SHORT REPORT

Placental disposition of eculizumab, C5 and C5-eculizumab in two pregnancies of a woman with paroxysmal nocturnal haemoglobinuria

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Eculizumab is known to cross the placenta to a limited degree, but recently therapeutic drug levels in cord blood were found in a single case. We report maternal, cord and placental levels of unbound eculizumab, C5 and C5-eculizumab in two pregnancies of a paroxysmal nocturnal haemoglobinuria patient who received 900 mg eculizumab every 2 weeks. In both pregnancies, cord blood concentrations of unbound eculizumab were below 4 µg/mL, while C5-eculizumab levels were 22 and 26 µg/mL, suggesting that a considerable fraction of C5 was blocked in the newborn. Concentrations in each placenta of unbound eculizumab were 41 ± 3 and 45 ± 4 µg/g tissue, of C5-eculizumab 19 ± 2 and 32 ± 3 µg/g, and of C5 20 ± 3 and 30 ± 2 µg/g (mean ± SD, in three tissue samples per placenta). Placental levels of unbound eculizumab were higher than those of C5-eculizumab complexes, while maternal concentrations were approximately equal, suggesting selective transport of unbound eculizumab across the placenta.

KEYWORDS

eculizumab, paroxysmal nocturnal haemoglobinuria, placental exposure, placental transfer, pregnancy

1 | INTRODUCTION

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare condition affecting women of reproductive age. Pregnancy is known to serve as a disease trigger and increases the risk for thrombosis and haemorrhage in these women. The management of PNH during pregnancy has become feasible with the introduction of eculizumab, a humanized IgG2/IgG4 anti-complement C5 antibody. Eculizumab treatment has reduced the typically high mortality rates associated

with PNH during pregnancy, and has greatly improved foetal and maternal pregnancy outcomes.[2] Given the fact that eculizumab is also administered during pregnancy in women with atypical haemolytic uremic syndrome[3] and its potential as a therapeutic option in preeclampsia treatment,[4,5] it has become increasingly relevant to report on its use during pregnancy. Eculizumab was reported to cross the placenta to a limited degree. Cord blood concentrations of eculizumab varying from undetectable up to 20% of the maternal levels have been reported in several studies.[1,6] To date, only one case has been described with eculizumab concentrations of 69 µg/mL in cord blood in which complement activation in the newborn was fully

blocked.[1] Although eculizumab levels in maternal and cord blood have been analysed previously, studies on the passage of the drug through the placenta have not yet been performed. Therefore, here we aimed to more extensively characterize placental disposition as compared to previous studies. Given the varying concentrations of eculizumab that have been reported in cord blood so far, investigating the fate of eculizumab within placental tissue will contribute to a better understanding of the mechanism of its placental transfer. Additionally, quantifying both eculizumab unbound and bound to C5 better reflects actual exposure to the drug. In the current study, we report maternal, cord and placental concentrations of unbound eculizumab, C5 and C5-eculizumab complexes in two pregnancies of a PNH patient who received 900 mg of eculizumab every 2 weeks throughout both pregnancies.

2 | METHODS

2.1 | Case presentation

We report two successive pregnancies of a PNH patient. Written informed consent was obtained at routine prenatal care visits at 30 weeks of pregnancy. The study was approved by the Regional Committee on Research involving Human Subjects (file no. 2016-2744). The patient received 900 mg of eculizumab every 2 weeks throughout both pregnancies and the last eculizumab administration was 4 days before both deliveries. The first neonate was born after a spontaneous vaginal delivery at 36^{+6} weeks, was healthy despite transient hypoglycaemia and had a weight at the 50th percentile. The second neonate was also born after a vaginal delivery at 38^{+0} weeks, was healthy and had a weight at the 5-10th percentile. Both neonates did not show signs of infection. In addition to eculizumab, the patient used the following medication for prolonged periods of time during the first pregnancy: nadroparine, meclozine/pyridoxine, metoclopramide, ferro fumarate, macrogol, folic acid, mesalazine, betametasone and nifedipine. During the second pregnancy mesalazine, bisacodyl, magnesium sulphate, nadroparine, meclozine/pyridoxine, metoclopramide, amoxicilline, beclometasone, nifidepine, progesterone, folic acid and paracetamol were used. We are not aware of any literature that demonstrate an effect of comedication on the disposition of eculizumab, C5 or eculizumab-C5 complex.

2.2 | Blood and placenta sample collection and preparation

The whole placenta and maternal and cord blood samples were stored at 4 °C directly after delivery. Serum was stored at −80 °C until analysis. Within 24 hours, 1 cm³ sized samples of villous tissue were excised at random areas midway between the umbilical cord and distal edge of the placenta. The samples were snap frozen and stored at −80 °C.

Twenty percent (weight/volume) tissue homogenates, based on tissue wet weight, were prepared in Radioimmunoprecipitation assay

What is already known about this subject

- Eculizumab crosses the placental barrier to a limited degree and in most cases cord blood levels of eculizumab are low. Recently, a case has been described in which therapeutic eculizumab levels were found in cord blood, indicating more extensive placental transfer.[1]

What this study adds

- In this case study, two pregnancies are described in which placental disposition of eculizumab has been investigated. Concentrations of unbound eculizumab, C5 and C5-eculizumab were quantified in cord and maternal blood, as well as in placental tissue.

(RIPA) buffer containing 5mM Tris-HCl buffer pH 7.4, 150mM NaCl, 1% Triton-X100, 0.5% sodium deoxycholate and one Roche complete proteinase inhibitor tablet per 10 mL of buffer (Roche, Penzberg, Germany). Homogenates were prepared on ice with a T10 basic Ultra-Turrax disperser (IKA, Staufen, Germany). Thereafter the samples were mixed and kept on ice for 1 hour, followed by mixing and centrifugation for 5 minutes at 5000 g. The supernatant was stored at −80 °C until analysis.

2.3 | Quantification of unbound eculizumab, C5 and C5-eculizumab complexes

Concentrations were measured with an in-house ELISA according to previously described methods for eculizumab,[7] C5[8] and C5-eculizumab complexes.[9] Standard curves for eculizumab measurements were prepared by dilution of eculizumab (Alexion Pharmaceuticals, Cheshire, CT, USA) (0.1-38 ng/mL) in phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA) and for C5 measurements by diluting purified C5 (3.9-150 ng/mL) (Calbiochem, San Diego, CA, USA) in PBS with 0.05% Tween (PBST), supplemented with 0.2% BSA. The standard curve of C5-eculizumab complexes (1-192 ng/mL) was prepared by adding eculizumab to a normal serum pool from 20 healthy blood donors, supplemented with 20 mM ethylenediaminetetraacetic acid (EDTA) and further dilution in PBST-EDTA. The intra- and interassay variabilities were 2.9% and 5.2%, 7% and 14%, 8.5% and 17.8% for eculizumab, C5 and C5-eculizumab complexes, respectively. The ELISAs were applied to measure concentrations in cord blood (serum), maternal blood (serum) and placental tissue homogenates (20% placental tissue in RIPA buffer). Three homogenate samples per placenta were analysed. For each serum and tissue homogenate sample, two technical replicates were used.

TABLE 1 Eculizumab, C5 and C5-eculizumab concentrations in serum and placental tissue

		[Eculizumab] µg/mL serum or µg/g tissue	[C5][a] µg/mL serum or µg/g tissue	[C5-Eculizumab] µg/mL serum or µg/g tissue
Pregnancy 1	Maternal	259	118	234
	Cord	3.5	73	26
	Placenta (mean ± SD)[b]	41 ± 3	20 ± 3	19 ± 3
Pregnancy 2	Maternal	232	152	252
	Cord	2.7	78	22
	Placenta (mean ± SD)[b]	45 ± 4	30 ± 2	32 ± 2

[a]The concentration C5 in cord blood relative to maternal blood was in agreement with a previously reported range of 54-61%.[16] Reference values of C5 in adults are 42-93 µg/mL.[8]

[b]Placental concentrations represent the mean ± SD of three samples per placenta, excised at random areas midway between the cord and distal edge of the placenta.

3 | RESULTS

Table 1 represents the concentrations of unbound eculizumab, C5 and bound eculizumab to C5 (C5-eculizumab) in maternal blood, cord blood and placental tissue from both pregnancies. Unbound eculizumab and C5-eculizumab complexes were detected in maternal blood and cord blood, suggestive of placental transfer. Maternal serum concentrations of unbound eculizumab and C5-eculizumab complexes were far above 100 µg/mL, which is the concentration necessary to fully block complement activity.[7] In both pregnancies, cord blood concentrations of unbound eculizumab were below 4 µg/mL while for C5-eculizumab concentrations of 22 and 26 µg/mL were found. In placental tissue, unbound eculizumab concentrations were substantially higher than those of C5-eculizumab complexes. Placental levels of C5 in exposed tissue did not differ from those in the placental tissue of healthy controls.

4 | DISCUSSION

The low levels of unbound eculizumab in cord blood that we report here indicate that most of the available eculizumab was bound to C5. This is supported by the presence of high concentrations of C5-eculizumab complexes (22 and 26 µg/mL) in cord blood. In the light of free C5 concentrations of 73 and 78 µg/mL, this suggests blockade of a considerable fraction of C5 in the newborns. Previously, Hallstensen et al reported C5-eculizumab concentrations of approximately 8 µg/mL in term neonatal blood corresponding to 6-7% of maternal levels.[9] While we found higher values, C5-eculizumab concentrations relative to maternal levels were similar in our study. To date, only one case report has been described with therapeutic eculizumab blood levels in a newborn and complete complement blockade, but unfortunately C5-eculizumab concentrations were not quantified in that study.[1] In cases with (partial) complement blockade by eculizumab, innate immunity in the newborn may be severely compromised and prophylactic treatment with antibiotics may be recommended. Also, during pregnancy, treatment with eculizumab may affect foetal outcomes. In a case series of 75 pregnancies, a high rate (29%) of premature births was observed.[2] However, as prematurity has been prominent in PNH pregnancies before the availability of eculizumab, it remains difficult to assess to what extent this is related to eculizumab treatment.

With respect to placental eculizumab levels, we observed higher placental concentrations of unbound eculizumab than of C5-eculizumab complexes, while maternal concentrations were approximately equal, suggesting selective transport of unbound eculizumab across the placenta. This indicates that either unbound eculizumab is taken up better into the placenta or the C5-eculizumab complex is more extensively degraded in lysosomes. Furthermore, partial dissociation of C5-eculizumab in endosomes may also contribute to higher placental eculizumab levels. Once taken up in the endosomes, transplacental transfer of IgGs occurs via binding to the neonatal Fc-receptor (FcRn).[10] The C5-eculizumab complex does not efficiently dissociate and may bind to the FcRn similarly as free eculizumab.[11] Therefore, it remains possible that placental transfer of C5-eculizumab complex is also driven by binding to FcRn, although to a smaller extent. Detectable levels of C5-eculizumab complexes coinciding with low levels of unbound eculizumab in cord blood may be explained by complete scavenging of eculizumab by C5 at low drug concentrations. At higher eculizumab concentrations, the proportion of measured unbound drug increases. It should therefore be noted that quantifying unbound eculizumab only, will underestimate the actual exposure in cord blood. More clinical data is needed to investigate the factors that influence the placental disposition of eculizumab and its binding to C5 in the placenta and foetal blood. For future cases it is recommended to report on C5-eculizumab levels in maternal blood, cord blood and placental tissue to study the relationship between treatment regimens, gestational age and eculizumab distribution across the placenta. It would also be important to perform histopathological studies of the placenta, as an affected syncytiotrophoblast layer can presumably lead to enhanced foetal eculizumab levels.

Placental passage of eculizumab is limited, particularly in comparison to transfer of full IgG1 therapeutics, such as infliximab, for which cord blood concentrations may even exceed maternal concentrations in the third trimester.[12] Eculizumab is an IgG2/4 hybrid with a similar

serum half-life and binding affinity to the FcRn as full IgG1 therapeutics.[9] FcRn prolongs the half-life of IgG1, IgG2 and IgG4 equally in endothelial cells, although placental transfer of IgG2 is significantly less efficient compared to that of IgG1 and IgG4.[13,14] Still, IgG2 transfer is more extensive than eculizumab transfer as cord-to-maternal ratios of endogenous IgG2 are on average 1,[15] which is not the case for eculizumab transfer ratios. Intracellular trafficking of eculizumab in the syncytiotrophoblast is therefore different from endogenous IgGs and requires further investigation.

Although challenging for this type of biological molecules, conducting *ex vivo* placental perfusions could be an approach to chart differences in placental handling between unbound eculizumab and C5-eculizumab complexes. This type of study would also allow the investigation of placental handling of these molecules separate from clearance processes taking place elsewhere in the foetus. To better understand the disposition and effects of eculizumab and C5-eculizumab complexes in the unborn child, future studies are needed on the mechanisms of placental uptake, trafficking and release.

COMPETING INTERESTS

All authors declare that they have no competing interests.

CONTRIBUTORS

G.E., P.vdB. and A.S. acquired and analysed the data. O.vdH. and S.L. had direct clinical responsibility for the patient described. G.E., J. vD., R.G., E.V. and F.R. contributed to the study idea, design and drafted the manuscript. All authors were involved in revising the manuscript and approved the final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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