Transfer of uremic solutes across the human term placenta: An ex vivo study in the dual-side perfused cotyledon

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ABSTRACT

Introduction: An increasing number of women becomes pregnant while suffering from chronic kidney disease (CKD). As a result of decreased renal function, uremic solutes circulate at high levels in the maternal circulation. This study aimed to acquire more knowledge about the placental transfer of uremic solutes across the human placenta.

Methods: Placental transfer was studied in healthy term placentas, via the ex vivo dual-side human cotyledon perfusion technique (closed-closed set-up for both maternal and fetal circulations). Uremic solute concentrations in maternal and fetal perfusates were measured via LC-MS/MS over 180 min of perfusion.

Results: We found that the studied compounds demonstrated different degrees of placental transfer. Fetal-to-maternal perfusate ratios at t = 180 min were for anthranilic acid 1.00 ± 0.02, indole-3-acetic acid 0.47 ± 0.08, hippuric acid 0.36 ± 0.18, l-arabinitol 0.33 ± 0.04, indoxyl sulfate 0.33 ± 0.1, neopterin 0.28 ± 0.14 and kynurenic acid 0.13 ± 0.03. All uremic solutes studied also emerged in the perfusates when cotyledons were perfused in the absence of uremic solute concentrations added to the maternal reservoir. For kynurenin these concentrations were so high, it complicated the calculation of a transfer ratio for the exogenously administered compound.

Discussion: After 180 min of exposure the extent of placental transfer differs substantially for the solutes studied, reflecting different transfer rates. Future studies should investigate to what extent specific uremic solutes reach the fetal circulation in vivo and how they may interfere with organ function and development of the unborn child.

1. INTRODUCTION

Management of chronic kidney disease (CKD) during pregnancy is feasible, but the condition is considered a risk factor for adverse pregnancy outcomes. Indirect estimates of the prevalence of CKD in pregnancy range from 1:30 for the earliest, often undiagnosed stage, to 1:750 pregnancies for the later stages of CKD [1-3]. In women with CKD, the degree of renal dysfunction correlates with severity and incidence of adverse pregnancy outcome [3]. Pregnant women on dialysis have a lower probability of delivering a live-born baby, and newborns are more often delivered via cesarean section, as compared to both transplant recipients and the general population. Intra-uterine growth retardation (IUGR) and maternal complications, such as pre-eclampsia, are the factors most often responsible for performing cesarean sections [4-8]. The clearance of circulating waste products appears to be directly related to pregnancy success [9]. Moreover, it has been shown that more intensive dialysis protocols improve maternal and fetal outcome [10, 11]. This suggests that high circulating concentrations of dialyzable uremic solutes may contribute to the adverse pregnancy complications observed.

Uremic solutes is the collective term used to indicate a wide variety of compounds that are retained in the body as a consequence of deteriorated renal function. While various uremic substances likely contribute to impaired renal function and further development of the uremic syndrome, the effect of only a few of them has been reported on pregnancy outcome and long-term development of off-spring. Still, data
from different lines of evidence demonstrate that uremic solutes have the potential to affect several fetal organ systems.

Neurodevelopmental effects of uremic solutes on the fetal brain have been reported in animal studies, particularly for metabolites originating from the kynurenine pathway of tryptophan metabolism. At least, two receptor targets of kynurenin metabolites have been identified in the cortex of the developing brain, namely the $\alpha_7$ nicotinic Acetyl choline ($\alpha_7nAch$) receptor and N-methyl-D-aspartate (NMDA) receptor [12,13]. Kynurenic acid may act as an antagonist of both receptors, and both receptors are implicated in neurodevelopment [14–17]. Moreover, both kynurenin and kynurenic acid are agonists of the Arylhydrocarbon receptor (AhR), e.g. expressed in fetal epithelial cells and the placenta [18]. Its activation may regulate a variety of processes such as cell division and cell differentiation [19]. The placenta has been implicated as an important source of kynurenin metabolites, and a tightly balanced homeostatic regulation of this metabolic pathway in mother, placenta and fetus appears crucial for normal fetal (neuro-)development, as comprehensively reviewed by Notarengalo and Pocivavsek [20]. Indeed, maternal overload of kynurenin metabolites in rodents has been associated with neurochemical and cognitive abnormalities in the offspring [21,22].

There are also indications that other solutes, such as indole-3-acetic acid and indoxyl sulfate, influence neurodevelopment by inducing neuronal apoptosis or oxidative stress in the fetal brain [23,24]. It appears that several of these molecules can elicit effects on organ systems other than the brain, as well. For indoxyl sulfate, disturbance of endothelial cell function has been associated with cardiovascular effects, which during pregnancy may possibly contribute to the development of pre-eclampsia [25]. Fuji et al. reviewed several effects on kidney, bone and the cardiovascular system [26]. For many of the molecules, however, the potential adverse effects on the human fetus remain uncharted yet.

An important question arising in this context is whether the human placenta constitutes a protective barrier for maternal-to-fetal transfer of uremic solutes circulating in high concentrations, and if so, for which solutes and to what extent? It is known that differences in cord-to-maternal plasma concentration ratios exist, but only for a small number of uremic solutes and to our knowledge no information is available for pregnancies of women with CKD [27–32]. For example, kynurenine, kynurenic acid, and indole-3-acetic acid exhibit cord-to-maternal ratios $>1$, while the uremic solute neopterin exhibits a ratio $<1$ [28,30,33,34].

Most uremic solutes are products of normal cellular metabolism and are therefore formed as well as catabolized in the fetus. Therefore, the cord-to-maternal ratios measured in vivo are always a composite of formation and clearance taking place in the fetus, as well as placental disposition processes. Hence, observed cord-to-maternal ratios do not solely reflect the differences in placental handling. As the placenta is known to express enzymes involved in the synthesis of several uremic solutes, it is likely that the ex vivo perfused placentas also synthesizes, catabolizes or releases these compounds. In any case, the ex vivo dual-side human cotyledon perfusion technique provides a relevant approach to study the placental disposition of uremic solutes, independent of fetal metabolic processes.

Here, we aimed to investigate the transfer of a mixture of small molecule uremic solutes in this system, at concentrations observed during CKD.

2. METHODS

Ethical approval

This study was approved by the Regional Committee on Research involving Human Subjects (file 2014-1397). Written informed consent was obtained at routine prenatal care visits, allowing the use of placentas for research.

2.1. Placenta perfusion procedure

Ex vivo dual-side cotyledon perfusions were performed as previously described by Eliesen et al. with modifications [35]. The maternal and fetal perfusion buffer consisted of 200 mL Krebs-Henseleit buffer, enriched with 2.0 g/L glucose, 2500 IU heparin and 34 g/L human serum albumin (maternal) or 29 g/L human serum albumin (fetal). The maternal buffer contained the uremic toxin mixture outlined below. In addition, the maternal buffer contained 100 mg/L antipyrine, a freely-diffusing marker molecule to check for overlap between the maternal and fetal circulation. Finally, 36 pg/mL FITC-dextran (40 kDa) was added to the fetal buffer, as a marker for integrity of the fetal capillary bed.

After collecting the placenta, a single intact cotyledon was selected and a matching fetal vein and artery were cannulated to establish a circulation of 6 mL/min. To establish a maternal circulation (12 mL/min), four cannulas were inserted into the intervillous space, while maternal buffer leaking from the cotyledon was collected in the perfusion chamber and subsequently recirculated. After cannulation, the placenta was flushed for 30 min with regular Krebs-Henseleit buffer in open circulation. Then, the solutions were changed to the maternal and fetal Krebs-Henseleit buffer containing the uremic toxins and control compounds, and the circulations were closed. During the perfusion, the maternal buffer was oxygenated with carbogen, and the fetal buffer was gassed with a nitrogen/carbon dioxide mixture (95% N2, 5% CO2). Both the buffers were kept at 37 °C, and at a pH between 7.3 and 7.4. The perfusion period lasted for 180 min during which samples of approximately 1.5 mL from maternal and fetal reservoir were taken at t = 0, 1, 5, 15, 30, 60, 90, 120, 150 and 180 min. Collected samples were stored on ice and were then centrifuged for 5 min at 5000g. The supernatant was transferred into a new tube and stored at −80 °C until analysis.

2.2. Uremic solutes

As uremic solutes are waste products of normal metabolism and circulate at low levels in plasma in healthy subjects, it is possible that these are released by perfused cotyledons over the 3-h perfusion period. To study the extent of this, a set of control perfusions was evaluated, in which placentas were not exposed to uremic solutes, ex vivo. For this, a series of perfusate samples from earlier-performed perfusion experiments were re-analysed but now for the uremic solutes under investigation. These data included perfusate samples obtained from placentas perfused with tacrolimus as described by Freriksen et al. [36], crizotinib as described by Eliesen et al. [37] and tofacitinib (unpublished data from our laboratory, manuscript in preparation). In these placenta perfusions, the same quality control/perfusion success criteria were used as in the current study, perfusion set-up (closed-closed), flows and duration of perfusion being the same.

Next, to study maternal-to-fetal transfer of uremic solutes, a new set of experiments was performed in which a mixture of these compounds was added to the maternal reservoir. Cotyledons of 4 individual placentas were perfused with this mixture. All compounds tested were obtained from Sigma-Aldrich and perfused cotyledons were exposed to the following concentrations (as verified by LC-MS/MS analysis at the start of the experiment): kynurenine (760 ng/mL), kynurenic acid (180 ng/mL), anthranilic acid (a low and high concentration of 200, n = 2 and high concentration of 2000 ng/mL, n = 2), indoxyl sulfate (48,000 ng/mL), indole-3-acetic acid (1100 ng/mL), hippuric acid (14,000 ng/mL), arabinitol (14,000 ng/mL), neopterin (120 ng/mL), inosine (1800 ng/mL), s-adenosyl homocysteine (1700 ng/mL). Stock solutions of mixed solutes were prepared, stored at −20 °C, and diluted in perfusion buffer just prior to the start of each perfusion experiment. The range of uremic solute concentrations observed in CKD patients is very wide. The concentrations investigated do not necessarily correspond to maximal concentrations reported in uremic patients, but fall within the wide range of elevated levels observed in these patients [38,39].
Before performing placenta perfusion experiments with uremic solutes, we studied whether the test compounds would adhere to the tubing of the perfusion system or evaporate from the perfusion buffers. To this end, we performed the same perfusion procedure as for the perfusions described above, but now without a cotyledon in the system. Buffers were recirculated for 180 min through the maternal side of the system.

2.3. Antipyrine and FITC-dextran assays

Samples were deproteinized by adding 6% perchloric acid in ultrapure water to the perfusate (1:1). The mixture was centrifuged for 3 min at 13,000g. Supernatant was transferred to a well with reaction mix (1:1) consisting of 0.2 g/L NaNO₂ + 0.6% H₂SO₄ in ultrapure water, in a 96 wells plate. After 20 min of incubation in the dark, the formed nitroantipyrine was measured at 350 nm using a Biarad Multitwell Plate reader and quantified using a calibration curve in ultrapure water. To evaluate the integrity of the fetal capillary bed, FITC-dextran levels were measured in all perfusates. The fluorescence was determined in duplicate using the PerkinElmer Multitwell Plate Reader (excitation/emission: 485/535 nm). Concentrations were calculated using a calibration curve constructd in perfusion buffer. Perfusions were considered successful when an antipyrine concentration in maternal and fetal circulations reached an equilibrium within 2.5 h. Fluid leak was approximated by measuring volume of the reservoir left after the experiment, correcting for sampling volumes taken.

2.4. LC-MS/MS analysis of uremic solutes

An Acquity UPLC (Waters, Milford, MA, USA) coupled to a Xevo TQ-S micro (Waters) triple quadrupole mass spectrometer was used for LC-MS/MS analysis of uremic solutes. For analysis of kynurenine, inosine, neopterin and low concentrations of kynurenic acid, perchloric acid was used as a protein precipitation step. For s-adenosylhomocysteine, indoxyl sulfate, indole-3-acetic acid, kynurenic acid, hippocric acid, anthranilic acid and arabininitol, methanol was used. Next, the samples were centrifuged at 13,000g for 3 min and 1 μl of the supernatant was injected onto the LC-MS/MS system. The compounds were detected [M + H]+ or deprotonated [M - H]- molecular ion and subsequent MS/MS fragmentations and a multi reaction monitoring (MRM) was carried out, of which the MRM transitions are given in Table 1. As internal standards, d4-kynurenine (Buchem), d5-kynurenic acid (CDN isotopes) and d5-Indole-3-acetic acid (Cambridge Isotopes) were used.

2.5. Exploratory stability studies in the presence of haemolytic blood

Given the rapid perfusate disappearance for inosine and s-adenosylhomocysteine, exploratory stability studies (n = 2) were performed. Fresh solutions of uremic solutes (uremic concentration) in perfusion buffer (1 mL) were mixed with a small aliquot of 50 μl placenta perfusate containing haemolytic blood and subsequently incubated for 30 min at 37 °C. Samples were taken before and 30 min after mixing, then processed for measurement of uremic solute concentrations, as described for the perfusates.

2.6. Data and statistical analysis

Graphpad version 8.3 was used for constructing graphs and data analyses. For the perfusion experiments, data are plotted either as individual data points and the associated mean, or they are depicted as the mean ± SD. For the data in Fig. 2, statistical significance was assessed via the two-sided Student’s t-test, assuming unequal variances. Means were considered to be significantly different when p < 0.05.

3. RESULTS

3.1. Stability of uremic solutes

We first assessed whether the selected uremic solutes were stable during perfusion conditions, as several of the investigated compounds are volatile to a small extent. Moreover, lipophilic compounds may display adherence to tubing of the system. In Fig. 1 it can be seen that the levels of uremic solutes studied remained constant over time in the perfusion system, without a placenta in situ. This indicated that no relevant adherence to tubing of the system, evaporation from the buffers, or chemical degradation took place over the 3-h perfusion interval, hence allowing us to further study placental disposition of these
Fig. 1. Stability of uremic solutes in the maternal circulation during 3 h of perfusion without a placenta in the system (n = 1).
Fig. 2. Appearance of uremic solutes in maternal and fetal perfusates after 180 min of perfusion. Data represent individual data points and mean from $n = 6-7$ perfusions. * indicates $p < 0.05$ between concentrations in maternal and fetal circulation (Student’s t-test). For all solutes, except kynurenine, the concentrations released are low compared to the concentrations added in perfusions with uremic levels of the solutes (see materials and methods section).
compounds in our system.

3.2. Release of uremic solutes in the perfused cotyledon

We then investigated if and to what extent uremic solutes were released in the perfusion set up by cotyledons that were not exposed to uremic solutes ex vivo. We found that all of the metabolites could be detected in the circulations over the 180 min perfusion period (Fig. 2), although not in every perfusate sample measurable concentrations were found. This points to secretion of these compounds by the placenta or possibly a presence in residual (hemolytic) blood. Arabinitol, inosine, hippuric acid and kynurenine were the most abundant solutes released. Kynurenic acid and neopterin were the least abundant. For kynurenic acid, concentrations in the fetal perfusate were significantly lower than observed in the maternal perfusate (p < 0.05), amounting to 1.5 ± 1.1 and 4.4 ± 2.3 ng/mL, respectively. For all compounds, except for kynurenine, the concentrations found in the perfusates after 180 min were low compared to the uremic solute concentrations added to the maternal circulation in subsequent experiments.

3.3. Quality control of placenta perfusions performed with uremic solutes

In order to assess maternal-to-fetal transfer of uremic solutes, the compounds were added to the maternal reservoir ex vivo in concentrations present in plasma of CKD patients. For analysis, only placental perfusions were used with overlapping cannulated maternal and fetal circulations and intact vascular integrity throughout the experiment. In Fig. 3, the mean concentration-time profile of antipyrine is depicted for all placentas used in the analyses. It shows that the fetal and maternal concentration of antipyrine reached an equilibrium at 180 min, confirming overlap between the circulations. The change in FITC-dextran concentrations and buffer volume over time is depicted in Fig. 3B and C, respectively. Vascular integrity was confirmed by the minimal fetal-to-maternal transfer ratio (0.01 ± 0.01) of the high molecular weight compound FITC-dextran, and could also be inferred from the fact that only limited volume loss occurred from the fetal circulation, aside from loss explained by sampling. We observed an initial decline in volume in the first 5–15 min of perfusion, after which volume loss averaged at a rate of 1.3 ± 1.0 mL/h (range 0.3–2.2 mL/h) during the last 165 min of perfusion.

3.4. Maternal-to-fetal transfer of uremic solutes

As depicted in Fig. 4, uremic solutes exhibited differences in the observed perfusate concentration-time profiles. Concentrations in the fetal and maternal circulations at the end of the 180 min perfusion interval are listed in Table 2, as well as the associated fetal-to-maternal ratios. On one end of the spectrum, anthranilic acid (uremic
Fig. 4. Placental transfer of uremic solutes added in uremic concentrations to the maternal reservoir (left hand side of the figure) compared to release of uremic solutes observed in perfusates from unexposed cotyledons (right hand side of figure). For the sake of comparison, y-axes are matched left and right. Logarithmic plots that allow better appreciation of the lower concentration ranges are included in Supplementary Fig. S1. Closed circles indicate maternal circulation, open circles the fetal circulation. Data represent mean ± SD, n = 4. For anthranilic acid a high (uremic) concentration (n = 2) and low concentration (n = 2) are shown.
concentration) displayed similar transfer characteristics as the passive diffusion marker antipyrine with fetal-to-maternal perfusate concentration ratios of $1.00 \pm 0.02$ and $1.00 \pm 0.27$, respectively. At the other extreme, kynurenic acid demonstrated a ratio of only $0.13 \pm 0.03$, indicating a particularly low placental permeability for this specific uremic solute. Indoxyl sulfate, indole-3-acetic acid, hippuric acid, L-arabinitol and neopterin had intermediate fetal to maternal ratios compared to anthranilic acid and kynurenic acid (Fig. 4I-R, and Table 2).

The concentration-time profiles of kynurenine, inosine and s-adenosylhomocysteine showed an unusual pattern (Fig. 4A, S and U). After exposure to the mixture of uremic solutes, kynurenine levels increased in the fetal circulation and also in the maternal circulation, after a small
Table 2
Perfusate concentrations and fetal-to-maternal concentration ratios for uremic solutes and antipyrine as a reference after 180 min.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fetal perfusate concentration (ng/mL)</th>
<th>Maternal perfusate concentration (ng/mL)</th>
<th>Fetal-to-maternal ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>antipyrine</td>
<td>29 ± 8</td>
<td>30 ± 8</td>
<td>1.00 ± 0.27</td>
</tr>
<tr>
<td>anthranilic acid (uremic)</td>
<td>810 ± 230</td>
<td>810 ± 240</td>
<td>1.00 ± 0.02</td>
</tr>
<tr>
<td>anthranilic acid (low)</td>
<td>83 ± 7</td>
<td>107.9 ± 0.1</td>
<td>0.77 ± 0.07</td>
</tr>
<tr>
<td>indole-3-acetic acid</td>
<td>250 ± 70</td>
<td>540 ± 80</td>
<td>0.47 ± 0.08</td>
</tr>
<tr>
<td>hippuric acid</td>
<td>23,000 ± 700</td>
<td>70,000 ± 30,000</td>
<td>0.36 ± 0.18</td>
</tr>
<tr>
<td>l-arabinose</td>
<td>3200 ± 1600</td>
<td>9400 ± 3600</td>
<td>0.33 ± 0.04</td>
</tr>
<tr>
<td>indoxyl sulfate</td>
<td>7200 ± 2200</td>
<td>21,700 ± 1,000</td>
<td>0.33 ± 0.11</td>
</tr>
<tr>
<td>neopterin</td>
<td>17 ± 9</td>
<td>62 ± 3</td>
<td>0.28 ± 0.14</td>
</tr>
<tr>
<td>kynurenic acid</td>
<td>16 ± 5</td>
<td>119 ± 13</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>s-adenosylhomocysteine</td>
<td>50 ± 70</td>
<td>30 ± 50</td>
<td>0.98 ± 1.19*</td>
</tr>
<tr>
<td>inosine</td>
<td>170 ± 70</td>
<td>474 ± 110</td>
<td>0.37 ± 0.15*</td>
</tr>
<tr>
<td>l-kynurenine</td>
<td>330 ± 130</td>
<td>950 ± 520</td>
<td>0.44 ± 0.25**</td>
</tr>
</tbody>
</table>

Data represent mean ± SD. For all solutes n = 4 except for anthranilic acid n = 2 + 2 (a high, uremic concentration + a low concentration). * Ratio is strongly influenced by the observed instability in the presence of haemolytic blood washed out of the placenta during perfusion. ** Ratio is largely influenced by the release of kynurenine into maternal and fetal perfusates.
initial decrease. For the fetal perfusate, the concentration increased to 330 ± 130 ng/mL. In the maternal perfusate, the starting concentration was 750 ± 30 ng/mL and increased to 950 ± 520 ng/mL. The release of kynurenine in the maternal and fetal perfusate was approximately 150 ng/mL (Fig. 4B), which can partly explain the increase in maternal perfusate concentration. It should be noted that the increase in maternal perfusate concentrations was observed in only 2 out of 4 of the placentas studied, as reflected in the high inter-placental variation observed. Maternal-to-fetal transfer of kynurenine did appear to occur, since only approximately 50% of the kynurenine measured in the fetal perfusate could be explained by placental release (Fig. 4A and B).

Inosine and s-adenosylhomocysteine concentrations decreased rapidly in the maternal perfusate. For inosine, levels decreased by 64% at t = 5 min and by 73% at t = 30 min, before stabilizing at approximately 470 ng/mL (27% of the initial value). The latter is in the same order of magnitude as observed in the experiments in which only release was studied (Fig. 4S, T and Supplementary Fig. S1). For s-adenosylhomocysteine a similar pattern was found. Levels of this solute were decreased by 46% at t = 5 min and by 95% at t = 30 min of perfusion, before ultimately stabilizing at approximate 30 ng/mL (only 2% of the initial value; Figure 4L, V and Supplementary Fig. S1).

As the flow rate is 12 mL/min in the maternal circulation, in 5 min time, only 60 mL of the total maternal buffer (200 mL) passes through the placenta. Hence, the rapid decline in concentrations of s-adenosylhomocysteine and inosine could not be fully explained by rapid uptake of these solutes by the placenta. Additional stability studies showed that in the presence of placental hemolytic blood, enzymatic or chemical degradation of s-adenosylhomocysteine and inosine occurred, which reduced the recovery of both compounds. For s-adenosylhomocysteine recovery at t = 30 min decreased to 69 ± 14% of starting values. For inosine, recoveries of the solute after addition of blood decreased even stronger, to 12.9 ± 0.1%. Despite flushing prior to the start of experiments, some washout of blood into the maternal and fetal buffer reservoirs is unavoidable during perfusion studies, which appears to be of influence here.

4. DISCUSSION

In this study, we found that uremic solutes demonstrate different degrees of placental transfer. Ranked in order of good-to-poor permeability this can be summarized as anthranilic acid > indole-3-acetic acid > hippuric acid = l-arabinitol = indoxyl sulfate = neopterin > kynurenic acid. Kynurenine, inosine and s-adenosylhomocysteine were difficult to rank, as a result of release of kynurenin into the perfusates and due to instability of the latter two compounds in the presence of hemolytic blood.

When searching for an explanation for the differences in placental permeability, differences in transmembrane diffusion, involvement of membrane transporters and metabolism need to be considered. Typically, unionized, lipophilic small molecules display the highest diffusion coefficients. In line with this, molecules exceeding 500–600 g/mol generally exhibit low placental transfer [40]. The compounds studied here ranged in molecular weight from 137 g/mol (anthranilic acid) to 253 g/mol (neopterin) and could be expected to cross the placenta to some extent. Secondly, lipophilicity (LogP) and molecular charge at physiological pH may further determine the extent of diffusion. However, negatively charged, lipophilic small molecules such as tolbutamide and chlorpropramide (molecular weight 270–277 g/mol, LogP of approximately 2) have been reported to successfully cross the placenta 
ex vivo [41]. We show this is also the case for anthranilic acid. For this compound, a LogP of 1.2 indicates a moderate degree of lipophilicity and despite a net negative charge at physiological pH the compound still demonstrated rapid transfer. In line with the diffusion hypothesis, uremic solutes with lower fetal-to-maternal transfer ratios did tend to exhibited lower LogP values and higher molecular weights. Next to diffusion, others have demonstrated that transport of small anionic molecules may be a result of naturally-occurring denudations in the syncytiotrophoblast cell layer, while transepidermal pores or trans epithelial channels are also hypothesized to play a role in the transplacental transport [42–44].

In this light it is noteworthy that kynurenic acid displays a much lower fetal-to-maternal transfer rate compared to the other molecules. Compared to anthranilic acid, kynurenic acid is only slightly higher in molecular weight (189 g/mol), also exhibits a net negative charge and a similar LogP. To our knowledge we are the first to describe the transfer characteristics of kynurenic acid across the human placenta. Our findings are in line with Goeden et al. who found similar results for kynurenic acid in mice. They demonstrated that kynurenine could cross the murine placenta, but not kynurenic acid [45]. Possibly, the difference in transfer follows from differences in plasma protein binding between these anions, or this points to regulation by transporters [46]. To our knowledge the complete vectorial transport pathway across the placenta remains to be elucidated for the compounds studied. Yet, indoxyl sulfate may be transported across the basal membrane of the syncytiotrophoblast by Organic Anion Transporter 4 (OAT4, SLC22A11), in a bidirectional manner [47]. At the apical side, both indoxyl sulfate and indole-3-acetic acid could be subject to efflux via Breast Cancer Resistance Protein (BCRP, ABCG2) [48]. In a recent paper, Anoschenko et al. determined the absolute membrane abundance of several placental transporters via quantitative proteomics, confirming high expression levels of OAT4 and BCRP, next to P-glycoprotein, Serotonin Transporter, Noradrenalin Transporter, Organic Cation Transporter 3 and Organic Anion Transporting Polypeptide 2B1 [49]. The low placental transfer of kynurenic acid may also be a result of active efflux via BCRP [37,48,50]. This would also be in line with the asymmetry in the appearance of kynurenic acid in maternal and fetal perfusates when no uremic solutes were added to the maternal reservoir, as we report in Fig. 2.

As outlined in Fig. 4, inosine and s-adenosylhomocysteine levels rapidly decreased as a result of instability in presence of blood. An interesting aspect of this observation is that these levels do not completely decrease to non-detectable levels, but stabilize at concentrations similar to those observed in the perfusates from cotyledons not exposed to uremic solutes 
ex vivo. This steady-state situation means that the placental release of inosine and S-adenosylhomocysteine has reached a rate that equals their respective degradation in perfusion buffer. This suggests that release rates for these compounds are very high, which could be due to high placental solute concentrations that have been taken up from the blood circulation in 
in vivo. In this respect, it would be relevant to compare concentrations of uremic solutes between placentas form healthy women and women with CKD. Alternatively, because many uremic solutes are waste products of normal metabolism, these compounds may be produced by the placenta itself. Particularly for kynurenine and associated metabolites kynurenic acid and anthranilic acid, it is known that the placenta expresses relevant enzymes for their synthesis. Expression and activity of the enzymes indoleamine 2,3-dioxygenase (IDO) for synthesis of kynurenine from tryptophan, and kynurenine aminotransferase (KAT) for the synthesis of kynurenine from tryptophan, are well described. In this respect, interindividual differences in IDO expression may account for the pronounced increase in maternal kynurenine concentrations observed in only 2 out of 4 placentas [51–53].

The 
ex vivo fetal-to-maternal ratios we report at t = 180 do not necessarily reflect the fetal-to-maternal steady-state concentration ratios observed in 
ex vivo, because for fetal drug exposure the maternal components of the components of the solutes in the fetus are not involved. In non-CKD pregnancies, neopterin cord plasma concentrations are lower than maternal levels, which is in line with our 
ex vivo findings as well [30–32]. However, kynurenine, kynurenic acid, and indole-3-acetic acid exhibit cord-to-maternal ratios of 5, 4 and 3, respectively, which is higher than the ratios we found after 180 min of perfusion [28,33,34].

To our knowledge no clinical data are available on how fetal uremic solute levels may have been altered by high maternal plasma...
concentrations during CKD. This information may be partly inferred from comparing normal cord concentrations with reported plasma concentrations in non-pregnant CKD patients. For kynurenine, the normal fetal concentration of approximately 1000 ng/mL is in fact similar to concentrations reported in non-pregnant CKD patients [29, 39]. As a result, fetal kynurenine levels may not be largely affected. Yet for other solutes, the concentrations found in non-pregnant CKD patients can largely exceed the normal fetal concentrations. For example, reported levels in non-pregnant CKD patients compared to reported cord concentrations in normal pregnancies can be as different as 300 vs 12 ng/mL for kynurenic acid, 9000 vs 300 ng/mL for indole-3-acetic acid and 120 vs 11 ng/mL for neopterin [28–30,39,54,55]. This suggests that fetal exposure to those solutes has increased significantly under CKD conditions. The ex vivo data presented in this paper provide insight into which solutes will more rapidly equilibrate between mother and fetus than others, in case of maternal uremic solute overload.

In conclusion, after acute (180 min) exposure to uremic solutes, the extent of placental transfer differs substantially for the compounds studied. Future studies should investigate to what degree fetal concentrations of specific uremic solutes are altered during CKD, and how these may interfere with organ function and development of the unborn child.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2020.12.015.

References


