Human Placental Transport of Cimetidine

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Abstract

This study addresses the mechanism of transport of the H_2-receptor antagonist, cimetidine, by the human placenta. A 4-h recycling perfusion of a single placental cotyledon of normal, term, human placenta was used. At a maternal concentration of 1 μg/ml, cimetidine clearance from the maternal circulation was 0.58±0.16 ml/min per g placenta, a rate about one third that of antipyrine. There was no evidence of cimetidine metabolism by the placenta. Transfer of cimetidine from maternal to fetal compartments showed no saturation kinetics and was not inhibited by putative carrier competitors. Cimetidine did not accumulate against a drug concentration gradient. Fetal clearance of cimetidine was similar to maternal clearance. Studies with placental apical vesicles confirmed lack of saturability of cimetidine transport and of its concentration within vesicles. Thus, (*a*) cimetidine is transported across the human placenta bidirectionally at a rate about one third that of antipyrine, (*b*) the drug is not metabolized by the placenta, and (*c*) the transport is a passive one.

Introduction

The H_2-receptor antagonists, of which cimetidine (Tagamet) and ranitidine (Zantac) are best known, are some of the most commonly prescribed therapeutic agents in current clinical use in the United States. These drugs are generally given for the treatment of peptic ulcer disease (1), but the indications seem to have been broadened recently (2).

Pregnant women often suffer from heartburn secondary to decreased esophageal tone and motility as well as the upward displacement and compression of the stomach by the uterus. They are also at risk of gastric acid aspiration (Mendelson's syndrome) during delivery under general anesthesia (3). The former problem is generally treated with antacids and for the latter some have suggested a brief use of cimetidine (3). Based on the latter experience and studies in animals, it is known that cimetidine can cross the placenta (4). However, the extent and mechanism of its transfer in humans are uncertain. Prolonged use of cimetidine in pregnancy is currently not sanctioned, except in compelling individual circumstances (5). In sheep, it has been determined that cimetidine (as well as ranitidine) crosses the placenta, that the binding on either side of the placenta is comparable, that the fetal kidneys excrete the drug and that the placenta apparently controls the rate of cimetidine transfer into the fetal circulation (6–8). The mechanism(s) of the transfer in sheep were not established, but the possibility of active transport of the drug from the fetal to the maternal circulation was strongly sugggested (8). The interpretation of these latter studies was hampered by the presence of the fetus in the system, rendering analysis of placental function alone difficult. Furthermore, sheep placenta differs morphologically from human placenta (9).

The purpose of the present study, therefore, was to assess comprehensively the mechanism(s) of human placental transport of cimetidine. To define the manner in which these drugs are transported by the placenta, we utilized the perfused human placental cotyledon system (10) and maternal-facing (apical) microvilli prepared from human placental syncytiotrophoblast (11).

Methods

Subjects

Placentas from 59 women were used. Of these, all but three were at term, the others were 30, 35, 36 wk. Of the 59 studies all but four were pregnancies without known complications. Three pregnancies were complicated by pregnancy-induced hypertension, one by class A (gestational) diabetes. In most instances, placentas were obtained by vaginal delivery under no anesthesia or only local anesthesia. In a few instances (5), the placenta was delivered by Cesarean section. No apparent difference was noted in studies with either delivery mode or in the four pregnancies with medical disorders. Hence, the data were pooled. The women ranged in age between 15 and 37 yr with a mean age of 22.6±4.4 yr. The study was approved by the local Institutional Committee for the Protection of Human Subjects.

Placental perfusion system

Perfusion technique. A single cotyledon placental perfusion model described by Brandes et al. (10) and used to good advantage by others (12–15) has been adopted for local use. Briefly, a normal human term placenta obtained immediately after delivery was used. The tissue was perfused immediately with heparinized cold Krebs-Ringer buffer, the fetal vessels supplying a well-defined cotyledon were cannulated with polyethelene catheter and after ascertaining good flow and lack of leakage via tears, the placenta was mounted in a plexiglass chamber with the maternal surface facing upward. The blanched cotyledon on the maternal side was perfused with two or three properly placed needles placed 2–3 mm below the maternal surface, in the intervillous space. The perfusion fluid consisted of Krebs-Ringer buffer, pH 7.4, containing fetal blood obtained from the cord (hematocrit ~ 20%) and human albumin (Alpha Therapeutic Corp., Los Angeles, CA) at a concentration of 2 g/100 ml. Temperature was maintained at 37°C, the pressure in the system was monitored and maintained at 40–60 mmHg, and the pH was maintained at 7.4 by adjustment of CO_2 in the system. The system was usually oxygenated with 21% O_2 and 5% CO_2. In some studies 95% O_2 was employed. These differences in O_2 delivery systems had no differential effect on placental viability or function. The fetal flow rate was usually 2–4 ml/min. This rate has been used by others (15) with maintenance of placental integrity. The perfusion lasts 2.5–4 h, depending on the approach used, and was successful in our hands in ~ 90% of instances.

Two types of perfusion systems were employed. In one system, the

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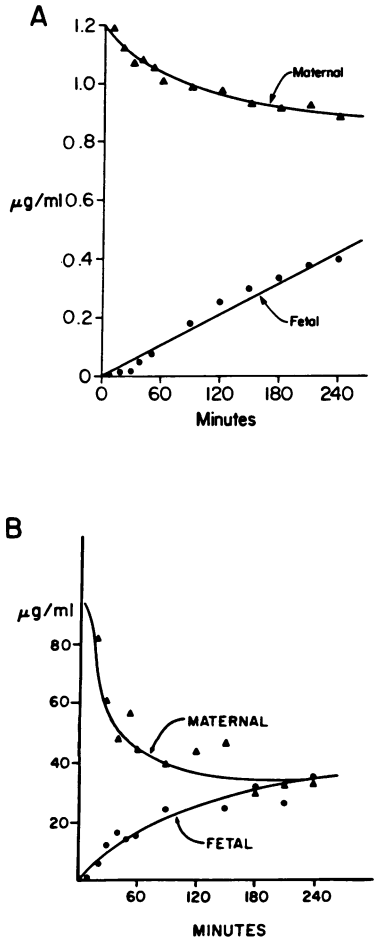

Figure 1. Comparison of cimetidine and antipyrine transport from maternal to fetal circuits via the human placenta. (*A*) A typical cimetidine study. 0.25 mg of [^{3}H]cimetidine was dissolved at the start of the study in 250 ml of buffer, constituting the maternal reservior. This gives a maternal compartment concentration of cimetidine of ~ 1 μg/ml, similar to values seen in patients on usual cimetidine therapy. Data show the decay of the drug from the maternal compartment and its appearance in the fetal circuit. No cimetidine metabolites were noted in either circuit or in the placental cotyledon at the end of the study. (*B*) A typical antipyrine study. 25 mg of antipyrine was dissolved at the start of the study in 250 ml of buffer, constituting the maternal reservior. The figure is a representative one of many experiments and shows the decay of the drug in the maternal circuit and appearance of antipyrine in the fetal reservoir. Equilibration occurred at ~ 3.5 h. No antipyrine metabolites were noted in either compartment.

recirculating one, both the maternal (250 ml) and fetal reservoirs (100 ml) were recirculated. The substance studied (i.e., cimetidine) was placed in the donor (maternal or fetal) reservoir at the start of the study (usually after 20 min of equilibration to verify proper function) and the appearance of the substance and any derivatives in the contralateral circuit was measured sequentially during the next 4 h in small aliquots (1 ml each) removed every 30 min from the recipient reservoir. The second perfusion model was an open one. (The solution was as described previously, except that erythrocytes were omitted.) In this approach either the fetal or maternal reservoir was again recirculated, while the opposite side was open. This permitted a steady-state administration of the agents. Thus, if a given concentration is infused continuously via the open circuit and the opposite reservoir concentration is initially adjusted to the same drug level, any subsequent net transfer and accumulation of the drug in that reservoir implies transport against a concentration gradient and is evidence for active transport. Additionally, clearance studies can be performed in which the drug concentration on the donor side can be maintained at a constant level, allowing optimal clearance calculations, especially for more rapidly removed drugs (i.e., antipyrine).

Because placental transport rates vary among individuals, it is customary to compare the transfer of test compounds in each placental preparation vs. a well-equilibrated reference marker such as antipyrine (10). This permits expression of individual transfer rates as the clearance index, i.e., cimetidine clearance/antipyrine clearance or transfer index, i.e. percent cimetidine/antipyrine transported per unit of time. In this study, both parameters were employed, although not in every perfusion, because in some experiments radiolabeled antipyrine was not used, and thus, accuracy of the assay was not optimal. Furthermore, in calculating the transfer index, 2-h time was set for the calculation because transfer of both antipyrine and cimetidine was linear until then. For calculation of maternal cimetidine and antipyrine clearances (*Cl*) the formula: $Cl = [(C_{FV} - C_{FA}) \cdot Q_F]/C_{MA}$ was used, wherein C_{FV} = concentration of drug in fetal vein, C_{FA} = concentration of drug in fetal artery, Q_F is the fetal perfusion rate, and C_{MA} is the maternal arterial drug concentration. To calculate fetal cimetidine and antipyrine clearances, the same procedure was used except maternal artery concentration C_{MA} was substituted for C_{FA}, maternal vein concentration C_{MV} was substituted for C_{FV}, maternal flow rate Q_M was substituted for Q_F and fetal arterial drug concentration C_{FA} was used instead of C_{MA}. A more detailed description of this procedure is given elsewhere (12). Because of its slower rate of transfer, cimetidine clearance in either direction was also defined by the formula: $Cl = (K \times V)/C$, where K = slope of drug appearance in the recipient reservoir, V = recipient perfusate volume, and C = mean drug concentration in the donor circuit. Another method involved calculation of cimetidine clearance by determining the drug area under the curve (AUC)[1] over 4 h in the maternal or fetal circuit and dividing the dose initially placed therein by the AUC. All three methods for cimetidine gave similar results. Clearances were expressed as milliliters per hour per gram of tissue.

Validation of placental integrity. Both perfusion systems previously described have been validated in the past for absence of nonspecific leakage, for viability by measurements of oxygen and glucose consumption and a predicted (low) lactate output, as well as by light and electron microscopy (12–16). In our studies, also, there was absence of transfer of bromosulphthalein (BSP), a water-soluble dye of 838 mol wt, in either direction. The spectrophotometric assay (17) permits exclusion of all but 2% of BSP transfer from either reservoir. Only 3.12±1.65% of ^{14}C-inulin (mol wt 5,000) crossed the placenta, as reported by others (10, 14). Maternal placental endogenous chorionic gonadotropin (mol wt 36,700) accumulated in the maternal reservoir but none was detected on the fetal side by radioimmunoassay (18). There was no leakage of fluid from fetal to maternal reservoirs or in the reverse direction as measured at the end of each study. In our studies, the pressure in the perfusion system consistently remained between 40 and 60 mmHg, glucose consumption was 7.04±0.562 mg/g placenta per h and remained constant throughout the study, and lactate output (combined maternal and fetal) was 1.35±0.559 μmol/g placenta per h. This glucose consumption is somewhat higher than that reported by others, and so is the lactate output (10, 15, 19). In a few studies with low lactate output (~ 0.5 μmol/g per h), the transport data for cimetidine were similar to those seen with ~ 1.5 μmol lactate/g per h output. When 21% O_2 was used, the fetal arterial O_2 partial pressure, measured with a 1302 pH/blood gas analyzer (Instrumentation Laboratory, Inc., Lexington, MA), was similar to those of others (14). There was no difference in lactate output, cimetidine transfer, or α-aminoisobutryic acid transport by the placenta with 21 or 95% oxygenations. Hence, 21% O_2 was used in most studies, as it permitted easier regulation of perfusate pH. Fetal blood flow in our system varied from ~ 2 to 4 ml/min, similar to that seen in other well-validated studies (15), but somewhat lower than in reports which generally perfused larger placental lobules (19). Maternal flow was 20 ml/min.

Specific perfusion experiments. A series of recirculating perfusion experiments were carried out with various concentrations of cimetidine (1–100 μg/ml placed in the maternal or fetal reservoir) with determination of transfer into the opposite compartment. In these experiments antipyrine was usually added as a well-defined transport marker. Antipyrine had no effect on the rate of cimetidine transport. In other studies, D- and L-glucose were added to verify the presence of

1. *Abbreviations used in this paper:* AIB, ^{14}C-α-aminoisobutyric acid; AUC, drug area under the curve; BSP, bromosulphthalein.

facilitated and passive transport, respectively, and thus validate the function of the system. In some experiments, ^{14}C-inulin (mol wt 5,000) was added to the perfusate as another validating marker of known placental transport. In some studies imidazole, a structural ring antagonist of cimetidine, in 10- or 100-fold excess, or ranitidine or quinine (putative organic cation competitors [20, 21]) in 10- and 100-fold excess, respectively, were added to the maternal perfusion circuit. At the conclusion of each study, the perfused placental lobule and contiguous nonperfused tissue were assayed for cimetidine and antipyrine.

A series of open perfusion studies were also carried out to determine if cimetidine and cycloleucine crossed from the maternal to fetal reservoirs against a concentration gradient, and for optimal comparative cimetidine vs. antipyrine clearances.

Other placental studies

Cimetidine metabolism. Metabolism of cimetidine by the placenta was assessed by assaying for the parent drug and its metabolites in both perfusates and the placenta in the perfusion experiments, as well as by incubating in vitro unlabeled and ^{3}H-labeled cimetidine with human placental microsomes and determining the recovery of the parent drug and formation of any metabolites over 60 min. In the perfusion studies, based on the radiolabeled cimetidine added and the sensitivity of the high-performance liquid chromatography (HPLC) system, all but 2–3% of any cimetidine metabolites present would have been detected. As a control for the in vitro studies, ^{14}C-caffeine, ^{14}C-aminopyrine, and ^{14}C-antipyrine were also incubated with the same microsomes. Placental microsomes were isolated and assayed for drug metabolism by methods reported by us earlier (22, 23).

Cimetidine binding in perfusate. < 20% of cimetidine is normally bound by plasma proteins in man (24). Nevertheless, binding of the various concentrations of drug by the perfusion solution with 2 g albumin/dl vs. that with 4 g albumin/dl was determined to assess whether this could vary enough to influence placental transport of the drug. Binding studies were carried out as described previously by us using equilibrium dialysis (25).

Vesicle studies. Maternal facing (apical) microvillous membrane vesicles were prepared from human placental syncytiotrophoblast using the method of Smith et al. (11). Validation of the microvillous membrane preparation as performed in our laboratory included electron microscopy and a 15-fold enrichment of alkaline phosphatase vs. placental homogenate. Using the nonmetabolizable amino acid ^{14}C-α-aminoisobutyric acid (AIB), we demonstrated that the amount of AIB retained by the vesicles was directly proportional to the amount of vesicle protein used, and that uptake of AIB occurs via an active (saturable) mechanism that was enhanced by sodium containing incubation media. Intravesicular water–space determinations were similar to those reported by Smith et al. (11), and variation of medium osmolarity demonstrated that AIB is taken up into the vesicle space rather than binding to the membrane protein. Using these vesicle preparations, we performed the following studies with [^{3}H]cimetidine: time course determinations, the effects of medium osmolarity and cimetidine concentration on cimetidine uptake, and calculation of extra- vs. intravesicular cimetidine concentration at equilibrium, using 3-*O*-methylglucose, a substrate that equilibrates between the incubation media and intravesicular water to measure vesicular space. Uptake studies were performed using timed incubations of 40 μl of membrane homogenate (3–5 mg protein/ml), to which cimetidine, dissolved in sodium containing Krebs-Ringer phosphate buffer, was added. All measurements were performed in quadruplicate.

H_2-Antagonist assays

^{3}H-labeled (10 Ci/mmol sp act) and unlabeled cimetidine (Amersham Corp., Arlington Heights, IL) were used. The radioactive drug's purity was checked periodically by HPLC and radioassay and it was used for binding, metabolism, as well as in low drug concentration perfusion studies. Unlabeled cimetidine and its metabolites were measured by HPLC (26, 27).

Statistics

Coefficients of correlation were calculated with linear regression analysis. Differences between data were calculated by Students *t* test with *P* value (two-tailed) of < 0.05 considered statistically significant.

Results

Cimetidine, placed in the maternal reservoir at a concentration of 1 μg/ml, readily crossed the human placenta into the fetal compartment (Fig. 1 and Tables I and II). This concentration of the drug is in the range seen in the serum of patients given conventional doses (250 mg three times a day per os) of the drug for the treatment of peptic ulcer (28). ~ 9% of the maternal cimetidine dose accumulated in the fetal compartment over 4 h on perfusion of a single cotyledon. Such cotyledons weigh 10 g on average and constitute ~ 2.2% of overall placental weight. The transfer of cimetidine over 4 h was about one third that seen for antipyrine, which equilibrates earlier between the two compartments as reported by others also (10, 16) (Fig. 1 and Tables I and II). As shown in Table II, the clearance of cimetidine at this concentration from the maternal circuit was 0.576±0.157 ml/h g placenta. When the clearance of cimetidine from the maternal circuit was studied at varying concentrations (from 1 to 100 μg/ml), the clearance was similar (Table II). Expression of these data as the antipyrine/cimetidine transfer ratio into the fetal circuit, to correct for interplacental variation in fetal blood flow also showed no differences in transfer of the cimetidine over a 100-fold concentration range in the maternal reservoir. Plotted in a different way, there was an increasing but linear transfer of the cimetidine from maternal to fetal compartments with increasing maternal cimetidine concentrations (r = 0.81251, P < 0.01). These data show absence of saturation kinetics of cimetidine transfer from maternal to fetal compartments and are consistent with passive transport. As is shown in Fig. 2, when cimetidine was infused at steady state into the maternal compartment and the same concentration of the drug was placed into a recirculating fetal compartment, the drug did not cross the placenta against a concentration gradient, i.e., it did not accumulate in the fetal reservoir. This is not consistent with active transport and differs from the transport of cycloleucine vs. a concentration gradient in two of these studies (Fig. 2). Cycloleucine is known to be transferred by the placenta by active transport (13). Similarily, as shown in Fig. 3, D-glucose (known to be transferred by the placenta by facilitated (carrier-mediated) transport was, in fact, transferred into the fetal reservoir more rapidly than L-glucose, which is known to be transferred passively (16). Interestingly, in the same studies, cimetidine, with a mol wt of 252.32, similar to that of glucose which is 180, was transferred at a rate very similar to that of L-glucose.

To examine further the possibility that cimetidine may be transferred via the placenta by a carrier-mediated transport, imidazole (the ring-structural analog of cimetidine) was added to the maternal reservoir in 10-fold higher concentration. However, no significant effect on cimetidine transfer was seen when comparing three control with two imidazole studies. In two other studies (not shown) with imidazole in 100-fold excess over a cimetidine concentration of 1 μg/ml, again no inhibitory effect of imidazole was noted. The possibility that ranitidine or quinine (21) may inhibit cimetidine transport via

Table I. Comparative Cimetidine and Antipyrine Clearances

	Cimetidine clearance*	Antipyrine clearance*	Cimetidine: Antipyrine clearance index	Cimetidine: Antipyrine transfer ratio
	ml/h per g	*ml/h per g*		*% at 2 h*
Maternal to fetal				
Open system	0.870	2.04	0.426	—
	0.585	2.25	0.379	—
	0.412	2.42	0.170	—
Mean±SE	0.622±0.133	2.24±0.110	0.325±0.079	
Closed system	0.162	0.91	0.177	0.230
	0.450	1.78	0.479	0.415
	1.20	4.93	0.243	0.384
Mean±SE	0.604±0.309	2.54±1.22	0.299±0.091	0.343±0.057
Fetal to maternal				
Open system	0.537	2.32	0.232	—
	0.241	0.81	0.296	—
	0.863	2.60	0.332	—
Mean±SE	0.547±0.179	1.91±0.556	0.287±0.029	
Closed system	0.628	1.86	0.338	0.490
	0.539	1.59	0.339	0.503
Mean	0.584	1.725	0.339	0.497

* Cimetidine studied at initial concentration of 1 μg/ml. Calculated using $Cl = [(C_{FV} - C_{FA}) \cdot Q_F]/C_{MA}$ for maternal to fetal or $Cl = [(C_{MV} - C_{MA}) \cdot Q_M]/C_{FA}$ for fetal to maternal transfer (see text for details).

competition for an organic cation carrier in the placenta also was not borne out.

In view of the studies carried out with sheep placenta (7, 8), the possibility was considered that fetal to maternal transfer of cimetidine may be greater than in the reverse direction. However, at a concentration of 1.0 μg/ml of the drug in the fetal circuit, clearance was similar (Table I and II) to that seen from the maternal circulation. In 4 h the maternal cimetidine concentration was ~ 0.11 μg/ml, i.e., 37.4% of the fetal dose had been transferred into the maternal reservoir. The similar clearance of the drug in both directions implies lack of any selectivity (preferred direction) of transport. Moreover, varying concentrations of cimetidine, over a 100-fold range, exhibited the same clearance from the fetal circuit. As expected for passive transport, the amount of cimetidine transferred from fetal to maternal circuits correlated linearly ($r = 0.8739$) with the dose of the drug placed in the fetal compartment. However, because

Table II. Effect of Cimetidine Concentration on Drug Transfer across the Placenta

Initial concentration	Cimetidine *Cl**	Cimetidine/antipyrine transfer ratio
μg/ml	*ml/h per g*	*% at 2 h*
Maternal to fetal transfer		
1 ($n = 6$)	0.576±0.157	0.381±0.068
10 ($n = 5$)	0.706±0.092	0.453±0.081
50 ($n = 2$)	0.414‡	0.390‡
100 ($n = 8$)	0.679±0.131	0.511±0.082
Fetal to maternal transfer		
1 ($n = 2$)	0.585‡	0.497‡
50 ($n = 2$)	0.860‡	0.415‡
100 ($n = 2$)	0.599‡	0.385‡

* Calculated using $Cl = (k \cdot V)/C$ (see text for symbol definition).
‡ Mean of two values.

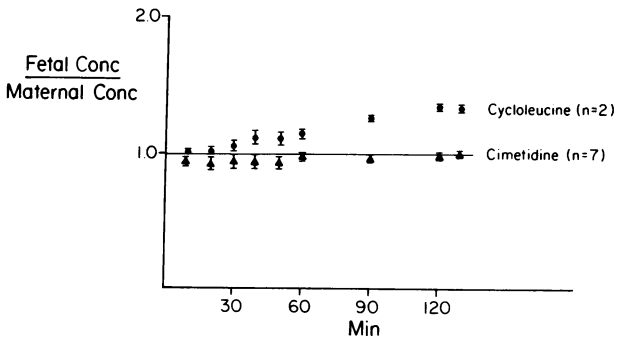

Figure 2. Differential transport of cimetidine vs. ^{14}C-cycloleucine from maternal to fetal compartments by perfused human placenta at steady state. An open perfusion system was used on the maternal side and a recirculating one for the fetus (see Methods). 150 mg of cimetidine was placed on the maternal side yielding a steady-state concentration of 50 μg/ml on that side. The same concentration of cimetidine was placed in the fetal reservoir. Cycloleucine concentrations of 12.9 μg/ml were set up in the maternal circuit (open system) and the same in the fetal (recirculating) reservoir. Data are given as mean±SE of the number of cimetidine studies listed. The study shows accumulation of cycloleucine ($n = 2$) in the fetal compartment against a concentration gradient (active transport), but the ratio was at ~ 1.0 for cimetidine (passive transport).

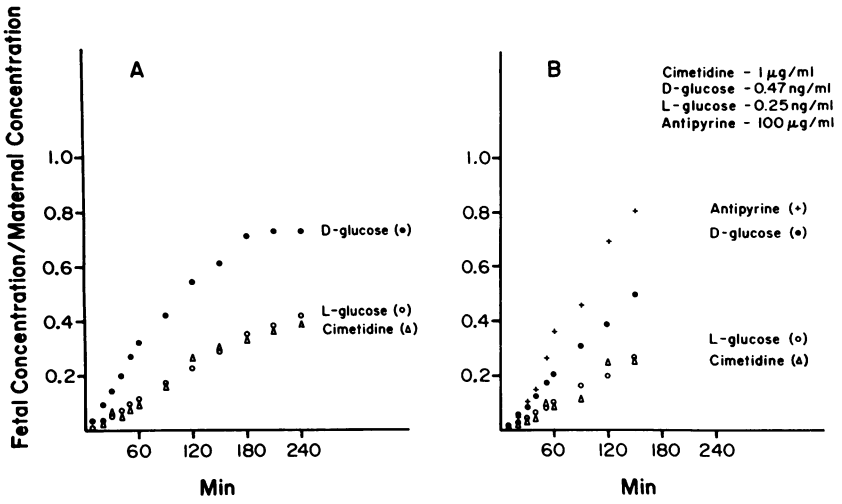

Figure 3. Relative rates of transfer of antipyrine, cimetidine, and D- and L-glucose by human placenta. (*A*) A more rapid (facilitated) transfer of D (than L) glucose. L-glucose mol wt 180 is transported more slowly, passively. Cimetidine with a similar mol wt 252 shows a rate of transport similar to L-glucose. (*B*) An experiment similar to that depicted in (*A*) is seen. In addition, it is shown that antipyrine is transported across the placenta faster than D-glucose. The initial concentrations of substrates used are given in (*B*).

only two studies were done at each of three concentrations, the correlation was not statistically significant. Also, we could not detect cimetidine accumulation in the maternal circuit against a concentration gradient.

The possibility was considered that the placenta may metabolize cimetidine, as it is known to biotransform various drugs (29). However, incubation of three concentrations of cimetidine, ~ 3, 15, 46 μg/0.7 ml of placental microsomal incubate over 90 min, failed to detect any metabolites of the drug by HPLC. Recovery of the parent drug as such after 90 min was 100%. This was comparable with control studies when microsomes were boiled (inactivated) and then incubated with 15 μg cimetidine/0.7 ml of microsomal incubate, and again full recovery of cimetidine was obtained by HPLC analysis. Furthermore, ~ 90% of ^{3}H-labeled cimetidine was recovered as such by combined HPLC and ^{14}C count after a 60-min incubation. No placental metabolism of antipyrine was detected either. By contrast, caffeine and aminopyrine metabolism, albeit in small amounts, was noted with these same microsomes. Aminopyrine was metabolized by human placenta at the rate of 0.188±0.012 pmol/mg microsomal proteins per min as compared with 81.9±4.2 pmol/mg protein per min in three rat livers. Caffeine was metabolized by human placenta at the rate of 0.124±0.008 pmol/mg microsomal protein per min as compared with 3.60±0.33 pmol/mg microsomal protein per min in rat liver. HPLC analysis of maternal and fetal perfusate, as well as of the placental lobule extract for cimetidine metabolites at the end of the studies, also was not rewarding despite the use of radiolabeled cimetidine sufficient to detect by HPLC all but 2–3% of any metabolite(s) present. These composite data strongly argue against any significant metabolism of this drug by the human placenta.

Considering the quantity of cimetidine retained in the perfused cotyledons at the end of the 4-h perfusion, only ~ 1.5–2.0% of the original dose of the drug was accounted for in the placenta. This did not vary with the dose of cimetidine used. Nonperfused cotyledons had essentially no cimetidine. Metabolites of cimetidine were not detected. Erythrocytes in blood-containing perfusions bound cimetidine only slightly, hence, no correction for this was needed.

Using the maternal-facing (apical) microvillous vesicles prepared from human placental syncytiotrophoblast, time course determinations with cimetidine revealed rapid vesicular uptake of this compound. The effect of altering medium osmolarity on cimetidine retention by the vesicles was consistent with intravesicular uptake rather than nonspecific binding of the drug to membrane protein. The correlation between cimetidine uptake and the reciprocal osmolarity was linear (r = 0.950). Incubation of the vesicles with increasing concentrations of cimetidine, from 0.1 to 100 μg/ml (identical to concentrations used in the perfusion experiments), revealed a direct correlation between cimetidine concentration and uptake by vesicles (Fig. 4). This lack of saturation of uptake with increasing dose suggests passive transport of cimetidine. Determinations of extra- and intravesicular concentrations of cimetidine were similar, also consistent with passive transport. Thus, the studies with apical microvillous vesicles corroborate the perfusion studies in describing transport consistent with passive transfer of cimetidine across this portion of the placental membrane.

Discussion

This study clearly demonstrates that cimetidine crosses the human placenta in both directions, maternal to fetal and vice versa. The clearance of the drug in the perfused placental cotyledon model from the maternal to fetal reservoirs is 0.576±0.157 ml/h per g of placenta. Expressed in terms of simultaneous transfer of a readily diffusible substance, antipyrine, the transport rate of cimetidine was about one third that of antipyrine. The clearance of cimetidine from the fetal

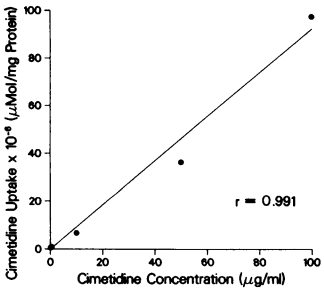

Figure 4. Effect of cimetidine concentration on uptake. 40-μl aliquots of microvillous vesicle homogenate (154 μg protein), in quadruplicate, were incubated with 20 μl of substrate containing cimetidine in concentrations from 0.1 to 100 μg/ml. Over this range, mean uptake of cimetidine by vesicles was essentially linear.

to maternal reservoirs was similar to that in the reverse direction, implying no selectivity (i.e., preferential) transfer from one to the other compartments.

The mechanism of cimetidine transfer via the human placenta is also elucidated by this study. The transfer had all the characteristics of passive transport. The evidence for this is (*a*) lack of saturation kinetics over a 100-fold cimetidine dose range in either direction (maternal to fetal and vice versa), (*b*) no inhibition of transport by a large excess of imidazole, a structural ring analog, or by quinine or ranitidine, likely organic base carrier inhibitors (20, 21), (*c*) similar rate of transport as for L-glucose, which has a similar molecular weight but is more polar and has no charge and is known to be transferred passively, and most important, (*d*) no transfer across the placenta against a drug concentration gradient. By contrast, in some of the same studies, cycloleucine, known to be transferred actively, did accumulate in the fetal compartment against a concentration gradient. Furthermore, in some of these experiments, facilitated (carrier-mediated) transfer of D-glucose and passive transport of L-glucose was demonstrated, indicating that the system was capable of such differential function. Data with apical (maternal-facing) placental vesicles confirm the absence of saturation kinetics (i.e., uptake is linear with increasing concentration), and lack of accumulation of the drug in the vesicles against a concentration gradient. These data are consistent with passive transport. The data also imply lack of binding of the cimetidine to this placental membrane.

It has been suggested from data in sheep that placenta may metabolize cimetidine (7). No evidence for this was found with human placental microsomes or during placental perfusion with labeled cimetidine over 4 h. Furthermore, binding to plasma proteins is unlikely to exert an important effect on cimetidine transport via the placenta, as binding was very low, only $\sim$ 4.8%, and it did not vary with a change in albumin concentration from 2 to 4 g/100 ml of buffer.

Two further comments are pertinent. First, because transport of cimetidine by the placenta appears to be passive, the drug is not metabolized by the placenta and plasma binding of cimetidine is minor, at steady state the concentrations of cimetidine in maternal and fetal compartments will be in equilibrium. With short-term or intermittent drug usage, however, fetal cimetidine concentration may depend not only on its rate of transport by the placenta but also on human fetal renal clearance of the drug. In sheep, the fetal renal secretion of cimetidine in absolute terms was low, but adjusted for creatinine clearance, extraction of cimetidine was comparable with that by the adult kidneys (7). No such data for the human fetus are available. In addition, the present data refer to mature, term placenta. It is uncertain whether transfer of the drug will be altered during human placental maturation, but the placenta is known to decrease in thickness during development (30) and this is one determinant of permeability (31). Preliminary studies in our unit, however, comparing term and ¾ term baboon perfused placenta show no difference in cimetidine transport at these two times. Moreover, the baboon placenta seems to transfer cimetidine at a similar rate as the human placenta (unpublished data). Second, one must consider the possible effects of cimetidine on the fetus. Antiandrogenic effects have been reported for male progeny in pregnant rats given large doses of cimetidine during gestation and lactation (32, 33). The relevance of this observation to pregnant patients and their offspring is uncertain.

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Note added in proof: Since submission of this paper, similar results have been obtained by another group (Ching, M. S., G. W. Mihaly, D. J. Morgan, N. M. Date, K. J. Hardy, and R. A. Smallwood. 1987. Low clearance of cimetidine across the human placenta. *J. Pharmacol. Exp. Ther.* 241:1006–1009).

References

1. Freston, J. W. 1982. Cimetidine 1. Developments, pharmacology and efficacy. *Ann. Intern. Med.* 97:573–580.

2. Cocco, A. E., and D. V. Cocco. 1981. A survey of cimetidine prescribing. *N. Engl. J. Med.* 304:1281.

3. McGowan, W. A. W. 1979. Safety of cimetidine in obstetric patients. *J. R. Soc. Med.* 72:902–907.

4. Wilson, J. 1980. Effect of intravenous cimetidine on intragastric pH at Caesarean section. *In* European Symposium on H_2-Receptor Antagonists. A. Torsoli, P. E. Lucchelli, and R. W. Brimblecombe, editors. Excerpta Medica, Amsterdam. 185–191.

5. Smith, Kline & French Pharmaceuticals. 1986. Usage in pregnancy. *In* Physicians Desk Reference. 40th ed. E. R. Barnhard, editor. Medical Economics Company, Inc., Oradell, NJ. 1726.

6. Mihaly, G. W., D. J. Morgan, A. W. Marshall, R. A. Smallwood, S. Cockbain, D. MacLeelan, and K. J. Hardy. 1982. Placental transfer of ranitidine during steady state infusions of maternal and fetal sheep. *J. Pharm. Sci.* 71:1008–1010.

7. Mihaly, G. W., D. B. Jones, D. J. Morgan, M. S. Ching, L. K. Webster, R. A. Smallwood, and K. J. Hardy. 1983. Placental transfer and renal elimination of cimetidine in maternal and fetal sheep. *J. Pharmacol. Exp. Ther.* 227:441–445.

8. Ching, M. S., D. B. Jones, D. J. Morgan, G. W. Mihaly, K. J. Hardy, and R. A. Smallwood. 1985. Fetal exposure to cimetidine following chronic administration to pregnant sheep. *Res. Commun. Chem. Pathol. Pharmacol.* 50:139–142.

9. Kaufmann, P. 1981. Functional anatomy of the non-primate placenta. *In* Transfer Across the Primate and Non-Primate Placenta. H. C. S. Wallenberg, B. K. van Kreel, and J. P. van Dijk, editors. W. B. Saunders Co., Philadelphia. 13–28.

10. Brandes, J. M., N. Tavoloni, B. J. Potter, L. Sarkozi, M. D. Shepard, and P. D. Berk. 1983. A new recycling technique for human placental cotyledon perfusion: application to studies of the fetomaternal transfer of glucose, inulin and antipyrine. *Am. J. Obstet. Gynecol.* 146:800–806.

11. Ruzycki, S. M., L. K. Kelley, and C. H. Smith. 1978. Placental amino acid uptake. IV. Transport by microvillous membrane vesicles. *Am. J. Physiol.* 3:C27–C35.

12. Schneider, H., M. Panigel, and J. Dancis. 1972. Transfer across the perfused human placental of antipyrine, sodium and leucine. *Am. J. Obstet. Gynecol.* 114:822–828.

13. Schneider, H., K.-H. Mohlen, and J. Dancis. 1979. Transfer of amino acids across the *in vitro* perfused human placenta. *Pediatr. Res.* 13:236–240.

14. Contractor, S. F., and P. J. Stannard. 1983. The use of AIB transport to assess the suitability of a system of human placental perfusion for drug transport studies. *Placenta.* 4:19–30.

15. Miller, R. K., P. J. Wier, D. Maulik, and P. A. di Sant'Agnese. 1985. Human placenta in vitro: characterization during 12 h or dual perfusion. *Contrib. Gynecol. Obstet.* 13:77–84.

16. Challier, J. C., M. Nandakumaran, and F. Mondon. 1985. Placental transport of hexoses: a comparative study with antipyrine and amino acids. *Placenta.* 6:497–504.

17. Gaebler, O. H. 1945. Determination of bromosulphtalein in

normal, turbid hemolysed or icteric serums. *Am. J. Clin. Pathol.* 15:452.

18. Siler-Khodr, T. M., G. S. Khodr, G. Valenzuela, and J. Rhode. 1986. Gonadotrophin-releasing hormone effects on placental hormones during gestation. I. Alpha-human chorionic gonadotropin, human chorionic gonadotropin and human chorionic somatomammotropin. *Biol. Reprod.* 34:245–254.

19. Schmidt-Sommerfeld, E., D. Penn, R. J. Sodha, M. Progler, M. Novak, and H. Schneider. 1985. Transfer and metabolism of carnitine and carnitine esters in the *in vitro* perfused human placenta. *Pediatr. Res.* 19:700–706.

20. van Crugten, J., F. Bochner, J. Keal, and A. Somogyi. 1985. Selectivity of the cimetidine-induced alterations in the renal handling of organic substrates in humans. Studies with anionic, cationic and zwitterionic drugs. *J. Pharmacol. Exp. Ther.* 236:481–487.

21. McKinney, T. D., P. Myers, and K. V. Speeg, Jr. 1981. Cimetidine secretion by rabbit renal tubules in vitro. *Am. J. Physiol.* 241:F69–F76.

22. Henderson, G., J. Secor, D. Heitman, and S. Schenker. 1986. Effects of age and sex on the hepatic monooxygenase system: a correlative approach. *Dev. Pharmacol. Ther.* 9:201–216.

23. Henderson, G. I., and S. Schenker. 1986. Effects of age, sex and cimetidine on acute ethanol-induced inhibition of the hepatic monooxygenase systems. *Alcohol. Clin. Exp. Res.* 10:259.

24. Griffiths, R., R. M. Lee, and D. C. Taylor. 1977. Kinetics of cimetidine in man and experimental animals. *In* Cimetidine. W. L. Burland and M. A. Simkins, editors. Excerpta Medical, Amsterdam. 38–51.

25. Johnson, R. F., S. Schenker, R. K. Roberts, P. V. Desmond, and G. R. Wilkinson. 1979. Plasma binding of benzodiazepines in humans. *J. Pharm. Sci.* 68:1320–1322.

26. Randolph, E. C., V. L. Osborne, S. S. Walkenstein, and A. P. Intoccia. 1977. High pressure liquid chromatographic analysis of cimetidine, a histamine H_2-receptor antagonist, in blood and urine. *J. Pharm. Sci.* 66:1148–1150.

27. Lorenzo, B., and D. E. Drayer. 1981. Improved method for the measurement of cimetidine in human serum by reverse-phase high-pressure liquid chromatography. *J. Lab. Clin. Med.* 97:545–550.

28. Redolfi, A., E. Borgogelli, and E. Lodola. 1979. Blood level of cimetidine in relations to age. *Eur. J. Clin. Pharmacol.* 15:257–261.

29. Juchau, M. R. 1980. Drug biotransformation in the placenta. *Pharmacol. & Ther.* 8:501–524.

30. Fox, H. 1979. Correlation between placental structure and transfer function. *In* Placental Transfer. G. V. P. Chamberlain and A. W. Wilkinson, editors. Pittman Press, Bath, England. 15–30.

31. Schneider, H., R. J. Sodha, M. Progler, and M. P. A. Young. 1985. Permeability of the human placenta for hydrophilic substances studied in the isolated dually in vitro purfused lobe. *Contrib. Gynecol. Obstet.* 13:98–103.

32. Anand, S., and D. H. Van Thiel. 1982. Prenatal and neonatal exposure to cimetidine results in gonadal and sexual dysfunction in adult males. *Science (Wash. DC).* 218:493–494.

33. Parker, S., R. R. Schade, C. R. Pohl, J. S. Gavaler, and D. H. Van Thiel. 1984. Prenatal and neonatal exposure of male rat pups to cimetidine but not ranitidine adversely affects subsequent adult sexual functioning. *Gastroenterology.* 86:675–680.