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Bidirectional transfer of methadone across human placenta

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Abstract

Methadone maintenance programs are considered the standard of care for the pregnant opiate addict. However, data on changes in methadone pharmacokinetics (PK) during pregnancy are limited and do not include its disposition by the placenta due to obvious ethical and safety considerations. Accordingly, investigations in our laboratory are focusing on human placental disposition of opiates including methadone. Recently, we reported on methadone metabolism by placental aromatase and provide here data on its bidirectional transfer across the tissue utilizing the technique of dual perfusion of placental lobule. The concentrations of the opiate transfused into the term placental tissue were those reported for its in vivo levels in the maternal serum of women under treatment with the drug. Data obtained indicated that the opiate has no adverse effects on placental viability and functional parameters and that it is retained by the tissue. Also, methadone transfer and its clearance index in the fetal to maternal direction (0.97 ± 0.05) was significantly higher ($P < 0.05$) than in the maternal to fetal (0.83 ± 0.09). The observed asymmetry in methadone transfer could be explained by the unidirectional activity of the efflux transporter P glycoprotein (P-gp) that is highly expressed in variable amounts in trophoblast tissue. Therefore, placental disposition of methadone might be an important contributor to the regulation of its concentration in the fetal circulation and consequently may affect the incidence and intensity of neonatal abstinence syndrome for women treated with the drug during pregnancy.

Keywords: Methadone; Human placenta; Pregnancy; Transplacental transfer

1. Introduction

Maternal and neonatal morbidity and mortality are significantly increased by opiate abuse during pregnancy [1,2]. The goal of methadone treatment programs is detoxification of the adult patient, but for the pregnant woman, the well-being of the fetus is an additional concern. Fetal distress and other complications can accompany fluctuations in maternal serum levels of the opiate [3,4]. Methadone, a long-acting opiate that minimizes changes in maternal serum levels, has been the drug of choice for treatment of the pregnant opiate addict, and is the only pharmacotherapy approved by the US Food and Drug Administration for this patient population. A combination of methadone maintenance therapy and adequate prenatal care reduces the incidence of adverse medical, obstetric, fetal, and neonatal outcomes [5,6]. Pregnant women not continuing in methadone treatment programs could relapse, with consequences for fetal development, and perinatal and neonatal outcome [7]. A major concern for prenatal exposure to opiates, therapeutically or abused, is the incidence of neonatal abstinence syndrome (NAS). Approximately 60–80% of the newborns exposed to methadone in utero experience NAS [8–10], but it is unclear whether its intensity is dependent on the dose of the opiate. A number of investigators were unable to demonstrate a dose–response relationship [8,9,11,12], while others reported a correlation between maternal methadone dose, its serum level, and intensity of NAS

Abbreviations: PK, pharmacokinetics; P-gp, P glycoprotein; NAS, neonatal abstinence syndrome; BUP, bupremorphine; LAAM, L-α-acetylmethadol; AP, antipyrine; M → F, maternal to fetal; F → M, fetal to maternal; EDDP, 2-ethyliolene-1,5-dimethyl-3,3-diphenylpyrrolidine; EMDP, 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline

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[13,14]. On the other hand, a preliminary report on a recent investigation indicated a lack of correlation between methadone dose and NAS [15]. In a review of the literature, this controversy was attributed, at least in part, to the lack of a defined causal relationship and to reports that are poorly controlled for confounding factors, thus leading the authors to correlate higher doses of methadone with severity of NAS [16].

The dose of methadone used for treatment of the pregnant opiate addict, according to a review of the literature by Warner et al. [17], ranges between 10 and 90 mg/day, with a mean of 50 mg/day. A recent investigation indicated that higher doses of the drug (between 50 and 150 mg/day) might be needed to achieve the maternal serum trough levels necessary to prevent withdrawal symptoms in both mother and fetus, with an occasional need for even higher doses towards the end of pregnancy or during the third trimester [18]. These data underscore the need to better understand the pharmacokinetics (PK) of methadone in the pregnant patient, a well-recognized issue for most therapeutic agents. An important contributor to the changes in the pharmacokinetics of a drug during pregnancy is the role of human placenta in its disposition, since one of the goals of the tissue is to protect the fetus from the effects of xenobiotics. Placental disposition of a drug, e.g., methadone, includes its transfer, distribution, and metabolism by trophoblast enzymes, but data on these processes and functions during pregnancy are scarce. There are at least two major reasons for the lack of data. The first is the obvious ethical and safety concern accompanying such in vivo investigations and the second is the unique structure of human placenta that has restricted extrapolation of the information obtained from laboratory animals [19]. Nevertheless, there is data on the concentrations of methadone at delivery in the amniotic fluid, maternal and cord blood, indicating that the opiate is readily transferred across the placenta [8,20,21]. This data, though valuable, addresses the concentration of methadone at a single time point only. Therefore, there is a lack of data on the kinetics for methadone transfer across the human placenta and its disposition by the tissue. This data is essential for better understanding of the factors affecting the amounts of the opiate that reach the fetal circulation and, consequently, its effect on neonatal outcome.

Data on the kinetics for transfer of a drug across human placenta can be obtained using the technique of dual perfusion of a placental lobule. This technique has proven a good predictor of in vivo conditions [22,23]. Recently, the technique was utilized in our laboratory to investigate the transplacental transfer of the opiates buprenorphine (BUP) and L-α-acetylmethadol (LAAM, a congener of methadone). The data obtained revealed that the extent of transplacental transfer of LAAM to the fetal circuit is higher than that for BUP [24,25]. Moreover, the major placental enzyme responsible for the metabolism of BUP, LAAM [26,27], and methadone [28] was cytochrome P 450 (CYP) 19 (known also as aromatase) and not CYP 3A4, as reported for human liver and intestine [29–32]. These data point to the role of the placenta in disposition of these opiates during pregnancy.

Therefore, the goal of this investigation is to determine the kinetics for the bidirectional transfer of methadone across term human placenta, its distribution between the tissue, maternal, and fetal circuits, and its effects on the viability and functional parameters of the tissue.

2. Materials and methods

2.1. Chemicals

Radioactive [^{3}H] methadone at specific activity of 14.1 Ci/mmol was a generous gift from the National Institute on Drug Abuse. All other chemicals were purchased from Sigma–Aldrich including radioactive [^{14}C] antipyrine at specific activity of 4.7 mCi/mmol.

2.2. Placentas

Term human placentas were obtained from healthy women, with no medical or obstetric complications during pregnancy, immediately after delivery from the labor and delivery ward of the John Sealy hospital, University of Texas Medical Branch in Galveston according to a protocol approved by the Institutional Review Board.

2.3. Placental perfusion

The technique of dual perfusion of a placental lobule was used as described in detail in reports from our laboratory [24,25] and originally by Miller et al. [22]. Each placenta was visually examined for tears, an intact peripheral cotyledon selected, and the fetal artery and vein cannulated and perfused within 20 min of delivery. The flow rate of the medium in the fetal circuit was 2.8–3.0 mL/min. The perfused cotyledon was excised from the placenta placed in the perfusion chamber, with the fetal side facing downwards and supported by phosphate-buffered saline. Two maternal artery catheters were inserted, within the region of fetal circulation, through the decidual plate and the intervillous space perfused at a flow rate of 12 mL/min. A large venous drain was connected to a peristaltic pump that removed the perfusate from the chamber and either returned it to the maternal reservoir (closed circuit) or not (open circuit). The perfusion medium was made of the tissue culture medium M 199 supplemented with the following: dextran (7.5 g/L in the maternal and 30 g/L in the fetal reservoir), 40 mg/L gentamicin sulfate, 80 mg/L sulfamethoxazole, and 16 mg/L trimethoprim. The maternal perfusate was gassed with 95% oxygen, 5% carbon dioxide, and the fetal with 95% nitrogen, 5% carbon dioxide, and both maintained at 37 °C. Sodium

bicarbonate was added to the maternal and fetal circuits to maintain the pH at 7.4 and 7.35, respectively.

2.4. Control period of perfusion

Each placenta was perfused with medium for a period of approximately 15 min to clear the fetal vessels and maternal villous space from residual blood. The control period of 120 min allowed the tissue to stabilize in its new environment and to determine the baseline levels for the viability and functional parameters of the tissue. Samples were collected every 15–30 min from the fetal and maternal veins and arteries and immediately analyzed for pH, pO_2, and pCO_2 using a pH/blood gas analyzer (Model 1620; Instrumentation Laboratory). Perfusion of a placenta was terminated if one of the following occurred: fetal arterial pressure exceeding 50 mmHg, a volume loss in the fetal circuit in excess of 2 mL/h, or a pO_2 difference between fetal vein and artery dropping to less than 60 mmHg.

2.5. Experimental period of perfusion

The experimental period followed the control, and was initiated by replacing the medium in the maternal and fetal reservoirs and addition of the drugs. Methadone was added to the final concentration specified for each experiment, together with antipyrine (AP) final concentration of 20 μg/mL. AP is an inert, non-ionizable, lipophilic marker compound with flow limited transfer across the tissue thus used to account for interplacental variability. AP and the opiates were added to either the maternal or fetal reservoir according to the transfer direction investigated, from the maternal to fetal (M → F) or fetal to maternal (F → M), respectively. The radioactive isotopes, 1.5 μCi, of [^{3}H] for methadone and [^{14}C] for antipyrine, were added to the reservoirs and co-transfused with the non-labelled drugs to enhance their detection limits. The range of methadone concentrations investigated was from 100 to 400 ng/mL. This range of concentrations corresponds to serum levels of methadone following the administration of a methadone dose of 30–120 mg/day to a pregnant woman [33].

The perfusion system was used in two configurations, either closed–closed, where the perfusates were recirculated, or open–open, i.e., without recirculation of the medium. The closed–closed configuration is used to investigate the distribution of a drug between the tissue and maternal and fetal circuits, as well as the accumulation of any metabolites formed during the experimental period. The open–open system is used to obtain data on the transfer of a drug across the placenta under steady-state conditions.

The concentration of methadone and AP was determined in 0.5 mL aliquots taken from the fetal and maternal veins and arteries every 5–15 min during the experimental period. Scintillating cocktail (4 mL) was added to each aliquot and the radioactivity of the two compounds determined simultaneously using a liquid scintillation analyzer (1900TR, Packard Instruments, Inc.).

A control experiment where the tissue was perfused for 120 min (control period), the medium changed, and the perfusion continued for another 240 min (experimental period) was performed every 3 weeks. These experiments were carried out to determine the effect of time and our experimental conditions, in absence of any drugs, on placental viability and functional parameters.

2.6. Accumulation of methadone by placental tissue

Methadone was added either to the maternal or fetal reservoirs to achieve a final concentration of 100 ng/mL. The amount of methadone retained by the tissue was determined after 60, 120, or 240 min of transfusion. Each experimental time period was repeated in five placentas. The transfused lobule was dissected, weighed, and homogenized in saline. One millilitre of 1 M NaOH was added to an aliquot (1 mL) of the homogenate and incubated overnight at 60 °C. Scintillation fluid (4 mL) was then added to each aliquot and the amount of radioactivity determined.

2.7. Assessment of placental viability and functional parameters

Aliquots of 200 μL were taken from the maternal and fetal circuits every 30 min during the control and experimental periods, centrifuged at 1000 × *g* for 10 min at 4 °C, and the supernatant stored at −80 °C until analyzed. The concentrations of glucose and lactate were determined using a glucose hexokinase kit (DMA, Inc.) and a lactate reagent kit (Sigma Chemical), respectively. The concentration of hCG was determined using an IRMA kit (Diagnostics Products Corp.).

2.8. Methadone metabolism

Methadone at a concentration of 200 ng/mL was cotransfused with its tritiated isotope (1.5 μCi) in the M → F direction while the system was in its closed–closed configuration to allow for accumulation of the metabolites formed. The duration of methadone transfusion lasted for 4 h and the experiment was repeated in six placentas. The perfusates of both circuits and tissue were analyzed for their content of methadone metabolites, namely, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP). The *N*-demethylated products formed retain the tritium label in the aromatic ring of methadone. Aliquots from the maternal and fetal reservoirs and the tissue homogenate were digested in 1 M NaOH and extracted twice with *n*-butyl chloride utilizing a method developed in our laboratory and described earlier in detail [24]. The organic layer was evaporated at 40 °C under a

nitrogen stream and the dried residue dissolved in 0.5 mL of the HPLC mobile phase. The HPLC system used consisted of a Waters 600E multisolvent delivery system, a Waters 2487 dual wavelength absorbance detector, and a Waters 717 autosampler controlled by Waters Millennium[32] chromatography manager. Chromatographic separation was performed on a C-18 column, 250 mm × 4.6 mm Luna 5 μm (Phenomenex). The mobile phase consisted of acetonitrile/water (33/67, v/v) containing 0.1%, v/v triethylamine. The pH was adjusted to 2.8 with orthophosphoric acid, and the analytes were eluted at a flow rate of 1 mL/min and monitored at a wavelength of 210 nm. The amount of radioactivity in each of the 1 mL fractions collected was determined as described above. Retention times for methadone, EDDP and EMDP standards were determined under identical experimental conditions.

2.9. Binding of methadone to dextran

The binding of [^{3}H] methadone to dextran was determined by gel filtration using Sephadex G-25 desalting columns as described earlier [24] and was found negligible.

2.10. Data and statistical analysis

Oxygen delivery, transfer, and consumption were calculated according to the methods of Wier and Miller [34]. Transfer of methadone (%) from the donor to the recipient circuit in the closed–closed system either in the M → F or F → M direction was calculated according to the following equations: %transfer (M → F) = $(F_c \times F_v) \times 100/[(F_c \times F_v) + (M_c \times M_v)]$; %transfer (F → M) = $(M_c \times M_v) \times 100/[(F_c \times F_v) + (M_c \times M_v)]$, where F_c is concentration of drug in fetal perfusate, F_v is volume of fetal perfusate, M_c is concentration of drug in maternal perfusate, and M_v is volume of maternal perfusate [35].

Transfer rate, clearance, and clearance index of the drug from the donor circuit under steady-state conditions were calculated utilizing the equations reported by Bourget et al. [36], and as applied in our laboratory [24]. Briefly, clearance index is the ratio of the transfer rate of a drug (methadone) to that of the marker compound AP.

The difference between the compared values was evaluated with the 2-tailed t test and considered significant when $P < 0.05$. One-way repeated-measures analysis of variance was applied to calculate statistical significance in continuous measurements as in the effect of the drug on placental viability and functional parameters with perfusion time.

3. Results

3.1. Placental viability and function

Placental viability and functional parameters were determined during the control and experimental periods as described in methods and in an earlier report from our laboratory [24]. The values determined for oxygen delivery, transfer and consumption, glucose utilization, hCG, and lactate release during methadone transfusion at its concentrations of 100, 200, and 400 ng/mL are shown in Tables 1 and 2. The values cited are within the normal range established in our laboratory for control placentas and indicate that methadone at the transfused concentrations does not adversely affect placental viability and functional parameters.

3.2. Bidirectional transfer of methadone

The bidirectional transfer (maternal to fetal and fetal to maternal) of methadone across term human placentas was investigated utilizing the technique of dual perfusion of an isolated lobule. The perfusion medium utilized is devoid of any macromolecules or compounds that binds methadone, as determined by gel filtration, hence the concentration of methadone is that of the free drug. The percent transfer of methadone and its distribution between the tissue, maternal, and fetal circuits were determined utilizing the system in the closed–closed configuration, while its clearance and clearance index under steady-state conditions were determined in the

Table 1
Effects of methadone concentrations on oxygen transfer, delivery, and consumption during the experimental period of 4 h

Concentration of zmethadone (ng/mL)	O_2 delivery		O_2 transfer		O_2 consumption	
	Experimental period (mL/min kg)	% of control period[a]	Experimental period (mL/min kg)	% of control period[a]	Experimental period (mL/min kg)	% of Control period[a]
Control placentas[b]	10.4 ± 2.5	83 ± 16	0.39 ± 0.1	81 ± 10	4.13 ± 1.6	103 ± 18
100	11.3 ± 2.6	95 ± 13	0.42 ± 0.1	91 ± 3	3.6 ± 0.57	104 ± 35
200	14.1 ± 4.8	102 ± 6	0.27 ± 0.15	96 ± 9	3.5 ± 0.7	87 ± 5
400	11.9 ± 1.1	107 ± 17	0.38 ± 0.18	97 ± 13	4.4 ± 1.4	108 ± 24

All values are expressed as mean ± S.D. of 5–8 placentas.

[a] The tissue was perfused with medium only during the control period. The values obtained during the experimental period were expressed as percent of that obtained during the control period.

[b] No drug was added in the experimental period of control placentas.

Table 2
Effects of methadone concentrations on placental tissue viability and functional parameters during the experimental period

Concentration of methadone (ng/mL)	hCG release % of control period[a]	Glucose consumption		Lactate production	
		Experimental period (μmol/min g)	% of control period[a]	Experimental period (μmol/min g)	% of Control period[a]
Control placentas[b]	87 ± 46	0.28 ± 0.13	92 ± 20	0.2 ± 0.04	83 ± 10
100	80 ± 31	0.30 ± 0.09	107 ± 35	0.2 ± 0.08	99 ± 49
200	81 ± 15	0.30 ± 0.09	124 ± 27	0.3 ± 0.1	105 ± 37
400	91 ± 6	0.25 ± 0.13	94 ± 15	0.2 ± 0.06	100 ± 21

All values are expressed as mean ± S.D.

[a] During control period the tissue was perfused with medium only. The values obtained during the experimental period were expressed as percent of the respective values obtained during the control period of each perfusion, which were set as 100%.

[b] No drug was added in the experimental period of control placentas.

open–open configuration. Antipyrine, an inert, lipophilic, and highly diffusible marker compound was cotransfused with methadone in all experiments to account for interplacental variability.

3.3. Maternal to fetal transfer of methadone

The decline in the initial concentration of methadone (100 ng/mL) in the recirculating maternal circuit was biphasic (Fig. 1A). The curve representing the concentration of the drug versus time reflects a steep slope during the initial 60 min. In the remainder of the experimental period, the decline in the concentration was slow and the representing curve had a shallow slope. Methadone appeared in the fetal circuit within 5 min of its addition to the maternal reservoir and initiation of its transfusion. It should be noted that the amounts of methadone appearing in the fetal circuit during the first 30 min of the experimental period were only a fraction of that lost from the maternal circuit, suggesting that the opiate could have been retained by the tissue. This was confirmed by the concentration gradient formed between the tissue and each of the maternal and fetal circuits at the end of the experimental period. The methadone concentration ratio in the tissue/maternal circuit was 6.5 ± 1 and in the tissue/fetal circuit, 9.9 ± 1.2. The fetal to maternal circuit concentration ratio of methadone was 0.71 ± 0.16.

The percent transfer of methadone from the maternal to fetal circuit during a 4-h period was investigated using three doses of the opiate added to the maternal reservoir to achieve the following final concentrations: 100, 200, and 400 ng/mL. The amounts of methadone in the fetal circuit were determined after 1, 2, and 4 h of its transfusion and calculated as a percent of that at "0" time (Table 3). The data revealed that approximately 84% of the total amount of methadone present in the fetal circuit at the end of 4 h was transferred during the initial 60 min of its transfusion. However, the increase in percent transfer of methadone after 2 and 4 h over that at 1 h was not statistically significant. This data was confirmed by the calculated concentration ratios for methadone to antipyrine being the same throughout the experimental period (Table 3). Moreover, a plot of the area under the curve (AUC) versus the dose of methadone added to the maternal reservoir (25, 50, and 100 μg/250 mL) exhibited a straight line (Fig. 2), indicating linear pharmacokinetics for transfer of the opiate in the concentration range tested.

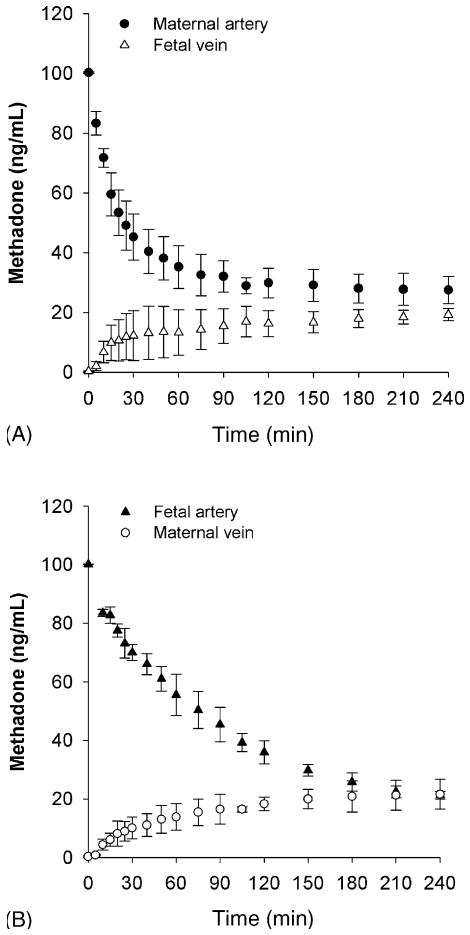

Fig. 1. (A) Plot of methadone concentration in the maternal artery and fetal vein with time. Methadone was transfused in the maternal to fetal (M → F) direction in a closed–closed system. The concentration of methadone in the maternal reservoir at "0" time was 100 ng/mL. At the end of the experimental period of 240 min, the transfer ratio of methadone to AP was 0.52 ± 0.07; (B) plot of methadone concentration in the fetal artery and maternal vein with time during its transfusion in the fetal to maternal direction (F → M) under the same experimental conditions mentioned above. The concentration of methadone in the fetal reservoir at "0" time was 100 ng/mL. At the end of the experimental period, the transfer ratio of methadone/AP was 0.81 ± 0.09. Each data point represents the mean of 5–9 placentas ± S.D.

Table 3
Methadone transfer across human placenta utilizing a closed–closed system in the maternal to fetal (M → F) direction

Time (min)	M → F					
	Methadone (100 ng/mL)		Methadone (200 ng/mL)		Methadone (400 ng/mL)	
	Transfer (%)	Methadone/antipyrine ransfer ratio	Transfer (%)	Methadone/antipyrine transfer ratio	Transfer (%)	Methadone/antipyrine transfer ratio
60	7.9 ± 3.7	0.58 ± 0.06	7.6 ± 2.0	0.47 ± 0.08	6 ± 2.3	0.40 ± 0.1
120	8.9 ± 1.5	0.52 ± 0.09	8.6 ± 1.8	0.48 ± 0.07	7.1 ± 2.3	0.43 ± 0.09
240	9.4 ± 0.7	0.52 ± 0.07	8.8 ± 0.9	0.47 ± 0.04	8.0 ± 2.3	0.45 ± 0.1

All values are expressed as a mean ± S.D.

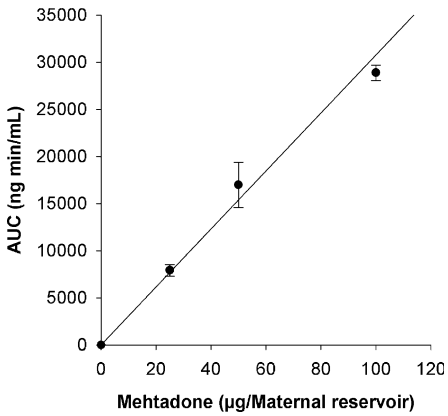

Fig. 2. A plot of the relation between the amount of methadone in maternal reservoir and area under the curve (AUC) for the maternal circuit revealed a strait line and indicates linear pharmacokinetics. Each data point is the mean ± S.D.

The pharmacokinetic parameters for transfer of methadone under steady-state conditions are shown in Table 4. Under the experimental conditions described, the clearance for methadone was 0.85 ± 0.14 mL/min, and its clearance index 0.83 ± 0.09.

An examination of the pharmacokinetic profile for transfer of the marker compound AP from the maternal to fetal circuit utilizing both configurations of the perfusion system is in agreement with earlier reports from our laboratory [24,25] and others indicating that its extent of transfer is independent of variations between individual placentas, but is affected by the degree of overlap between the two circuits in each experiment.

3.4. Fetal to maternal transfer of methadone

The transfer of methadone from the fetal to maternal circuit was investigated by the addition of the opiate to the fetal reservoir at a final concentration of 100 ng/mL. The concentration of methadone in the fetal artery (Fig. 1B) declined gradually during the initial period, reaching a plateau after 150 min. The gradual decrease observed was different from the distinct biphasic decline in the concentration of methadone in the maternal circuit when methadone was transfused in the opposite direction, i.e., from the maternal to fetal circuit (Fig. 1A). On the other hand, the appearance of methadone and the increase in its concentration in the maternal vein was immediate during the initial 90 min. The amount of methadone appearing in the maternal circuit was not equal to that lost from the fetal, suggesting that the opiate was retained by the tissue. Indeed, a concentration gradient for the opiate between the tissue and both maternal and fetal circuits was formed, as evident from the ratios of methadone concentrations in the tissue/maternal circuit and tissue/fetal circuit of 6.5 ± 1.0 and 6.5 ± 0.64, respectively.

The concentration ratio of methadone in the maternal/fetal circuit at the end of the experiment was 1.02 ± 0.29, indicating a statistically significant ($P < 0.05$) higher transfer of the opiate in the fetal to maternal than the maternal to fetal direction (0.71 ± 0.16) cited earlier.

Under steady-state conditions, clearance of methadone from the fetal to maternal circuit was approximately 50% higher than its clearance from the maternal to fetal (Table 4). These observed differences in the transfer of methadone, when normalized to the transfer rates of AP and expressed as clearance index, were statistically significant ($P < 0.05$). On the other hand, there was no difference in the kinetics for AP transfer from the maternal to fetal versus the fetal to maternal circuits.

Taken together, these data suggest that methadone transfer across human placenta is higher in the fetal to maternal than maternal to fetal direction.

Table 4
Pharmacokinetics parameters for methadone in open–open system

Parameters	Methadone (200 ng/mL)		Antipyrine (20 μg/mL)	
	M → F	F → M	M → F	F → M
Lag time (min)	6.36 ± 1.8	12.3 ± 2.9	1.25 ± 1.07	5.2 ± 1.7
Clearance (mL/min)	0.85 ± 0.14	1.29 ± 0.48	1.01 ± 0.24	1.3 ± 0.41
Clearance index	0.83 ± 0.09	0.97 ± 0.05*	–	–

All values are expressed as the mean ± S.D.
* $P < 0.05$.

3.5. Rates for methadone distribution between the tissue, maternal, and fetal circuits

Rates of methadone distribution between the tissue and both circuits during its transfer from the maternal to fetal and fetal to maternal directions were investigated utilizing the model system in its closed–closed configuration. In each experiment, methadone was transfused at a concentration of 100 ng/mL for either 1, 2, or 4 h, and its con-

centrations in the tissue and both circuits determined and presented as a percent of its amount at "0" time. Each time point represents the mean for five placentas.

The amount of methadone in the tissue after 60 min of its transfusion from the maternal to fetal direction represented 52.2 ± 12% of its initial dose. A slight increase in the tissues' retention of methadone was observed after 120 and 240 min, and accounted for 57.8 ± 9% and 60.1 ± 7.5%, respectively (Fig. 3A). These data indicate that, during the initial hour of methadone transfusion, the amount of methadone in the tissue represented 87% of that at the end of the experimental period. The amount of methadone retained by the tissue affects that available for transfer to the fetal circuit. Thus, after 4 h, the amount of methadone transferred to the fetal circuit represented only 10% of its dose at "0" time.

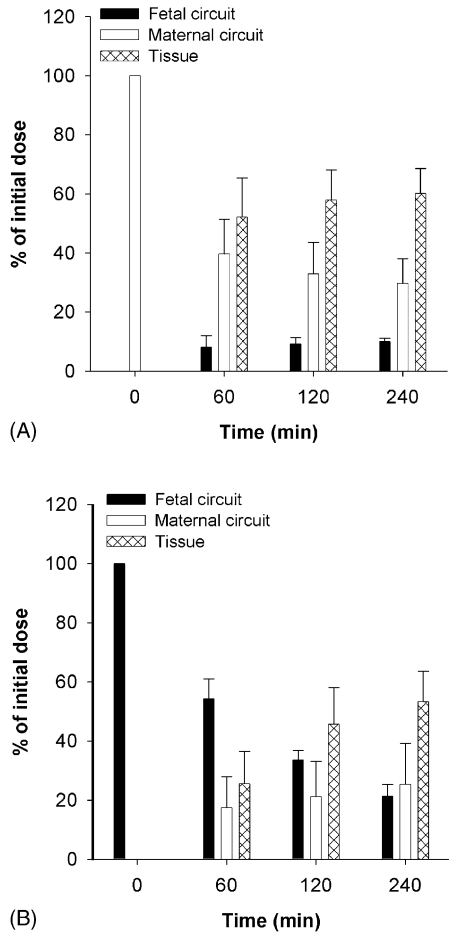

Fig. 3. Rate of methadone distribution between the tissue, maternal, and fetal circuits after 60, 120, and 240 min of its transfusion in a closed–closed system. (A) The concentration of methadone in the maternal reservoir at "0" time was 100 ng/mL when the opiate was transfused in the (M → F direction) for a period of 4 h. Most of the opiate was distributed between the tissue, maternal, and fetal circuits during the initial 60 min of the experimental period. Very little change in the amounts of methadone in the three compartments was observed during the remaining 3 h; (B) The concentration of methadone at "0" time in the fetal reservoir was 100 ng/mL when it was transfused in the (F → M direction). Transfer of methadone from the fetal to maternal circuit, as well as its accumulation in the tissue, was gradual during the experimental period. All values are expressed as mean ± S.D.

On the other hand, the rates of methadone distribution during its transfusion from the fetal to the maternal circuit were different from those in the maternal to fetal direction cited above. The amount of methadone retained by the tissue at the end of its initial hour of transfusion represented only 25.6 ± 11% of the dose added to the fetal reservoir (Fig. 3B). This quantity of methadone retained by the tissue represents only half of that present when the opiate was transfused in the maternal to fetal direction. Moreover, an apparent gradual increase in the quantity of methadone retained by the tissue was observed after 2 and 4 h of its addition to the fetal reservoir, and represented 45.7 ± 12.5% and 53.3 ± 10.3% of its initial dose, respectively. These data indicate that the tissue retained half the quantity of methadone accumulated during the initial 60 min and the other half in the following 3 h of the experimental period.

The rate of methadone distribution between the two circuits during its transfusion in the fetal to maternal direction is shown in Fig. 3B. During the initial hour of transfusion, the amount of methadone transferred from the fetal to maternal circuit represented 17% of the initial dose added to the fetal reservoir and reached 24% by the end of the experimental period. The amount of methadone present in the recipient (maternal) circuit was almost twice that observed when the opiate was transfused in the opposite direction (maternal to fetal) for the same duration of time (60 min). A gradual and low increase in the amount of methadone in the maternal circuit was observed during the following 3 h.

The above data indicate that the rates of methadone distribution between the tissue and the two circuits during the initial hour depend on the direction of transfusion being in the M → F or F → M. Moreover, the quantity of methadone transferred from the fetal to maternal circuit was twice that transferred from the maternal to fetal.

3.6. Methadone metabolism during its transfusion

The amounts of methadone metabolites namely, EDDP and EMDP, were determined in the tissue and in the media of both circuits. The amount of EDDP determined was approximately 1% that of methadone and was present in the tissue extract, maternal and fetal reservoirs at the end of the experimental period. EMDP was not detected in any of the samples under our experimental conditions.

4. Discussion

Methadone is the only agonist approved in the US for pharmacotherapy of the pregnant opiate addict. Methadone treatment programs, worldwide, are considered the standard of care for this patient population, but data on

associated adverse effects have been reported. The most controversial of the reported data is whether the incidence and intensity of NAS is associated with the administered dose of methadone. However, it is generally accepted that NAS should correlate with the concentration of the opiate in the fetal circulation and the duration of exposure, especially whether it has occurred during early or late gestational ages.

The lack of data on the PK of methadone during pregnancy and its concentrations in the fetal circulation is due to obvious ethical and safety considerations. The only available data are those obtained at delivery for maternal plasma levels of methadone, its concentration in the newborn circulation, and in the amniotic fluid. However, this data represents one point in time and does not reflect any dynamic changes. An understanding of the ability of a compound to cross the ''barrier'' between the maternal and fetal compartments, the extent of its metabolism by the placenta, and its effects on functions of the tissue should lead to better knowledge of the factors regulating its concentration in the fetal circulation and, consequently, the ability to improve neonatal and maternal outcome. Therefore, the goal of this investigation was to determine the role of the human placenta in the bidirectional transfer of methadone, its metabolism by the tissue and effects on the viability and functional parameters of the tissue. The concentrations of methadone transfused into the tissue were those reported in maternal plasma following the administration of therapeutic doses of the opiate.

The bidirectional transfer of methadone, a lipophilic compound with a molecular weight of 345 Da, was determined utilizing the technique of dual perfusion of a placental lobule. In view of the physicochemical properties of methadone and its molecular weight being <600, it should readily permeate across the placenta [37]. Data cited here indicate that the transfer of methadone in the maternal to fetal direction is less than that in the fetal to maternal, whether the model system was used in the closed–closed or open–open configuration. A plot of the concentration of methadone in the donor circuit versus time, when the drug was transfused in the M → F direction in a closed–closed system, indicated that the decline in its concentration in the maternal circuit was distinctly biphasic: initially fast, then gradual (Fig. 1A). On the other hand, when methadone was transfused in the fetal to maternal direction, the decline in its concentration in the donor (fetal) circuit was slow and gradual and not biphasic (Fig. 1B). The decline in the concentration of methadone in the donor circuit represents the sum of the quantities of the drug retained by the tissue and that transferred to the recipient circuit. The observed higher amounts of methadone retained by the tissue after 1 h of its transfusion in the maternal to fetal direction as opposed to the fetal to maternal is likely due to the flow rate of the drug being four times higher in the former than in the latter, as well as the insertion of the catheters directly into the maternal intervillous space (Fig. 3A and B). Consistent with this explanation is the higher amount of methadone remaining in the donor circuit when it was transfused in the F → M than in the M → F direction. However, when methadone was transfused in the maternal to fetal direction, its amount in the recipient circuit was half that observed after its transfusion in the opposite direction. Therefore, the transfer of methadone from the fetal to maternal direction (M/F concentration ratio: 1.02 ± 0.29) was higher than the maternal to fetal (F/M concentration ratio: 0.71 ± 0.16) using the model system in its closed–closed configuration, which is considered the best available simulation of in vivo conditions.

The above data obtained utilizing an ex vivo model system is in agreement with the limited number of reports on the concentrations of methadone in the maternal and fetal circulations and amniotic fluid at time of delivery, where the umbilical vein/maternal plasma concentration ratio was 0.5 [8,21]. The observed higher ratio of 0.71 ± 0.16 for the concentration of methadone in the fetal/maternal circuit when it was transfused in the maternal to fetal direction could be explained by the lower levels of the ''free'' methadone in vivo due to its binding to plasma proteins, fetal elimination, and placental metabolism that are absent under our experimental conditions.

The transfer of methadone was also investigated under steady-state conditions to determine its clearance and the clearance index. Under these conditions, the clearance of the marker compound AP in the maternal to fetal direction was within the experimental value determined for that in the fetal to maternal, i.e., the transfer of AP was independent of the transfusion direction (Table 4). However, methadone clearance when it was transfused in the fetal to maternal was higher (1.29 ± 0.48 mL/min) than that in the maternal to fetal (0.85 ± 0.14 mL/min) direction. Clearance of methadone, in absence of its metabolism by the placenta or fetus, represents the transfer of the opiate to the recipient circuit. Moreover, the clearance index for methadone, which is the ratio between the transfer rate of methadone to that for AP, is a parameter for the opiate that has been normalized for variations between placentas was also higher ($P < 0.05$) in the fetal to maternal (0.97 ± 0.05) than maternal to fetal (0.83 ± 0.09) direction. Taken together, it is evident that the transfer rates for methadone from the fetal to maternal direction, after normalization of the data to that for the transfer of AP, are higher than those from the maternal to fetal. This data could be explained by the activity of the unidirectional efflux transporter P glycoprotein. P-gp is a product of the multidrug resistance gene (MDR1 or ABCB1), and methadone is one of its substrates [38]. P-gp is highly expressed in the apical membranes of the syncytiotrophoblast [39] and its function is to extrude drugs and xenobiotics from the tissue to the maternal circulation to protect the fetus [40,41]. A recent preliminary report from our laboratory provided data on P-gp

expression in 81 term placentas and indicated that the amount of the protein relative to that of β-actin varied widely between individuals [42]. Therefore, it is reasonable to suggest that P-gp plays a role in the efflux of methadone from the placental tissue to the maternal circuit to counteract its diffusion from the maternal to fetal circulation.

The amount of methadone retained by the tissue during the initial period of 1 h was higher in the M → F direction, but by the end of the experimental period, the tissue had accumulated the same amounts of the opiate irrespective of direction of transfusion (Fig. 3A and B). However, the concentration gradient formed for methadone between the tissue and the two circuits after 4 h was dependent on the direction of its transfusion. When methadone was transfused in the maternal to fetal direction, the ratio for its concentrations in the tissue/fetal and tissue/maternal circuits were 9.9 ± 1.2 and 6.5 ± 1, respectively, but when the opiate was transfused in the F → M direction, its concentration ratios were almost the same (6.5 ± 0.6 for tissue/fetal and 6.5 ± 1.0 for tissue/maternal). The formation of a concentration gradient between the tissue and each of the two circuits, at the end of 4 h of transfusing L-acetylmethadol, a congener of methadone, has been reported. The concentration ratios for LAAM in the tissue/fetal and tissue/maternal circuits were 20.2 ± 4.6 and 15.8 ± 1.7, respectively [25]. Buprenorphine, the partial opiate agonist used for treatment of the pregnant opiate addict in many countries, was also retained by placental tissue. Its concentration ratios in the tissue/fetal and tissue/maternal circuits were 27.4 ± 0.4 and 13.1 ± 6.5, respectively [24]. Taken together, it is apparent that the concentration ratio between the tissue/fetal circuit is highest for BUP and lowest for methadone, with LAAM in between. The order in which the tissue accumulated the three opiates is consistent with their log *P* values of 5.1, 4.9, and 3.9 for BUP, LAAM, and methadone, respectively. Therefore, these data indicate that placental tissues act as a depot for the three opiates, but their capacity to retain each is different. Other in vitro perfusion studies have shown that the rapid disappearance of highly lipophilic drugs from the maternal circuit might result in restricting their fetal transfer relative to less lipophilic drugs [43]. Indeed, the maternal to fetal transfer rates of the three opiates were 11.6 ± 2.5% for BUP, 26.9 ± 1.5% for LAAM, and 29.4 ± 4.6% for methadone. These data are in agreement with the hypothesis that the transfer of a lipophilic drug across the human placenta is a 2-step process: the first is its uptake by the tissue and the second is its release to the recipient circulation [44].

In vivo, hepatic microsomal cytochrome P 450 (CYP) 3A4 was identified as the major enzyme responsible for the metabolism of methadone to EDDP and EMDP [45]. Recent investigation in our laboratory utilizing placental subcellular fractions identified CYP 19 (aromatase) as the major enzyme responsible for the metabolism of methadone to EDDP only, with apparent K_m and V_{max} values of 424 ± 92 μM and 420 ± 89 pmol mgprotein^{-1} min^{-1} [28]. However, after 4 h of methadone transfusion in a closed–closed system to allow for accumulation of the metabolites formed, negligible amounts of EDDP were detected. This is due to the limited area of the tissue being transfused which represents less than 5% of that available in vivo and is a limitation of the technique used. These data are in agreement with reports on the very low metabolism of BUP and LAAM during their transfusion into placental tissue, than that observed in vitro using placental subcellular fractions [26,27].

Data in Tables 1 and 2 indicate that all of the methadone concentrations investigated had no effect on the determined placental tissue viability and functional parameters, namely, glucose utilization, lactate production, and hCG release. The values obtained for these parameters were within the acceptable ranges obtained in our laboratory and others, as well as the ranges for control placentas, i.e., those perfused with medium only [22,24,25,46]. These data suggest that methadone, in the concentrations investigated, which are also comparable to those present in maternal plasma during pregnancy, does not cause adverse effects on placental viability and functional parameters. This data is consistent with the lack of reports, to the best of our knowledge, on pregnancy complications in women under treatment with methadone attributable to placental functions being adversely affected by the opiate.

In summary, the transfer of a low molecular weight lipophilic compound, such as methadone, across the human placenta is by passive diffusion and is flow limited. However, this transfer could be affected by placental carrier mediated proteins, efflux transporters, and metabolic enzymes. Data reported here indicate that, in the ex vivo model system utilized, the transfer of methadone across the placenta favored the fetal to maternal direction, a characteristic of P-gp activity. Reports from our laboratory and by others indicate that P-gp expression in human placentas varies over a wide range and aromatase is the major enzyme responsible for metabolism of methadone in the tissue. Also, the activity of these two proteins is subject to change with gestational age and is under genetic and metabolic control. Therefore, it is reasonable to assume that placental disposition, transfer and metabolism, of methadone during pregnancy is one of the important factors affecting the concentration of the opiate in the fetal circulation and hence the incidence and intensity of neonatal abstinence syndrome.

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