Brief Communications

Placental trophoblast transfer of opioids following exposures to individual or mixtures of opioids *in vitro*

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Impact statement

Gaining knowledge about opioid exposure and placental transfer of opioids is an important part of providing clinicians with the necessary information for therapeutic strategies during pregnancy in the rising opioid epidemic. Opioids exposure often includes a mixture rather than an exposure to an individual opioid. We investigated placental trophoblast permeability to opioids *in vitro* for six individual opioids and selected mixtures. The rate and concentration of opioid translocation across placental trophoblast monolayer varied considerably between the six investigated opioids. Oxycodone had the highest level of translocation. We also found that the transfer of heroin decreased when administered together with fentanyl, while no change was observed for fentanyl transfer in the mixture. This suggests that opioid mixtures may decrease translocation of some opioids while others are unaffected.

Abstract

Infants born with neonatal abstinence syndrome increased 5-fold between 2000 and 2012. Prenatal exposure to opioids has been linked to altered brain development, congenital heart defects, neural tube defects, and clubfoot. In the study presented here, placental transfer rate of six opioids; morphine, heroin, fentanyl, methadone, oxycodone, and naloxone was compared *in vitro* using a human trophoblast cell monolayer model, BeWo b30. The rate and concentration of opioid translocation were investigated individually and in mixtures. The opioid transfer was quantified at 5, 15, 30, 60, and 120 min, using target liquid chromatography-mass spectrometry. The transfer was lowest for heroin (<1.2% of administered dose at 120 min; the permeability across the cells [P]: 0.21×10^{-5} cm/s) and highest for oxycodone (13.2% of administered dose at 120 min; P: 2.46×10^{-5} cm/s). As oxycodone is a preferred drug for both short- and long-term pain treatment, more knowledge is needed to understand the potential adverse impact on fetal development, growth, and well-being. Opioid exposure is likely to happen as mixtures, and we therefore investigated the transfer of selected mixtures. Permeability was significantly decreased for heroin when administered together with fentanyl. This study demonstrates that the transfer rate between individual opioids varies significantly and that some mixtures may impact the transfer of some individual opioids like heroin.

Keywords: Opioid transfer, trophoblasts, BeWo b30, opioid mixtures, transfer kinetics, *in vitro*

***Experimental Biology and Medicine* 2019; 244: 846–849. DOI: 10.1177/1535370219851109**

Introduction

The opioid epidemic in the U.S. is accelerating. In 2012, an estimated 21,732 infants were born with neonatal abstinence syndrome due to maternal opioid use, a 5-fold increase since 2000.[1,2] In laboratory animal studies, prenatal exposure to opioids has been demonstrated to affect myelination in the developing brain,[3,4] to modulate the fetal hypothalamic-pituitary-adrenal axis,[5,6] and to have neurobehavioral consequences in offspring; including hyperactivity,[7] elevated startle response,[6] and depression-like neurobehavior.[8] Clinical data have also linked prenatal opioid exposure to altered brain development,[9] as well as congenital heart defects, neural tube defects, and clubfoot (reviewed in Yazdy *et al*. and Lind *et al*.[10,11]).

The placenta is one of the least understood human organs and perturbations in cell growth and signaling can disrupt temporally sequenced developmental processes in the developing fetus and result in long-term functional deficits and detrimental health impacts for offspring. A challenge with studying placental transport using laboratory animals is the inter-species variation which can make the translational aspects of animal studies to human health outcome complicated (as reviewed in literature[12–15]). The *ex vivo* human placenta perfusion model addresses several of these challenges; however, the perfusion model require

access to human placental tissue and a sophisticated instrumental setup which makes it challenging to use for comparison studies of multiple individual and mixture of compounds. The *in vitro* model of monolayer forming immortalized human trophoblastic cell lines, like BeWo B30, is therefore more suited for larger comparison studies of transport rate and permeability.[16–18]

Opioid peptides have been suggested to cross the placental trophoblast monolayer models, BeWo b30 cells[19,20] mainly via a paracellular route.[19] However, transporter proteins like ABC transporters and solute carrier transporters are also involved in opioid transport (as reviewed in Yang *et al.*[21]). Data from a dually perfused human placental lobule model suggest that levo-alpha-acetylmethadol and methadone, but not buprenorphine, is extruded by the ABC transporter P-glycoprotein (PGP).[22,23]

In this brief communication, the placental transfer kinetics for six individual opioids and selected opioid mixtures were investigated *in vitro* using a BeWo b30 monolayer model to compare the permeability across the cells (P) between opioids and to understand how P changes when cells are exposed to a mixture of opioids.

Methods

Materials

Cell culture medium DMEM:F12, 50/50 Mix (Cellgro, Corning, NY), MEM nonessential amino acids (NEAA) (ATCC, Manassas, VA), and 200 mM L-glutamine, 100 U penicillin–streptomycin (P/S) and fetal bovine serum (FBS) (Gibco, Gaithersburg, MD). Transwell 12-well plates, polycarbonate, 0.4 μm pore size (Corning, NY). Human placental collagen type IV and opioids were purchased from Sigma-Aldrich (St Louis, MO). Chemicals and solvents for LC-MS analysis were purchased from Thermo Fisher Scientific (Hampton, NH) and an Acquity UPLC HSS C18 column (2.1 × 50 mm with VanGuard HSS C18 1.8 μm) was purchased from Waters, (Milford, MA).

Cell culture

The choriocarcinoma human trophoblast cell line BeWo b30 was obtained from Dr. Erik Rytting (University of Texas Medical Branch, Galveston, TX, USA) and was cultured as described.[24] Briefly, cells were cultured at 37°C with 5% CO_2 in a humidified atmosphere in DMEM:F12 medium supplemented with 1% NEAA, 1% L-glutamine, 1% P/S, and 10% FBS.

Opioid transport studies

Cells between passages 25–45 were seeded onto permeable membrane inserts coated with collagen at 2.0×10^5 cells/cm^2 and cultured for 10 days. Transepithelial electrical resistance (TEER) was measured with an Endohm 12 chamber and EVOM voltohmeter (World Precision Instruments Ltd, Sarasota, FL) to ensure a confluent cell monolayer prior to transport studies. TEER was measured in DMEM:F12 media and calculated by subtracting the resistance of a blank transwell without cells. Only transwells with a cell monolayer measuring between 35 and 70 ohm cm^{-2} were used.[17,25] Six opioids were investigated: heroin, methadone, morphine, naloxone, fentanyl, and oxycodone. Cells were exposed to opioids on the apical side at a final concentration of 10 μg/mL and samples collected from the basolateral side at 5, 10, 15, 30, 60, and 120 min after administration. Vehicle, methanol (1%) was used as control. Studies were conducted in biological triplicates and experimental duplicates. The permeability across the cells (P [cm/s]) was calculated as described;[19] P (cm/s)=X/(A×t×Cd), where X is the amount of substance (mol) in the receiver chamber at time t (s), A is the diffusion area (1.12 cm^2), and Cd is the concentration of the substance in the donor chamber (mol/cm^3). An unpaired, two-tailed t-test was used to evaluate significant differences using GraphPad Prism 7.04.

Ultra-performance liquid chromatography mass spectrometry

All liquid chromatography was performed on a waters Acquity ultra-performance liquid chromatography (UPLC). For reversed phase analysis, 2 μL of each sample were injected into an Acquity UPLC HSS C18 column at 30°C. Water with 0.1% formic acid (mobile phase A) and acetonitrile (mobile phase B) were the mobile phases. The elution gradient consisted of initial conditions at 98% mobile phase A, held for 0.75 min, then linearly increased B to 80% over 3.25 min, and to 95% over 1 min, and then returned to initial conditions. MS analysis was performed using an API 5000 (Applied Biosystems/MDS SCIEX, Foster City, CA) triple quadrupole mass spectrometer. Multiple reaction monitoring was conducted with the following transitions used for quantitation: morphine: 285.8→152.1, naloxone: 328.2→212.0, oxycodone: 316.3→241.2, heroin: 369.9→165.2, fentanyl: 336.9→188.2, and methadone: 309.7→265.2.

Results and discussion

The opioid transfer kinetics across human placental trophoblast, BeWo b30, monolayer of six opioids were tested individually and in combination over a time frame of 120 min (Figure 1). The transfer rate varied between the individual opioids. Permeability for individually tested opioids ranked as following oxycodone > naloxone ~ fentanyl >methadone ~ morphine ~ heroin (Table 1).

In the study presented here, oxycodone had the highest level of transfer. Studies of oxycodone's impact on birth weight have been inconclusive (as reviewed in Yazdy *et al.*[10]). As oxycodone is a commonly prescribed drug for both short- and long-term pain treatment, more knowledge is needed to understand the potential adverse impact on fetal development, growth, and well-being. Furthermore, it has been suggested that the transfer rate is slower in the BeWo b30 transwell model compared to the human placenta perfusion system,[25] which could indicate that the transfer of oxycodone in the human placenta is even higher than reported in this study.

The permeability was significantly decreased for heroin when administered together with fentanyl. Our findings

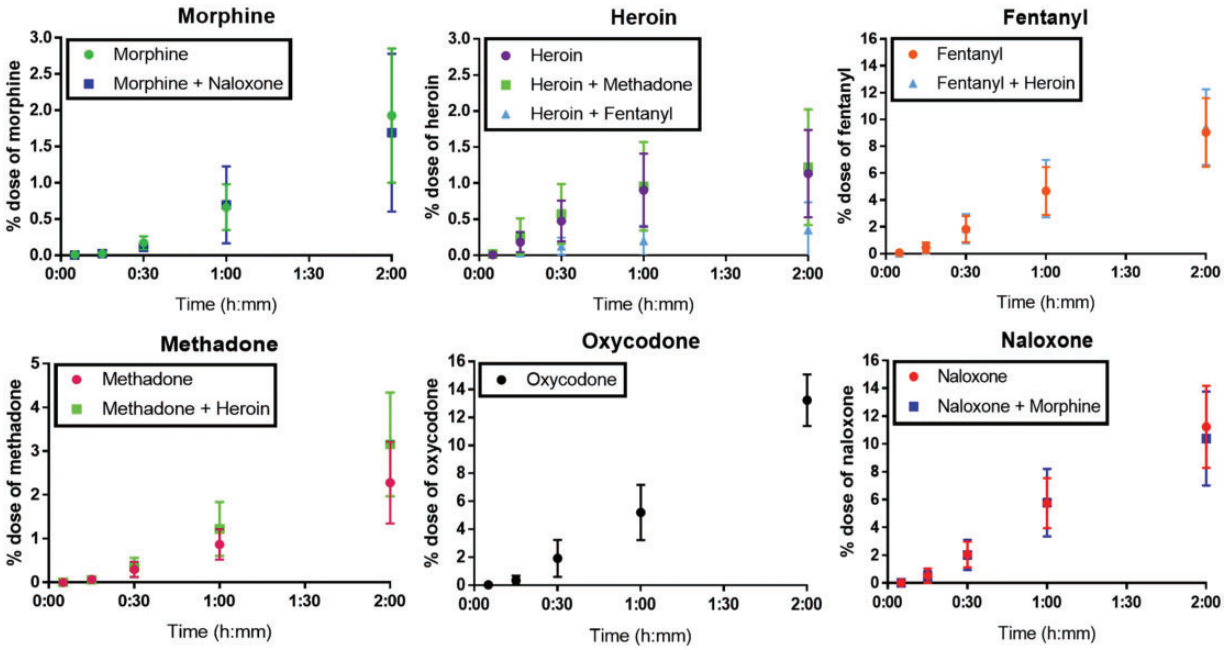

Figure 1. Translocation of opioids across the trophoblast monolayer over time expressed as percentage of dose, showing mean ± standard deviation. The doses were either individual opioids (morphine, heroin, fentanyl, methadone, oxycodone, or naloxone) or mixtures of opioids (morphine + naloxone, heroin + methadone, or heroin + fentanyl). (A color version of this figure is available in the online journal.)

Table 1. Permeability [cm/s] across the cells at 120 min for opioids tested individually or in mixtures.

	Permeability (cm/s)*10^{-5}
Single doses	
Morphine	0.36 ± 0.16
Heroin	0.21 ± 0.10
Fentanyl	1.76 ± 0.48
Methadone	0.58 ± 0.22
Oxycodone	2.46 ± 0.031
Naloxone	2.09 ± 0.50
Mixed doses	
Heroin + fentanyl	H: 0.066* ± 0.066; F: 1.76 ± 0.48
Heroin + methadone	H: 0.23 ± 0.14; M: 0.59 ± 0.20
Morphine + naloxone	M: 0.32 ± 0.19; N: 1.93 ± 0.57

Note: Permeability was significantly decreased ($P < 0.05$) for heroin when administered together with fentanyl as indicated by '*'.

suggest that heroin transfer was decreased by fentanyl, while fentanyl transfer was not affected by heroin. It is also possible that fentanyl increases the heroin metabolism. Fentanyl is a fast-acting opioid with a potency of 100:1 to morphine, and it is therefore concerning that the permeability of fentanyl is the third highest for individual opioid exposure tested here and does not seem affected by the presence of another opioids. The drug–drug interactions between heroin and fentanyl need to be investigated further.

With the exception of heroin, which is metabolized by carboxylesterases,[26] Cytochrome P450 CYP3A4 is involved in the metabolism of all the investigated opioids.[27,28] While other CYPs are also involved, the overlap in CYPs makes it unlikely that the explanation for the difference in transfer rate is due to differences in metabolism. Transporter proteins involved in opioid translocation include ABC transporters (PGPs, multidrug resistance proteins (MRPs), and breast cancer resistance protein (BCRP)) and solute carrier transporters (as reviewed[21]). Methadone, morphine, and fentanyl[21,23,29] are PGP substrates, while oxycodone, naloxone, and heroin are not,[21,29,30] suggesting that PGP-mediated efflux is not the determining factor in the transfer rates observed in BeWo b30 cells. The data presented here demonstrate the need to better understand the role of PGPs, MRPs, and BCRPs in placental opioid transport, as well as how potential activation of nuclear receptors and modulated expression of ABC transporters may influence the placental transfer of opioids.

Authors' contributions: Mortensen designed the studies, analyzed the data and wrote manuscript. Moreno Caffaro performed the *in vitro* studies. Snyder and Yueh developed and validated the LCMS methods for detection of opioids. Fennell has extensive experience in small molecule research including ADME and PK studies and contributed with his experience in this field.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Erik Rytting, University of Texas Medical Branch, for the BeWo b30 cells. The authors would like to thank RTI International for financial support.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by RTI International Internal Research and Development grant.

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(Received February 13, 2019, Accepted April 25, 2019)