Placental Transfer of Ranitidine During Steady-State Infusions of Maternal and Fetal Sheep

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Abstract □ The placental transfer of ranitidine was studied at pharmacokinetic steady state in anesthetized, full-term, pregnant sheep. Ranitidine was administered to the ewe in three preparations and to the fetus in three other sheep. In all experiments, dose size was based on the combined maternal–fetal weight. Steady-state plasma levels were reached within 4 hr by using an initial intravenous bolus dose followed by continuous infusion. Following maternal dosage, the mean maternal (jugular vein) steady-state concentration ($C_{M_{ss}}$) at 4 hr was 842 ± 66 ng/ml (*SEM*), the mean fetal (carotid artery) steady-state concentration ($C_{F_{ss}}$) was 26.5 ± 4.2 ng/ml, and the mean fetal umbilical venous steady-state concentration was 28.9 ± 3.5 ng/ml. Both the fetal and umbilical plasma concentrations were significantly less than the maternal plasma concentration ($p < 0.01$). With fetal dosage, mean $C_{M_{ss}}$ was 414 ± 42 ng/ml at 4 hr and was significantly less than the mean $C_{F_{ss}}$ value at the same time, which was 6890 ± 360 ng/ml ($p < 0.005$). Ranitidine was not bound extensively to plasma proteins in the ewe or the fetus (range 12–55% bound). The reversal of the $C_{M_{ss}}/C_{F_{ss}}$ gradient with the change from maternal to fetal administration and the low binding of the drug shows that the gradient following maternal dosage cannot be explained by ion-trapping or differential plasma protein binding. As active placental transport is considered unlikely, the low fetal plasma concentrations are probably due to the presence of significant fetal elimination of ranitidine. Furthermore, the substantial gradient between maternal and umbilical venous plasma concentrations suggests that placental elimination of ranitidine should also be considered.

Keyphrases □ Ranitidine—placental transfer during steady-state infusions of maternal and fetal sheep □ Placental transfer—ranitidine, during steady-state infusions, maternal and fetal sheep

The use of H_2-receptor antagonists in pregnancy is becoming widespread (1, 2), although little is known of their placental transfer. Cimetidine has been shown to cross the placenta (1) but ranitidine, a new H_2-receptor antagonist, has not been studied.

Since opportunities to make observations in pregnant women are necessarily limited, we have used a pregnant sheep model to study the placental transfer of ranitidine. A similar model has been used recently to examine the placental transfer of meperidine (3), indomethacin (4), and aspirin (5). In contrast to earlier work, the bidirectional transfer of drug has been studied in this report. Results establish that ranitidine does cross the placenta. They also indicate that the comparatively low drug concentrations found in the fetus after maternal dosage are probably accounted for by fetal and/or placental elimination.

EXPERIMENTAL

Methods—Experiments were carried out in pregnant Merino or Dorset-Horn sheep, 2–5-years old, during the last 2 weeks of gestation (term = 147 days). The animals were treated with fenbendazole[1] to clear intestinal parasites. Anesthesia was induced with sodium thiopental[2] (15 mg/kg iv) and maintained with a mixture of halothane and oxygen using intermittent positive pressure respiration. The partial pressure of oxygen was kept in excess of 100 mm of Hg, while the partial pressure of carbon dioxide was 25–35 mm of Hg. Surgery was performed with strict sterile techniques.

The uterus was incised and the fetus was delivered onto a small draped platform. The fetus was kept on the platform through the experiments and its rectal temperature was maintained at 38–39° with a heating pad. The fetus and umbilical cord were kept moist with saline packs.

The maternal jugular vein was cannulated with a dimethicone cannula (1.2-mm i.d.) to allow collection of maternal blood samples. The fetal carotid artery was cannulated with a dimethicone catheter (0.76-mm i.d.) to allow fetal blood sampling. In fetal dosage experiments, the internal jugular vein was cannulated with a dimethicone catheter (0.76-mm i.d.) for drug administration.

Liver function tests were carried out in both ewe and fetus before and after every experiment. No abnormalities were encountered. Fetal and maternal hematocrits changed by <3% over the course of the experiment.

Calculation of Bolus Dose and Infusion Rate—Steady-state plasma levels of ranitidine were achieved by the combination of an initial bolus dose together with a continuous intravenous infusion that was maintained throughout the experiment. The bolus dose for both maternal and fetal administration was calculated as follows (6):

$$\text{Bolus Dose} = C_{ss} \times V_\beta \times W$$

where C_{ss} (μg/liter) is the desired steady-state plasma concentration, V_β (liter/kg) is the total apparent volume of distribution, and W is the animal's weight (kg) (*i.e.*, the combined weight of sheep and fetus). Maternal and fetal infusion rates were calculated as follows (6):

$$\text{Infusion rate} = C_{ss} \times Q \times W$$

where Q (liter/hr) is the systemic plasma clearance of ranitidine. The desired steady-state concentrations in maternal plasma following maternal dosage was between 500 and 1000 ng/ml (500 ng/ml was used in the calculations). The values used for V_β (1.6 liter/kg) and Q (0.5 liter/hr/kg) were previously determined from a pilot pharmacokinetic study in a single nonpregnant sheep.

Since $t_{1/2\beta}$ for the adult sheep was ~2 hr, a constant infusion would not have achieved steady-state concentrations until 8–10 hr. Using this procedure, steady-state plasma concentrations were approached within 2 hr and were then maintained for the duration of the experiment.

Maternal Dosage Experiments—Ranitidine[3] was administered intravenously to three pregnant ewes *via* a foreleg vein. A predose blood sample was collected from the maternal jugular vein (8 ml) and from the fetal carotid artery (5 ml) and further samples were then collected from both the ewe and the fetus at 2, 4, 10, 15, 120, 180, 210, and 240 min postbolus dose. A sample was taken from the umbilical vein of each fetus at 240 min.

Fetal Dosage Experiments—Ranitidine was administered to three fetuses *via* the internal jugular vein. The same dosage regimen as used for maternal administration was applied, using the weight of the pregnant ewe for calculations. Blood samples were collected as described above from both the ewe and the fetus. Additional samples were collected at 240 min from umbilical artery and vein.

Protein Binding—Plasma protein binding of ranitidine in maternal and fetal plasma samples collected at 240 min was estimated by ultrafiltration (7).

Drug Analysis—Plasma concentrations of ranitidine were determined

[1] Panacur, Hoescht, Melbourne, Australia.
[2] Pentothal, Abbott Pharmaceuticals, Australia.
[3] Glaxo Group Research Ltd.

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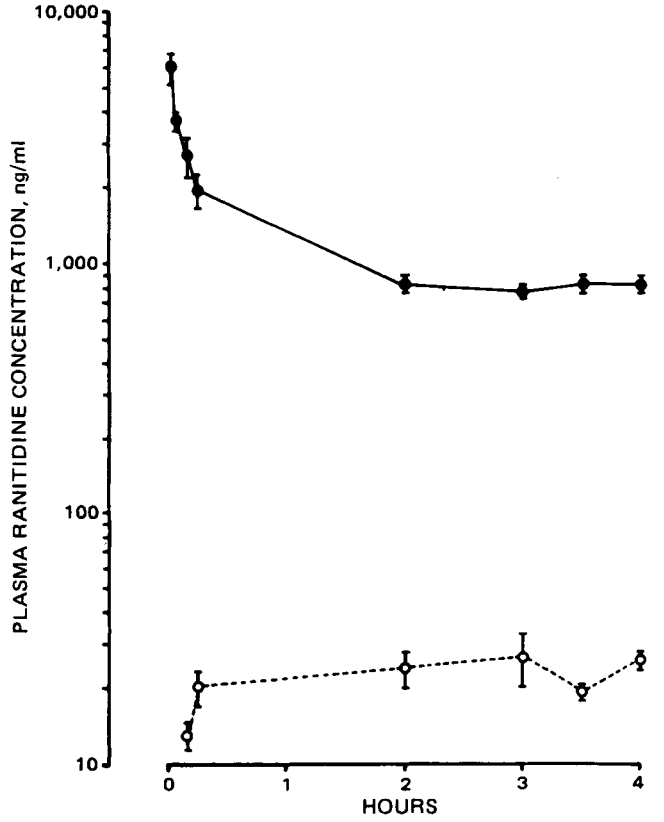

Figure 1—*Mean plasma ranitidine concentrations in ewes and fetuses during maternal administration (intravenous bolus plus constant rate infusion). Key: (●) maternal, (○) fetal.*

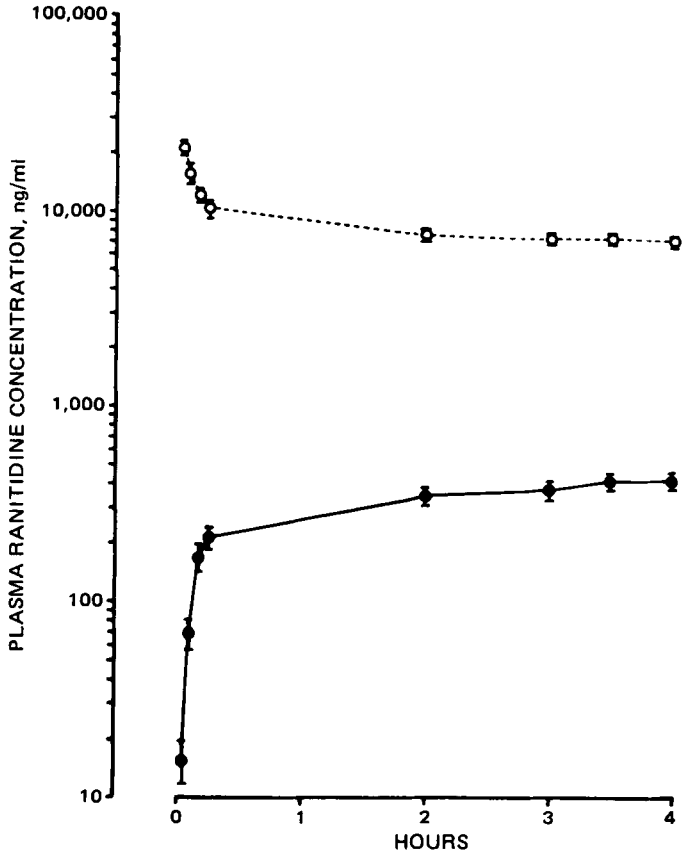

Figure 2—*Mean plasma ranitidine concentrations in ewes and fetuses during fetal administration (intravenous bolus plus constant rate infusion). Key: (●) maternal, (○) fetal.*

by a high-performance liquid chromatographic (HPLC) method (8). This method was specific for ranitidine and had a sensitivity of 5 ng/ml of compound in plasma.

Statistical Analysis—Data are presented as mean ±*SEM*. Statistical comparisons were made using the Student's t test or linear regression analysis and statistical significance accepted when $p < 0.05$.

RESULTS

Maternal Dosage Experiments—The mean plasma ranitidine concentration *versus* time curves in both the ewe and the fetus, following maternal dosage, are shown in Fig. 1. The initial bolus dose produced an early peak in maternal plasma levels and steady state was achieved by 4 hr, since the slope of the linear regression line through the plasma concentration–time data from 2 to 4 hr was not significantly different from zero ($p > 0.05$). The mean steady-state concentration of ranitidine at 4 hr was 842 ± 66 ng/ml. Ranitidine was detected in fetal plasma within 10 min and fetal plasma concentrations also achieved steady state by 4 hr ($p > 0.05$). The mean fetal steady-state concentration of ranitidine at 4 hr was 26.5 ± 4.2 ng/ml and this value was significantly less than the maternal concentration at 4 hr (paired t test, $p < 0.01$). The mean umbilical venous concentration of ranitidine at 4 hr was 28.9 ± 3.5 ng/ml and was also significantly less than the corresponding maternal concentration (paired t test, $p < 0.01$) but did not appear to differ from the fetal concentration.

Fetal Dosage Experiments—The mean plasma ranitidine concentration *versus* time curves in both the ewe and the fetus, after fetal dosage, are shown in Fig. 2. Placental transfer was detected within 2 min, and a steady state was again reached in both the ewe and the fetus by 4 hr ($p > 0.05$). Fetal mean carotid arterial, umbilical arterial, and umbilical venous plasma concentrations of ranitidine at 4 hr were 6890 ± 360 ng/ml, 8010 ± 544 ng/ml, and 6990 ± 433 ng/ml, respectively. The mean maternal steady-state plasma concentration of ranitidine at 4 hr was 414 ± 42 ng/ml, which was significantly less than each of the above three mean fetal concentrations (paired t test, $p < 0.005$).

Plasma Protein Binding of Ranitidine—Plasma protein binding experiments are summarized in Table I. Approximately 30% of plasma was bound in both maternal and fetal plasma.

DISCUSSION

Studies of placental drug transfer and fetal pharmacokinetics cannot be readily undertaken in humans, in whom observations have been largely confined to the comparison of drug concentrations in maternal and umbilical vein plasma at birth. Such observations contribute little to the understanding of placental transfer, or of fetal drug distribution and elimination. The pregnant sheep preparation is not limited in this way, and the increased flexibility of experimental design allows much greater insight into the fetal handling of drugs.

The present study was carried out in anesthetized sheep. General anesthesia may cause hemodynamic changes (9), and this should be borne in mind when considering these results. However, all maternal and fetal sheep remained in excellent condition, and previous work suggests that anesthesia does not significantly alter propranolol disposition in this pregnant sheep preparation (10). Blood gases, pH, biochemical, and hematological profiles remained within normal physiological values (11) in mothers and fetuses in all experiments. The comparatively large doses of ranitidine administered to fetuses produced no detectable adverse effects, despite steady-state fetal plasma levels which were tenfold greater than the maternal levels obtained after maternal dosage. Fetal dosage was based on the combined maternal–fetal rather than fetal weight, because it was presumed that most of the drug would be eliminated by the mother, and a small fetal dose might have resulted in undetectable maternal plasma concentrations.

Following maternal dosage, steady-state levels of ranitidine in the ewe were 30 times that in the fetus. This cannot be explained by a delay in placental transfer, since a steady state existed. Furthermore, ranitidine was detected within 10 min in fetal plasma, suggesting that any hold up at the placenta is short lived.

It has been argued (12) that, after maternal dosage, the fetal–maternal steady-state concentration ratio should equal one, unless one of the following applies: (*a*) there is active transport of drug across the placenta, (*b*) there is differential binding of drug to fetal and maternal plasma proteins, (*c*) there is differential ionization of drug in maternal and fetal plasma due to differences in plasma pH, (*d*) the fetus eliminates the drug, or (*e*) the placenta eliminates the drug. The fact that fetal dosage reversed the placental gradient obtained after maternal dosage (Figs. 1 and 2)

Table I—Percentage of Total Plasma Ranitidine Bound to Proteins in Both Maternal and Fetal Plasma after Both Maternal and Fetal Administration

Sheep Number	Maternal		Fetal	
	Total Plasma Concentration, ng/ml	Bound, %	Total Plasma Concentration, ng/ml	Bound, %
M1	838	45	[a]	[a]
M2	606	40	[a]	[a]
M3	714	21	1110[b]	36
Mean	719	35	—	—
SEM	67	7	—	—
F1	374	31	6300	12
F2	481	31	7680	40
F3	370	55	6630	20
Mean	408	39	6870	24
SEM	36	8	415	8

[a] Plasma not available for ultrafiltration. [b] Sample supplemented with pure ranitidine to ~1100 ng/ml.

effectively rules out *a*, *b*, and *c* above. These three mechanisms would tend to maintain a gradient in the same direction, whatever the route of administration. Furthermore, the plasma protein binding data (Table I) shows that ranitidine is not extensively bound in maternal or fetal plasma. This also rules out any possibility that differential binding of drug in maternal and fetal plasma could be responsible for the placental gradient, because the unbound fraction of drug in fetal plasma would need to exceed that in maternal plasma 30-fold. It could be argued that since active transport is likely to be unidirectional (*e.g.*, fetus → mother) the higher fetal concentrations produced by drug administration to the fetus might saturate an active transport system and alter the maternal–fetal ratio. Such an active transport mechanism is unlikely (13), although there is insufficient data to allow us to rule it out completely.

The placental transfer of the other drugs has been studied in pregnant sheep. Following maternal administration, meperidine (3), indomethacin (4), and aspirin (5) were found to have lower fetal than maternal steady-state plasma concentrations of drug. Szeto *et al.* (3) used a pharmacokinetic model to propose that the difference in fetal and maternal steady-state meperidine concentrations was due partly to fetal drug elimination. This proposal was subsequently supported when it was demonstrated that renal excretion of meperidine does take place in the ovine fetus (14, 15).

Following maternal dosage, the fact that umbilical venous plasma concentrations of ranitidine were substantially less than maternal concentrations suggests that the gradient in concentration occurs across the placenta. Hence, the possibility of placental drug elimination in addition to fetal drug elimination should be considered. Several drug biotransformation processes have been identified in animal and human placentas (16), but the role of the placenta in xenobiotic metabolism still remains unclear (17). A previous study (13), in discussing the theoretical relationships between metabolism and fetal plasma levels of drugs, concluded that enzymes in the fetus would usually be more effective than those in the placenta in protecting the fetus against lipid-soluble drugs. Therefore, the relative importance of placental compared with fetal elimination of ranitidine requires further investigation. It may be that fetal exposure to ranitidine encountered during pregnancy is reduced by placental metabolism, at a time when the fetus' own capacity to eliminate this foreign substance is greatly limited.

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