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Clearance and disposition of indometacin in chronically instrumented fetal lambs following a 3-day continuous intravenous infusion

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Abstract

Indometacin is used in pregnancy for the treatment of premature labour, but there are limited data on the disposition of the drug in the fetus. In order to elucidate fetal indometacin pharmacokinetics at plasma levels and duration comparable with those occurring with use of the drug for tocolysis in humans, indometacin was administered at doses of 1.9 (low dose, LD; n = 5) or 7.5 (high dose, HD; n = 9) μg min^{-1} to steady state over a 3-day period in chronically instrumented fetal lambs. Indometacin concentrations in biological fluid samples were analysed by a sensitive capillary gas chromatography-electron capture detection method. The mean steady-state fetal arterial plasma indometacin concentrations were 68.6$\pm$16.5 ng mL^{-1} in the LD infusion and 230.3$\pm$28.8 ng mL^{-1} in the HD infusion. Indometacin concentrations in amniotic fluid were $\sim$10% of those in fetal plasma, and below assay detection limits in tracheal fluid. Total body clearance (TBC) in the LD and HD infusions were not different and the overall mean was 11.3$\pm$1.2 mL min^{-1} kg^{-1}. In the 11 experiments where paired fetal arterial and umbilical venous samples were collected, the extraction of indometacin across the placenta averaged only 5.2$\pm$1.1%, indicating low placental permeability to the drug in sheep. However, fetal placental clearance (CL_{pl}) of indometacin (10.0$\pm$2.5 mL min^{-1} kg^{-1}, n = 10) averaged 115.1$\pm$41.2% of TBC in these animals and the calculated value for fetal non-placental clearance (0.6$\pm$2.8 mL min^{-1} kg^{-1}) was not significantly different from zero. Fetal renal clearance of intact indometacin (3.8$\pm$1.1 μL min^{-1} kg^{-1}; n = 12) was also very low. However, treatment of fetal urine with glucuronidase indicated the presence of glucuronide conjugates and these comprised 69.9$\pm$8.2% of the total drug concentration (i.e. intact+conjugated) in urine. Thus, the fetal lamb appears to be able to glucuronidate indometacin, but the contribution of this and other non-placental routes to overall fetal elimination of the drug appear minimal. CL_{pl} of the drug is also low owing to the physicochemical properties of indometacin (high polarity) and the permeability characteristics of the sheep placenta.

Introduction

Indometacin (1-[*p*-chlorobenzoyl]-5-methoxy-2-methyl-indole-3-acetic acid) is used in maternal/fetal medicine for several purposes, but primarily for the treatment of preterm labour (Macones et al 2001). It appears to cross the human placenta easily with a fetal to maternal plasma indometacin ratio of approximately 1.0, and fetal plasma drug concentrations averaging 219$\pm$13 ng mL^{-1} following maternal indometacin administration for premature labour (Moise et al 1990). Although some studies have indicated that indometacin crosses the placenta in

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rabbits (Parks et al 1977; Harris & Van Petten 1981), rats (Klein et al 1981), and sheep (Harris & Van Petten 1981), these studies have been acute, evaluating short-term drug effects (< 20 h), and there are no data on chronic indometacin effects or pharmacokinetics.

We have used chronically instrumented pregnant sheep in studies of placental drug transfer and maternal/fetal drug pharmacokinetics (Rurak et al 1991). This preparation allows for serial sampling to an extent that is impractical in small animal species, such as rats, rabbits and guinea-pigs. Using pregnant sheep, we have employed direct fetal administration of indometacin to provide plasma concentrations comparable with those observed in studies of human tocolysis. Moreover, we utilized the steady-state drug concentrations in fetal arterial and umbilical venous blood, and in fetal urine, to estimate fetal total body, placental and renal clearances of the drug.

Materials and Methods

Animals and surgical preparation

The studies were carried out on 14 time-dated pregnant sheep (Dorset/Suffolk breeds). Ethical approval for these studies was obtained from the UBC Animal Care Committee and the guidelines of the Canadian Council on Animal Care were followed. The animals were studied in the animal unit at the BC Research Institute for Children's and Women's Health and were allowed at least 1 week after arrival to acclimate to the new environment. They were housed in groups of 2–4 in different, but adjacent, pens in full view of one another. The animals were fed a standard diet of grain, alfalfa cubes and hay and had free access to water. They were subjected to aseptic surgical preparation at 121–125 days of gestation (mean 124.2±0.3 days, term ~145 days). Anaesthesia was induced with intravenous sodium pentothal (1 g) and maintained, following intubation, with halothane (1–2%), nitrous oxide (60%) and oxygen. Following a midline abdominal incision, access to the fetus was gained through an incision of the uterine wall, free of placental cotyledons and major blood vessels. Catheters were placed in the inferior vena cava and descending aorta through the femoral and tarsal vessels, respectively. The fetal bladder was cannulated through a suprapubic incision, followed by non-occlusive catheterization of the common umbilical vein at the umbilicus. Two catheters were placed in the amniotic cavity and anchored to the abdominal skin of the fetus. A carotid artery was also catheterized, via a separate uterine incision, and a non-occlusive catheter was inserted into the trachea. A catheter was also placed in the amniotic cavity and anchored to the skin on the neck. The hysterotomy and laparotomy incisions were then closed. The maternal inferior vena cava and descending aorta were cannulated via maternal femoral vessels. All catheters were passed subcutaneously to a small incision in the maternal abdominal wall on the left flank where they exited. They were stored in a denim pouch attached to the flank. Postoperatively, ampicillin (500 mg) was administered as an intramuscular prophylactic antibiotic for 3 days to the ewe, and intra-amniotically to the fetus at the time of surgery and daily into the amniotic fluid for the duration of the preparation.

Experimental protocol

Indometacin infusion

At least 24 h before each experiment, the ewe was placed in an experimental pen in full view of companion ewes, with access to standard diet and water. Each experiment lasted 5 days, with the first day being the control day when no drug was given. Indometacin in a solution of 1.1% ethanol and 0.75% sodium bicarbonate in normal saline was infused (2 mL h^{-1}) into the fetal vein at doses of 1.9 (low dose, LD; n = 5) or 7.5 (high dose, HD; n = 9) μg min^{-1} or vehicle alone (control) on Days 2–4. When normalized to fetal weight, the LD and HD infusion rates averaged 0.65±0.02 and 2.36±0.08 μg min^{-1} kg^{-1}, respectively. The infusion was terminated on Day 5 and the fetus was monitored during the 24-h recovery period. Stability studies indicated that indometacin was stable in the pH 7.8 infusion vehicle for at least 96 h. At the end of the experiment, the ewe and fetus were euthanized with sodium pentobarbital and fetal and placenta tissues were obtained for microsphere processing.

Sampling schedule

Samples (2 mL) of fetal arterial blood, umbilical venous blood, amniotic and tracheal fluids, and fetal urine were collected twice daily at 6-h intervals. Blood samples were collected in heparinized Vacutainer collection tubes, centrifuged at 3000 *g* for 10 min at −10°C, and the plasma transferred into Pyrex 15-mL culture glass tubes for storage and analysis. Samples (0.6 mL) of fetal arterial and umbilical venous blood were also collected at the same intervals for blood gas analysis. Withdrawn fetal blood was promptly replaced with an equal volume of heparinized drug-free maternal blood via the tarsal vein catheter.

Physiological measurements

Fetal and maternal arterial pressure, amniotic pressure, and tracheal pressure were continuously monitored using strain-gauge manometers (Statham Model P23Dd; Gould Inc., Oxnard, CA) or disposable DTX transducers (Spectramed, Oxnard, CA). Fetal heart rate was measured using a cardiotachometer (Model 9857; Sensormedics, Anaheim, CA). These variables were recorded using a Beckman R-711 polygraph recorder and a computerized data acquisition system. Fetal arterial pressure and venous pressure were adjusted to zero reference by subtracting amniotic fluid pressure continuously using the computer. Initial studies involved determination of urinary flow rate twice daily using an intermittent measurement system. In later studies, fetal urine flow rate was measured continuously by the computer by means of a bladder catheter, which drained by gravity into a sterile reservoir, the hydrostatic pressure of which was monitored by the computer. When the preset pressure ($\sim$3 mmHg) was exceeded, a calibrated peristaltic pump (DIAS, Ex 154; DIAS Inc., Kalamazoo, MI) was activated to pump urine from the reservoir into the amniotic cavity via an amniotic catheter. The volume pumped each minute was stored on diskette and provided a measurement of urine flow. Fetal arterial blood samples (0.7 mL) were collected for measuring pH, pCO_2, pO_2, HCO_3^-, and base excess at 39.5°C (Instrument Laboratory System 1306 pH/Blood Gas Analyser; Lexington, MA). Glucose and lactate concentrations in fetal blood samples were also determined using a YSI 2300 Glucose/Lactate Analyser (YSI Inc., Yellow Springs, OH). Haematocrit was estimated using a microcapillary centrifuge. Blood O_2 saturation and haemoglobin concentrations were determined using an OSM-2 Hemoximeter. Plasma protein concentration was determined using an American Optical hand-held refractometer. All these physiological data will be reported elsewhere. Umbilical blood flow and fetal regional blood flows (not reported here) were measured using 15-μM radioisotope labelled (^{153}Gd, ^{85}Sr, ^{51}Cr, ^{46}Sc and ^{95}Nb) microspheres as previously described (Rurak et al 1990). One set of flow measurements was obtained each day of the experiment, at the time of the morning blood sampling.

Indometacin measurement

Indometacin concentrations were determined for each sample in duplicate using a sensitive gas chromatography-electron capture detection method (Krishna et al 1995). Briefly, fetal fluids (plasma, urine, amniotic fluid and tracheal fluid), typically 0.1 mL, were pipetted into clean 15-mL borosilicate Kimax culture tubes with polytetrafluoroethylene-lined screw caps. To the biological fluid samples, 50 μL of internal standard, α-methyl indometacin and 2 mL acetate buffer (pH 5.0) were added and the mixture was adjusted to a final volume of 3.0 mL with water (final pH of the aqueous phase $\sim$5.00). The mixture was gently vortex-mixed and 5 mL ethyl acetate was added. The aqueous phase was extracted for 20 min on a rotary shaker. The samples were then placed in a freezer at -5°C for 10 min to facilitate breakage of any emulsion formed during the extraction step. This was followed by centrifugation for 10 min at 3000 *g*. The upper organic layer was transferred to clean 15-mL tubes and evaporated to dryness in a water bath maintained at 37°C under a gentle stream of nitrogen gas. To the residue was added 100 μL toluene containing 8 μL *N*-methyl-*N* (*tert*-butyldimethylsilyl)-trifluoroacetamide; this was vortex-mixed and placed in an oven at 60°C for 50 min. The samples were allowed to cool to room temperature, after which 200 μL of toluene was added and vortex-mixed; the samples were then transferred to automatic sampler injection vials. Aliquots of 2 μL were injected onto the gas chromatograph. The limit of quantitation of the method is 1 ng mL^{-1} of biological fluid sample, requiring as little as 0.1 mL. The inter- and intraday variability was within acceptable limits (i.e. $< 10\%$ over the 2–32 ng mL^{-1} standard curve concentration range and $< 20\%$ at the lower limit of quantitation). Recoveries from all fluids were $> 80\%$ (Krishna et al 1995).

Estimation of indometacin glucuronide conjugates in fetal urine

The presence of indometacin glucuronide conjugates in fetal urine was assessed by incubating urine samples with bovine liver β-glucuronidase (5000 U mL^{-1}, pH 5.0; Sigma Chemical Co.) for 3 h at 37°C. The concentration of glucuronide-conjugated indometacin was estimated as the difference in drug concentration in each sample before and after hydrolysis.

Pharmacokinetic calculations

Fetal total body clearance (TBC) of indometacin was estimated as:

$$TBC = k_o/C_{ss}$$

where k_o is the infusion rate and C_{ss} is the apparent fetal arterial steady-state indometacin concentration.

Fetal placental clearance (CL_{pl}) was estimated as:

$$CL_{pl} = Q_{um} \times (C_{fa} - C_{um})/C_{fa}$$

where Q_{um} is the umbilical blood flow rate, C_{fa} is the fetal femoral arterial indometacin concentration, and C_{um} is the umbilical venous indometacin concentration at apparent steady state.

Fetal non-placental indometacin clearance (CL_{npl}) was then calculated as:

$$CL_{npl} = TBC - CL_{pl}$$

Fetal renal clearance (CL_r) was estimated by plotting the average urinary excretion rate versus the drug concentration at the mid point of the collection interval and fitting the least squares straight line forced through the origin. The slope of the line provided the CL_r value in mL min^{-1}.

Fetal weight in-utero was estimated from the weight at euthanasia and the interval between then and the day of the experiment, for which weight was determined using the method of Koong et al (1975).

Statistical calculations

All data are reported as mean ± s.e. unless otherwise indicated. The coefficient of variation is reported as a percentage. The paired *t*-test was used to determine whether fetal umbilical venous and femoral arterial indometacin concentrations were significantly different. All high dose versus low dose pharmacokinetic parameters were also compared using the unpaired *t*-test. The significance level was $P < 0.05$ in all cases.

Results

Pre-infusion values for fetal physiological parameters were within the normal range and were as follows: PaO_2 19.1 ± 0.9 mmHg, $PaCO_2$ 52.5 ± 0.7 mmHg, pHa 7.340 ± 0.008, base excess 2.6 ± 0.6 meq L^{-1}, arterial glucose concentration 0.72 ± 0.04 mM, arterial lactate concentration 1.19 ± 0.07 mM, umbilical blood flow 187.3 ± 11.8 mL min^{-1} kg^{-1}, urine flow 0.17 ± 0.03 mL min^{-1} kg^{-1}. Changes in the variables during the infusion period will be reported elsewhere. At the end of the experiment, gestational age and fetal weight averaged 134.7 ± 0.4 days and 3.371 ± 0.096 kg, respectively.

The concentrations of indometacin in fetal arterial and umbilical venous plasma, amniotic fluid and fetal urine in the five LD and nine HD experiments are shown in Figure 1; the steady-state plasma concentrations and clearance data are given in Table 1. Steady-state plasma concentrations appeared to be attained by 5 h of infusion, and after termination of the infusion the drug levels fell rapidly, although the sampling frequency was insufficient to allow for accurate determination of the elimination half-life. Umbilical venous concentrations were generally lower than the arterial concentrations, and the mean difference of 9.0 ± 2.8 ng mL^{-1} was significantly greater than zero (Table 1). However, the placental extraction of the drug (5.2 ± 1.1%) was low, indicating low permeability of the sheep placenta to indometacin. Throughout the infusion period, indometacin levels in amniotic fluid and fetal urine were substantially lower than in fetal plasma. The amniotic fluid/fetal arterial plasma ratio for the drug was 0.14 ± 0.01. Indometacin levels in fetal tracheal fluid samples were below the detection limit of the assay.

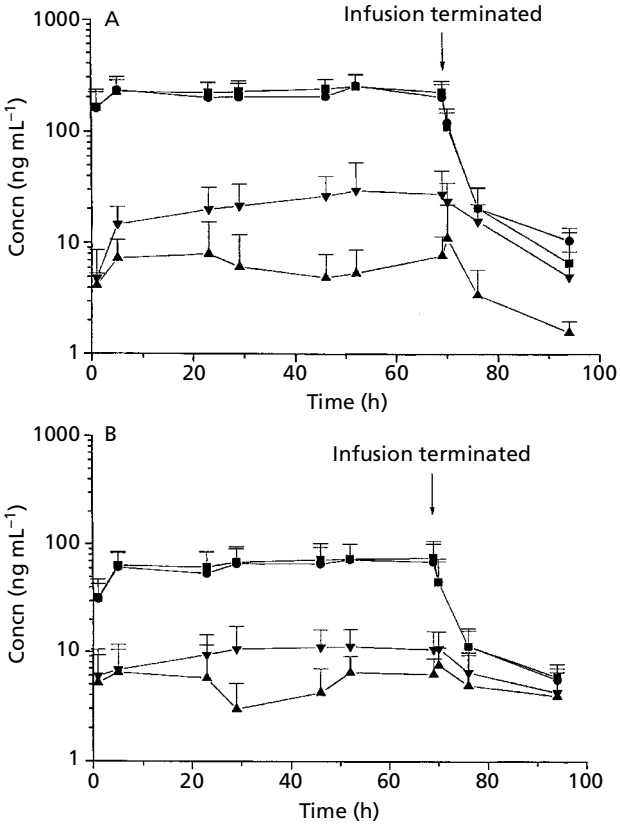

Figure 1 Mean ± s.d. concentration–time profiles for indometacin in fetal arterial (■) and umbilical venous (●) plasma, and in fetal urine (▲) and amniotic fluid (▼), during and after a 3-day continuous intravenous infusion at 7.5 (A; n = 9) and 1.9 (B; n = 5) μg kg^{-1} min^{-1}.

Table 1 Steady-state indometacin plasma concentrations and clearance values in the low (1.9 μg min^{-1}) and high (7.5 μg min^{-1}) dose infusions.

Parameter	High dose	Low dose	Overall mean
C_{fa} (ng mL^{-1})	230.2±28.8 (9)	68.6±11.5 (5)	–
C_{um} (ng mL^{-1})	221.2±35.5 (7)	71.6±12.1 (4)	–
C_{fa-um} (ng mL^{-1})	12.1±3.9 (7)	3.4±2.2 (4)	9.0±2.8 (11)
TBC (mL min^{-1} kg^{-1})	11.5±1.5 (9)	10.9±2.1 (5)	11.3±1.2 (14)
Q_{um} (mL min^{-1} kg^{-1})	221.0±20.5 (8)	218.5±15.4 (5)	220.0±13.5 (13)
CL_{pl} (mL min^{-1} kg^{-1})	10.4±2.7 (6)	9.5±5.5	10.0±2.5 (10)
CL_{npl} (mL min^{-1} kg^{-1})	0.8±2.8 (6)	0.3±6.3 (4)	0.60±2.8 (10)
CL_{npl}/TBC×100	109.3±41.2	122.8±91.8	115.1±41.2
CL_r (μL min^{-1} kg^{-1})	3.6±1.4 (9)	4.4±0.6 (3)	3.8±1.1 (12)
CL_{rtot} (μL min^{-1} kg^{-1})	8.2±2.3 (4)	23.0±14.5 (3)	14.5±6.5 (7)

Data are means±s.e., numbers in parentheses represent the number of experiments. C_{fa}, fetal femoral arterial indometacin concentration; C_{um}, umbilical venous indometacin concentration; TBC, total body clearance; Q_{um}, umbilical blood flow; CL_{pl}, placental indometacin clearance; CL_{npl}, non-placental indometacin clearance; CL_r, renal clearance of intact indometacin; CL_{rtot}, total renal clearance of intact and glucuronidated indometacin.

Fetal TBC was 11.5±1.5 mL min^{-1} kg^{-1} in the HD experiments and 10.9±2.1 mL min^{-1} kg^{-1} in the LD experiments (Table 1). These values were not significantly different and the overall mean value was 11.3±1.2 mL min^{-1} kg^{-1}. In some animals, umbilical venous samples could not be collected or umbilical blood flow was not measured. Thus, CL_{pl} could not be calculated in all animals. In the remaining HD (n = 6) and LD (n = 4) experiments, the mean CL_{pl} estimates were similar and the overall mean value was 10.0±2.5 mL min^{-1} kg^{-1}. This value is very similar to the estimate of TBC (11.3±1.2 mL min^{-1} kg^{-1}). The estimate of CL_{npl} of 0.60±2.8 mL min^{-1} kg^{-1} is thus very low and not significantly different from zero, indicating that fetal elimination of the drug is largely or wholly via placental transfer to the mother.

As shown in Figure 1, the levels of intact indometacin in fetal urine are much lower than in plasma. The estimates of CL_r of intact drug are also very low (Table 1), averaging 3.6±1.4 and 4.4±0.6 μL min^{-1} kg^{-1} for the HD and LD experiments, respectively. Overall, CL_r of the drug composed only 0.04±0.009% of TBC. In four of the HD and three of the LD experiments, there was sufficient fetal urine to allow for treatment with β-glucuronidase. In all these experiments, the indometacin concentration in the enzyme-treated samples was higher than in the control samples, indicating the presence of glucuronide conjugates. In the individual experiments, the conjugates accounted for 26.1–89.3% of the total urinary drug concentration, with a mean of 69.9±8.3%. CL_r of the total drug (i.e. intact indometacin + glucuronide) averaged 14.5±6.5 μL min^{-1} kg^{-1}, but this still represents a very small fraction (0.19±0.09%) of TBC.

Discussion

The fetal indometacin concentrations in the current study are similar to those reported in human fetuses. Moise et al (1990) measured indometacin in cord blood samples (obtained via cordocentesis) and in maternal venous samples from 26 pregnant women who received a 50 mg oral dose of the drug 6.1 h before sampling. The fetal drug concentration ranged from 87 to 496 ng mL^{-1}, with a mean of 219 ng mL^{-1}. This is very similar to the mean fetal arterial (230.2±28.8 ng mL^{-1}) and umbilical venous (221.2±35.5 ng mL^{-1}) indometacin concentrations in the present HD experiments. The steady-state fetal arterial indometacin concentration in the LD experiments was 29.8% of the HD concentration, whereas the weight-normalized LD infusion rate is 27.5% of the HD rate. The similarity of these values suggests that, over the dose range studied, the drug exhibits linear pharmacokinetics in the fetal lamb.

Indometacin concentrations in amniotic fluid were much lower than in fetal plasma, with the average amniotic/plasma ratio being 0.14±0.01. This is similar to the value of 0.10 reported for indometacin in human pregnancy (Moise et al 1990), and also to the situation with other organic acids in sheep, such as valproic acid (Gordon et al 1995) and diphenylmethoxyacetic acid (DPMA), a diphenhydramine metabolite (Tonn et al

1995), which are also found in low concentrations in amniotic fluid. The very low fetal renal excretion of these and other acidic compounds (see below) could be involved, since fetal urine is a major contributor to amniotic fluid. This possibility is supported by the fact that the fetal renal excretion of amine drugs, such as diphenhydramine, is higher than in the adult (Kumar et al 1997), and it and other amine drugs, including metoclopramide (Riggs et al 1987) and ritodrine (Wright et al 1991), accumulate in amniotic fluid. However, the intramembranous pathway is also important in fluid and solute exchange between amniotic fluid and fetal blood (Gilbert & Brace 1989). The low amniotic concentration of indometacin suggests that it does not utilize this pathway either. Its high degree of protein binding in fetal plasma (~ 98% ; Anderson et al 1980a) could be a factor here, since DPMA is also highly bound (Kumar et al 1999). Moreover, fluoxetine, an amine drug, is also highly bound (~ 93%) in fetal sheep plasma and it does not accumulate in amniotic fluid after maternal or fetal drug administration (Kim 2000).

Indometacin was not detected in fetal tracheal fluid, and this is similar to the findings with DPMA (Tonn et al 1995) and valproic acid (Gordon et al 1995). It is in contrast to the results for amine drugs such as diphenhydramine, metoclopramide and ritodrine, which are present in high concentrations in tracheal fluid following either maternal or fetal drug administration (Riggs et al 1987; Wright et al 1991). Such amphiphilic drugs are taken up by the adult lung (Bend et al 1985) and there is evidence for a similar process in the fetal lamb (Rurak et al 1991), with some of the drug taken up by the lung being transferred to the fluid secreted into the airways. Thus, the lack of indometacin in tracheal fluid suggests that accumulation of the drug in the fetal lung may also be low.

CL_{pl} and CL_{npl} of indometacin have been measured previously in the fetal lamb by Anderson et al (1980a). They used a fetal bolus injection of ^{14}C-labelled indometacin coupled with maternal intravenous infusion of ^{3}H-labelled drug to steady state, and calculated fetal TBC from the rate of loss of the ^{14}C-labelled form and CL_{pl} as TBC times the fetal to maternal concentration ratio for the tritiated indometacin. The values for CL_{pl} and CL_{npl} are 2.1 ± 0.3 and 3.6 ± 0.6 mL min^{-1} kg^{-1}, respectively. These compare with the estimates for CL_{pl} of 10.0 ± 2.5 mL min^{-1} kg^{-1} and CL_{npl} of 0.60 ± 2.8 mL min^{-1} kg^{-1} in the current study. Given the different experimental approaches employed in the two studies, the differences in the clearance estimates are not large, particularly when they are compared with the clearance values for most of the other drugs studied in pregnant sheep, which are generally much higher. Thus in terms of CL_{pl}, the values for diphenhydramine (214 mL min^{-1} kg^{-1}; Kumar et al 1997), metoclopramide (103 mL min^{-1} kg^{-1}; Riggs et al 1990), methadone (168 mL min^{-1} kg^{-1}; Szeto et al 1982), paracetamol (31 mL min^{-1} kg^{-1}; Wang et al 1986), labetalol (23 mL min^{-1} kg^{-1}; Yeleswaram et al 1993), morphine (19 mL min^{-1} kg^{-1}; Szeto et al 1982) and valproic acid (17 mL min^{-1} kg^{-1}; Kumar et al 2000a) are all higher than that for indometacin. In contrast, the estimates for ritodrine (5 mL min^{-1} kg^{-1}; Wright et al 1991) and acetylsalicylic acid (0.5 mL min^{-1} kg^{-1}; Anderson et al 1980b) are similar to the values for indometacin. These compounds with low CL_{pl} are polar compounds, with a low octanol/water partition coefficient (e.g. ritodrine; Wright et al 1991). The sheep epitheliochorial placenta has a low permeability to such compounds (Rurak et al 1991). In contrast, the drugs mentioned above that have higher CL_{pl} in sheep are more lipophilic (Rurak et al 1991). The low placental permeability of indometacin in sheep was also demonstrated with maternal administration of the drug, with the fetal to maternal concentration ratio being reported as 0.28 (Anderson et al 1980a) and 0.04 (Harris & Van Petten 1981). However, the situation is different in species with haemochorial placentae, since the fetal to maternal indometacin concentration following maternal drug administration is 0.57 (Parks et al 1977) or 0.80 in the rabbit (Harris & Van Petten 1981), and 1.0 in the human (Moise et al 1990). Again, this is consistent with the permeability characteristics of the haemochorial placenta, which is more permeable to hydrophilic drugs compared with epitheliochorial placentae (Rurak et al 1991).

The CL_{npl} of indometacin is also much lower than that reported for most other drugs in the fetal lamb, which range from 11 mL min^{-1} kg^{-1} for paracetamol (Wang et al 1986) to 110 mL min^{-1} kg^{-1} for diphenhydramine (Kumar et al 1997). However, there are other drugs with a low CL_{npl}, including acetylsalicylic acid (Anderson et al 1980b) and valproic acid (Kumar et al 2000a), although, in the latter case, there may be metabolism of the drug by the placenta. In adults, the main pathways of indometacin metabolism involve *O*-desmethylation, *N*-deacylation and glucuronide conjugation of the resulting metabolites and of intact indometacin as well (Duggan et al 1972). In the current study, glucuronide conjugates of indometacin were detected in fetal urine, and given the very low permeability of the sheep placenta to glucuronide conjugates (Olsen et al 1988), it is very likely that they were produced in the fetus. However, their contribution to overall fetal indometacin elimi-

nation appears minimal. The low estimate of CL_{npl} suggests that the other metabolic pathways for the drug also have no or minimal function before birth. This is consistent with the limited metabolism of the drug in neonatal rabbits and rats (Evans et al 1981; Clozel et al 1986) and prolonged indometacin elimination half-life in preterm human newborns (Thalji et al 1980; Vert et al 1980; Yaffe et al 1980). Our finding that CL_{pl} accounts for all of TBC suggests that placental transfer to the mother is the primary route of indometacin elimination in the fetus. This route is of course lost at delivery, which could result in the persistence of the drug in newborns exposed in-utero.

The very low CL_r of indometacin in the fetal lamb is similar to the findings with other organic acids, including valproic acid (Kumar et al 2000b), DPMA (Kumar et al 1999), and *p*-aminohippuric acid (Elbourne et al 1990). The very weak organic acid, paracetamol, also exhibits a very low CL_r (Wang et al 1986). This is in contrast to the situation with organic bases, such as diphenhydramine, where the CL_r value is higher than in the adult (Kumar et al 1997). It seems likely that the renal tubular transporters involved in excretion of organic acids are not developed or functional before birth and develop in the postnatal period (Wong et al 2000). Thus, renal excretion plays no significant role in the overall elimination of indometacin in the fetal lamb.

In conclusion, this study is the first to examine the fetal disposition of indometacin in any species, employing a clinically relevant drug concentration range and duration of administration. The results suggest that the fetal lamb has a very limited ability to eliminate the drug via non-placental routes, including the kidney. Thus, the placenta is the main route of elimination. However, CL_{pl} of indometacin is also low owing to the physicochemical properties of the drug and the permeability characteristics of the sheep placenta. There is also limited accumulation of indometacin in amniotic fluid and none in tracheal fluid.

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