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Deoxynivalenol transport across the human placental barrier

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ABSTRACT

Deoxynivalenol (DON) is the most commonly detected mycotoxin contaminant of cereal crops and cereal based food products in temperate regions of the world. DON causes adverse health effects in animals, passes through to the foetus and causes foetal abnormalities in animals. Biomonitoring for DON has revealed frequent human exposure. This study reports on DON transfer across the human placenta. Firstly, *in vitro* studies with the BeWo b30 clone were used as a rapid screening model showing transfer of DON through a stable confluent cell monolayer. Five term placentas were then used to study DON transfer with the *ex vivo* dual perfusion model. The concentration of DON on the foetal side after 4 h was about 21% of that on the maternal side at $t = 0$. These results support the data from the BeWo monolayer model in respect to the transport rate of DON, and are consistent with our hypothesis of foetal exposure to DON during pregnancy.

1. Introduction

Mycotoxins are secondary metabolites produced by fungi and are natural and frequent contaminants of cereal crops throughout the world (CAST, 2003). Deoxynivalenol (DON) is a secondary metabolite of the fungi's *Fusarium culmorum* and *Fusarium graminearum*, and the most commonly detected mycotoxin contaminant of wheat, barley and maize based food products in temperate regions of the world (Pestka, 2010; Turner, 2010). The stability of DON makes it survive both storage as well as food processing including heating (Jackson and Bullerman, 1999), and therefore, human exposure to DON will be frequent (SCOOP, 2003; Turner, 2010c; Turner et al., 2008a). DON has been associated with a number of adverse health effects in exposed animals, including reduced food consumption, vomiting, weight loss, diarrhoea and both immunological and neurological dysfunction (Rotter and Oh, 1996; Schlatter, 2004). At a cellular level, DON binds to ribosomes that inhibits protein synthesis (Ehrlich and Daigle, 1987), and activates a signalling pathway known as the ribotoxic stress response (Pestka and Smolinski, 2005). Epidemiological studies strongly support association of exposures to mixtures of mycotoxins including DON, with numerous food poisoning incidences in China (1961–1981) (Luo, 1988) and India (1987) (Bhat et al., 1989), where on occasions thousands and even tens of thousands of people were affected, with symptoms typically observed in DON exposed animals (Pestka, 2010).

The effect of DON on growth and immune suppression is of particular concern, thus understanding exposure in early life is clearly important. DON transfer via the placenta to the foetus has been observed in different species. After exposure to pregnant sows, DON was detected in foetal plasma (Goyarts et al., 2007), liver and kidney (Tiemann et al., 2008b). Further, the exposure was associated with growth restriction (Tiemann et al., 2008a). In rats, relatively high doses of up to 5 mg/kg DON given to pregnant females on days 5–19 of gestation impaired foetal development (Collins et al., 2006). DON passes the placenta in animals, and it is therefore likely that the human foetus will also be exposed following maternal ingestion. A Norwegian epidemiological study revealed an association between grain farming and late-term abortions, and in particular seasons with poor quality harvests, data was supportive of a link with mycotoxins (Kristensen et al., 1997), and later studies have indicated that grain and or silage handling can lead to mycotoxin exposure (Garon et al., 2006; Turner et al., 2010a). As there is limited information on the transfer of DON to the human foetus, placental transport experiments with the placental perfusions system (Mathiesen et al., 2010) are valuable tools to better understand such events.

Several models are available for investigating the kinetics of xenobiotic placental transport. Studies in animal models can be used for the assessment of different uptake, route, and accumulation in organ of the mother and the foetus though there are differences in the placental structures and in the gestational lengths between commonly used laboratory animals and humans (Enders and Carter, 2004). Therefore, the use of human cell lines and human placentas are preferred for human risk assessment. The *ex vivo* perfusion model, using human placentas, is a validated model, as it contains the complexity of the whole organ.

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The rate-limiting barrier for the exchange of compounds between the maternal and foetal circulations is the syncytiotrophoblast cell layers, formed by fusion of cytotrophoblast cells, in the placental cotyledon. The BeWo cell line is an immortalized trophoblastic cell line of human origin, used in the *in vitro* BeWo cell transport model (Poulsen et al., 2009). In contrast to *ex vivo* perfusion, this model is suitable for fast screening of kinetics with regard to transport of compounds. The b30 clone, originated from the human BeWo cell line, forms a confluent monolayer consisting of tight junctions and thereby mimics the rate-limiting barrier (Bode et al., 2006; Poulsen et al., 2009).

The *in vitro* and the *ex vivo* models are used for studies of placental transport of DON in this report. Firstly, *in vitro* studies with the BeWo b30 clone were used as a screening model to assess DON transfer kinetics. As a supplement, trophoblast function was investigated by measuring β-hCG secretion from the DON exposed BeWo cells (Rama et al., 2004). Secondly, DON concentrations in five cord blood and placental tissue samples were measured, alongside *ex vivo* dual perfusion models of DON transport in these human placentas (Mathiesen et al., 2010).

2. Material and Methods

2.1. BeWo b30 cell culture

BeWo b30 clone (hereafter referred to as b30 cells) (kindly provided by Dr. Margaret Saunders, Bristol, UK, with permission from Dr. Alan Schwartz) was used for transport experiments, following previously described protocol (Bode et al., 2006; Poulsen et al., 2009). Cells were cultured in DMEM F12 Ham (Sigma–Aldrich, Ayrshire, UK) containing 10% Foetal bovine serum (FBS), (*In vitro*, Copenhagen, Denmark), 2% glutamine (Panum Institute, University of Copenhagen, Denmark) and 1% penicillin/streptomycin (100 U/ml) (Panum Institute, University of Copenhagen, Denmark) at 37 °C and 5% CO_2. Cells were sub-cultured when 80–90% confluent, using 0.25% EDTA-trypsin (*In vitro*, Copenhagen, Denmark). When the b30 cells were confluent, 10^{-4} cells/well were seeded for MTT in a 96 well microtiter plate (4 replicates) in DMEM F12 Ham, grown overnight and then incubated with DON (Sigma–Aldrich, Ayrshire, UK) (50 nM–50 μM) or medium with vehicle (0.1% ethanol) for 24 h.

2.2. BeWo b30 cell viability

Cell viability was measured by MTT assay (Mosmann, 1983). After 24 h of incubation with DON, MTT (5 mg/ml) (Sigma–Aldrich, Ayrshire, UK) was added at 1:10 ratio to each well and incubated for 4 h. Medium was removed, cells re-suspended in 100 μl DMSO and absorbance was measured at 540 nm. Wells containing medium only were used as background and data was expressed as percentage of viability of the control cells.

2.3. BeWo b30 Transport assay

When b30 cells were 80–85% confluent, they were seeded on human placenta collagen (Sigma–Aldrich, Ayrshire, UK) pre-coated Transwell® filters (pore size 0.4 μm, 1.12 cm^2, polyester, Corning Costar, NY, USA) at a density of 112,000/0.5 ml and grown for 5 days until confluent. Collagen 2.9 mg/ml in 0.1% acetic acid was diluted 1:3 with 70% EtOH and filters were coated with 1 ml of diluted collagen, dried (3 h at room temperature), and then placed under UV light (1 h). Transepithelial electrical resistance (TEER) was measured with an Endohm chamber and Voltohmmeter (EVOM, World Precision Instruments Inc.) to assess integrity of the monolayer before starting the assay. 0.5 ml media containing DON (final concentration 337 nM or 100 ng/ml in 0.1% EtOH) was added to the apical compartment. Samples (150 μl) taken over a 12 h period from the basolateral compartment were replaced by media. The mass transported at each sampling point was corrected for the mass removed during the previous sampling periods as previously described (Poulsen et al., 2009) using the following equation: $\Delta Q_n = c_n V_w + \sum_{j=1}^{n-1} V_s \cdot C_j$ For analysis of DON uptake and retention in collagen and filters, cells were lysed overnight at 5 °C, and lysing buffer was collected and stored at −20 °C. An ELISA was used to measure DON, as described in Section 2.4.

2.4. ELISA measurement of DON

Samples from the b30 cell monolayer transport assay were analysed with High sensitivity Veratox DON test kit (Neogen, Lansing, USA). The test kit was developed for quantitative analysis of DON contamination in food and therefore a modified protocol was used. DON standards (0–100 ng/ml, detection limit 5 ng/ml) were prepared in cells culture media and absorbance was read at 630 nm.

2.5. Protein concentration

Protein concentration from cell lysates were measured at the end of the transport experiment with BSA assay (Thermo Scientific, Rockford, USA) as a control for amount of cells in each well.

2.6. BeWo cell culture

BeWo cells (ATCC, Cat. No. CCL-98), were used for *in vitro* experiments to assess cell viability and β-hCG secretion. BeWo cells were cultured in DMEM (Sigma–Aldrich, Ayrshire, UK) supplied with 10% FBS, 2% glutamine and 1% penicillin/streptomycin (100 U/ml) at 37 °C and 5% CO_2. BeWo cells were seeded for MTT in the same manner as the b30 clone and culture media was replaced after ~4.5 h with media containing 2% FBS and grown overnight. Cells were exposed for 24 h with the same concentrations of DON as detailed in Section 2.1, and MTT assay conducted as in Section 2.2.

2.7. β-hCG secretion

Fifty μl of medium was collected from the 24 h BeWo cell viability assay immediately before adding MTT. β-hCG secretion was assessed using an immunoenzymometric assay (Radim Dianostic, Italy).

2.8. Cord blood and tissue samples

The study protocol for placenta perfusions has been approved by the regional Ethics Committee (KF 01-145/03 + KF(11) 260063) and the Data Protection Agency. Human placentas were collected after informed consent from the donors. Five term placentas were collected after vaginal delivery or elective caesarean at the University Hospital of Copenhagen, Denmark. Umbilical cord blood was collected immediately after birth; plasma was isolated by centrifugation (4000*g*, 5 min) and stored at −20 °C for later analysis.

Placenta tissue from before and after perfusion (perfused cotyledon and tissue surrounding the perfused cotyledon) was homogenized by blending (Dispo mix, Xiril, Switzerland in Gentle MACS Tubes, Miltenyi, Germany). DON was extracted from 1 g tissue with acetonitrile twice and dried *in vacuo*; the pellet was re-suspended in 1 ml PBS pH 7.4 and analysed, see Section 2.11.

2.9. Placenta Perfusions

The *ex vivo* dual perfusion model was used, as previously described (Mathiesen et al., 2010). Placentas were collected immediately after delivery and flushed with Krebs Ringer Buffer (Mose et al., 2007) containing glucose (9 mM) and heparin (5000 IU/ml). In the laboratory a cotyledon was cannulated and perfused with RPMI cell medium (Panum Institute, University of Copenhagen, Denmark) supplied with physiological concentrations (30 g/L in maternal and 40 g/L in foetal compartment) of human albumin, and two cannula's were gently placed in the maternal side. The lobule containing the perfused cotyledon was separated from the placenta and placed in a perfusion chamber. The circulation in the maternal and foetal compartments was sustained by pumps at 9.0 and 2.8 ml/min, respectively. The system was kept at 37 °C and pressurised with oxygen and nitrogen to maintain oxygen level between 20–60 kPa on the maternal side and 10–15 kPa on the foetal side throughout the perfusion. Antipyrine (Aldrich–Chemie, Steinheim, Germany) (final concentration 100 μg/ml in maternal reservoir) was added to the maternal compartment at the start of the perfusion, together with DON, and used as a quality control parameter together with fluid loss, oxygen transfer, stable flow and stable pH, in the classification of successful perfusions (Mathiesen et al., 2010). Samples (1.3 ml) were collected before and immediately after addition of DON (final concentration 100 ng/ml in ethanol vehicle (final concentration 1 μl/ml)) ($t = 0$) and at several time points over the 4-h perfusion. Samples were centrifuged (4000*g*, 5 min) and supernatants stored at −20 °C until analysed, see Section 2.11. As a control for unspecific binding to the tubes and perfusion chamber, a perfusion was conducted with a closed maternal circulation, without foetal circulation or the placenta in the chamber. DON and antipyrine were added to RPMI and the control perfusion conducted with sampling as described above.

2.10. Antipyrine analysis

Antipyrine was used as a positive control in placenta perfusion assays (Mathiesen et al., 2010). The concentration of antipyrine was analysed by high-pressure liquid chromatography (HPLC) as previously described (Mathiesen et al., 2010). Prior to analyses, albumin was removed by adding 200 μl ice-cold acetonitrile containing phenacetine (10 μL/ml) as internal standard (IS). Samples were centrifuged (10 min, 4000*g*) and 200 μl of the supernatant was used for antipyrine analysis.

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2.11. DON extraction and analysis in placenta perfusion

DON was extracted from tissue and perfusion samples, and then quantified by liquid chromatography–mass spectrometry (LC–MS) as described (Turner et al., 2008a). The samples were analysed in six batches with two positive and one negative control run with each batch. The negative control was PBS and the positive QCs contained DON (10 ng/ml). In brief, to 1 ml of sample was added 1 ml of phosphate buffer (pH 6.8), and the solution adjusted to pH 6.8. $^{13}C_{15}$ DON (125 μl, 160 ng/ml, Sigma) as an IS was added to all tests and controls and samples and incubated for 18 h at 37 °C with β-glucuronidase (7000 U, Type IX-A from *E. coli*; Sigma). Following digestion samples were centrifuged (2000*g*, 10 min), and the supernatants were diluted to 8 ml with phosphate buffered saline (pH 7.4) prior to passage through DON wide bore IAC columns (Analytical Ventures, UK). After loading IAC columns, a wash step with 10 ml of water removed unbound material, and DON was subsequently eluted with 4 ml of methanol, dried *in vacuo* and reconstituted in 250 μl of 10% (v/v) ethanol for analysis. LC–MS was conducted as previously described (Turner et al., 2008b) using a Waters 2795 separations module (Milford, MA) connected to a Micromass Quattro Micro triple quadrupole mass spectrometer (Manchester, UK). The separation of DON was achieved using a Luna C18 column (150*4.6 mm, 5 μm particle size, Sigma). The LC–MS standard curve was constructed using seven concentrations of DON (Sigma), range 2–250 ng/ml, each spiked with the IS. Selective ion recording was used to quantify the individual DON levels by reference to the IS. The limit of quantification (LOQ) in urine and plasma was 0.5 ng DON/ml. Test samples were quantified by reference to a response-ratio calibration curve generated by Quanlynx software. No DON was detected for any of the control samples ($n = 6$) and DON was detected for all positive QC samples (mean: 10.1 ng/ml, 95% CI: 9.8–10.4 ng/ml).

2.12. Statistical analysis

All data were analysed using GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA) Data from the cell viability studies were analysed by a one-way ANOVA followed by a Dunnett's test. Placenta perfusion data comparing foetal and maternal levels were analysed by student's *t*-test. Placenta perfusion data comparing antipyrine and DON was analysed by one-way ANOVA followed by a Tukey's test. *P*-values $\leqslant 0.05$ were considered statistically significant. Indication Permeability coefficient (IPC_{30}) was calculated by linear regression of the FM ratio curve in the first 30 min of the experiment. Effective Concentration for 50% reduction in viability (EC50) was calculated by converting concentration to logarithms and performing nonlinear regression.

3. Results

3.1. DON functional effect on BeWo cells

b30 cells were exposed to DON in different concentrations (50 nM–50 μM) or medium with vehicle (0.1% ethanol) in the controls. After 24 h exposure, cell viability was negatively correlated to DON concentrations (Fig. 1), with an apparent EC50 at 40 μM. BeWo cells gave a similar response, though they were slightly more sensitive than the b30 clone with EC50 at 21 μM (see Appendix). In contrast to the b30 clone, BeWo cells secrete β-hCG. β-hCG showed a dose response decrease with increasing concentrations of DON at high DON concentrations. There was no effect on β-hCG secretion at 337nM (100 ng/ml) DON (see Appendix).

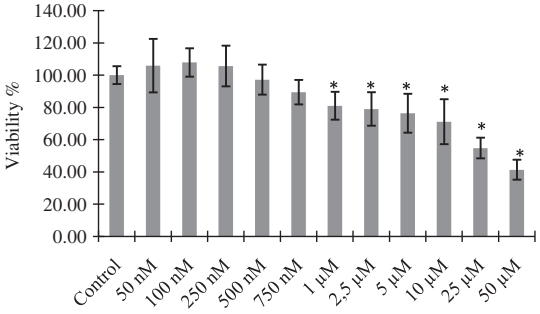

Fig. 1. Cell viablity of BeWo b30 clone, after 24 h exposure to DON in concentrations from 50 nM to 50 μM. Viability is expressed in % of control and data are mean of 4 replicates from 3 experiments. Control cells were exposed to 0.1% ethanol in cell media and set to 100%. $^{*}p < 0.05$. Values are expressed as mean ± SD.

3.2. Transcellular transport across BeWo b30 cell monolayer

The integrity of the b30 cell barrier was measured by TEER and visually inspected by light microscopy. On day 5 of seeding the cells were confluent. TEER was 50.7 ± 3.6 Ω cm^2 prior to adding of DON and 54.8 ± 9.6 Ω cm^2 at the end of the experiment, showing that the cells barrier was intact during the experiment and the concentration of DON did not cause gross toxicity to the b30 cells. Protein concentration was 369 ± 50 ng/well for the wells with cells and 247 ± 38 ng/well for the control wells. All wells were coated with placenta collagen, thus protein was present in controls.

In the absence of cell monolayer the amount of DON in the basolateral compartment increased after 12 h such that it represented 107% of that in the apical compartment at the start of the experiment. In seeded wells the basolateral compartment contained 59% of the initial DON, indicating that the cell monolayer was able to transport DON (Fig. 2). For comparison with other compounds transported across the cell monolayer, the transported mass of compound was calculated as described under methods (Section 2.3).

3.3. Transplacental transport

Five placentas were collected after caesarean section and one from vaginal birth. The average age of the women was 33.2 years, one smoked during pregnancy and two took prescribed medicine, a single dose of morphine and the drug ondansetron to prevent nausea and vomiting, respectively.

The concentration of DON remained constant during the control perfusion (mean 72.1 ng/ml, 95%CI: 70.6–73.6 ng/ml), and showed no non-specific binding of DON to the experimental system during the study time course. Four placentas were successfully perfused according to quality criteria previous described by (Mathiesen et al., 2010). Perfusion on one of the caesarean section placentas lost 22.7 ml (4.7 ml/h) from the foetal circulation (Table 1), but 188 min into the perfusion the loss was 10 ml (2.6 ml/h) and the flow was steady. The results from the perfusion were considered successful until 3 h and data does not differ from the 4 successful perfusions. Passage of DON across the placenta barrier was slower than the reference compound antipyrine (Fig. 4), as indicated by the FM ratio (foetal/maternal ratio). This is also reflected, when comparing the IPC_{30} (Indication Permeability coefficient; the slope of the FM ratio curve in the first 30 min of the experiment) (Table 1) for the 2 compounds, being 0.0163 for antipyrine and 0.0036 for DON. IPC_{30} values are useful when ranking compounds in regard to the transport rate. At the end of the perfusion, 21 ± 6% of the

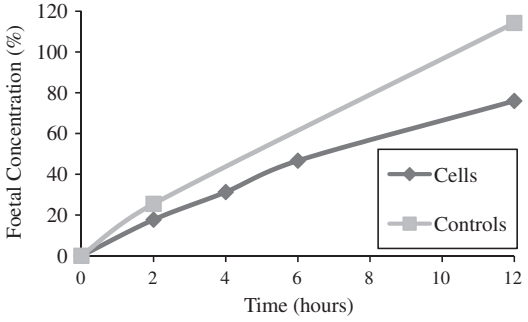

Fig. 2. Accumulation of DON in the foetal compartment (cells, $n = 6$) during 12 h transport experiment across BeWo b30 cell monolayer. The transport data are represented as % of added DON to the maternal compartment at time zero and compared to the transport across coated transwell insert without cells (controls, $n = 3$). Each point is one measurement of pooled samples from transwells with cells or controls.

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Table 1
Data collected from the individual donors (1–5) of placentas used for perfusion with DON, selected quality criteria and data on recovery of added amount DON. Recovery is expressed as % of initially added DON.

Parameters	1	2	3	4	5	Mean ± SD
Volume loss foetal compartment (ml/h)	2.0	1.4	1.6	0.7	5.7[a]	1.4 ± 0.5[b]
Foetal flow (ml/2 min)	2.8	2.9	3.1	2.8	2.8	2.9 ± 0.1
Time from birth to laboratory (min)	20	25	21	25	22	22.8 ± 2.6
Pre-perfusion (min)	30	31	32	44	48	34 ± 6.6
Maternal age (years)	36	39	33	26	32	33.5 ± 5.6
Placenta weight (g)	632	942	724	638	580	734 ± 145
Cotelydon weight (g)	20.8	31.1	22.5	30.1	11	26.1 ± 5.2
Perfused tissue weight (g)	100.8	146.7	86	99.6	102.7	108.3 ± 26.5
Caesarean section (Yes/No)	Yes	Yes	No	Yes	Yes	–
Gestation age (days)	273	262	288	280	275	276 ± 11
Prescribed medicine (Yes/No)	No	Yes	No	No	Yes	–
DON recovery [c] (%)	76	63	88	90	80	79 ± 12
Accumulation in cotelydon (ng DON/g tissue)	31.4	69.9	29.6	42.3	31.5	41 ± 17
Accumulation in surrounding tissue (ng DON/g tissue)	7.4	13.8	25.9	0.9	33.4	16 ± 13
IPC_{30}[d] for DON	0.0044	0.0037	0.0032	0.0028	0.0038	0.0036 ± 0.0001
IPC_{30} for antipyrine	0.0147	0.0159	0.0144	0.0160	0.0208	0.0163 ± 0.003

[a] Foetal volume loss was 10 ml (2.6 ml/h) at 188 min and thereafter the volume loss dropped to 22.7 ml at 240 min. Additional quality parameters and data from this perfusion do not differ from the rest of the perfusions and data are considered to be valid.

[b] Data from perfusion #5 is excluded from the mean and SD.

[c] Recovery was calculated by the following equation, using the mass of DON re-found in tissue (cotyledon and after tissue), in samples taken from maternal and foetal reservoir during the perfusion, and in foetal and maternal reservoir at the end of the perfusion:

$$\text{Recovery} = \frac{M_{\text{tissue}} + M_{\text{reservoir samples}} + M_{\text{foetal reservoir end}} + M_{\text{maternal reservoir end}}}{M_{\text{maternal reservoir start}}} * 100$$

[d] IPC_{30} (Indication Permeability coefficient) is the slope of the FM curve in the first 30 min of the experiment. The slope was calculated by linear regression using the FM values from the first 30 min of the perfusion.

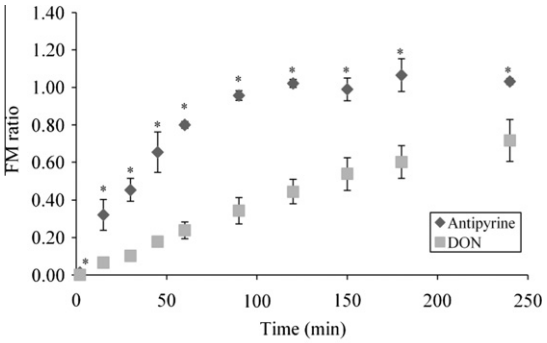

Fig. 3. Foetal-maternal (FM) ratio of DON and antipyrine. FM ration reached approximately 1 within 120 min, indicating equilibrium in the foetal and maternal compartment. DON reaches equilibrium at the 4 h point, indicating a less rapid transport rate across the placenta barrier, than seen for antipyrine. *$p < 0.05$. Values are expressed as mean ± SD.

initiated added DON was in the foetal compartment and 31 ± 6% in the maternal compartment (Fig. 3). The rest was bound to tissue or components in fluids. The recovery, in regard to initially added amount of DON, was 79 ± 11% for the five perfusions. Accumulation in the placenta tissue was 41 ± 17 ng DON/g tissue in the cotyledon and 16 ± 13 ng DON/g surrounding tissue (Table 1), giving a accumulation of 17 ± 6% of the added DON in the perfused tissue during the four hour experiment. DON was not detected in umbilical cord blood plasma from any of the five placentas, indicating that DON did not accumulate in the foetal circulation during the pregnancy of the five mothers.

4. Discussion

Studies on transport across the placental barrier are important in order to assess knowledge of the unborn child's exposure to environmental pollutants and other harmful substances. The mycotoxin DON has been associated with food poisoning in both humans and animals (Pestka, 2010) as well as with immunological and neurological dysfunctions in animals (Rotter and Oh, 1996; Schlatter, 2004). This report provides novel data on DON transfer through the human placenta. Transport across the BeWo cell monolayer was used as a preliminary *in vitro* study providing information on transport kinetics and toxicity of DON, before performing *ex vivo* placenta perfusion.

Transport studies across the b30 cell monolayer were performed at non-toxic concentrations based on results from the viability assay. β-hCG is considered to be a marker of endocrine activity of syncytiotrophoblast cells, which covers the epithelial of the chorionic villi (Rama et al., 2004). Though, the b30 clone was used for the actual transport assay, it does not secrete β-hCG and therefore the BeWo cells were used to estimate DONs effect on cell function. The β-hCG secretion assay indicated no effect at 337 nM, and subsequently DON at 337 nM was to assess transport across the b30 monolayer. TEER analysis additionally confirmed both a lack of gross toxicity and monolayer 'leakiness' via paracellular routes during transport studies.

Previous studies comparing the BeWo b30 cell model with placental perfusions, showed positive correlations in transport rates between the two models (Correia Carreira et al., 2011; Poulsen et al., 2009). Studies with antipyrine in the b30 cell model indicate that the transport rates with and without cells are almost the same (Poulsen et al., 2009); antipyrine cross the placenta barrier via passive diffusion (Schneider et al., 1972). As the b30 study with DON indicated a lower transport rate with than without cells, we decided to run the perfusion for 4 h and not 2.5 h, which is usually used for compounds with rapid transport rate (Mathiesen et al., 2010; Poulsen et al., 2009). Further, we also used albumin for the perfusion to mimic the *in vivo* situation. A physiological concentration of albumin has been shown to promote transport of benzo(a)-pyrene across the placental barrier and thereby increasing the foetal exposure to the toxin (Mathiesen et al., 2009).

For the actual placenta perfusions, five sets of data were available. Over the time course of the study DON levels reduced in the maternal chamber, and were mirrored by an increase in the foetal chamber. There was good reproducibility in the measures at every time point of sampling in the placenta perfusion. The

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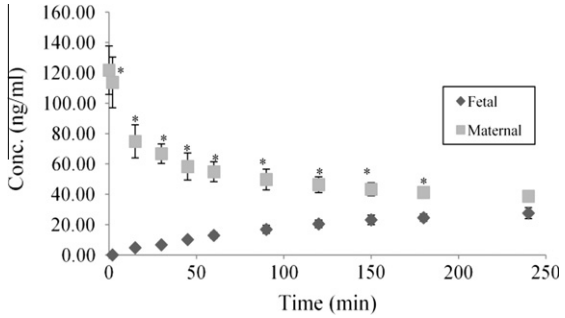

Fig. 4. Foetal and maternal concentrations of DON during 4 h perfusion. DON concentrations quickly declined in the maternal reservoir within the first hour, probably due to accumulation in the tissue; cotyledon and the surrounding tissue, and after 4 h the concentration was 31.8% of the initially added DON. DON slowly increased the foetal reservoir and after 4 h 22.6% of the initially added DON was transported to the foetal side. $^{*}p < 0.05$. Values are expressed as mean ± SD.

FM ratio for DON after 2.5 h was 0.54 ± 0.09. Compared to other substances tested in the placental perfusion system (Frederiksen et al., 2010; Mathiesen et al., 2009; Morck et al., 2010; Mose et al. 2008), DON was ranked as a slower transported compound, similar to benzo(a)pyrene (FM ratio between 0.5 and 0.6) (Mathiesen et al., 2009). By contrast, substances like ethanol (FM ratio [2.5 h] = 1.03 ± 0.07) and bisphenol A (1.00 ± 0.22) were transported more rapidly across the placental barrier (Morck et al., 2010). This is also reflected when comparing the IPC_{30} values for DON (0.0036 ± 0.0006) and antipyrine (0.0163 ± 0.0025); DON was transported 4.5 times more slowly within the first 30 min, than antipyrine, a compound that is passively diffused across the placental barrier (Schneider et al., 1972). It is known that DON crosses the epithelial barriers via paracellular diffusion through the tight junctions in Caco-2 cell monolayer, an intestinal *in vitro* model (Sergent et al., 2006). Further, drug efflux proteins expressed in cell apical membranes can transport DON from the basolateral to the apical side of the monolayer. This has been shown to be the case in Caco-2 monolayer, where accumulation on the basolateral side was increased when blocking ABC transporters (P-gp and MRP2) (Videmann et al., 2007). This may be similar in the b30 model, that DON is transported though the tight junction. Also, P-gp are present in BeWo cells (Evseenko et al., 2006) and are known to be involved in foetal to maternal transport of several other compounds (Morck et al., 2010; Myllynen et al., 2008).

DON in perfusion fluid samples and tissue samples obtained from the placental perfusion experiments were measured by modification of a urinary assay for DON (Turner et al., 2008a). It should be noted that the analytical method was used for the first time to measure DON from extracted tissue samples and the assay has not been specifically optimised for these measurements. However, samples were spiked with an IS at the outset and thus every sample was adjusted for DON recovery throughout the process. QC data were excellent and further, there was a good recovery (79 ± 11% of the initial concentration) (Table 1) of DON at the end of the perfusions. The concentration of DON on the foetal side after 4 h was 21% of that on the maternal side at $t = 0$. These results support the data from the BeWo monolayer model in respect to the transport rate of DON, and are consistent with our hypothesis that the unborn child is being exposed to DON during pregnancy. That no DON was detected in cord bloods is consistent with the short terminal half-lives of DON determined in plasma to between 2 and 12 h dependent on administration route (oral, intravenous) and animal model (cheep, swine, mouse) (Pestka et al., 2008; Prelusky et al., 1988, 1985).

The frequent DON contamination of cereal grains throughout the world has caused concern about the possible public health implications, not only due to acute toxicity at high exposures but also as a result of chronic intakes at moderate levels (Pestka, 2010). Intake estimates within the European Union were made based on average contamination levels of cereal based foods and average intakes of those foods, (>11,000 samples) and average intakes of those foods within 12 countries (SCOOP, 2003). Whilst estimated mean intakes were below the maximum tolerable daily intake (TDI) of 1 μg/kg bw/day, intake may on occasions exceed the TDI, particularly in younger children. In 1997–1998, approximately 1700 US children became ill with symptoms normally associated with DON intoxication. The children were thought to be exposed by burritos, served as school lunch. Analyses revealed DON levels of 0.5 ppm, which is less than the Food and Drug Administration (FDA) advisory level of 1.0 ppm. However, the level is set for adults and it may not be applicable to children; hence DON could not be eliminated as the causal agent (Etzel, 2002; WR, 1999).

DON has been shown to be transported over the placenta in swine (Goyarts et al., 2007). We have presently studied DON transfer over the human placenta at a concentration (337 nM, approximately 200 μg/perfusion) that is relevant for human exposure, considering the TDI of 1 μg/kg bw/day (SCF, 2002) and a mean body weight of 70 kg. The unborn child has been shown to be exposed to other mycotoxins, for instance aflatoxin B1 that showed transfer and metabolic activation when studied with the placenta perfusion model (Partanen et al., 2010). High levels of aflatoxins have been reported in maternal and cord bloods in highly contaminated areas of the world (Abdulrazzaq et al., 2002; Denning et al., 1990). Further, maternal exposure to aflatoxin during pregnancy has been linked to growth faltering in Gambian infants during the first year of life (Turner et al., 2007).

There are few studies using DON exposure biomarkers in humans. However, urinary metabolites have recently been favourably evaluated as a biomarker for DON exposure by comparison to estimated intakes in adults, using a duplicate diet and individual food measure for DON, in the UK (Turner et al., 2010a). DON intake was strongly correlated with the urinary biomarker in this study. Urinary DON has been observed frequently in UK, French, Swedish and Chinese samples to date (Hepworth et al., 2011; Meky et al., 2003; Turner, 2010c; Turner et al., 2008a,b,c, 2009, 2010a,b, 2011) with levels of exposure in some individuals expected to exceed the TDI (SCF, 2002). Some of the highest levels of urinary DON yet recorded were reported for pregnant UK women from Bradford, UK (Hepworth et al., 2011). Therefore, it is important to further understand the levels and consequences of foetal exposure to DON.

5. Conclusion

This work shows that DON was relatively slowly transported across b30 cell monolayer from the maternal side to the foetal side. The more complex *ex vivo* dual perfusion model supported these data, and in this model 21% of the initially incubated toxin was transported to the foetus. DON is known to have an effect on foetal growth and is immunosuppressive (Pestka, 2010). Maternal exposure through diet will lead to foetal exposure to DON. DON requires no activation, and foetal detoxification mechanisms in general are poorly developed, and therefore, foetal exposure should be taking into consideration when evaluating the limit of DON in food.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fct.2011.05.016.

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