Journal of Pharmacological and Toxicological Methods 88 (2017) 25–31

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

How to article

Integration and validation of the *ex vivo* human placenta perfusion model

Sigrid Conings[a], Frédéric Amant[b,1], Pieter Annaert[c], Kristel Van Calsteren[a,*]

[a] Organ Systems, KU Leuven, Department of Development and Regeneration, Leuven, Belgium
[b] Gynaecological Oncology, KU Leuven, Department of Oncology, Leuven, Belgium
[c] Drug Delivery and Disposition, KU Leuven, Department of Pharmaceutical and Pharmacological Sciences, Leuven, Belgium

ARTICLE INFO

Keywords:
Antipyrine
Ex vivo
HPLC
High performance liquid chromatography
Human
Methods
Placenta perfusion model

ABSTRACT

Introduction: The *ex vivo* human placenta perfusion model is an effective and non-invasive method to study transplacental passage of drugs and environmental compounds in humans. Due to many challenges and its high complexity it remains difficult to incorporate it routinely into laboratories.

Methods: This article describes a step-by-step protocol for the implementation and validation of a closed-closed *ex vivo* perfusion model. Antipyrine, a small molecule that passes the placental barrier by passive diffusion, was used as a measurement of overlap between foetal and maternal circulation. The pressure and the flow rate in the foetal circulation, glucose consumption and pH were implemented to ensure the integrity, viability and functionality of the method.

Results: In total 89 placenta were collected of which 34 placentas were successfully perfused with antipyrine and fulfilled all quality control measurements. A foetal/maternal antipyrine concentration ratio of 0.75 was reached within 89 ± 21 min, while 210 min were required to achieve equilibrium. The foetal pressure remained under 70 mmHg during the entire experiment. The end foetal flow was 98% of the foetal starting flow. The average glucose consumption was 0.30 ± 0.15 μmol/min/g. Every 30 min the maternal pH declined to 7.29 ± 0.06 and was adjusted to 7.4. The foetal pH stayed stable at 7.30 ± 0.05.

Discussion: Based on the assessment of multiple quality control measurements, the described method of a closed human ex-vivo placenta perfusion model was validated. The success rate (38%) was more than twice the success rate reported in literature (15%).

1. Introduction

The *ex vivo* human placenta perfusion model is one of the methods to study transplacental transport of drugs and environmental compounds across the human placenta. Other methods include human *in vivo* methods (sampling of umbilical cord blood *via* cordocentesis or at delivery, amniocentesis, chorionvillous sampling), animal models and *in vitro* models like placental explants, primary placenta cells and placental derived cell lines.

These techniques with their advantages and disadvantages are extensively described in reviews by Sastry, Myllynen and Kovo (Myllynen & Vähäkangas, 2012; Sastry, 1999; Kovo & Golan, 2008).

The *ex vivo* human placenta perfusion model with perfusion of a single cotyledon of the placenta was first described in 1967 by Panigel and later modified by Schneider in 1972 (Panigel, Pascaud, & Brun, 1967; Schneider, Panigel, & Dancis, 1972). It can be conducted in two set-ups: an open circulation (single pass or non-recirculating) for calculations of drug clearance at steady-state concentrations and a closed circulation (circulating) for calculations of drug transfer, xenobiotic metabolism and the maternal-placental-foetal distribution (Vähäkangas & Myllynen, 2006). It is the only method that retains the full structure of a full term human placenta, making it possible to study transplacental passage without harming the foetus or the mother.

Although the method exists for a long time, it is still difficult to implement the method in laboratories due to the many challenges including the availability of fresh human placental tissue and the high complexity of maintaining the integrity, viability and functionality of the placenta during the perfusions. In this paper we give a step-by-step protocol for the implementation and validation of the *ex vivo* human placental perfusion model.

* Corresponding author at: Herestraat 49, box 7003, 3000 Leuven, Belgium.
E-mail addresses: sigrid.conings@kuleuven.be (S. Conings), frederic.amant@uzleuven.be (F. Amant), pieter.annaert@kuleuven.be (P. Annaert), kristel.vancalsteren@uzleuven.be (K. Van Calsteren).
[1] Present address: Center for Gynaecologic Oncology Amsterdam (CGOA), Netherlands Cancer Institute, Amsterdam (The Netherlands).

http://dx.doi.org/10.1016/j.vascn.2017.05.002
Received 1 March 2017; Received in revised form 10 May 2017; Accepted 12 May 2017
Available online 15 May 2017
1056-8719/ © 2017 Elsevier Inc. All rights reserved.

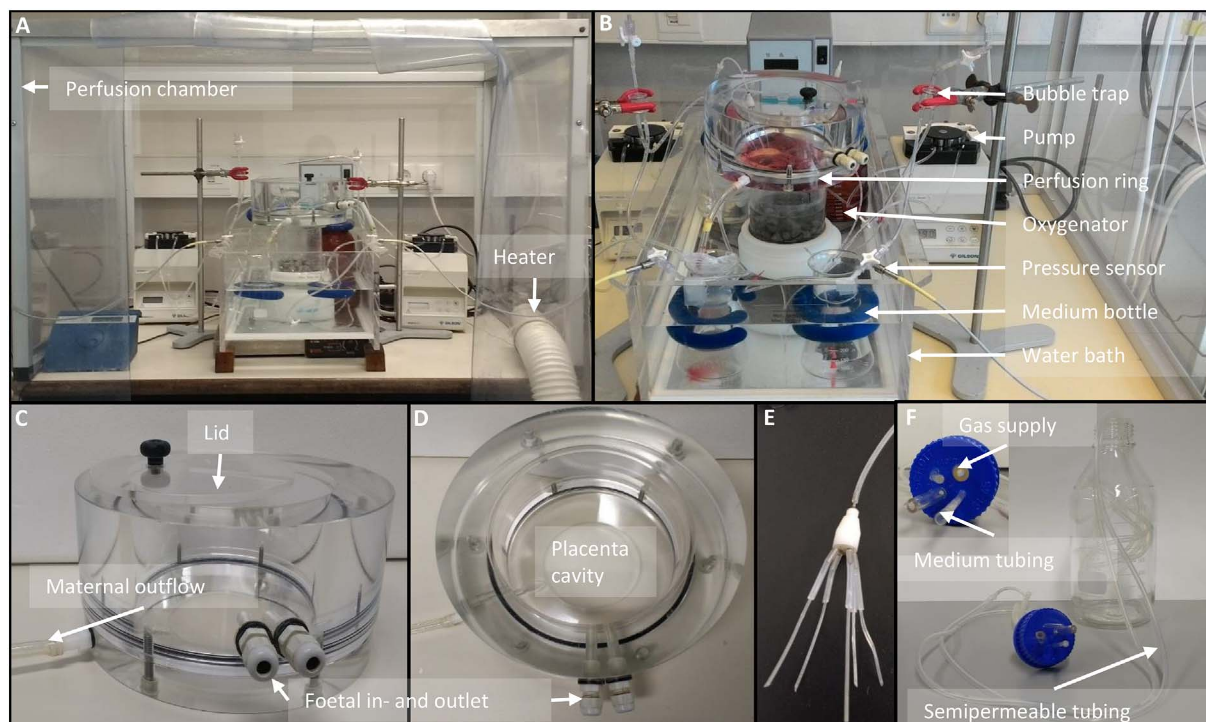

Fig. 1. Placenta perfusion setup. A: perfusion chamber, B: close-up of the set up with bubble traps, pumps, perfusion ring, oxygenators, pressure sensors, medium bottles and warm water bath, C: side view of the perfusion ring, D: top view of the perfusion ring, E: close-up of the maternal perfusion head, F: close up of the oxygenator and the oxygenator lid.

2. Material and methods

2.1. Patient accrual

The placentas were obtained in collaboration with the Department of Obstetrics of the University Hospital Gasthuisberg of Leuven. Only placentas of uncomplicated full term pregnancies (37–41 weeks) were collected. Informed consents were signed by the mothers prior to the delivery. Placentas from both vaginal births ($n = 14$) and elective caesarean sections ($n = 20$) were included. The study was approved by the ethical commission of the UZ Leuven (s54819). EudraCT number 2012–004580-51 (clinicaltrails.gov).

2.2. Dual placenta perfusion model

2.2.1. Placenta perfusion equipment

Our model is adapted from the model described by Schneider (Schneider & Huch, 1985).

The experimental setup (Fig. 1) consists of a heated flow bench (37 °C) with two minipuls peristaltic roller pumps (Gibson, England), a warm water bath at 37 °C (VWR, Belgium), two magnetic stirrings (Hanna Instruments, Belgium), a perfusion chamber (manufactured at the University of Leuven), a nanologger pressure measuring instrument (Gaeltec, the Netherlands), two glass bubble traps (manufactured at the University of Leuven), two oxygenating systems consisting of two 1 L bottles filled with 4 m of mono-lumen semipermeable tubing with internal diameter of 2.64 mm. In the lid 4 holes were made, two for the in- and outlet of the media tubing and two for the in- and outlet of the gasses (all manufactured at the University of Leuven), a perfusion ring consisting of a Plexiglas cylinder with a diameter of 13 cm (manufactured at the University of Leuven), maternal perfusion head consisting of perfusion manifold MPP5 and tubing with internal diameter 1.02 mm (Harvard Apparatus, England), maternal tubing (Tygon, internal diameter 2.79 mm, Harvard apparatus, England), foetal tubing (Tygon, internal diameter 2.29 mm, Harvard apparatus) and connectors (Altec, England).

2.2.2. Media

Media for tissue preparation and washing procedures were prepared with Krebs Ringer buffer (2.52 g/l $NaHCO_3$, 8.30 g/l NaCl, 0.42 g/l KCl, 0.19 g/l KH_2PO_4, 0.35 g/l $MgSO_4$, 0.44 g/l $CaCl_2$), supplemented with glucose (2.61 g/l) and heparin (25,000 UI/l). The Krebs Ringer buffer was bubbled for 15 min until the pH reached 7.2–7.4 with 95% O_2 and 5% CO_2 for the maternal medium and 95% N_2 and 5% CO_2 for the foetal medium.

For the experimental phase Earle's balanced salt solution (EBSS) supplemented with glucose (1.6 g/l), L-glutamine (1%), penicillin and streptomycin (1%) and heparin (25,000 UI/l) was used. For both the maternal and foetal reservoir 250 ml was used. The perfusion media were first bubbled for 15 min until the pH reached 7.2–7.4 with 95% O_2 and 5% CO_2 and 95% N_2 and 5% CO_2 for the maternal and foetal medium respectively. When a pH of 7.2–7.4 was reached, 40 mg/ml of bovine serum albumin (BSA) was added to the foetal medium and 30 mg/ml to the maternal medium to mimic physiological levels of albumin (Syme, Paxton, & Keelan, 2004). To obtain a physiological pH, the pH was adapted to 7.4 on the maternal side and 7.3 on the foetal side. During the entire experiment the foetal and maternal medium were continuously gassed with respectively 95% O_2 and 5% CO_2 and 95% N_2 and 5% CO_2.

Table 1 summarizes the different media used with their specific characteristics.

2.2.3. Tissue preparation

The placenta was collected and weighed at the delivery ward. Within 10 min after the delivery, the placenta was flushed through the umbilical cord arteries with 20–40 ml medium A and transported to the laboratory in an isolated box.

Upon arrival in the laboratory, the maternal side was carefully inspected for ruptures and an intact cotyledon was selected and the amnion membrane was removed. Of the intact cotyledon, the corresponding foetal artery and vein were cannulated (Flocare Pur sonde-MP Ch 6/60, Pharmacy, UZ Leuven, Belgium) while the foetal artery catheter was connected to a dripping bag and the catheters were fixed

Download English Version:

https://daneshyari.com/en/article/5556488

Download Persian Version:

https://daneshyari.com/article/5556488

Daneshyari.com