**The transfer of everolimus into colostrum of kidney transplant mother**

**Authors:** Kociszewska-Najman B (1), Szpotanska-Sikorska M (1), Mazanowska N (1), Paczek L (2), Samborowska E (3), Dadlez M (3), Wielgos M (1), Pietrzak B (1)

Affiliation:

1) 1st Department of Obstetrics and Gynecology, Medical University of Warsaw, Pl. Starynkiewicza 1/3, 02-015 Warszawa, Poland

2) Department of Immunology, Transplant Medicine and Internal Diseases, Transplantation Institute, Medical University of Warsaw, Warsaw, Poland.

3) Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland.

Corresponding author: Monika Szpotanska-Sikorska, MD, PhD

Address: 1st Department of Obstetrics and Gynaecology,

 Medical University of Warsaw

 1/3 Starynkiewicza Sq, 02-015 Warsaw, Poland

Mobile phone: +48 698 676 551 e-mail: mszpotanska@wp.pl

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*SUMMARY:*

*Background:*

Transplanted women are increasingly expressing their desire to breastfeed. 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 kidney transplant mother.

*Case:*

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 delivered vaginally a healthy infant. The highest concentration of everolimus in the colostrum was observed 4 hours after drug administration and was 0.066 ng/ml. The estimated maximal dose of everolimus in colostrum would amount to 0.38% of the mother’s dose.

*Conclusion*:

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.

*KEYWORDS*: TOR Serine-Threonine Kinases, everolimus, colostrum, kidney transplantation

***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. Breastfeeding 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 recommendation of various societies, exclusive breastfeeding 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 breastfeeding 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 increasing number of organ donors, who decide to breastfeed their infants during the immunosuppressive treatment and the percentage of these women reaches 35% [2]. In addition, children of transplanted mothers can achieve even greater breastfeeding benefits because of the increased risk of preterm birth. However, data is lacking of studies assessing the risk of immunosuppressive use by breast-feeding mothers after organ transplants.

Everolimus, which belongs to the mammalian target of rapamycin inhibitors (mTOR), reduces cell proliferation, angiogenesis and glucose uptake. Currently, in the literature there is a lack of studies on the use of everolimus in pregnant women. However, there are 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, i.e. 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 should be advised to use effective birth control methods, while receiving everolimus and up to 8 weeks after stopping this therapy.

In the literature, there are limited data presenting the excretion of everolimus into rats’ milk at a concentration higher than that found in the rat’s maternal blood. Due to the unknown effects that may occur in newborns after exposing to everolimus in breast milk, it is recommended that breastfeeding should be avoided, when receiving this immunosuppressant by the mother.

This report presents the 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 this drug during and after the delivery.

***Case report***

 A white 28-year-old primipara was admitted to the hospital at 38 weeks of gestation due to the increasing proteinuria and deterioration of blood pressure measurements. In the interview at the age of 18 years, the woman was diagnosed with nephrotic syndrome in the course of the focal segmental glomerulonephritis and two years after the first kidney transplant was performed. At the age of 26 years, she underwent a second kidney transplantation because of the recurrence of the underlying disease. Twenty three months later she conceived unintentionally. At that time the women 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 nor fetal growth restriction. At 37th week of gestation due to proteinuria of 1.5g/24h and moderate hypertension the woman was admitted to the hospital and qualified for preinduction and subsequent induction of labor. The woman 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 two milliliters and was stored in sterile tubes. The colostrum samples were collected before the next dose of everolimus, followed by two, four, six, eight and twelve hours after drug administration. The result was multiplied by the amount of milk that 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 hours. After all samples were collected, the mother's lactation was inhibited by bromocriptine.

 The highest concentration of everolimus in colostrum, measured on the second postpartum day, was observed after 4 hours from the drug administration and was equal 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/24h and amounted to 0.38% of the mother's dose. The concentration of everolimus in umbilical vein was higher than in the neonatal blood. 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, USA) and analytical grade ammonium acetate (POCH, Gliwice, Poland), Everolimus and Everolimus13C2D4 (Toronto Research Chemicals, Inc., North York, Canada). Ultra-pure water was obtained from a water purification system (Mili-Q, Millipore, Milford, MA, USA). Commercial kit *Chromsystems MassCheck® Immunosuppressants whole blood control* was obtained from Chromsystems Instruments & Chemicals GmbH (Munich, Germany).

Sample extraction procedure. 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. A 3 ml of methyl-tert-butyl ether was used to extract everolimus. Samples were vortexed for 10 mins and centrifugated for 10 mins at 3000 rpm. The organic layer was transferred into clean test tube and evaporated under a stream of nitrogen in a water bath Turbo-Vap evaporator (Caliper Life Sciences, Hopkinton, MA, USA). Samples were solubilized in 100 µl 60% methanol and 10 µl was injected into LC/MS/MS. Whole blood samples were prepared by protocol described before [4].

Analyzes Instrumentation consisted of Waters Acquity Ultra Performance Liquid Chromatograph coupled with 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 a positive electrospray ionization mode (ESI) and the mass spectrometer was operated in a 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 = 200ºC, cone gas flow = 150 L/h, desolvation gas flow = 800 L/h, source temperature = 150°C. Chromatographic separation of analytes was performed using a Waters BEH C18 column (1.7µm, 2.1mm x 50mm) thermostatted at 50 ºC. 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. For concentration determination in whole blood and breast milk own calibration standards were prepared. The concentration of everolimus was calculated using Everolimus13C2D4 as internal standard. The calibration curve range was 0.22-33.4 ng/ml and 0.03-1 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 breastfeeding 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, until now there is a lack of standards regarding feeding of neonates and infants by transplant mothers. According to our knowledge, only one case report, assessing the concentration of everolimus in colostrum of 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. In this publication, the everolimus concentration in colostrum was measured once at 48 hours postpartum and was under lower limit of detection. As in our case, the women decided not to breastfeed 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 described woman after heart transplantation, thus 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. 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 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 infant born to heart transplant mother [5, 6]. Our results, concerning the amount of everolimus excreted in colostrum, are similar to 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 immunosuppressants’ concentrations to which the fetus is exposed [7, 8].

***Conclusions***

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

*Disclosure of interest:* The authors report no conflicts of interest.

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