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Evaluation of the maternal–fetal transfer of granisetron in an *ex vivo* placenta perfusion model

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ABSTRACT

The objective of this study was to estimate maternal–fetal transplacental passage of granisetron in an *ex vivo* placental perfusion model. Term human placentas ($N = 8$) were collected immediately after delivery. A single cotyledon from each placenta was perfused granisetron concentration to mimic systemic maternal peak plasma concentrations following either IV (50 ng/mL) or transdermal administration (5 ng/mL). To assess drug transfer and accumulation, samples were collected from maternal and fetal compartments.

In the 50 ng/mL open model, the mean transport fraction was 0.21 ± 0.08 with clearance index of 0.53 ± 0.66. Fetal peak concentrations achieved was 5.6 ± 6.6 ng/mL with mean accumulation of 5.35 ± 6.4 ng/mL. No drug was detected in the fetal compartment with the 5 ng/mL models.

Transplacental passage of granisetron was inconsistent at the 50 ng/mL concentration that achieved with IV dosing. However, there consistently was no detectable passage in all the placentas evaluated of the granisetron at 5 ng/mL concentration that would be achieved after transdermal patch administration.

1. Introduction

Nausea and vomiting is common in the first trimester of pregnancy and can vary greatly in frequency and intensity and typically resolves by week 14 in most women [1]. The reported incidence is between 50% and 80% of women who will experience some level of nausea during their pregnancy [2]. Severe disease, referred to as hyperemesis gravidarum, is reported to affect 0.5–2% of pregnancies and is associated with significant maternal morbidity and even loss of pregnancy [3,4]. The exact causes of nausea and vomiting in pregnancy (NVP) remain unknown, although, it is thought to arise secondary to elevations in human chorionic gonadotropin (hCG) and estrogen [5].

Typical pharmacotherapy for NVP includes medications such as antihistamines, phenothiazines and serotonin receptor antagonists. Antihistamines and phenothiazines agents are often associated with undesirable side effects such as excessive sedation and phenothiazines can cause extra pyramidal symptoms which have led to a widespread adoption of the use of serotonin receptor ($5HT_3$) antagonists, primarily ondansetron [6,7]. All of the marketed $5HT_3$ antagonist agents are pregnancy category B and offer a favorable adverse event profile. A new formulation of granisetron as a transdermal patch (Sancuso®, ProStrakan, Bedminister, NJ) may offer additional utility for NVP because it can deliver granisetron continuously to achieve steady state concentration of 5 ng/mL or less for up to six days without fluctuating peak concentrations observed with IV and PO intermittent administration once every 12 h. Often the challenge in the outpatient pharmacologic management of NVP is dependency on the oral route which is often unavailable in patients with severe nausea and vomiting. This leads to inpatient admission for intravenous (IV) administration of medication and/or replacement hydration until the NVP or hyperemesis is controlled. A transdermal patch delivery of an effective antiemetic would overcome this barrier and facilitate convenient and less expensive outpatient management of NVP. To date there

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has been limited formal, prospective evaluation of the safety of the class of serotonin receptor antagonist agents antiemetic during pregnancy, with most data available being extracted from retrospective studies or case reports [8–10].

The objective of the current study was to estimate the maternal to fetal transplacental passage of granisetron in the *ex vivo* human placental perfusion model.

2. Methods

2.1. Placenta collection

Term, 37–40 weeks, placentas were obtained from uncomplicated pregnancies immediately after delivery from either cesarean or vaginal deliveries in accordance with the University of Texas Health Sciences Center (UTHealth) at Houston Medical School Institutional Review Board for Human Studies between February 2011 and March 2012. The IRB committee granted a waiver of informed consent for use of the discarded placenta tissues from uncomplicated pregnancies. All placentas were confirmed to be hepatitis A/B/C and HIV negative based prior to collection. Placentas were transported to the laboratory in warmed 0.9% saline solution immediately after delivery.

2.2. Placental perfusion

The single cotyledon placenta perfusion system was used as described by Challier [11–13]. Briefly, a fetal artery and vein on the chorionic plate were cannulated with a 3.0 F and a 5.0 F catheter, respectively to re-establish a vascular circuit. The fetal circulation of the selected placenta cotyledon was gently perfused with Eagle's minimum essential medium containing 1 U/mL heparin from a porcine source (Sigma, St Louis, MO), and 3 g/dL bovine serum albumin (Sigma, St Louis, MO). Adjustment of pH to 7.40 was achieved with sodium hydroxide and 2.2 g/L sodium bicarbonate as indicated throughout the experiment. After confirmation of circulatory integrity, the single cotyledon and its surrounding placental tissue were placed on placenta capsule and secured in place, then transferred to perfusion chamber that was temperature-controlled to 37 °C by the Digi-Sense temperature controller R/S (Cole Parmer, Veron Hills, IL). The fetal circulation was perfused in an open-system at a rate between 4.5 and 4.8 mL/min for approximately 20 min to remove any residual blood and to allow stabilization of circuit. Throughout the experiments perfusion chamber pressure was monitored by a BP-1 Pressure Monitor (World Precision Instruments, Sarasota, FL) and maintained below 60 mmHg. Visual inspection for leaks measurement of any loss of fluid was also monitored and the experiment is aborted if leaks occur that accumulated to greater than 2 mL/h. Once stable pressure was confirmed, to initiate maternal circulation, three 18-guage needle probes were inserted into intervillous space of the cotyledon with perfusion at a maternal flow rate of 17 mL/min. The placental cotyledon preparations that did not achieve a stable pressure secondary to lack of vascular integrity or leaks were discarded. Due to the necessity to establish circulation and perfuse placenta within 30 min of delivery, if the selected single cotyledon failed, there were no secondary attempts for different cotyledon within the same placenta.

The maternal and fetal compartments consisted of 150 mL of Eagle's minimal essential media (pH 7.2–7.4), aerated with 95% oxygen and 5% carbon dioxide and continually mixed by a magnetic stirring bar to reached desired oxygenation levels of 10–15 kPa in fetal and 225–35 kPa in maternal media throughout the study [12]. After system was equilibrated determined by steady pressure with continuous perfusion with media alone, the media with drug was added to the maternal side. Volume loss from fetal chamber was also monitored and ensured to be less than 2 mL/h throughout all experiments.

Placenta transfer studies were performed with granisetron (99% purity, Sigma, St Louis, MO). Granisetron was added to the maternal medium, at two selected concentrations: low: 5 ng/mL which is the maximum plasma concentration (Cmax) achieved with the transdermal administration of granisetron in non-pregnant patients that is being explored as alternative formulation to use in management of NVP and high: 50 ng/mL which is the Cmax achieved with the intravenous (IV) granisetron administration in non-pregnant patients but is also often used in management of NVP. Antipyrine at a concentration of 100 mg/L was added to the media on the maternal side of the model as the quality control of perfusion to evaluate placenta transfer as described in the reference method by Challier [11–13]. Fluid aliquots from both the fetal and maternal compartments were collected at time zero then every 10 min for a total of 70 min for analysis in each experiment. The experiments were first conducted in an open-open (non-recirculation) set-up to determine the transport fraction (TF) (portion of drug that crossed placenta from maternal to fetal side). Experiments were then repeated in a closed-closed recirculation system to determine clearance index (Ci) and accumulation of granisetron that could occur based on a balance of the transport fraction and the rate of clearance from fetal side to maternal side. When transport fraction from maternal side to fetal side exceeds the clearance index from fetal side to maternal side, it leads to accumulation on the fetal side. Studies were completed first in an open system for 1 h and then completed in a closed system for 1 h. All experiments containing the low and high concentrations of granisetron were performed in quadruplicate in separate placenta cotyledon collections.

The transport fractions, clearance, and clearance index of granisetron and antipyrine were determined based on the Challier formula for placental transfer [11–13]. The following equations were used for determination of placental transport: $TF = (CFv - CFa)/(CMa - CFa)$, where TF = the transport fraction; C = concentration (ng/mL); M = maternal perfusate; F = fetal perfusate; a = artery; and v = vein. $Cl = TF^{*}Q$, where Cl = clearance of the compound (mL/min); TF = transport fraction; and Q = flow rate of the fetal circulation (mL/min). Ci = Cl of the granisetron/Cl of antipyrine, where Ci = clearance index. Criteria for successful perfusion required that the antipyrine Ci had to be >0.75 [11–13].

2.3. Analytical detection

2.3.1. Granisetron assay

Granisetron concentrations were quantified by validated high pressure liquid chromatography (HPLC) assay according to parameters described in the CDER Guidance for Industry Bioanalytical Assay Method Validation [14]. Placental perfusion samples were kept in the sub-freezer (−80 °C) until time of analysis and then were thawed at room temperature for analysis. Granisetron was isolated from placental perfusion samples by liquid:liquid extraction with dichloromethane. Liquid chromatographic separation was achieved by isocratic mobile phase of: acetonitrile:0.05 M KH_2PO_4 buffer:triethylamine:phosphoric acid (220:1000:1.5:0.750), adjusted to pH of 4.3 with flow rate 1 mL/min with a Thermo BDS Hypersil C8, 4.6 mm × 250 mm, 5 μm particle size analytical column (ThermoFisher Scientific, Waltham, MA). The granisetron peak eluted at 14.4 min identified by photodiode array detector at a wavelength of 305 nm. The granisetron assay was found to be linear over 2.5–50 ng/mL with a correlation coefficient (r) of 0.998. The coefficient of variation for granisetron assay ranged from 0.16% to 6.3%.