Assessment of Placental Disposition of Infliximab and Etanercept in Women With Autoimmune Diseases and in the *Ex Vivo* Perfused Placenta

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Tumor necrosis factor (TNF) inhibitors are increasingly applied during pregnancy without clear knowledge of the impact on placenta and fetus. We assessed placental transfer and exposure to infliximab (n = 3) and etanercept (n = 3) in women with autoimmune diseases. Furthermore, we perfused healthy term placentas for 6 hours with 100 µg/mL infliximab (n = 4) or etanercept (n = 5). In pregnant women, infliximab transferred into cord blood but also entered the placenta (cord-to-maternal ratio of 1.6 ± 0.4, placenta-to-maternal ratio of 0.3 ± 0.1, n = 3). For etanercept, a cord-to-maternal ratio of 0.04 and placenta-to-maternal ratio of 0.03 was observed in one patient only. In *ex vivo* placenta perfusions, the extent of placental transfer did not differ between the drugs. Final concentrations in the fetal compartment for infliximab and etanercept were 0.3 ± 0.3 and 0.2 ± 0.2 µg/mL, respectively. However, in placental tissue, infliximab levels exceeded those of etanercept (19 ± 6 vs. 1 ± 3 µg/g, P < 0.001). In conclusion, tissue exposure to infliximab is higher than that of etanercept both *in vivo* as well as in *ex vivo* perfused placentas. However, initial placental transfer, as observed *ex vivo*, does not differ between infliximab and etanercept when administered in equal amounts. The difference in placental tissue exposure to infliximab and etanercept may be of clinical relevance and warrants further investigation. More specifically, we suggest that future studies should look into the occurrence of placental TNF inhibition and possible consequences thereof.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ The extent of placental transfer of structurally different types of tumor necrosis factor (TNF) inhibitors differs, but the underlying mechanisms are unknown. Also, it is unknown to what extent placental tissue is exposed to these drugs.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ The aim was to quantify placental exposure to infliximab and etanercept in women with autoimmune diseases, in relation to maternal and cord blood concentrations at the time of delivery. In addition, we studied the placental handling of these drugs by means of *ex vivo* placenta perfusion experiments.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Tissue exposure to infliximab is higher than that of etanercept both in placentas exposed *in vivo* as well as in *ex vivo* perfused placentas. The initial placental transfer, as observed *ex vivo*, does not differ between infliximab and etanercept when applied in equal amounts.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The difference in placental tissue exposure may translate into differential TNF inhibition, which warrants future studies that look into the possible consequences thereof.

Autoimmune diseases, such as rheumatoid arthritis and inflammatory bowel disease (IBD) typically affect women in their reproductive years. Both rheumatoid arthritis and IBD are associated with adverse pregnancy outcomes, such as preterm birth and low birth weight.[1,2] In women with IBD, the occurrence of disease flares during pregnancy further increases these risks.[3,4] To suppress disease activity, a trend is seen towards continuing pharmacotherapy during pregnancy. Current guidelines state that immunosuppressive drugs, such as sulfasalazine, azathioprine, and cyclosporin are considered compatible with pregnancy, whereas methotrexate and cyclophosphamide are teratogenic and should be discontinued. For novel biologics, such as anakinra and

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ustekinumab, discontinuation is also suggested due to a lack of human safety data.[5]

In women with severe disease activity, treatment with tumor necrosis factor (TNF) inhibitors may include infliximab, a full immunoglobulin G1 (IgG1) chimeric mouse/human monoclonal antibody, and etanercept, a fusion protein composed of two extracellular TNF receptor domains and an fragment crystallizable region (Fc) portion of human IgG1. Both TNF inhibitors are examples of biologics for which experience exists with use during pregnancy from more than 1,000 reported pregnancies for infliximab and approximately 300 for etanercept.[5]

In general, anti-TNF therapies are considered safe during pregnancy.[5,6] Some concerns exist, however, regarding the immune status in newborns that were exposed[7] and the long-term effects on development. An increased risk of major birth defects, preterm birth, and decreased birth weight were found in women exposed to anti-TNF therapy reported to Teratology Information Services, but it is unclear whether these are associated with anti-TNF therapy or inadequate disease control.[8] Other data on drug safety of TNF inhibitors in pregnancy are limited to small case series, which do not reflect our overall clinical experience with these types of drugs because of underreporting and selective reporting.

During pregnancy, therapeutic levels of TNF inhibitors in the mother are necessary, while absence of fetal exposure would be ideal. For infliximab, however, the fetal drug levels towards the end of pregnancy exceed maternal levels,[9,10] since IgG molecules are actively transported across the placenta via Fc-receptor mediated transport. Recently, higher placental transfer of infliximab compared with adalimumab was described in 94 cases.[10] In only two cases treated with etanercept, minimal placental transfer was observed.[11,12] Altogether, this points to differences in placental transfer which we do not fully understand mechanistically.

In addition, placental exposure to TNF inhibitors has not been investigated, while it may be another important determinant of adverse pregnancy outcome. As the placenta is extensively vascularized, it may be more exposed to drugs than other tissues, making it a potential target organ for toxicity. Also, TNF is highly regulated during pregnancy and plays an important role in placental function.[13]

As our understanding of placental transfer and, in particular, placental tissue exposure to structurally different TNF inhibitors is limited, it is important to support the existing data with mechanistic studies that also involve preclinical models on their placental handling. In this study, we investigated placental transfer and placental exposure in six patients treated with infliximab or etanercept throughout pregnancy and compared these findings with those of short-term exposure in *ex vivo* placenta perfusion experiments.

METHODS

Study population

Six pregnant women treated with either infliximab (n = 3) or etanercept (n = 3) for autoimmune disease throughout pregnancy were enrolled in this study, which was approved by the Regional Committee on Research involving Human Subjects (file no. 2016-2744). Written informed consent was obtained at routine prenatal care visits from 30 weeks of pregnancy onwards. Descriptive data on medication use and pregnancy outcome were retrieved from medical files retrospectively.

Nine healthy pregnant women were enrolled in this study for *ex vivo* placenta perfusion experiments with infliximab (n = 4) or etanercept (n = 5). Medical-ethical approval was also acquired for these experiments (file no. 2014-1397), and data on pregnancy outcome were retrieved from medical files (**Table S1**).

Blood and placenta sample collection

Maternal and cord blood samples were collected on the day of delivery. Serum was stored at −80°C until analysis. The placenta was stored at 4°C directly after delivery and 1 cm^3 sized samples of villous tissue were excised, snap frozen, and stored at −80°C within 24 hours.

Ex vivo placenta perfusions

Placentas from healthy women were used for *ex vivo* placenta perfusions based on the method described by Mathiesen *et al*.[14] Briefly, a branch of the umbilical artery and an associated vein from a single cotyledon were cannulated to reestablish the fetal circulation. The fetal flow rate was slowly increased to 6 mL/minute. If no measurable leakage occurred, the placental cotyledon was cut from the placenta and placed in the perfusion setup. Then, the maternal circulation of 12 mL/minute was established by inserting four cannulas on the maternal side of the cotyledon and recirculating the fluid that leaked from the intervillous space. During cannulation, the placentas were flushed for 30–60 minutes with Krebs-Henseleit buffer enriched with 10.1 mM glucose and 0.5 mL/L heparin 5,000 IU in open circulation. The Krebs buffer was then changed to the experimental perfusion solutions, and the circulations were closed. When changing the buffers, approximately 20 mL of Krebs buffer mixed with each experiment buffer, since this is the dead volume of the perfusion system. The maternal solution consisted of RPMI 1640 culture medium (catalog no. R8758, Sigma, St Louis, MO) with infliximab (Remsima, Mundipharma, Amersfoort, The Netherlands) (10 mg/mL) or etanercept (Enbrel, Pfizer, Dublin, Ireland) (10 mg/mL) in a final concentration of 100 mg/L, antipyrine (100 mg/L), human albumin (29 g/L) (Albuman, Sanquin, Amsterdam, The Netherlands), and 1% penicillin and streptomycin. Infliximab and etanercept have an equal molecular weight and for comparison of their disposition, equal amounts were applied in the placenta perfusions. The fetal solution consisted of RPMI culture medium with human albumin (34 g/L) (Albuman, Sanquin, Amsterdam, The Netherlands) and 1% penicillin and streptomycin. Maternal perfusion solutions were gassed with 95% oxygen /5% carbon dioxide and fetal perfusion solutions with 95% nitrogen /5% carbon dioxide. All solutions were continuously kept at 37°C, while pH was monitored and kept at 7.4. Placentas were perfused for 6 hours, during which the buffer volume was monitored and 15 samples were taken over time of both maternal and fetal buffers. These were centrifuged for 5 minutes at 3,000 *g* and the supernatant was stored at −20°C. In addition, three samples of 1 cm^3 of villous tissue from the perfused cotyledon were collected, snap frozen, and stored at −80°C. Infliximab and etanercept levels were quantified as outlined below. Control experiments were performed for both TNF inhibitors by carrying out the perfusion experiment in absence of a placenta.

Quantification of infliximab and etanercept in serum, perfusion buffers, and tissue homogenates

Infliximab and etanercept levels in serum were quantified by enzyme-linked immunosorbent assay performed by Sanquin Diagnostic Services (Amsterdam, The Netherlands). Over the investigated concentration range, the method for infliximab exhibited an accuracy and precision of 94–108% and 4–8%, respectively. For etanercept, the respective accuracy and precision in serum were 92–103% and 10–17%. The lower limits of quantification were 0.03 μg/mL and 0.1 μg/mL

for infliximab and etanercept, respectively.[15,16] Perfusate samples were analyzed in the same manner, and we found recoveries for infliximab and etanercept from the perfusion buffer of 76 ± 16% and 73 ± 8% in the starting solutions prepared at a concentration of 110 μg/mL. Briefly, microtiter plates were coated with a mouse monoclonal antibody against TNF and recombinant TNF was added. Samples were added and subsequently incubated with a horseradish peroxidase conjugated monoclonal antihuman IgG (infliximab assay) or idiotypic specific monoclonal antibody (etanercept assay). Color reaction with tetramethylbenzidine was related to a standard curve and samples were back-calculated.[15-17]

The above method was also applied to the tissue homogenate supernatants. Briefly, 20% placental tissue homogenates (based on tissue wet weight) were prepared in RIPA buffer containing 5 mM Tris-HCl buffer pH 7.4, 150 mM sodium chloride, 1% Triton-X100 (catalog no 23,472-9, Sigma, St Louis, MO), 0.5% sodium deoxycholate, and one Roche complete proteinase inhibitor tablet per 10 mL buffer (catalog no. 04693116001, Sigma, St Louis, MO). Homogenates were prepared with a T10 basic Ultra-Turrax disperser (IKA, Staufen, Germany), and the samples were kept on ice during homogenization. After homogenization, the samples were mixed and kept on ice for 1 hour, after which they were mixed again and centrifuged for 5 minutes at 5,000 *g*. The supernatant was stored at −80°C until analysis. To investigate the performance of the analytical method, 20% blanc homogenates were spiked in triplicate with 1, 3, and 10 mg/L concentrations of infliximab and etanercept after which the accuracy and precision of the analysis method were assessed. Additionally, we studied the stability of etanercept and infliximab in tissue homogenates stored at 4°C for 24 hours, which would conveniently allow for storage of the placenta at 4°C after birth, before further processing the next day. Infliximab and etanercept could be measured accurately and with good precision and remained stable for 24 hours at 4°C using the sample preparation procedure described (**Table S2**). To allow for measuring within the range of the assay, homogenates for etanercept were also prepared as a 50% homogenate.

Antipyrine measurements

In all placenta perfusion experiments, antipyrine transfer was used as a parameter for free diffusion to check if the cannulation procedure was successful. To quantify antipyrine in placental tissue, a 20% homogenate in ultrapure water was prepared. Next, placental homogenate samples or perfusion buffer samples were deproteinized with 6% perchloric acid and spun. A reaction mix consisting of 0.2 mg/mL sodium nitrite and 0.6% concentrated sulfuric acid was added to the supernatants (1:1). The nitroantipyrine that was formed was determined via absorbance measurements at 350 nm against calibration curves constructed in the corresponding matrix.

Data analyses

Placental tissue samples consist by definition of placental tissue as well as fetal and maternal blood or buffer that is present within the tissue. Hence, the concentrations measured in the homogenates were corrected for TNF inhibitor concentrations present in buffer/blood, to better reflect the biological concentrations in the actual tissue. We assumed that the homogenate consisted of 80% placental tissue, 12% maternal blood or buffer, and 8% fetal blood or buffer, based on earlier work by Barker *et al*. This resulted in the following formula for correction:[18]

$$[\text{Tissue, corrected}] = \frac{[\text{Tissue, total}] - 0.12 \cdot [\text{Maternal}] - 0.08 \cdot [\text{Fetal}]}{0.8}$$

Serum concentrations were first recalculated to maternal or fetal whole blood concentrations by multiplying with the serum fraction relative to hematocrit in whole blood, which is 0.65 for maternal samples and 0.54 for fetal samples.[19,20] We assumed that partition of biologicals into hematocrit did not occur.

To express placental transfer, cord-to-maternal ratios were calculated using serum levels. To express tissue exposure, placenta-to-maternal ratios were calculated by dividing the average corrected placental tissue concentration by the maternal whole blood or buffer concentrations. For placenta perfusion experiments, fetal volume loss was estimated by comparing the measured buffer volume with the nominal volume that should be present in the fetal or maternal perfusate circulation, taking into account the sampling volume. Graphs were constructed using GraphPad Prism software version 5.03 (GraphPad, San Diego, CA). Results are presented as mean ± SD or the individual data points and the means are plotted. For statistical analysis of the placenta perfusion data, a one-way analysis of variance was used to compare placenta-to-maternal ratios and an unpaired *t*-test was performed to compare drug concentrations in placenta tissue.

RESULTS

Subject characteristics

We included six pregnant patients treated with either infliximab or etanercept. In **Table 1**, the patient characteristics are presented. Age was between 25 and 37 years, and none of the patients smoked. Most pregnancies were uncomplicated and reached full-term delivery. One patient had a caesarean section. All neonates were born with a birth weight within normal limits (10^{th}–90^{th} percentile) and had an uncomplicated hospital stay, although one neonate was suspected for infection.

In vivo placental transfer and exposure

Infliximab. All patients were on standard infliximab treatment of 5 mg/kg every 8 weeks and received the last infliximab dose 23, 31, or 57 days prior to delivery. Infliximab levels in cord blood exceeded the maternal levels. Infliximab was detectable in all placentas and was distributed homogeneously within the tissue with average drug levels of 5.8 ± 0.9, 4.8 ± 1.5, and 1.8 ± 0.0 μg/g tissue per placenta. Placental concentrations were lower than maternal or cord blood concentrations at delivery and placenta-to-maternal ratios were on average 0.34 ± 0.11. The data are summarized in **Table 2**, and represent tissue concentrations after correction for blood-associated infliximab concentrations. The uncorrected results can be found in **Table S3**.

Etanercept. All patients received 50 mg etanercept every 7–12 days. Etanercept drug levels were detectable in maternal blood, cord blood, and placental tissue of one patient who received the last etanercept dose 4 days before delivery. The cord-to-maternal ratio was 0.04, indicating limited placental passage. Etanercept concentrations in the placenta were 0.1 ± 0.1 μg/g when corrected for serum levels, equaling a placenta-to-maternal ratio of 0.03. In the second patient, the final etanercept administration was 29 days before delivery and etanercept was not detected in any sample. In the third patient, final etanercept administration was 16 days before delivery. The maternal etanercept concentration was 0.2 μg/mL, but no levels could be detected in the placenta. For this patient, cord blood was not collected (**Table 2** and **Table S3**). In patients 5 and 6, discontinuation of etanercept before delivery may have been intentional to limit fetal exposure.

Table 1 Subject characteristics

Patient	Age (years)	Pre-pregnancy BMI (kg/m^2)	Auto immune disease	Comorbidity	Pregnancy complications	Medication during pregnancy	Gestational age at delivery (weeks)	Birth weight neonate (percentile range)	Neonatal complications
1	37	21.5	Rheumatoid arthritis	Primary hypothyroidism Partial androgen insensitivity syndrome Uveitis Vitiligo	None	Infliximab Azathioprine Levothyroxine	40^{+1}	4050 g (p50–90)	None
2	27	22.3	Crohn's disease	Asthma	None	Infliximab	41^{+6}	3715 g (p10–50)	None
3	25	24.7	Crohn's disease	None	None	Infliximab, Hydroxocobalamin	38^{+6}	3100 g (p10–50)	None
4	30	21.5	Ankylosing spondylitis	Attention deficit disorder	Preeclampsia	Etanercept Nitrofurantoin Methyldopa	38^{+5}	3305 g (p50–90)	Suspicion for infection
5	31	24.4	Rheumatoid arthritis	None	None	Etanercept	40^{+6}	3780 g (p10–50)	None
6	37	23.1	Psoriasis vulgaris	None	None	Etanercept	39^{+1}	3780 g (p50–90)	None

p, percentile.

Table 2 Placental transfer and placental exposure to infliximab and etanercept in patients with autoimmune diseases

				TNF inhibitor				
				(μg/mL serum)		Mean ± SD (μg/g tissue)		
Patient	TNF inhibitor	Dosing regimen	Time from last dose to delivery (days)	Maternal	Cord	Placenta	Cord-to-maternal ratio	Placenta-to-maternal ratio
1	Infliximab	400 mg per 8 weeks (5 mg/kg)	23	25.3	29.8	5.8 ± 0.9	1.18	0.35
2	Infliximab	400 mg per 8 weeks (5 mg/kg)	57	12.0	24.0	1.8 ± 0.0	2.00	0.23
3	Infliximab	400 mg per 8 weeks (5 mg/kg)	31	17.0	29.0	4.8 ± 1.5	1.71	0.44
4	Etanercept	50 mg per 12 days	4	3.0	0.1	0.1 ± 0.1	0.04	0.03
5	Etanercept	50 mg per week	29	<0.1	<0.1	<0.1	NA	NA
6	Etanercept	50 mg per week	16	0.2	NA	<0.1	NA	NA

Placental transfer is represented as cord-to-maternal ratios based on serum levels, and placental exposure is calculated as placenta-to-maternal ratios based on placental tissue concentrations corrected for serum levels and maternal calculated whole blood concentrations. Cord blood of patient 6 was not available.
NA, not assessed; TNF, tumor necrosis factor.

Ex vivo placental transfer and exposure to infliximab and etanercept

Nine successful *ex vivo* placenta perfusions were conducted (infliximab $n = 4$, etanercept $n = 5$). **Figure 1** displays (**a**) the placental transfer of infliximab and (**b**) the corresponding final concentrations in each compartment. The initial average maternal infliximab concentration was 83 ± 18 μg/mL, and the final maternal concentration (72 ± 12 μg/ml) did not substantially differ from that at 1 hour. Still, placental exposure could be measured with infliximab concentrations in tissue reaching 19 ± 6 μg/g, corrected for infliximab concentrations associated with perfusion buffer remaining in the tissue. Infliximab was first detected in the fetal circulation after 2–4 hours, and final concentrations were 0.3 ± 0.3 μg/mL, which points to slow placental transfer.

Placental transfer of etanercept and final etanercept concentrations per compartment can be found in **Figure 2a,b**, respectively. Initial maternal etanercept concentrations were 80 ± 9 μg/mL, and final concentrations were 43 ± 22 (range: 5.7–61) μg/mL. Etanercept concentrations in placental tissue were on average 1 ± 3 μg/g. In four placenta perfusions, tissue concentrations ranged from 1.2 to 4.9 μg/g, whereas in one placenta perfusion measured tissue levels were negligible and could be explained by the concentrations in the maternal and fetal buffers (**Figure 2b**). In two experiments, fetal etanercept remained below the detection limit of 0.1 μg/mL for 6 hours. In the other experiments, the first detectable fetal etanercept levels were seen after 1–3 hours. On average, final etanercept concentrations in the fetal compartment were 0.2 ± 0.2 μg/mL, again pointing to a slow but detectable placental transfer comparable to infliximab.

Antipyrine concentrations reached an equilibrium between the maternal and fetal circulations within 2.5 hours in all placenta perfusions (**Figure 3a**), confirming successful cannulation. After 6 hours of perfusion, antipyrine concentrations in maternal, fetal, and placental compartments were 44 ± 5 μg/mL, 39 ± 3 μg/mL, and 32 ± 6 μg/g, respectively, showing that antipyrine distributes across all three compartments (**Figure 3b**). Since both infliximab and etanercept could be detected in placental tissue from *ex vivo* perfusion experiments, placenta-to-maternal ratios were calculated to evaluate differences in placental exposure using antipyrine as a reference (**Figure 4**). Placenta-to-maternal ratios of antipyrine,

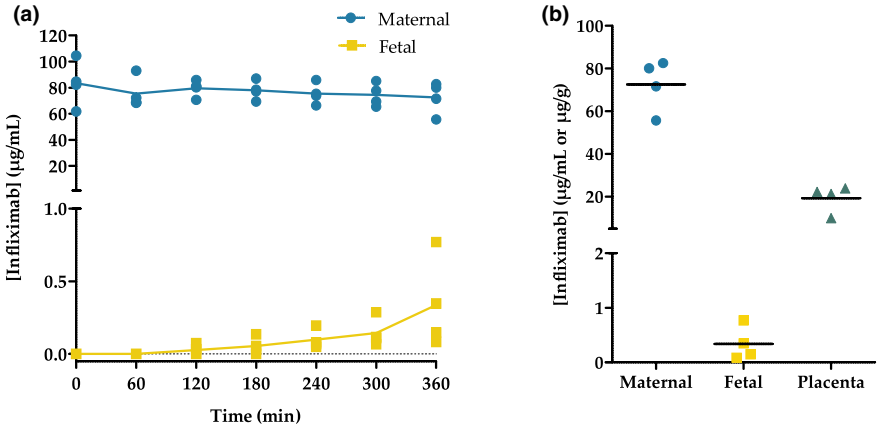

Figure 1 *Ex vivo* placental transfer of (**a**) infliximab and (**b**) final infliximab distribution across the different compartments after 6 hours placenta perfusion. Mean and individual experiments are represented. Placental concentrations are the average of three samples per perfused placenta and are corrected for buffer-associated drug concentrations. [Colour figure can be viewed at wileyonlinelibrary.com]

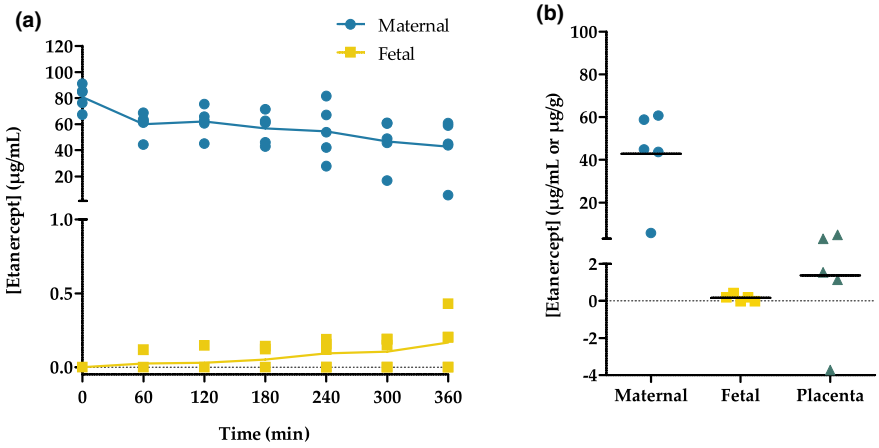

Figure 2 *Ex vivo* placental transfer of (**a**) etanercept and (**b**) final etanercept distribution across the different compartments after 6 hours placenta perfusion. Mean and individual experiments are represented. Placental concentrations are the average of three samples per perfused placenta and are corrected for buffer-associated drug concentrations. [Colour figure can be viewed at wileyonlinelibrary.com]

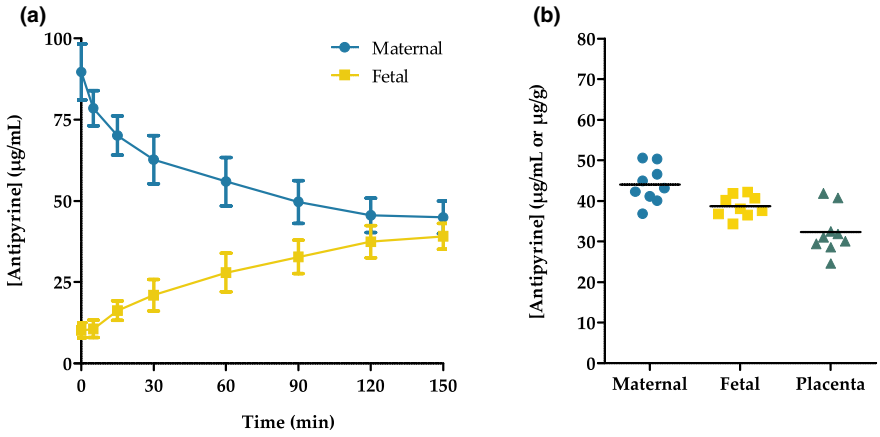

Figure 3 *Ex vivo* placental transfer of (**a**) antipyrine in all placenta perfusions and (**b**) its final distribution across the perfusion compartments after 6 hours of perfusion (n = 9). Data points represent **a** mean ± SD or **b** the average of three samples per perfused placenta which are corrected for buffer-associated drug concentrations. In all placenta perfusions, antipyrine levels show good overlap within 2.5 hours of perfusion, confirming a successful cannulation procedure. [Colour figure can be viewed at wileyonlinelibrary.com]

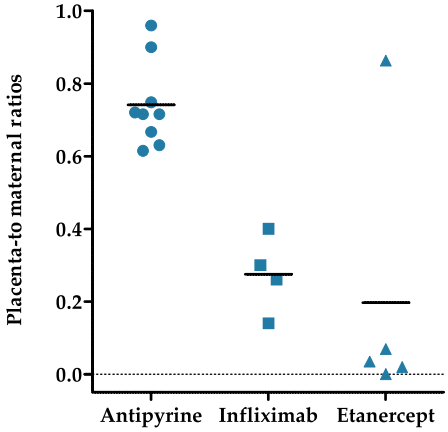

Figure 4 Placental exposure to antipyrine, infliximab, and etanercept as reflected by placenta-to-maternal ratios for each placenta perfusion experiment. [Colour figure can be viewed at wileyonlinelibrary.com]

infliximab, and etanercept were 0.74 ± 0.12, 0.28 ± 0.11, and 0.2 ± 0.4, respectively. The placental exposure to antipyrine largely exceeded the exposure to infliximab and etanercept ($P < 0.001$). Absolute placental tissue concentrations of infliximab were higher than those of etanercept ($P < 0.001$).

Quality control criteria in placenta perfusion experiments

All placenta perfusions had fetal volume loss to some extent, mostly occurring after three hours of perfusion with total volume loss ranging from 4 to 28 mL (**Figure S1**), corresponding to on average 0.7–4.7 mL/hour, which we found acceptable. A certain loss of volume is inherent to the perfusion system and may point to an impaired vascular integrity. If this were the case, it would impact both drugs equally as mean volume loss did not significantly differ between infliximab and etanercept placenta perfusions. In control perfusions without the presence of a placenta, we found that infliximab levels remained stable in the perfusion system up to 6 hours. However, etanercept concentrations decreased to approximately 50% of the initially added concentration (**Figure S1**), indicating that etanercept is either unstable in 6-hour placenta perfusions or displays adherence to components of the perfusion system. To this end, we assessed the stability of etanercept at 37°C in perfusion buffer and found recoveries of 113%, 112%, and 114% of control, after 1, 3, and 6 hours of incubation in standard reaction vials, respectively.

DISCUSSION

Exposure of placental tissue to TNF inhibitors may be an important determinant of adverse pregnancy outcome. To this end, we studied placental drug concentrations in relation to maternal and cord blood concentrations in women who continued TNF inhibitor therapy during pregnancy. In addition, we used *ex vivo* placenta perfusions to study placental handling of TNF inhibitors in the first hours after initial exposure. We found that at delivery, infliximab can be readily detected in the placenta, whereas etanercept concentrations were much lower to undetectable. In *ex vivo* perfused placentas, tissue concentrations of both TNF inhibitors were found. Here, infliximab levels exceeded those of etanercept, while placental transfer of both biologics was comparable.

Placental concentrations of TNF inhibitors may be placed in perspective by comparison to levels observed in other tissues, in which therapeutic responses are observed upon clinical dosing. Tissue levels of TNF inhibitors have not been studied widely, but infliximab levels in gut biopsies obtained 2 weeks after administration were determined by Yoshihara *et al.*[21] They observed median drug levels of 1.7 and 1.0 µg/g in inflamed and uninflamed bowel tissue, respectively.[21] We found 1.8–5.8 µg/g in exposed placentas (final dose: 23–57 days before delivery), indicating pharmacologically relevant placental exposure levels, which are also in line with target serum trough levels of 5 µg/mL.[22] Consistent with previous studies, we found that infliximab cord blood levels largely exceed maternal drug levels.[9,10] Thus, in pregnant women treated with infliximab, both fetal and placental exposure may be of pharmacological relevance.

For etanercept, we were able to detect a placental concentration in one patient only, who received etanercept shortly (4 days)

before delivery. Tissue distribution of etanercept in rats was found to be 7–15% of plasma levels, depending on the type of tissue.[23] No placental tissue was studied, but the order of magnitude of tissue exposure appears in line with the placental distribution of approximately 3% of the plasma concentration that we report here. To our knowledge, no data are currently available regarding tissue distribution in humans, except that etanercept levels in synovial fluid of three rheumatoid arthritis patients were found to be approximately equal to serum levels.[24] The placental exposure we quantified here amounted to 0.1 ± 0.1 μg/g tissue, which is lower than the target serum etanercept trough levels of 2.1 μg/mL.[25] Still, from these data it is difficult to conclude whether etanercept tissue levels may be within the therapeutic range. In the literature, only two cases with etanercept measurements in cord blood have been described with intervals between last treatment and delivery of 1–7 days.[11,12] Cord-to-maternal ratios were 0.04 in the first case[12] and 0.07 in the second case,[11] confirming the limited placental passage observed in the current study.

For small molecule drugs, *ex vivo* placental transfer correlates well with *in vivo* cord-to-maternal ratios.[26] For antibody-based biologicals, however, results are more difficult to interpret, as placental transfer of *IgG*'s is slow compared with small molecules and only minimal transfer is expected to occur after 6 hours.[27] Therefore, only the initial phase of IgG transport can be studied via this technique. Additionally, it is not clear yet how *in vivo* and *ex vivo* placental drug levels and placenta-to-maternal ratios relate to one another. We found that infliximab placenta-to-maternal ratios were similar *in vivo* and *ex vivo*, which could imply that placental infliximab levels are largely determined by maternal drug concentrations and that infliximab does not accumulate in the placenta. We also observed that *in vivo* and *ex vivo* etanercept placenta-to-maternal ratios were lower than those of infliximab. This could be explained by differences in affinity for the neonatal Fc-receptor (FcRn), which is the driving factor for IgG transport across the placental barrier.[28] Also, the FcRn saves IgG molecules from degradation, prolonging their half-life. Porter *et al*. determined that infliximab has a ten-times-higher affinity for the FcRn than etanercept, which could explain the higher infliximab tissue concentrations.[29] The observed lower placental and maternal concentrations of etanercept could result from spontaneous etanercept degradation in buffer and tissue, but may also be due to enzymatic placental degradation by, for example, metalloproteinases.[30] Although etanercept levels in maternal perfusate declined to 50% and placental tissue concentrations were lower compared with infliximab, we found comparable *ex vivo* transfer patterns of infliximab and etanercept. However, in two out of five placenta perfusions, etanercept was not detected in the fetal compartment. This could reflect a true absence of etanercept transfer in these placentas, but may also be due to a 10-fold higher detection limit of the etanercept enzyme-linked immunosorbent assay compared with the infliximab assay. In future experiments, it may be relevant to perform placenta perfusions with different biologics simultaneously to correct for interindividual differences, provided that they do not interfere with each other's disposition. For reasons of comparison, the concentration of etanercept used was equal to that of infliximab. For etanercept, this is a supratherapeutic concentration, which may explain why etanercept transfer was seen *ex vivo* in the initial transfer phase, but not *in vivo* after long-term exposure to therapeutic levels of etanercept.

Despite the increasing use of therapeutic anti-TNF antibodies during pregnancy, placental transfer of and exposure to infliximab and etanercept have not been studied in a placenta perfusion model before. Recently, Roy *et al*. did study placental transfer of the TNF inhibitor adalimumab, a full IgG1 molecule and thus comparable in chemical structure to infliximab, but not etanercept.[30] They found placental transfer rates of adalimumab similar to the values we found for infliximab, with average fetal concentrations after 6 hours of 0.5 ± 0.5 and 0.3 ± 0.3 μg/mL, respectively. Adalimumab placenta concentrations were not assessed in this study.

When prescribing anti-TNF therapy to women who wish to conceive, the extent of placental transfer and placental exposure could be taken into consideration. However, further research is needed to better understand the consequences of placental exposure to anti-TNF therapy. TNF is highly expressed in term placental tissue and is known to play a role in placental development as well as in major placental functions, such as maintenance of the syncytiotrophoblast layer and hormone production.[13] Reducing TNF levels may interfere with placental function, but it may also be important to decrease elevated cytokine levels in autoimmune diseases. Treatment with TNF inhibitors may even improve live birth rates in women with recurrent miscarriage.[31] Furthermore, it would be interesting to investigate whether infliximab and etanercept are bound to TNF in placental tissue and whether differences exist in localization between the two inhibitors. For example, full antibodies such as infliximab, but not etanercept, can bind to monocytes and activated T cells, inducing apoptosis, which may also occur in placental tissue.[32] As etanercept is a TNF receptor Fc-portion fusion protein, it also binds other ligands of the TNF superfamily, such as lymphotoxin α and ß,[32] of which messenger RNA expression was found in the placenta.[33]

In conclusion, our data showed significant placental tissue concentrations of infliximab after both *in vivo* and *ex vivo* exposure studies. For etanercept, tissue concentrations were much lower, which may be due to different recycling of etanercept by the FcRn. Interestingly, the extent of *ex vivo* placental transfer during the 6-hour perfusion period did not differ between these two drugs. Our findings with regard to placental tissue exposure to infliximab and etanercept may be of clinical relevance and warrant further investigation. More specifically, we suggest that future studies should look into the occurrence of placental TNF inhibition and possible consequences thereof.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST
The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS
G.A.M.E., J.v.D., A.d.V., N.R., F.G.M.R., and R.G. wrote the manuscript. G.A.M.E., J.v.D., N.R., F.G.M.R., and R.G. designed the research. G.A.M.E., H.v.H., N.I.K., and P.v.d.B. performed the research. G.A.M.E., J.v.D., H.v.H., N.I.K., P.v.d.B., and R.G. analyzed data. A.d.V. contributed new analytical tools.

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