Insulin Glargine Safety in Pregnancy: a Transplacental Transfer Study

Short Title: Insulin glargine placental transfer

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**Objective:** Insulin glargine (Lantus, Aventis Pharmaceuticals) is an extended-action insulin analogue with greater stability and duration of action compared to 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 is 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 following elective cesarean section delivery of non-complicated term pregnancies. Insulin glargine, at a therapeutic concentration of 150 pmol/L (20µU/ml) was added to the maternal circulation. Additional experiments were carried out at insulin glargine concentrations 1000-fold higher than therapeutic levels (150, 225, 300 nmol/L). A subsequent perfusion was completed for further confirmation of findings where the maternal circuit remained open and insulin glargine was continuously infused at 150pmol/L. The appearance of insulin glargine in the fetal circulation was analyzed by a chemiluminescence immunoassay.

**Results:** Results from perfusions carried out at therapeutic concentrations (150pmol/L) of insulin glargine showed no detectable insulin glargine in the fetal circuit. Following perfusion with very high insulin glargine concentrations of 150, 225, and 300 nmol/L, the rate of transfer remained low, 0.079 ± 0.01, 0.14 and 0.064 pmol/min/g tissue respectively.

**Conclusions:** Insulin glargine, when used at therapeutic concentrations, is not likely to cross the placenta.
Several new long-acting insulin analogues 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 hrs), avoiding a peak in insulin concentrations (1,2). The absence of a peak with the use of these insulin analogues has led to a decreased incidence of symptomatic, overall and nocturnal hypoglycemia, in patients with type 1 diabetes (3). As well, these analogues are increasingly used in patients with type 2 diabetes, where 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 as 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 attempting to achieve tight glycemic control in pregnancy (11). These insulin analogues would help patients achieve excellent glycemic control without the risk of maternal hypoglycemia.

Insulin Glargine (Lantus, Aventis Pharmaceuticals) is a long-acting insulin analogue that differs from regular human insulin by the addition of 2 molecules of arginine to the C-terminal of the Beta 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 due to 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). While 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 analogues, 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 insulin-like growth factor (IGF-1) receptor (15). Although the evidence to date is conflicting (16), one study demonstrated that glargine has a 6 to 8-fold 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 the insulin cross the placenta.

It is well known that excellent glucose control throughout pregnancy while minimizing maternal hypoglycemia is essential in 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 analogues, such as insulin glargine, for use in pregnancy. While there are some case reports and case series describing patients who have gone through a pregnancy using glargine (17-20), there are no studies to date looking 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 following elective cesarean section delivery of non-complicated term pregnancies. The placentae were transported to the lab in heparanized ice-cold phosphate-buffered saline (PBS). Within 30 minutes of delivery, maternal and fetal circulations were established independently to a peripheral lobule.

The fetal and maternal perfusates were maintained at 37°C and consisted of heparin (2000 U/L), kanamycin (100mg/L), glucose (1.0g/L), 40,000 molecular weight dextran (7.5 g/L maternal; 30g/L fetal). Antipyrine (1mM) was added to the maternal perfusate for determination of tissue viability.

A single perfusion experiment consisted of a 1 hour closed control period followed by a 3 hour closed experimental period and a final 1 hour post control 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.

**Pre-experimental 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 re-circulated. Maternal and fetal samples were taken every 15 minutes 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 3mL per hour. In addition, the rate of hCG secretion was determined from a concentration-time plot as an additional marker of physical integrity.

Prior to beginning the experimental period, the perfusates in the fetal and maternal reservoirs were replaced with fresh media and the circulations were closed and re-circulated.

**Experimental Period.** In a closed circuit experiment, insulin glargine was added at a therapeutic concentration of 150 pmol/L (20µU/ml) to the maternal circulations (n=4) (22). Additional closed circuit experiments (n=4) were also carried out at insulin glargine concentrations 1000-fold higher than therapeutic levels (150, 225, 300 nmol/L). A subsequent perfusion was completed for further confirmation of findings where the maternal circuit remained open and insulin glargine was continuously infused at 150pmol/L. Samples (2mL) were drawn from the maternal and fetal reservoirs every 10 minutes for the first half-hour and every half-hour following 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, pO2, and pCO2 using a blood gas analyzer (Radiometer ABL 625, Copenhagen, Denmark).

**Post-experimental Control Period.** The perfusates in the fetal and maternal reservoirs were replaced with fresh media and both circulations were closed and re-circulated for the final 1-hour control period. Samples were taken from the fetal and maternal reservoirs every 15 minutes 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 pre-control and experimental periods.

**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, pO2, and
pCO2, throughout the perfusion were analyzed simultaneously by a blood gas analyzer (Radiometer ABL 625, Copenhagen, Denmark).

Insulin in the maternal and fetal perfusate samples was measured using a 1-step chemiluminescent immunoassay (Architect i2000 analyzer, Abbot 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 following analysis. The detection limit of this method is 0.5µU/mL (23).

RESULTS

The mean mass (± SE) 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 consumption and delivery, as indicators of metabolic viability, did not vary significantly between the experimental and control periods. Measures of hCG remained stable throughout the perfusions and indicated a preferential secretion into the maternal compartment. Lactate production was maintained throughout the perfusions. The rate of antipyrine disappearance from the maternal circuit and appearance into the fetal circuit was indicative of an optimal overlap between the maternal and fetal circulations.

Results from the 3-hour perfusions carried out at maternal therapeutic concentrations (150pmol/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 (1000-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-hour perfusions (Fig 2,3). 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/g tissue respectively (Table 2). A final perfusion was carried out using an open maternal circuit with continuous insulin glargine infusion. Concentrations were maintained in the maternal compartment at 137pmol/L ± 11.8 throughout the 180 minute perfusion. Levels of glargine were not detectable in the fetal compartment despite continuous infusion in the maternal compartment (Fig 1).

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 1000-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 likely 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 1000-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. While 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 (IDE) (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) following 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 placenta 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 carried out 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 carried out 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 hours, which mimics physiological insulin secretion typically seen in non-diabetic patients (12). The peakless activity of insulin glargine decreases the risk of hypoglycemia in non-pregnant patients, and for this reason is particularly attractive for use in pregnancy. While 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). While 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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Disclosure: The authors have no relevant conflict of interest to disclose.
REFERENCES


Table 1. Placental Physical Parameters.

<table>
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<th>Parameter</th>
<th>Value</th>
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<tr>
<td>Lobule Weight (g)</td>
<td>12.6 ± 3.2</td>
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<tr>
<td>Fetal flow rate (mL/min)</td>
<td>1.6 ± 0.1</td>
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<tr>
<td>Maternal flow rate (mL/min)</td>
<td>13.6 ± 3.2</td>
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<tr>
<td>Fetal arterial inflow pressure (mm Hg)</td>
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<tr>
<td>Precontrol</td>
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<td>Experimental</td>
<td>37.4 ± 5.7</td>
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<tr>
<td>Postcontrol</td>
<td>38.9 ± 6.0</td>
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</table>

Data are means ± SD (n=9).

Table 2. Maternal to fetal transport of insulin glargine.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Maternal concentration (pmol/L)</th>
<th>Lobule Weight (g)</th>
<th>Fetal Concentration (pmol/L)</th>
<th>Rate of Transfer (pmol/min/g tissue)</th>
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<tr>
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Figure Legends

Figure 1. Maternal and fetal insulin concentrations during 3 hour perfusions in the presence of therapeutic levels (150 pmol/L) of insulin glargine (n=4). Inverted triangle = maternal open circuit, square = maternal closed circuit, circle = fetal.

Figure 2. Disappearance of insulin from the maternal compartment over 180 minutes of perfusion in the presence of insulin glargine concentrations 1000-fold greater than therapeutic (150-300nmol/L) (n=4). MR = maternal reservoir concentration, square = MR 150nmol/L, circle = MR 300nmol/L, triangle = MR 300nmol/L, diamond = MR 225nmol/L.

Figure 3. Appearance of insulin in the fetal compartment following perfusions in the presence of maternal insulin glargine concentrations 1000-fold greater than therapeutic (150-300nmol/L) (n=4). MR = maternal reservoir concentration, square = MR 150nmol/L, circle = MR 300nmol/L, triangle = MR 300nmol/L, diamond = MR 225nmol/L.
Figure 1

![Figure 1 Image]

Figure 2

![Figure 2 Image]
Figure 3