PLACENTAL PERFUSION STUDIES WITH 2,3,7,8-TCDD

Tina Mose[1], Line Mathiesen[1], Lisbeth E. Knudsen[1] and Harrie Besselink[2]

[1]Department of Environmental Health, Institute of Public Health, Copenhagen, Denmark
[2]BioDetection Systems BV (BDS), Kruislaan 406, 1098 SM Amsterdam, The Netherlands

Introduction

Dioxin is a generic term for a group of polychlorinated dibenzo-para-dioxines (PCDD) that are formed e.g. during combustion of chlorine containing materials. Dioxins exhibits a high acute toxicity and long term effects such as neuro toxicity, reduced immuno response, cancer, and endocrine disturbances[1]. The group consists of 75 dioxins where 2,3,7,8-TCDD is the most toxic congener. The other dioxins are given a toxic equivalent factor according to their relative toxicity compared to 2,3,7,8-TCDD making it possible to calculate the total toxic equivalence (TEQ) of PCDD in a given sample. Dioxins are persistent in nature and accumulate in fatty matter from animals and fish.

Exposure of the unborn-child to dioxins occurs through transport via the placenta[2,3]. To study the transport of dioxins over the placenta, an *in vitro* placental perfusion experiment was conducted in which 2,3,7,8-TCDD was introduced at the maternal circulation. The concentration of 2,3,7,8-TCDD was then determined using the DR CALUX® bioassay by BDS in both the maternal and fetal circulation and in the cotyledon.

Methods and materials

Human placental perfusion method . Immediately after birth, the fetal circulation in a single cotyledon was re-established by cannulation of the fetal vein and artery (Fig 1)[4]. The cotyledon was placed in the perfusion chamber, with maternal side up. Maternal arteries were connected to the intervillous space. Perfusion media was RPMI cell culture media supplemented with heparin, glucose, glutamine, albumine, and dextran. Antipyrine (100 µg/ml) and TCDD (6.04 pg/ml) were added to the maternal reservoir and samples (5.3 ml) collected from both circulations during 6 hour perfusions. Antipyrine was used as a positive control of proper connection between the established maternal and fetal circulation. Fetal venous outflow, pO_2 uptake in fetal circulation and pH were controlled regularly during the perfusions. Umbilical cord serum samples and placenta samples before and after perfusion were collected and analyzed.

Determination of 2,3,7,8-TCDD TEQ. The DR CALUX® assay was used to measure the total 2,3,7,8-TCDD TEQ in perfusion samples, in plasma, and placental tissue. Approximately 5 g perfusion fluid, 10 g placental tissue or 2 g umbilical cord serum was extracted by shake-solvent extraction (hexane:diethylether, 97:3). Extracted fat was used for clean-up on an acid silica column

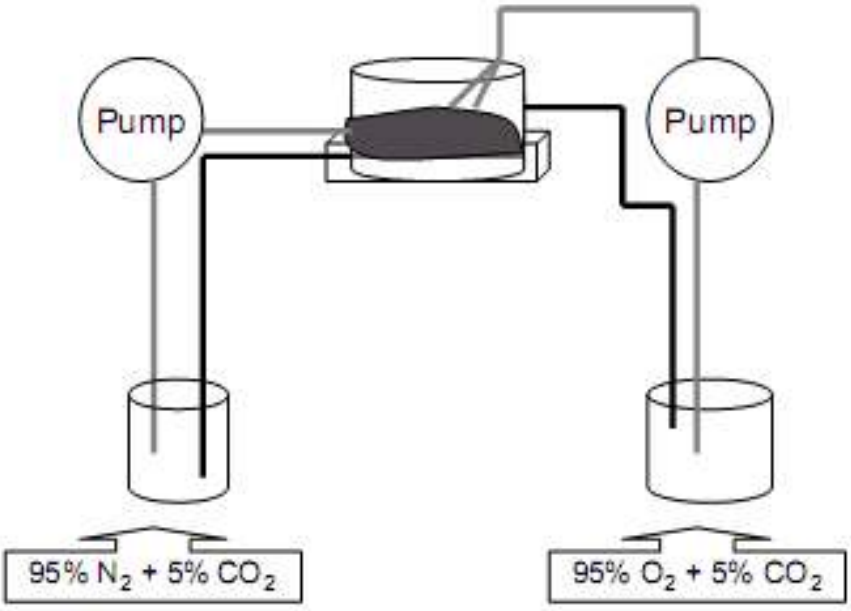

Figure 1. The ex-vivo placental perfusion system. The maternal circulation is on the right hand side aerated with O_2 and the fetal circulation is on the left hand side aerated with N_2. The perfusion chamber containing the perfused placental cotyledon is shown in the middle.

(20% and 33% H_2SO_4), topped with Na_2SO_4. Cleaned extracts were dissolved in DMSO (8 µl); the DR CALUX® activity is determined following 24hr of exposure. Data were corrected for internal reference sample and procedure blanks.

Results

Three successful 6 hour perfusions were performed. The result showed that the total TEQ decreased in maternal circulation from 0.65 pg 2,3,7,8-TCDD TEQ/g media after 2 minutes of perfusion to 0.12 pg 2,3,7,8-TCDD TEQ/g media after 360 minutes of perfusion. No TCDD TEQ was detected in the fetal circulation during or after end perfusion (Fig. 2). In contrast, the positive control compound antipyrine decreased in the maternal circulation and increased in the fetal circulation indicating that transplacental passage was enabled in the present placental perfusion study (Fig.3). In placental tissue from before perfusion 17 pg TEQ TCDD/g fat was found whereas 61 and 46 pg TEQ TCDD/g fat was found in the cotyledon and the surrounding tissue after end perfusion, respectively (Fig.4). The results showed that TCDD accumulated in placental tissue and that TCCD was not measured to cross the placenta under the present experimental setup. The results are supported by data from three other perfusions lasting only 3-4 hours. The total lipid content was approximately 0.6% in placental tissue, 0.25% in umbilical cord serum and no lipids were found in perfusion media. Fetal plasma (cord-plasma) level was measured in newborns from a Danish cohort

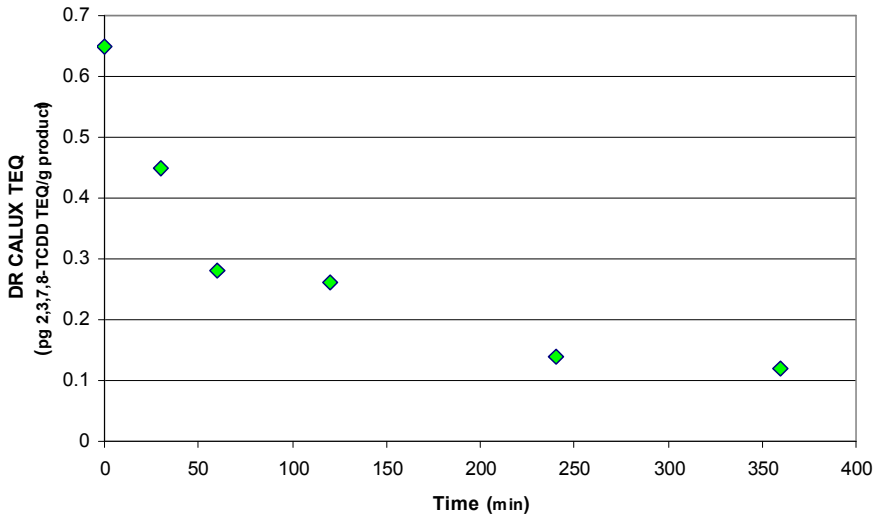

Figure. 2. The level of TCDD in maternal circulation is decreasing from 0.65 to 0.12 pg 2,3,7,8-TCDD TEQ/g media during the perfusions. No detectable levels in fetal circulation were found.

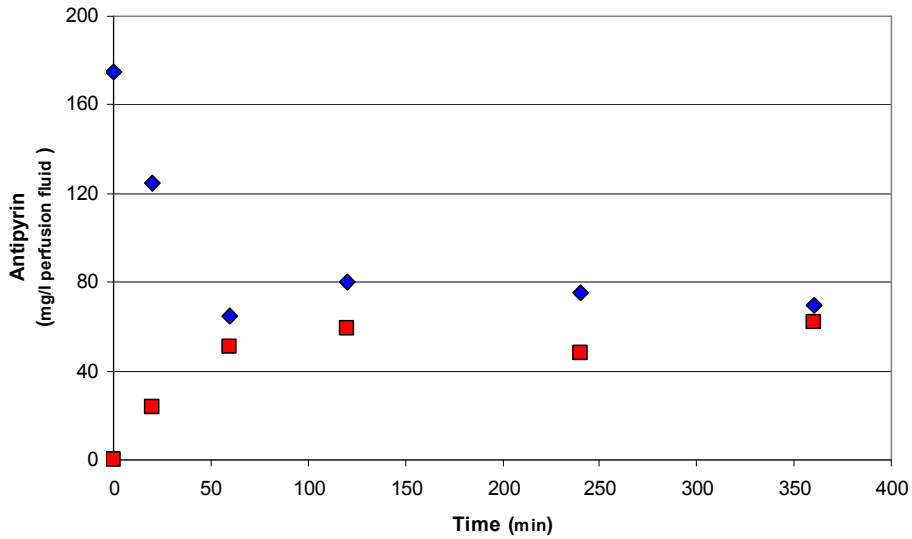

Figure. 3. The transplacental passage of the positive control compound antipyrine (n=2). The level is decreasing in maternal circulation (blue) and increasing in fetal circulation (red). Fetal/maternal concentration ratio after 2 hour of perfusion is 0.73.

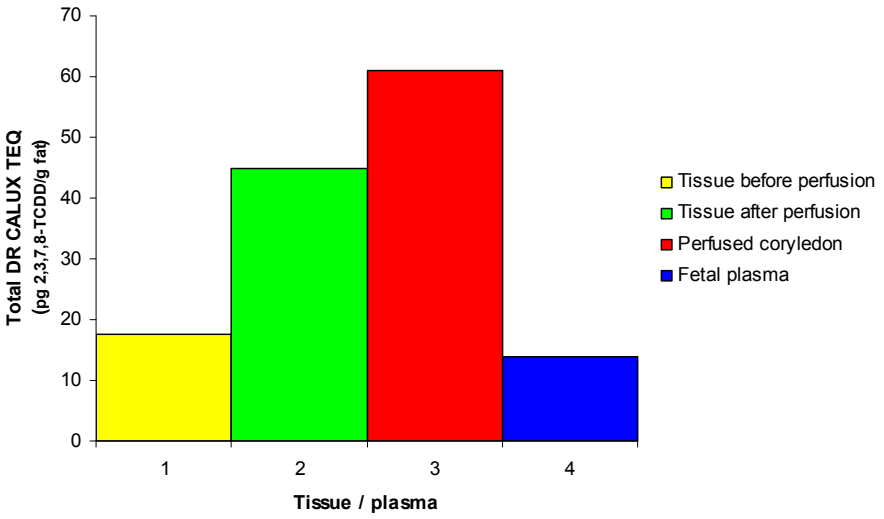

Figure 5. Total TEQ of TCDD in placental tissue before perfusion (yellow), perfused tissue (green), tissue surrounding the perfused tissue during the study (red), and in umbilical cord serum (blue) measured by DR CALUX® bioanalysis

Discussion

It is well known that TCDD is detectable in umbilical cord serum samples indicating that TCDD in individuals is transferred from maternal compartment to fetal compartment. The detection of TCDD in some umbilical cord serum samples suggests that components present in blood but not present in perfusion media, such as lipids and/or proteins might facilitate placental transfer or that longer perfusion times are needed to study this transfer. Adding fetal serum to the fetal perfusion media and thereby increasing the lipid content may enhance the transplacental transfer of TCDD in combination with longer duration of the perfusion.

Acknowledgements

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