Reproductive Toxicology 53 (2015) 92–98

Contents lists available at ScienceDirect

Reproductive Toxicology

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

Evaluating the potential effect on fetal tissue after exposure to granisetron during pregnancy

Judith A. Smith [a,*], Justin M. Julius [b], Anjali Gaikwad [a], Pamela D. Berens [a], Joseph Alcorn [c], Kenneth J. Moise [a], Jerrie S. Refuerzo [a]

[a] Department of Gynecology, Obstetrics & Reproductive Sciences, UT Health-University of Texas Medical School at Houston, Houston, TX, United States
[b] Division of Pharmacy, The University of Texas MD Anderson Cancer Center, Houston, TX, United States
[c] Department of Pediatrics, UT Health-University of Texas Medical School at Houston, Houston, TX, United States

ARTICLE INFO

Article history:
Received 27 September 2013
Received in revised form 19 February 2015
Accepted 27 February 2015
Available online 24 March 2015

Keywords:
5-HT_3 receptor antagonists
Fetal toxicology
Granisetron
Pregnancy
Safety

ABSTRACT

The objective of this study was to elucidate the possible toxic effects on the fetal tissues after exposure to two clinically relevant concentrations of granisetron. Primary cells were isolated from human fetal organs of 16–19 weeks gestational age and treated with 3 ng/mL or 30 ng/mL of granisetron. Cell cycle progression was evaluated by flow cytometry. ELISA was used to detect alterations in major apoptotic proteins.

Up to 10% apoptosis in cardiac tissue was observed following treatment with 30 ng/mL granisetron. Neither concentration of granisetron caused alteration in cell cycle progression or alterations in apoptotic proteins in any of the other tissues.

At 30 ng/mL granisetron concentration had the potential to induce up to 10% apoptosis in cardiac tissue; clinical significance needs further evaluation. At granisetron 3 ng/mL there was no detectable toxicity or on any fetal tissue in this study. Further research is needed to confirm these preliminary findings and determine if clinically significant.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Nausea and vomiting in pregnancy (NVP) is a common symptom that affects 80% of pregnant women often manageable with over-the-counter medications and non-pharmacological interventions however some women will require use of prescription medications. In more severe cases resulting in serious health complications for both the mother and the developing fetus [1]. While there are no large studies to support their use, 5-hydroxytryptophan receptor type 3 antagonists (5-HT_3) are considered to be safe during pregnancy [2,3]. This consideration is driven by the use of the 5-HT_3 antagonist, ondansetron. Despite consensus agreement, some literature still gives rise to suspicion and reports increased likelihood of some birth defects such as cleft palate [4]. It is known that ondansetron crosses the placenta and is present in fetal tissue at a ratio of 0.41 to maternal plasma concentrations [5]. The effect of these concentrations in developing fetal tissues is unknown.

Granisetron is a 5-HT_3 antagonist commonly used for the prevention and treatment of nausea and vomiting associated with chemotherapy however data on the utilization of granisetron for management of NVP in limited. One case report described successful use of granisetron along with cytotoxic chemotherapy in pregnant women with Ewings sarcoma [6]. To date, no prospective or retrospective human studies have been published which can establish safe use of granisetron for NVP. Recently, granisetron has been formulated into a transdermal delivery system (TDS) by Prostraken, Inc (Galashiels, UK) which allows for sustained drug administration over a period of 5–7 days without reliance on continuous or repeated oral or intravenous (IV) administration [7]. These features demonstrated an improvement in patient compliance and reduction the need for hospital admissions for the management of nausea [8]. Because of the ease of use in the outpatient setting along with low sustained plasma drug concentrations, granisetron TDS (Sancuso, Prostraken, INC, Galashiels, UK) is an excellent candidate for the potential use in the management of NVP.

We recently reported the findings from a dual placental perfusion model that demonstrated the granisetron transferred from maternal to fetal circulations was dependent upon the on maternal

* Corresponding author at: UT Health-University of Texas Medical School at Houston, Department of Obstetrics, Gynecology & Reproductive Sciences, 6431 Fannin Street, Houston, TX 77030, United States. Tel.: +1 713 500 6408; fax: +1 713 500 5474.
E-mail address: Judith.Ann.Smith@uth.tmc.edu (J.A. Smith).

http://dx.doi.org/10.1016/j.reprotox.2015.02.011
0890-6238/© 2015 Elsevier Inc. All rights reserved.

peak plasma drug concentrations. Peak maternal plasma concentrations following IV administration of granisetron resulted in fetal drug transfer of up to 30 ng/mL. The 3 ng/mL concentration which is similar to the peak plasma concentration achieved with administration of granisetron transdermal delivery system did not show attributable fetal drug transfer in an *ex vivo* human placenta perfusion model [9].

The objective of this study was to determine the effect of granisetron concentrations achieved after maternal IV drug administration (30 ng/mL) compared to granisetron concentrations achieved after maternal transdermal patch administration (3 ng/mL) on alterations in cell cycle progression and cell death in human primary fetal organ cells and ultimately to define the effect on the fetal tissues after exposure to granisetron in this study.

2. Methods and materials

Granisetron was purchased from Sigma–Aldrich Co. (St. Louis, MO). Weymouth's MB 752/1 medium, Neurobasal medium, Hepatozyme medium, Hank's balanced salt solution (HBSS), chicken serum, HEPES buffer, B-27 supplement and human epidermal growth factor (EGF) were purchased from GIBCO Invitrogen Co. (Carlsbad, CA). Fetal bovine serum (FBS), Eagle's minimal essential medium (EMEM), Iscove's DMEM, Trypsin EDTA 0.25%, PBS, Antibiotic–Antimycotic solution were purchased from the media core facility of The UT MD Anderson Cancer Center (Houston, TX). Bt2cAMP, DEAE–Dextran, propidium iodide (PI) solution, collagenase 1A, collagenase 1, DNase 1 and RNase A were purchased from Sigma–Aldrich Co. (St. Louis, MO). The BCA Protein estimation kit was purchased from Pierce (Rockford, IL). PathScan® Sandwich ELISA kits for the detection of total p53, total Bax, cleaved PARP and cleaved Caspase-3 were purchased from Cell Signaling Technology, Inc. (Danvers, MA). Sixteen to nineteen week old fetal organs including brain, heart, lungs, kidneys and small intestine were purchased from Advanced Bioscience Resources, Inc. (Alameda, CA).

2.1. Assay buffer solutions

Digestion buffer consisting of 2.5 mL chicken serum, 1.25 mL of 500 mM HEPES buffer, 50 mg collagenase 1A, 50 mg collagenase 1, 2–3 mg DNase 1 diluted to 25 mL with respective medium was used to isolate the primary cells from fetal organ tissue. DEAE–dextran buffer consisting 1.25 mL of 500 mM HEPES, 250 μL of 25 mg/mL DEAE–dextran in distilled water diluted to 25 mL of respective medium was used for fibroblasts elimination. NP40 lysis buffer was used to extract the total proteins from cells using standard procedure.

2.1.1. Isolation of primary cells from fetal organs

The primary cells were isolated from 16 to 19 week old fetal brain, heart, lungs, kidneys and small intestine according to an explants fetal tissue culture model described by Snyder and colleagues [10]. After rinsing the fetal organs once with the respective media, they were finely chopped and mixed with 3–5 mL of media. This minced tissue was then placed into five 100 mM dishes on sterile lens paper supported by four sterile stainless steel grids and maintained in organ culture for 3 days by changing the medium daily. After 3 days, all tissue was pooled aseptically and suspended in pre-warmed (37 °C) 30 mL digestion buffer. Tissue was then triturated continuously with 10 mL pipette for 15–20 min until all clumps were broken. The digestion buffer tissue suspension was immediately centrifuged at 1400 rpm for 5 min and the supernatant was removed. The pellet was re-suspended into 25 mL dextran buffer and incubated at 37 °C with 5% CO_2 on a shaker at 200 rpm for 1 h for the fibroblast removal. After 1 h, the dextran buffer tissue suspension was immediately centrifuged at 1400 rpm for 5 min and supernatant was removed. The pellet was re-suspended in 30 mL medium, after mixing 1 mL cell suspension was plated each on thirty 60 mM collagen coated dishes and 2 mL respective medium supplemented with 10% FBS, 1 mM Bt_2cAMP and 3% antibiotic antimycotic solution was added into each plate. Cells were then incubated overnight at 37 °C with 5% CO_2. After overnight incubation, cells were cleaned up by washing vigorously three times with HBSS to remove all cell debris. Fresh medium supplemented with 1 mM Bt_2cAMP and 3% antibiotic/antimycotic solution was added daily for 5 consecutive days to accelerate the differentiation of primary cells. Weymouth's MB 752/1 medium was used to isolate primary cells from fetal lungs. Whereas neurobasal medium with 2% B-27 supplement was used to isolate primary cells from human brain. Eagle's minimal essential medium (EMEM) was used to isolate cells from lungs as well as kidneys. Iscove's DMEM was used for isolation of primary cells from fetal heart.

2.2. Drug treatment

To evaluate the toxic effect of granisetron on fetal brain, heart, lung, kidney and small intestine, the primary cells isolated from human fetal organs were treated concentrations of granisetron used for the treatment were determined to mimic systemically achieved maternal peak concentrations following IV administration (30 ng/mL) and transdermal administration (3 ng/mL) with samples obtained at baseline, 12 h, 24 h, 48 h and 168 h. No treatment controls were set up for each time point and each concentration. All experiments were repeated in triplicate for each time point and each drug concentration.

2.2.1. ELISA for apoptotic markers

Following the respective time point treatment of primary cells isolated from fetal organs with low and high concentrations of granisetron, cells were harvested and protein extracts were prepared by lysing cells on ice in 100–200 μL of NP40 lysis buffer. Pierce Micro BCA Protein Assay Kit was used to determine the protein concentration. For each series of protein determinations, a standard curve was constructed with known concentrations of bovine serum albumin (BSA). Sandwich ELISAs for the detection of total p53, total Bax, cleaved PARP and cleaved caspase-3 were performed according to the manufacturer's protocol.

2.2.2. Flow analysis of cell cycle

Following the respective time point treatment of primary cells isolated from fetal organs with low and high concentrations of granisetron, both floating and adherent cells were pooled, and washed once in ice-cold PBS. Cells were then fixed in ice-cold 70% ethanol overnight. After fixing in ethanol, cells were again washed in ice-cold PBS and stained with 500 μL of 20 μg/mL propidium iodide (PI) in the presence of 10 μg/mL RNase A and 0.1% tween 20 at 37 °C for 15 min. Cell cycle distribution was analyzed on 10,000 cells for each experimental condition. Data analysis was performed using FACS Caliber at the UTHealth- University of Texas Medical School at Houston (Houston, TX). Cell cycle analysis was completed using Cell Quest Pro program. Data analysis was done using FlowJo software. Quantitative data from cell cycle analysis were graphed to illustrate the accumulation of the percentage of cells in sub-G_0 phase of cell cycle to evaluate the extent of apoptosis occurring in cells at various time points.

2.3. Sample size and statistical considerations

All tissue drug exposure experiments were completed in duplicate. Analysis for immunoblotting for changes in expression of markers of apoptosis and flow cytometry were completed in triplicate. Hence there were six samples per dose level per time point in

Download English Version:

https://daneshyari.com/en/article/2593475

Download Persian Version:

https://daneshyari.com/article/2593475

Daneshyari.com