Insulin Glargine Safety in Pregnancy

A transplacental transfer study

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OBJECTIVE — Insulin glargine (Lantus) is an extended-action insulin analog with greater stability and duration of action than regular human insulin. The long duration of action and decreased incidence of hypoglycemia provide potential advantages for its use in pregnancy. However, the placental pharmacokinetics of insulin glargine have not been studied. Therefore, the objective of this study was to determine whether insulin glargine crosses the human placenta using the human perfused placental lobule technique.

RESEARCH DESIGN AND METHODS — Placentae were obtained with informed consent after elective cesarean section delivery of noncomplicated term pregnancies. Insulin glargine, at a therapeutic concentration of 150 pmol/l (20 μU/ml) was added to the maternal circulation. Additional experiments were carried out at insulin glargine concentrations 1,000-fold higher than therapeutic levels (150, 225, and 300 nmol/l). A subsequent perfusion for which the maternal circuit remained open and insulin glargine was continuously infused at 150 pmol/l was completed for further confirmation of findings. The appearance of insulin glargine in the fetal circulation was analyzed by a chemiluminescence immunoassay.

RESULTS — Results from perfusions carried out at therapeutic concentrations (150 pmol/l) of insulin glargine showed no detectable insulin glargine in the fetal circuit. After perfusion with very high insulin glargine concentrations of 150, 225, and 300 nmol/l, the rate of transfer remained low at 0.079 ± 0.01, 0.14, and 0.064 pmol · min^{-1} · g $tissue^{-1}$, respectively.

CONCLUSIONS — Insulin glargine, when used at therapeutic concentrations, is not likely to cross the placenta.

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Several new long-acting insulin analogs, such as glargine and detemir, are currently available for the treatment of diabetes. These long-acting insulins have the advantage of a very long elimination half-life (24 h), avoiding a peak in insulin concentrations (1,2). The absence of a peak with the use of these insulin analogs has led to decreased incidence of symptomatic, overall, and nocturnal hypoglycemia in patients with type 1 diabetes (3). In addition, these analogs are increasingly being used in patients with type 2 diabetes, for whom they provide improved glycemic control and reduced hypoglycemia (4). With their increased use, more women with diabetes may find themselves pregnant while taking these insulins or find they are taking these insulins while planning a pregnancy. Such insulins may be particularly useful in pregnancy because tight glycemic control during gestation decreases the risk of maternal and fetal complications (5–10) and attenuates their severity (9). Studies have shown, however, that severe hypoglycemia is often a consequence of attempts to achieve tight glycemic control in pregnancy (11). Use of these insulin analogs would help patients achieve excellent glycemic control without the risk of maternal hypoglycemia.

Insulin glargine (Lantus; Aventis Pharmaceuticals, Bridgewater, NJ) is a long-acting insulin analog that differs from regular human insulin by the addition of two molecules of arginine to the COOH terminus of the β-chain and the replacement of aspartic acid with glycine in position A21. These molecular changes cause the drug to precipitate upon subcutaneous injection, increasing stability and duration of action (12).

It is believed that insulin does not cross the placental barrier because of its large molecular size. However, beef/pork insulin has been shown to cross the placenta via the formation of insulin-antibody complexes, leading to fetal macrosomia despite excellent glycemic control (13). Whereas insulin uptake into cellular compartments is mainly by receptor-mediated endocytosis, there are other mechanisms in place that may allow its transfer across biological membranes, such as pinocytosis and the involvement of membrane transporters (14). The possible consequences of transplacental transfer of insulin analogs, such as insulin glargine, include teratogenicity, immunogenicity, and mitogenicity. Specifically, structural modifications to insulin have been shown to cause altered affinity for the insulin and IGF-1 receptor (15). Although the evidence to date is conflicting (16), one study demonstrated that glargine has a six- to eightfold increased affinity for the IGF-1 receptor in the osteosarcoma cell line Saos/B10 (15). Concern exists that such growth-promoting properties may lead to increased fetal growth and other mitogenic effects should insulin cross the placenta. It is well known that excellent glucose control throughout pregnancy while minimizing maternal hypoglycemia is essential for the safe and effective treatment of women with diabetes in pregnancy. Consequently, there is a need to address the issues of fetal exposure and safety with the introduction of new and potentially beneficial insulin analogs, such as insulin glargine, for use in pregnancy. Although there are some case reports and case series describing patients who have gone

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through a pregnancy using glargine (17–20), there are no studies to date that have looked at the placental pharmacokinetics of glargine. The objective of the present study was to examine whether insulin glargine crosses the placenta into the fetal circulation using the ex vivo technique of human placental lobule perfusion.

RESEARCH DESIGN AND METHODS

In vitro perfusion of human placental cotyledon

Placentae were obtained with informed consent after elective cesarean section delivery of noncomplicated term pregnancies. The placentae were transported to the laboratory in heparinized ice-cold PBS. Within 30 min of delivery, maternal and fetal circulations were established independently to a peripheral lobule (21).

The fetal and maternal perfusates were maintained at 37°C and consisted of heparin (2,000 units/l), kanamycin (100 mg/l), glucose (1.0 g/l), and 40,000 molecular weight dextran (7.5 g/l maternal; 30 g/l fetal). Antipyrine (1 mmol/l) was added to the maternal perfusate for determination of tissue viability.

A single perfusion experiment consisted of a 1-h closed control period followed by a 3-h closed experimental period and a final 1-h postcontrol period. During the control and experimental periods, the perfusates were maintained at physiological pH by the addition of small volumes of sodium bicarbonate and hydrochloric acid. The maternal perfusate was equilibrated with 95% oxygen and 5% nitrogen, and the fetal perfusate was equilibrated with 5% oxygen and 95% nitrogen.

Preexperimental control period

The fetal and maternal circulations were maintained until residual blood was cleared out of the vessels. At this point the maternal and fetal circuits were closed and the perfusates were recirculated. Maternal and fetal samples were taken every 15 min to analyze glucose and oxygen consumption and lactate production to confirm tissue viability. The integrity of the placenta was also analyzed by monitoring fetal perfusion pressure and fetal reservoir volume. The perfusion was terminated if there was a loss in fetal reservoir volume greater than 3 ml/h. In addition, the rate of human chorionic gonadotropin secretion was determined from a concentration-time plot as an additional marker of physical integrity. Before the experimental period was begun, the perfusates in the fetal and maternal reservoirs were replaced with fresh media, and the circulations were closed and recirculated.

Experimental period

In a closed-circuit experiment, insulin glargine was added at a therapeutic concentration of 150 pmol/l (20 μU/ml) to the maternal circulations ($n = 4$) (22). Additional closed-circuit experiments ($n = 4$) were also performed at insulin glargine concentrations 1,000-fold higher than therapeutic levels (150, 225, and 300 nmol/l). A subsequent perfusion in which the maternal circuit remained open and insulin glargine was continuously infused at 150 pmol/l was completed for further confirmation of findings. Samples (2 ml) were drawn from the maternal and fetal reservoirs every 10 min for the first half-hour and every half-hour thereafter for the measurement of insulin concentrations as well as for the measurement of antipyrine, glucose consumption, and lactate production. Additional samples were taken for monitoring of pH, P_{O_2}, and P_{CO_2} using a blood gas analyzer (ABL 625; Radiometer, Copenhagen, Denmark).

Postexperimental control period

The perfusates in the fetal and maternal reservoirs were replaced with fresh media, and both circulations were closed and recirculated for the final 1-h control period. Samples were taken from the fetal and maternal reservoirs every 15 min for analysis of glucose and oxygen consumption and lactate production to confirm tissue viability. In addition, the integrity of the placenta was also analyzed by monitoring fetal perfusion pressure and fetal reservoir volume as in the precontrol and experimental periods.

Table 1 —*Placental physical parameters*

Parameter	Value
Lobule weight (g)	12.6 ± 3.2
Fetal flow rate (ml/min)	1.6 ± 0.1
Maternal flow rate (ml/min)	13.6 ± 3.2
Fetal arterial inflow pressure (mmHg)	
Precontrol	39.2 ± 6.9
Experimental	37.4 ± 5.7
Postcontrol	38.9 ± 6.0

Data are means ± SD ($n = 9$).

Sample analysis

Perfusate samples were kept at −80°C until they were analyzed. Glucose and lactate concentrations, as well as samples taken for monitoring of pH, P_{O_2}, and P_{CO_2}, throughout the perfusion were analyzed simultaneously using a blood gas analyzer (ABL 625).

Insulin in the maternal and fetal perfusate samples was measured using a one-step chemiluminescent immunoassay (Architect i2000 analyzer; Abbott Laboratories) that has been shown to have a high degree of cross-reactivity with insulin glargine (83–105%) (23). Standard curves were prepared for insulin glargine in perfusate and used to calculate insulin levels after analysis. The detection limit of this method is 0.5 μU/ml (23).

RESULTS — The mean mass ± SEM of the perfused cotyledons was 12.6 ± 3.2 g. The fetal arterial pressure remained constant throughout the control and experimental periods (Table 1). The rate of placenta glucose and oxygen consump-

Table 2 —*Maternal to fetal transport of insulin glargine*

Experiment no.	Maternal concentration (pmol/l)	Lobule weight (g)	Fetal concentration (pmol/l)	Rate of transfer (pmol · min^{-1} · g tissue^{-1})
1	150	15.17	Below LOQ	—
2	150	9.8	Below LOQ	—
3	150	12.83	Below LOQ	—
4	150	19.51	Below LOQ	—
5	150	12.24	Below LOQ	—
6	150,000	9.64	802.5	0.069
7	150,000	12.46	906.3	0.09
8	225,000	9.08	1,475.2	0.14
9	300,000	12.94	1,001.9	0.064

LOQ, limit of quantitation.

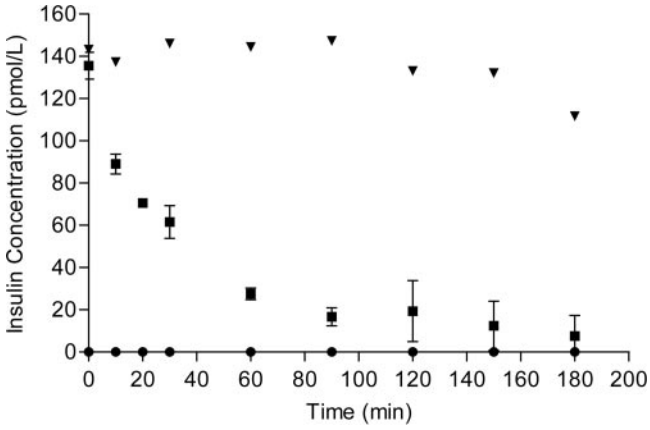

Figure 1—*Maternal and fetal insulin concentrations during 3-h perfusions in the presence of therapeutic levels (150 pmol/l) of insulin glargine* (n = 5). ▼*, maternal open circuit;* ■*, maternal closed circuit;* ●*, fetal.*

tion and delivery, as indicators of metabolic viability, did not vary significantly between the experimental and control periods. Measures of human chorionic gonadotropin remained stable throughout the perfusions and indicated a preferential secretion into the maternal compartment. Lactate production was maintained throughout the perfusions. The rates of antiypyrine disappearance from the maternal circuit and appearance into the fetal circuit were indicative of an optimal overlap between the maternal and fetal circulations.

Results from the 3-h perfusions performed at maternal therapeutic concentrations (150 pmol/l) of insulin glargine showed a decline in insulin concentration from the maternal circuit over time with no detectable insulin glargine in the fetal circuit (Table 2, Fig. 1). At higher concentrations of insulin glargine (1,000-fold), there was an observed decrease in maternal insulin glargine concentrations and a detectable accumulation of insulin glargine in the fetal circuit over the 3-h perfusions (Figs. 2 and 3). However, even at the excessive concentrations of 150, 225, and 300 nmol/l, the rate of transfer to the fetal circulation remained low (0.079 ± 0.01, 0.14, and 0.064 pmol · min^{-1} · g $tissue^{-1}$, respectively) (Table 2). A final perfusion was performed using an open maternal circuit with continuous insulin glargine infusion. Concentrations were maintained in the maternal compartment at 137 ± 11.8 pmol/l throughout the 180-min perfusion. Levels of glargine were not detectable in the fetal compartment despite continuous infusion in the maternal compartment (Fig. 1).

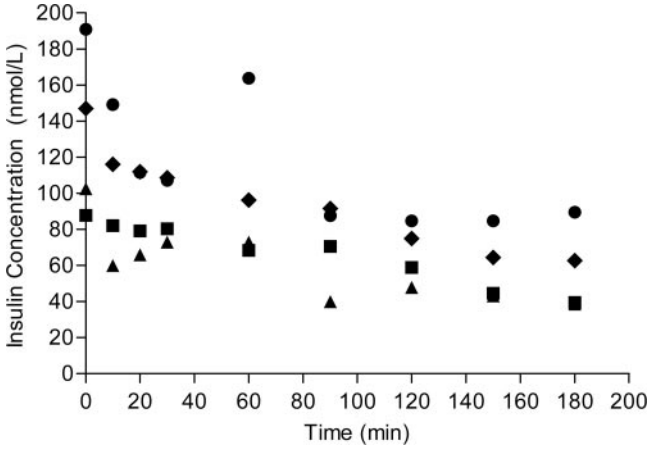

Figure 2—*Disappearance of insulin from the maternal compartment over 180 min of perfusion in the presence of insulin glargine concentrations 1,000-fold greater than therapeutic (150–300 nmol/l)* (n = 4). ■*, maternal reservoir concentration 150 nmol/l;* ●*, maternal reservoir concentration 300 nmol/l;* ▲*, maternal reservoir concentration 150 nmol/l;* ◆*, maternal reservoir concentration 225 nmol/l.*

CONCLUSIONS — Our results obtained from perfusions carried out at therapeutic insulin glargine concentrations suggest that insulin glargine does not cross the human placenta to a measurable extent. Transport across the placenta was demonstrated at concentrations 1,000-fold higher than therapeutic levels. Even at these very high levels, there was a 100-fold difference in the rate of disappearance from the maternal compartment and the rate of appearance in the fetal compartment. This difference between insulin uptake and insulin transferred to the fetal compartment probably corresponds to the clearance of insulin by placental tissue. These data suggest that the placenta is able to sequester and/or metabolize insulin glargine at concentrations up to 1,000-fold higher than therapeutic levels, thereby limiting its entry into the fetal compartment. The limited transfer of insulin glargine across the placenta is supported by previous research findings. Although the liver and kidney are the major sites of insulin clearance, the placenta has been shown to possess receptors for insulin as well as a capacity for rapid degradation by insulin-degrading enzymes (24). The insulin receptors have been located on the syncytiotrophoblast membrane of the placenta where they interact with the maternal circulation (24). The mechanism of clearance by the placenta most likely involves insulin binding to its receptor, internalization, and degradation. However, at very high concentrations, it has been suggested that non–receptor-mediated processes, such as pinocytosis, may also be involved in insulin transport across cell membranes in other tissues (14).

In the current study, we chose insulin glargine levels of 150 pmol/l (20 μU/ml) to mimic typical therapeutic levels achieved and maintained after administration of a single dose of insulin glargine given by subcutaneous injection (0.3 U/kg) (22). Levels of insulin glargine have been shown to remain relatively stable (13–21 μU/ml) after subcutaneous injection (25). Therefore, the results obtained from perfusions completed at concentrations of 150 pmol/l (20 μU/ml) are clinically relevant in showing no placental transfer. Results obtained from perfusions with excessive levels of insulin glargine (150–300 nmol/l) are important in terms of determining the capacity of the pla-

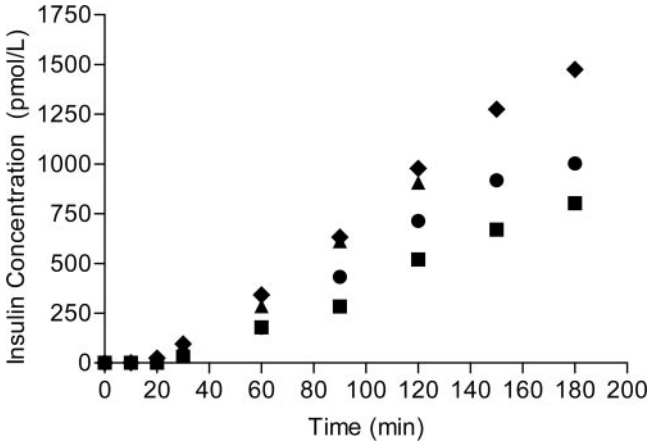

Figure 3—*Appearance of insulin in the fetal compartment after perfusions in the presence of maternal insulin glargine concentrations 1,000-fold greater than therapeutic (150–300 nmol/l) (*n *= 4).* ■*, maternal reservoir concentration 150 nmol/l;* ●*, maternal reservoir concentration 300 nmol/l;* ▲*, maternal reservoir concentration 150 nmol/l;* ♦*, maternal reservoir concentration 225 nmol/l.*

centa to degrade insulin glargine at supratherapeutic levels; however, these levels would not occur in the clinical setting.

The use of the human placental perfusion model has its limitations in that it only allows for the study of transport in term placenta. Therefore, our conclusions regarding the placental transfer of insulin glargine cannot be directly extrapolated to first-trimester use. In addition, our studies were performed using placentae from healthy term pregnancies. Placentae of diabetic mothers, particularly those with poorly controlled diabetes, may exhibit structural or physiological abnormalities; however, the consequence of these abnormalities on the transfer of regular human insulin or insulin glargine is not known. Furthermore, these studies were performed on placentae delivered by cesarean section. During active prolonged labor, the mixing of fetal and maternal blood can result in fetal exposure to drugs circulating in maternal blood. Despite these limitations, the perfusion model is unparalleled by any other in vitro placental preparations for the study of transplacental transfer of drugs in pregnancy. This model most closely resembles the in vivo situation, without the ethical dilemma with clinical studies in pregnancy or the confounding effects of maternal metabolism. Importantly, there are wide differences in placental structure and function in other mammals, making the ex vivo use of human placenta ideal.

The structural modifications to insulin glargine allow a smooth action profile over 24 h, which mimics physiological insulin secretion typically seen in nondiabetic patients (12). The peakless activity of insulin glargine decreases the risk of hypoglycemia in nonpregnant patients and for this reason is particularly attractive for use in pregnancy. Although there have been few reports on the safety of insulin glargine in pregnancy, several case series and small case-control studies have been reported (17–20). The largest was a case series of 115 women with type 1 diabetes who took glargine during pregnancy (17). There were no "unexpected" adverse events. A small case-control study of 15 women who took glargine and an equal number who took NPH throughout pregnancy did not show a significant difference in any maternal or fetal outcomes (18). Although no randomized clinical trials of insulin glargine use during pregnancy are currently available, data obtained from these case-control studies and case series further support the findings demonstrated by our placental perfusion studies, suggesting that insulin glargine may be safe for use in pregnancy.

In summary, when used at therapeutic concentrations, insulin glargine is not likely to cross the placenta. Our results indicate wide capacity of the human placenta to block insulin glargine transfer to the fetal compartment.

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