a stream of nitrogen in a Turbo-Vap evaporator water bath (Caliper Life Sciences, Hopkinton, MA). Samples were solubilized in 100 µl 60% methanol and 10 µl was injected into LC/MS/MS. Whole-blood samples were prepared by a protocol described previously [4].

Analyzes instrumentation consisted of a Waters Acquity Ultra Performance Liquid Chromatograph coupled with a Waters TQ-S triple-quadrupole mass spectrometer. For the instrument control and data acquisition, MassLynx software was used. LC/MS/MS analysis was performed in positive electrospray ionization mode (ESI) and the mass spectrometer was operated in multiple-reaction monitoring (MRM) mode. The ion transitions were 975.6157>908.6 and 975.6157>926.6 for everolimus and 975.6>914.6 for internal standard. The first ion transition was used for quantification. For all analytes, mass spectrometer optimized settings were as follows: capillary voltage=1.5 kV, desolvation temperature=200°C, cone gas flow=150 L/h, desolvation gas flow=800 L/h, and source temperature=150°C. Chromatographic separation of analytes was performed using a Waters BEH C18 column (1.7 µm, 2.1×50 mm) thermostatted at 50°C. Mobile phase A consisted of 2 mM ammonium acetate with 0.1% formic acid (v/v) in water and mobile phase B consisted of 2 mM ammonium acetate with 0.1% formic acid (v/v) in methanol. The total analysis time was 3 min. We prepared calibration standards for concentration determination in whole blood and breast milk. The concentration of everolimus was calculated using Everolimus$^{13}C_2D_4$ as the internal standard. The calibration curve range was 0.22–33.4 ng/ml and 0.03–1.00 ng/ml for whole blood and colostrum, respectively. To ensure control of the method for the determination of analytes in whole blood, external control samples were used.

Discussion

The benefits of breast-feeding are well documented, especially in premature infants. Until now, it has not been established whether the benefits of breast-feeding outweigh the potential risk associated with the transfer of immunosuppressive drugs into breast milk; therefore, there has been no standards for feeding of neonates and infants of transplant mothers. To the best of our knowledge, only 1 case report, assessing the concentration of everolimus in colostrum of a heart transplant mother, is available in the literature [5]. In this case, a woman was diagnosed with unplanned pregnancy at the 21st week of gestation and everolimus dosages were increased from 1 to 2 mg/day. The everolimus concentration in colostrum was measured once at 48 h postpartum and was under the lower limit of detection. As in our case, the woman decided not to breast-feed due to limited data on the transfer of everolimus into breast milk. Our kidney transplant mother received a lower dose of everolimus (0.5 mg/d) than that described in the woman after heart transplantation; therefore, the drug concentration was lower in all tested samples: maternal (1.1 ng/ml *vs.* 1.4 ng/ml, respectively), umbilical venous (1.0 ng/ml *vs.* 1.5 ng/ml, respectively), and neonatal blood (0.56 ng/ml *vs.* 1.4 ng/ml, respectively). The above data, as well as the results of other publications, indicate that the fetus appears to be exposed to everolimus concentrations comparable to the therapeutic levels achieved in mothers after organ transplants.

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However, until now, no complications related to prenatal exposure to everolimus have been reported in neonates of organ transplant mothers [5,6].

Unlike in the case of the heart transplant mother, in our case, despite lower doses of everolimus administered to the mother, the concentration of this drug was estimated several times on the second day after delivery, and in every measurement the presence of everolimus was confirmed in colostrum [5]. Given the possible implications for immature liver metabolism in neonates, particularly premature neonates, it should be noted that the observed bilirubin concentrations in our infant were within the range of physiological neonatal hyperbilirubinemia, as in the aforementioned case of the infant born to the heart transplant mother [5,6]. In our case, the amount of everolimus excreted in colostrum was similar to that of other reports, evaluating the same issue in lactating mothers on tacrolimus therapy, and indicate that the concentrations of both drugs in the colostrum appear to be several times lower than the weight-adjusted maternal dose and immunosuppressant concentrations to which the fetus is exposed [7,8].

Conclusions

In conclusion, we would like to emphasize that our report provides new information that might be useful in the ongoing debate and discussion over the safety of breast-feeding, as well as perspectives that can be used in the future for counseling patients. It is important to reconsider the need for discontinuation of breast-feeding by transplanted mothers treated with everolimus, particularly in cases in which the mother chooses to breast-feed her neonate, especially in the first postpartum days when immunoglobulin levels in the colostrum are high and concentrations of immunosuppressant appear to be low.

Conflicts of interest

None.

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