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Transplacental Transfer of Oseltamivir Carboxylate

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Abstract

Objectives—Determine the bidirectional transfer of oseltamivir carboxylate (OC) across term human placenta and its distribution between the tissue, maternal and fetal circuits.

Methods—The technique of dual perfusion of placental lobule (DPPL) in its recirculating mode was utilized to determine the transfer of the drug. OC (350 ng/ml) was co-perfused with its [³H]-isotope and the marker compound antipyrine (AP, 20 µg/mL) together with its [¹⁴C]-isotope. The concentrations of OC and any of its metabolite(s) formed during perfusion were determined in the tissue, maternal and fetal circuits by liquid scintillation spectrometry following their separation by High Performance Liquid Chromatography (HPLC).

Results—The distribution of OC following its perfusion in the Maternal-to-Fetal direction for 4 hours was as follows: 21 ± 4% of the drug was transferred to the fetal circuit, 13 ± 5% was retained by the perfused lobule, and 66 ± 4% remained in the maternal circuit. The normalized transfer of OC to that of AP (Clearance index) in the Maternal-to-Fetal direction was (0.47 ± 0.11) and was not different from its transfer from the Fetal-to-Maternal direction (0.47 ± 0.06) suggesting that involvement of placental efflux transporters is unlikely.

Conclusions—OC crosses human placenta. Since the transfer rate of OC is 47% of the freely diffusible AP, it is likely that fetus could be exposed to OC during pregnancy.

Keywords

Oseltamivir Carboxylate; Transplacental Transfer; H1N1 influenza virus; Tamiflu; Pregnancy

INTRODUCTION

Pregnant patients with influenza have higher rates of severe morbidity and mortality for seasonal as well as for pandemic outbreaks of flu, including H1N1, than the general adult population. The primary pharmacological agent for prophylaxis and treatment of seasonal and pandemic flu strains in non pregnant women is the oral antiviral drug oseltamivir. Despite the relative lack of information on the use of oseltamivir during pregnancy, the increased rate of H1N1 influenza-related morbidity and mortality in pregnant women led the Centers for Disease Control and Prevention to recommend the use of oseltamivir for pregnant patients with suspected or confirmed H1N1 infection.

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DECLARATION OF INTEREST:

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Oseltamivir phosphate (OP) is an orally administered pro-drug that is extensively (~80%) hydrolyzed by hepatic esterases to the single active metabolite oseltamivir carboxylate (OC) [1, 2]. In patients with influenza, OC selectively inhibits the enzyme neuraminidase that is critical for viral assembly thus allowing for the dissemination and subsequent infection of other host cells necessary for propagation of the infection [3].

The prophylactic and treatment efficacy of oseltamivir in the management of influenza infection was demonstrated by several clinical trials [3, 4]. However, due to the limited available data on the adverse effects of OC to the fetus as well as its pharmacokinetics during pregnancy, the drug is designated as Class C and is currently not approved by the FDA for management of influenza infection in pregnant patients.

Therefore, the potential use of oseltamivir for treatment of the pregnant woman with suspected or confirmed influenza infection requires additional preclinical and clinical information on its effectiveness and safety during pregnancy. Of major safety concern is the extent of fetal exposure to the drug. The first step to obtain this information is to determine the role of human placenta in the disposition of OC and, consequently, the concentration of the drug in the fetal circulation.

The relatively low molecular weight (284) of OC and its extremely low (3%) binding to plasma proteins [2] suggest that it should be transferred across human placenta from the maternal to the fetal circulation and consequently intra uterine fetal exposure is plausible. Indeed, utilizing the technique of dual perfusion of placental lobule (DPPL) and OC in a concentration similar to that in the circulating blood of non-pregnant patients, Berveiller et al. demonstrated that OC is transferred to the fetal circuit [5]. On the other hand, Worley et al., utilizing the same experimental technique was unable to detect OC in the fetal circuit following the perfusion of OP in the maternal to fetal direction in the concentration range 5–6 fold above of its therapeutic level [6]. Even using OP in the concentration range of 600–800 fold higher than therapeutically relevant concentrations, transplacental transfer of OC was incomplete [6]. Thus, data reported to date on the extent of OC transfer across dually perfused human placental lobule are controversial [5,6,].

Although, OP undergoes extensive metabolism and at least 75% of an oral dose enters the systemic circulation as the active metabolite, OC is not metabolized further by either human cytochrome P450 isoforms or glucuronosyltransferases [2]. The formed OC exclusively (99%) eliminated by renal excretion. Renal clearance of OC exceeds the glomerular filtration rate, suggesting that renal tubular secretion contributes to the elimination of this compound [1,2,7]. Since kidney transporters are important components of active tubular secretion, the interaction of OC with transporters in kidney and other tissues, including placenta is plausible. Also, to the best of our knowledge, there is no published data on the interaction between placental transporters and OC, Berveiller et al does not exclude their role in the observed transplacental transfer of OC [5].

Therefore, the aim of this investigation was to determine bidirectional transfer of OC across term human placenta and its distribution between placental tissue, maternal and fetal circuits. These parameters were determined by using the *ex vivo* technique of dual perfusion of placental lobule. This technique retains the anatomic and functional integrity of placental tissue and has been validated in determining the bidirectional transfer and distribution of numerous drugs.

METHODS

Chemicals

[³H]-OC (specific activity, 59 Ci/mmol) was purchased from Perkin Elmer (Boston, MA). The non-radioactive OC was a gift from F. Hoffmann-La Roche Ltd (Pharma Research, Basel, Switzerland). All other chemicals including radioactive [¹⁴C]-antipyrine (specific activity, 6.5 mCi/mmol) were purchased from Sigma-Aldrich (St. Louis, Mo).

Clinical Material

Placentas from uncomplicated term (37 – 42 weeks) pregnancies (n = 21) were obtained immediately after vaginal or abdominal deliveries from the Labor and Delivery Ward of the John Sealy hospital, the teaching hospital of University of Texas Medical Branch, Galveston, Texas, according to a protocol approved by the Institutional Review Board. Exclusion criteria included maternal infections, systemic diseases, and drug or alcohol abuse during pregnancy.

Dual Perfusion of Term Human Placental Lobule

The technique of DPPL was used as previously described by Miller et al. and an earlier report from our laboratory [8, 9]. Briefly, each placenta was examined for tears followed by choosing 2 chorionic vessels (an artery and a vein) supplying a single intact peripheral cotyledon that were cannulated with 3F and 5F umbilical catheters, respectively. The cotyledon was trimmed and placed in the perfusion chamber with the maternal surface upward. The intervillous space on the maternal side was perfused by 2 catheters piercing the basal plate. The flow rate of the perfused medium in the fetal and maternal circuits was 2.8 and 12 mL/min, respectively. The perfusion medium was made of tissue culture medium M199 (Sigma, St. Louis, MO) supplemented with: Dextran 40 (7.5 g/L in the maternal and 30 g/L in the fetal reservoir), 25 IU/mL heparin, 40 mg/L gentamicin sulfate, 80 mg/L sulfamethoxazole, and 16 mg/L trimethoprim. The maternal perfusate was equilibrated with a gas mixture made of 95% O₂, 5% CO₂, and the fetal perfusate with a mixture of 95% N₂, 5% CO₂. Sodium bicarbonate was added to the maternal and fetal circuits to maintain the pH at 7.4 and 7.35, respectively. All experiments were carried out at a temperature of 37 °C.

Each placenta was perfused for an initial period of one hour (control). The perfusion experiment was terminated if one of the following occurred: fetal arterial pressure exceeded 50 mmHg, a volume loss in fetal circuit in excess of 2 mL/h, or a pO₂ difference between fetal vein and artery less than 60 mmHg, indicating inadequate perfusion overlap between the two circuits.

Transplacental Transfer and Distribution of OC

Following the control period, the maternal and fetal perfusates were replaced with fresh medium. Human serum albumin, at a concentration of 3 mg/mL, was added to the media of both the maternal and fetal circuits. The non-ionizable, lipophilic marker compound antipyrine (AP) 20 µg/mL and its [¹⁴C]-isotope (1.5 µCi) were co-transfused with OC to account for interplacental variations and to normalize the transfer of OC to that of AP.

AP and OC were added either to the maternal or fetal reservoirs according to the transfer direction investigated i.e., from the maternal to fetal (M→F) or fetal to maternal (F→M), respectively. The initial concentration of OC in the donor circuit was 350 ng/mL. This concentration corresponds to serum levels of OC following the administration of 75 mg dose of oseltamivir twice a day [10].

The perfusion system was utilized in its closed-closed configuration (re-circulation of the media). The concentrations of compounds were determined in 0.5 mL aliquots taken from the maternal and fetal arteries and veins at 0, 5, 10, 15, 30, 40, 50, 60, 90, 120, 150, 180, 210 and 240 minutes. The amount of radioactivity in both the maternal and fetal perfusate were determined by liquid scintillation spectrometry in the [³H] and [¹⁴C]-channels simultaneously (1900TR; Packard Instruments, Inc, Shelton, CT).

At the end of experiment, the perfused area was dissected from the adjoining placental tissue, weighed, and homogenized in a volume of saline equal to four times its weight. 1 mL of 1M NaOH was added to 1 mL of the homogenate and the samples were incubated for 12 hours at 60 °C in the dark to allow for luminescence decay. Scintillation cocktail was added to each sample and the concentration of OC determined.

Metabolism of OC

The biotransformation of OC during its perfusion was investigated at the end of the 4 hour experimental period. OC and its metabolites, if formed, were extracted from the maternal and fetal perfusates using solid phase extraction (SPE). Samples (1 mL aliquots of the perfusate) were pretreated with 250 µM of 1M citric acid. Cartridges Sep-Pak Plus tC18, 400 mg (Waters, Milford, MA) were preconditioned with 3 mL of 0.01M HCl in acetonitrile:H₂O (5:95), 3 mL of acetonitrile:H₂O (75:25). After loading 2 mL of samples, the unwanted components were eluted by 3 mL of 0.01M HCl in acetonitrile:H₂O (5:95) and OC was eluted by acetonitrile:H₂O (75:25). Eluates containing OC from 5 cartridges were combined and dried by air. The dry residue was reconstituted in 250 µL of mobile phase, filtered and 200 µL was injected to the column. The recovery of OC under these experimental conditions was more than 60%.

Instrumentation and Chromatographic Conditions

The HPLC system consisted of a Waters 600E Multisolvant Delivery System, (Waters Corporation, Milford, MA), a 717 autosampler, and 2487 dual λ absorbance detector coupled with the β-RAM flow-through in-line detector (model 4, IN/US Systems, Tampa, FLA). The HPLC system was controlled by Empower pro 2154 chromatography manager (Waters, Milford, MA) and ScintFlow SA (Version 1.3.3, IN/US Systems, Inc, FLA).

The mobile phase consisted of Ammonium acetate (10 mM, PH 3.5):Acetonitrile: (90:10). The separation was achieved on Agilent Eclipse XDB-C18 column (150×4.6 mm, 5 µm) connected with a Phenomenex C18 guard column (4×3.0 mm) at ambient temperature. The flow rate of mobile phase was 1 mL/min and wavelength of UV detector was set at 205 nm. The split ratio of eluent to radioactive detector was 100% and the ratio of scintillation cocktail (Ecoscint Flow, National diagnostics, Georgia) to eluent was 3:1.

Data and Statistical Analysis

The maternal to fetal (M→F) and fetal to maternal (F→M) transfer rates (%) were calculated as follows: $(C_{\text{recipient}} / C_{\text{donor}}) \times 100$, where $C_{\text{recipient}}$ is the final concentration of drug in recipient circuit and C_{donor} is the initial concentrations of drug in donor circuit. The clearance index (Cl_{index}) is the ratio between transfer rate of the drug to that of the marker compound antipyrine.

Statistical analyses were performed using GraphPad Prism version 5.01. All reported values are expressed as mean ± S.D. Statistical significance of the differences observed between groups were calculated by two-tailed t test and considered significant when $P < 0.05$.

RESULTS

Metabolism of OC

Under our experimental conditions, the retention time of OC radiolabeled standard was 8 minutes. Preliminary analyses were performed to determine the purity and chemical stability (radiation damage) of [^3H]-OC. Chromatograms revealed that the retention time of eluted [^3H]-isotope was identical to that of the non radioactive OC standard. No additional peaks of tritiated OC compounds were detected in the perfusion medium that had been re-circulated in the perfusion system for 4 hours. Therefore, [^3H]-OC was chemically stable and was not degraded during the experimental period.

The elution profile of aliquots from placental tissue homogenates, maternal and fetal perfusates revealed one peak of the [^3H]-nuclide at 8 minutes that corresponded to that of the OC standard. Therefore, OC was not metabolized by placental lobule during 4 hours of perfusion.

Placental Transfer of OC in the Maternal to Fetal Direction and its Distribution

In this investigation, the transplacental transfer of AP was approximately 50%, and is consistent with previous reports from our laboratory [9]. OC crossed the placenta and appeared in the fetal circuit within the initial five minutes of perfusion (Figure 1). The concentration of OC in the fetal circuit at the end of the experimental period was 79 ± 14 ng/mL. Furthermore, at the end of the 4-hour experimental period, the fetal/maternal concentration ratio of OC was 0.35 ± 0.075 . The normalized transfer of OC (Clearance index) to AP in the Maternal-to-Fetal direction was 0.47 ± 0.11 .

The distribution of OC, at the end of 4-hour perfusion, between the perfused tissue, the maternal and fetal circuits was as follows: $13 \pm 5\%$ of the drug was retained by the tissue, $66 \pm 4\%$ remained in the maternal circuit, and $21 \pm 4\%$ was transferred to the fetal circuit.

Placental Transfer of OC in the Fetal to Maternal Direction and its Distribution

Following the addition of OC to the fetal (donor) reservoir, the drug rapidly crossed the placenta and appeared in the maternal (recipient) circuit (Figure 2). At the end of the 4 hours of perfusion period, the concentration of OC in the maternal circuit reached 45.9 ± 4.07 ng/mL which represents $13 \pm 1\%$ of its initial concentration in the donor reservoir. The maternal/fetal concentration ratio of OC was 0.2 ± 0.03 , however the normalized transfer of OC to AP in the Fetal-Maternal direction was 0.47 ± 0.06 and did not differ from its transfer from the Maternal to Fetal direction (Figure 3).

Furthermore, the retention of OC by the perfused lobule at the end of the 4-hour experimental period reached 31 ± 6 ng/g (10% of its initial concentration in the donor circuit).

DISCUSSION

The aim of this investigation was to determine the bidirectional transfer of oseltamivir carboxylate (OC) across term human placenta and its distribution between the tissue, maternal and fetal circuits of the dually perfused placental lobule.

The data obtained in this investigation revealed that OC rapidly crosses the human placenta in both the Maternal to Fetal (Figure 1) and Fetal to Maternal (Figure 2) directions. Although the transfer rate of OC in the Maternal to Fetal direction was different from that in the Fetal to Maternal, the observed difference is attributed to the following experimental conditions: (1) differences in the flow rates between donor and recipient circuits; (2) the

volume of the donor and recipient circuits; and (3) the localization of the catheters inside of the trophoblast tissue vs inside of the vessels on the fetal side of the placenta. However, these differences in the experimental conditions between perfusing the drug in the Maternal to Fetal versus Fetal to Maternal direction is accounted for by the transfer of the marker compound antipyrine since its transfer rate is proportionately affected by the same variables in the experimental conditions. Thus, normalized OC transfer from the Fetal to the Maternal circuit was not different from the transfer in the Maternal to Fetal direction (Figure 3). The lack of difference between Fetal to Maternal and Maternal to Fetal transfer of OC suggests that a placental efflux transporter is not involved in this process. These results are in agreement with previous reports indicating that OC is neither a substrate of P-glycoprotein (P-gp) [11], or breast cancer resistant protein (BCRP), or multidrug resistance-associated proteins 1, 2, or 3 (MRP1, MRP2, MRP3) [12] which are expressed in the placenta [13]. On the other hand, the distribution of OC across the blood-brain barrier is regulated, to some extent, by the organic anion transporter 3 (OAT3) and the multidrug resistance-associated protein 4 (MRP4) [14] but these proteins are not expressed in human placenta [13].

The transplacental transfer of OC to the fetal circuit (Cl_{index} range 0.2–0.6) utilizing the perfusion system in its closed-closed (re-circulating) mode revealed that it is in agreement with the reported data utilizing the open-open mode of the perfusion system (Cl_{index} range 0.2–0.5) [5]. The re-circulated mode of the perfusion system most closely simulates the *in vivo* conditions, especially for drugs that do not undergo further biotransformation by maternal, placental, and fetal metabolic enzymes, such as in the case of OC. Earlier reports, mentioned in the introduction, indicated that OC is not metabolized by human cytochrome P450 isoforms (phase I) or by glucuronosyltransferases (phase II) [2]. Furthermore, data obtained in this investigation revealed that OC was not metabolized by the perfused placental lobule under the conditions that would favor accumulation of any metabolites formed. Although the absence of extraplacental maternal and fetal biotransformation reactions and their effect on drug concentrations in the maternal and fetal circuits is one of the limitations of perfusion system, this is not the case for OC. Therefore, it is evident that the fetal to maternal concentration ratio of OC (0.35 ± 0.075) calculated at the end of the *ex vivo* perfusion experiment should correlate closely with fetal (umbilical blood) to maternal concentration ratios determined *in vivo*. Moreover, the low octanol / water partition coefficient of OC (0.45) favors its distribution into the aqueous maternal and fetal circuits over the tissue as confirmed in this investigation by its minimal (approximately 10%) retention by the placental lobule.

In summary, the transfer of OC across the dually perfused placental lobule to the fetal circuit, its inert metabolic nature, low retention by placental tissue and extremely low binding to plasma proteins suggest that intrauterine fetal exposure to OC is likely.

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Figure 1. Maternal to Fetal transfer of OC

The maternal to fetal transfer of OC ($n = 11$) was determined in the closed-closed mode of the perfusion system. The transfer of OC was compared to the transfer of the freely diffusible marker compound AP. The concentration of the OC in the maternal reservoir at “0” time was 350 ng/ml. In the maternal to fetal direction, OC rapidly crossed the human placenta at a transfer rate of 47% that of AP. This data indicate that in vivo fetal exposure to OC is plausible.

Figure 2. Fetal to Maternal transfer of OC

The fetal to maternal transfer of OC ($n = 10$) was determined in the closed-closed mode of the perfusion system and was compared to the transfer of the marker compound AP. The concentration of OC in the fetal reservoir at “0” time was 350 ng/ml. The data revealed that approximately 10% of the initial concentration of OC is transferred back to the maternal circuit and 80% remained in the fetal circuit.

Figure 3. Normalized Maternal to Fetal and Fetal to Maternal transfer of OC

In order to compare M-F and F-M rate of OC transfer the initial concentration of the OC in the donor circuits was similar. Normalized (to AP) transplacental transfer of OC in the Fetal to Maternal (n=10) direction was not statistically different from its transfer in the opposite Maternal to Fetal (n=11) direction.