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Methimazole and Propylthiouracil Equally Cross the Perfused Human Term Placental Lobule*

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ABSTRACT

Propylthiouracil (PTU) is widely believed to cross the placenta less freely than methimazole (MMI) and is therefore regarded as the preferred drug for treatment of hyperthyroidism in pregnancy. Clinical studies comparing the two drugs show, however, no differences in maternal or fetal thyroid function. We investigated transfer from the maternal to the fetal circuit in the isolated perfused term human placental lobule of low and high doses of PTU (4 μg/mL and 40 μg/mL) and MMI (1.5 μg/mL and 15 μg/mL) in protein-free perfusate and low doses of both drugs with addition of 40 g/L of bovine albumin. Both drugs readily crossed the placenta, reaching equilibrium in all experiments in about 2 h. Drug concentrations in the two circuits fitted a two compartmental model. Transfer kinetics for the two drugs were similar, nonsaturable, and unaffected by addition of albumin. Clearances ($mL \cdot min^{-1} \cdot g^{-1}$, means $\pm$ SD) of PTU from maternal to fetal circuits were: 0.229 $\pm$ 0.110, 0.216 $\pm$ 0.065, and 0.170 $\pm$ 0.032; and for transfer of MMI: 0.165 $\pm$ 0.025, 0.232 $\pm$ 0.153, and 0.174 $\pm$ 0.009 (for low doses without, low doses with, and high doses without albumin, respectively). Clearances of PTU from fetal to maternal circuits were: 0.147 $\pm$ 0.072, 0.109 $\pm$ 0.014, and 0.116 $\pm$ 0.028; and for transfer of MMI: 0.095 $\pm$ 0.029, 0.122 $\pm$ 0.088, and 0.12 $\pm$ 0.005 (in the same experiments). There was no significant difference between drugs or drug doses and no effect of addition of albumin. We conclude that PTU and MMI have similar placental transfer kinetics. (*J Clin Endocrinol Metab* **82:** 3099–3102, 1997)

PREGNANT hyperthyroid women are at risk of miscarriage and premature labor, whereas in those whose hyperthyroidism is caused by Graves' disease, transplacental passage of thyroid-stimulating antibodies also may cause fetal and/or neonatal hyperthyroidism (1). The thionamide antithyroid drugs, either carbimazole (CMI, or its active metabolite methimazole, MMI) or propylthiouracil (PTU) are widely used in pregnant thyrotoxic women. Although these drugs freely cross the placenta and can cause fetal and neonatal hypothyroidism, an early study (2) suggested that the placenta, at least in the first half of pregnancy, is less permeable to PTU than to MMI. PTU is regarded accordingly as the preferred drug in pregnancy. Despite this, no differences have been demonstrated between MMI and PTU treatment (3, 4) in terms of maternal and fetal thyroid function. We report here studies, in the isolated perfused term human placental lobule, which show similar transfer of MMI and PTU from the maternal to fetal circuit.

Materials and Methods

Placental perfusions

Perfusion method. These studies were approved by the Royal Women's Hospital Research Ethics Committee. The placentas used were from normal women without a history of drug ingestion, delivered at term by cesarean section. The indication for this form of delivery was a previous cesarean section. Perfusions were established within 20 min of delivery.

The perfusion techniques, materials, conditions, and the viability of the perfused isolated human placental lobule have been described previously (5). Briefly, a fetal circuit was established by cannulation of a paired chorionic artery and vein supplying a lobule. A maternal circuit was achieved by piercing the chorionic plate to allow arterial inflow to the maternal sinusoids. Venous outflow was collected by gravity from the maternal surface of the lobule. The maternal perfusate was recirculated at 25 mL/min and the fetal perfusate at 3 mL/min.

Experimental design. Nine perfusions were carried out. In three, PTU and MMI were together added to maternal perfusate at the beginning of perfusion in doses calculated to produce concentrations similar to those found in plasma after ingestion of the drugs (PTU final concentration: 4 μg/mL, MMI final concentration: 1.5 μg/mL) and samples taken from the maternal and fetal circuits at 30-min intervals for 6 h for measurement of PTU and MMI levels and for measurement of pO_2, pH, lactate, and β hCG levels (indices of tissue viability). This experiment was repeated with 10-fold higher drug concentrations to test for saturability of transfer (PTU final concentration: 40 μg/mL, MMI final concentration: 15 μg/mL). In the final three perfusions, of 3-h duration, PTU (final concentration: 4 μg/mL) and MMI (final concentration: 1.5 μg/mL) were again used with BSA (final concentration: 40 g/L, Sigma Chemical Company, St. Louis, MO) added to maternal and fetal perfusate. BSA, rather than human serum albumin, was used because of the high cost of the human material. An average of 21.3 mL perfusate was removed from the maternal circuit and 27.7 mL from the fetal circuit during the course of a perfusion.

Drug assays

PTU. PTU, (6-propyl-2-thiouracil), was measured in perfusate by selective liquid chromatography, after chloroform extraction, using 5-propyl-2-thiouracil as an internal standard, as previously described (6).

PTU; protein binding. Binding of PTU to the BSA used in the perfusions and, for comparison, to human serum albumin (Sigma Chemical Company) was measured by ultrafiltration. Then 4-μg/mL solutions of PTU

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were prepared in maternal perfusate solutions containing: 1) no albumin; 2) 40 g/L BSA; and 3) 40 g/L human serum albumin. Then 400 μL of each solution was placed in a Millipore Ultrafree-MC Low Binding Membrane Unit, 10K nominal molecular weight limit (Millipore Products Division, Bedford, MA) and centrifuged at 6,500 rpm for 15 min or at 13,000 rpm for 5 min (results identical) in an MSE Microcentrifuge (MSE Scientific Instruments, Crawley, UK). PTU was measured in the ultrafiltrates. Absorptive loss of PTU to the filter was about 3%. Protein leak across the membrane, measured by a biocinchoninic acid technique (BCA Protein Assay Reagent Kit, Pierce Chemical Company, Rockford, IL) was negligible. The unbound fraction of PTU was calculated as the ratio of the concentration of PTU in ultrafiltrate from protein containing perfusate, to the concentration of PTU in the ultrafiltrate from protein-free perfusate.

MMI. MMI, (1-methylimidazole-2-thiol), in perfusate was measured by gas chromatography-mass spectrometry (GCMS) using a method adapted from that of Floberg *et al.* (7). MMI was obtained from Sigma Chemical Company and the internal standard, [1-CD_3]-MMI, was a generous gift from Professor B. Lindstrom (National Board of Health and Welfare, Uppsala, Sweden). Isotopic purity of the deuterated methyl on the internal standard was determined as 98.7%. N, O-bis(trimethylsilyl)-trifluoroacetamide, and silylation-grade acetonitrile were obtained from Pierce Chemical Company.

The GCMS system consisted of a Hewlett Packard model 5985B mass spectrometer and model 5840 gas chromatograph (Hewlett Packard, Palo Alto, CA), equipped with a 12.5-m HP1 capillary column (0.2-mm inside diameter, 0.25-μm stationary phase thickness). The ion source temperature of the mass spectrometer was 200 C, the ion source pressure was 3×10^{-6} torr, and the direct capillary inlet transfer line was maintained at 290 C. The instrument was calibrated with perfluorotributylamine and operated in electron impact mode (70 electron volts). The GC was operated in a split injection mode (10:1), with the compounds separated, using helium gas at a flow rate of 1 mL/min.

Perfusate samples (1 mL), to which the internal standard (50 ng) had been added, were shaken gently with chloroform (2 mL) for 5 min, centrifuged (700 (times] *g*) for 5 min, the organic phase separated, and the chloroform evaporated (60 C) under a gentle stream of nitrogen. The residue was dissolved in N,O-bis(trimethylsilyl)-trifluoroacetamide (50 μL) and acetonitrile (50 μL), and the mixture heated at 100 C for 1 h. This solution was analyzed directly by GCMS.

Complete mass spectra were acquired over the range 50–500 atomic mass units. Quantitative analyses were performed using selected ion monitoring (MMI, m/z 186; internal standard, m/z 189) with a dwell time of 100 msec/ion. The injection port temperature was set at 250 C. The column oven was programmed from 90–290 C (10 C/min for 5 min followed by 15 C/min to 290 C). Peak area ratios of MMI to internal standard in samples and standards were measured from selected ion-monitoring spectra. Both MMI and the internal standard produced significant molecular ions (approximately 50% relative abundance) in total ion mass spectra as the trimethylsilylated derivatives. MMI concentrations were determined from a calibration curve derived from the corresponding ratios for a series of standards.

The assay was linear for concentrations of MMI ranging from 10 ng/mL to 2 μg/mL, whereas extraction recovery, tested at 50 and 500 ng/mL, averaged 25%. The specificity of the assay was investigated by extracting samples of perfusates collected from placental perfusion experiments not containing MMI. In addition, two samples of perfusate were prepared, containing a range of drugs used in pregnancy at the upper limit of their respective therapeutic concentrations, one sample being spiked with MMI and an aliquot of each sample extracted and analyzed, as described above, for any interference. Analysis of used perfusate and perfusate containing the drugs showed no interference caused by coelution or production of ions at either m/z 186 or 189.

The lower limit of detection, determined as the concentration of MMI which gave a signal to noise response of 2:1 for selected ion monitoring at an m/z of 186 Dalton, was 5 ng/mL. Intraassay coefficients of variation at concentrations of 20 and 150 ng/mL were 7.2 ± 1.5 and 4.1 ± 6.3%, respectively, and the corresponding interassay coefficient of variation for a concentration of 50 ng/mL was 5.6%.

Mathematical analyses

ANOVA. Drug levels in maternal and fetal circuits, expressed as a percentage of the initial concentration in the maternal circuit and calculated elimination-rate constants with and without addition of albumin to the perfusion medium, were compared by a two-way repeated-measures unbalanced ANOVA using banded covariance with BMDP Program 5V (BMDP Statistical Software, Los Angeles, CA) (8). The model included change in drug concentration with time, type of drug, presence or absence of albumin, and interaction of drug and albumin. A probability value less than 0.05 was regarded as significant.

Compartmental analysis. Drug concentration data were fitted to a two-compartment model (maternal and fetal circuit) by solving differential equations (dM/dt − change in maternal compartment and dF/dt − change in fetal compartment drug concentrations with time) using the BMDP program AR (9). In the equations below, the elimination rate constants k1 and k2 represent transfer from the maternal to fetal circuit and transfer from the fetal to maternal circuit. Mconc and Fconc refer to initial concentrations of drug in the maternal and fetal circuits, respectively: dMconc/dt = −k1·Mconc + k2·Fconc; dFconc/dt = k1·Mconc − k2·Fconc.

Quality of fit of the two-compartment model was measured by the pseudo R-square (1.0 − ratio of the weighted residual sum of squares to (N-1) times the weighted variance). Clearances were calculated from the product of the elimination-rate constants (normalized for perfused

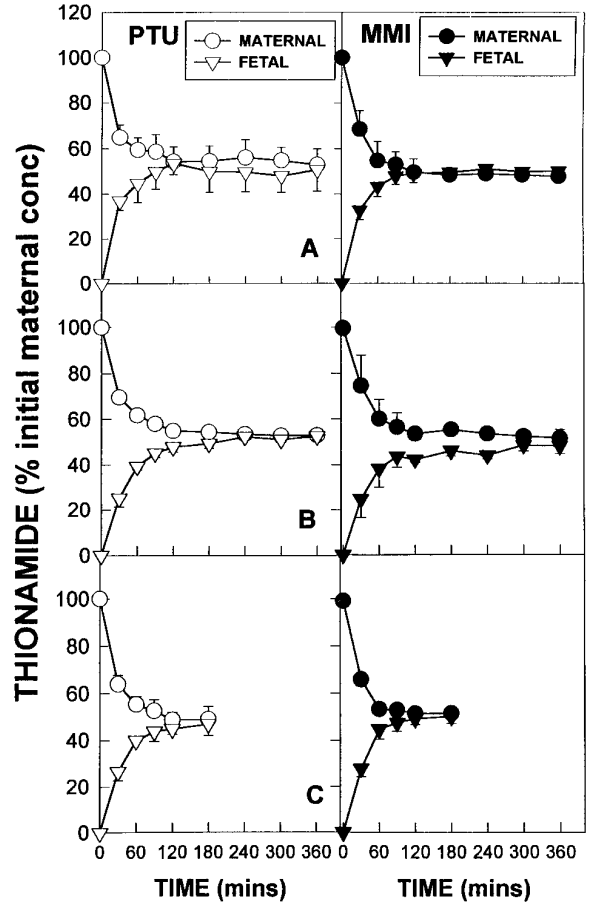

FIG. 1. PTU and MMI levels (±SD), expressed as a percentage of initial concentration in maternal (*circles*) and fetal (*triangles*) circuits of the perfused human placental lobule. Panel A, initial concentration PTU (4 μg/L) and MMI (1.5 μg/L), without added albumin; panel B, initial concentration PTU (4 μg/L) and MMI (1.5 μg/L) with 40 g/L BSA; and panel C, initial concentration PTU (40 μg/L) and MMI (15 μg/L) without albumin.

TABLE 1. Maternal and fetal elimination rate constants and clearances, normalized to lobule weight, derived from a two-compartment model of thionamide transfer in the perfused human placenta

		Maternal		Fetal	
		K1 ($hr^{-1} \cdot g^{-1}$)	CL ($ml \cdot min^{-1} \cdot g^{-1}$)	K2 ($hr^{-1} \cdot g^{-1}$)	CL ($ml \cdot min^{-1} \cdot g^{-1}$)
PTU	A	0.103 ± 0.048	0.229 ± 0.110	0.111 ± 0.046	0.147 ± 0.072
	B	0.088 ± 0.021	0.216 ± 0.065	0.093 ± 0.019	0.109 ± 0.014
	C	0.071 ± 0.013	0.170 ± 0.032	0.074 ± 0.018	0.116 ± 0.028
MMI					
	A	0.075 ± 0.011	0.165 ± 0.025	0.073 ± 0.017	0.095 ± 0.029
	B	0.095 ± 0.062	0.232 ± 0.153	0.104 ± 0.074	0.122 ± 0.088
	C	0.072 ± 0.004	0.174 ± 0.009	0.071 ± 0.003	0.112 ± 0.005

Initial maternal circuit thionamide concentrations of (A) 4 μg/L PTU and 1.5 μg/L MMI without albumin, (B) 4 μg/L PTU and 1.5 μg/L MMI with 40 g/L BSA, and (C) 40 μg/L PTU and 15 μg/L without BSA. There is no significant difference, by two-way, repeated-measures ANOVA, between drugs or drug dose and no effect of addition of albumin. There were three placentas in each group. Mean lobule mass was 12.5 ± 4.4 g. Values are means ± SD.

lobule wet weight) and the mean circuit volume over the course of a perfusion

Results

Maternal circuit perfusate thionamide levels fell, and fetal perfusate thionamide levels rose in a biexponential manner, reaching equilibrium between 120–180 min (Fig. 1). ANOVA revealed that while maternal circuit levels fell, and fetal circuit levels rose, in a highly significant manner with time (maternal and fetal $P < 0.0001$); there were no significant differences between maternal circuit PTU and MMI levels or between fetal circuit PTU and MMI levels within or between the three groups. There was no significant effect of albumin or statistical interaction between drug and albumin.

Maternal and fetal circuit thionamide levels fit well to a simple two-compartmental model, with mean pseudo R-squares ranging from 0.948–0.996 (mean 0.982). Mean drug elimination rate constants and clearances (Table 1) did not differ significantly between PTU and MMI within or between the three experimental groups.

The binding of PTU (mean ± SD) to BSA was 94.5 ± 0.2% (n = 11) and to human serum albumin, 60.6 ± 2.4% (n = 12).

Discussion

This study was done using sensitive and specific drug assays and a well-established model of drug transfer across the human placenta. The results show no difference between the transfer of MMI and PTU across the isolated perfused-term human placental lobule. They differ, therefore, from the data of Marchant and co-workers, which were derived from administration of a single dose of radiolabeled drug to a small group of women before pregnancy termination (2) and suggested less transfer of PTU than MMI. Unfortunately, such single-dose studies may be misleading, because the maternal-fetal distribution of a drug at steady state is not determined by the ratio between the postdistribution maternal and fetal plasma drug levels after a single dose of drug (10). Marchant and co-workers invoked differences in drug binding to protein to explain their results. Unlike MMI, which does not bind to plasma proteins, PTU is reported to be about 67% albumin bound, using an equilibrium dialysis technique (11). We used BSA in this study for cost reasons and found significantly greater binding of PTU to bovine than to human serum albumin (94% *vs.* 61%). Although protein binding of PTU might be expected to reduce its placental clearance, in our study, addition of 40 g/L BSA to perfusate had no significant effect on PTU transfer, suggesting that there is a short dissociation time from albumin and relatively efficient placental extraction of the drug (12). Addition of a 10-fold higher dose of PTU or MMI did not alter clearance, indicating that transport is not saturable.

The present study does not confirm relatively limited placental transfer of PTU and helps explain the failure to find a difference in fetal outcome between maternal PTU and MMI treatment of hyperthyroidism in pregnancy (3, 4). Other arguments for preferring PTU in pregnancy, however, have been invoked. Maternal MMI treatment may be associated with the very rare condition, aplasia cutis congenita, although the causality is not certain (13). Even though low maternal doses of MMI do not seem to cause significant hypothyroidism in a suckling infant, less PTU than MMI is excreted into breast milk (14). Although there may be reasons, therefore, to prefer PTU over MMI in pregnancy, the choice should not be based on the belief that the placenta is relatively impermeable to PTU.

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