Insulin Glargine Safety in Pregnancy

A transplacental transfer study

ERIKA K. POLLEX, BMSC1,2
DENICE S. FEIG, MD3
ANGELIKA LUBETSKY1

PAUL M. YIP, PHD4
GIDEON KOREN, MD1,2

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.

From the 1Division of Clinical Pharmacology and Toxicology, the Hospital for Sick Children, Toronto, Ontario, Canada; the 2Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, Canada; the 3Division of Endocrinology, Mount Sinai Hospital, Department of Medicine, University of Toronto, Toronto, Ontario, Canada; and the 4Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada.

Corresponding author: Gideon Koren, gkoren@sickkids.ca.

Received 8 June 2009 and accepted 14 September 2009. Published ahead of print at http://care.diabetesjournals.org on 6 October 2009. DOI: 10.2337/dc09-1045.

© 2010 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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 as in the precontrol 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 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 consum-

**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, PO2, and PCO2 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 the circulations were closed and recirculated. 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.

**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 consumption and lactate production was confirmed by monitoring fetal perfusion pressure and fetal reservoir volume as in the precontrol 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 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 consumption was confirmed by monitoring fetal perfusion pressure and fetal reservoir volume as in the precontrol and experimental periods.
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).

**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 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.

**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 300 nmol/l; ◆, maternal reservoir concentration 225 nmol/l.
over 24 h, which mimics physiological inplacenta ideal. There are wide differences in placental structure and function in other mammals, making the ex vivo use of human placenta to degrade insulin glargine at supra-therapeutic 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 placenta 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.

Acknowledgments—G.K. and E.K.P. are supported by a grant from the Canadian Institute of Health Research. E.K.P. is also supported by the Hospital for Sick Children Research Institute.

No potential conflicts of interest relevant to this article were reported.

We thank the clinical researchers at Mount Sinai Hospital for their help obtaining placenta tissue.

References

1. Owens DR, Coates PA, Luzio SD, Timbergren JP, Kurzhals R. Pharmacokinetics of $^{125}$I labelled insulin glargine (HOE 901) in healthy men: comparison with NPH insulin and the influence of different subcutaneous injection sites. Diabetes Care 2000;23:813–819
2. Plank J, Bodenlenz M, Sinner F, Magnes C, Gorzer E, Regitz W, Endahl LA, Draeger E, Zdravkovic M, Pieber TR. A double-blind, randomized, dose-response study investigating the pharmacodynamic and pharmacokinetic properties of the long-acting insulin analog detemir. Diabetes Care 2005;28:1107–1112
3. Gough SC. A review of human and analogue insulin trials. Diabetes Res Clin Pract 2007;77:1–15
4. Yki-Jarvinen H, Dressler A, Zoetendal, the HOE 901/300s Study Group. Less nocturnal hypoglycemia and better post-dinner glucose control with bedtime insulin glargine compared with bedtime NPH insulin during insulin combination therapy in type 2 diabetes: HOE 901/3002 study group. Diabetes Care 2000;23:1130–1136
5. Rosenstock J, Dailey G, Massi-Benedetti M, Fritsche A, Lin Z, Saltzman A. Reduced hypoglycemia risk with insulin glargine: a meta-analysis comparing insulin glargine with human NPH insulin in type 2 diabetes. Diabetes Care 2005;28:950–955
6. Kitzmiller JL, Gavin LA, Gin GD, Jovanovic-Peterson L, Main EK, Zigrang WD. Preconception care of diabetes: glycemic control prevents congenital anomalies. JAMA 1991;265:731–736
7. Mello G, Parretti E, Mecacci F, Pratesi M, Lucchetti R, Scarselli G. Excursion of daily glucose profiles in pregnant women with IDDM: relationship with perinatal outcome. J Perinat Med 1997;25:488–497
8. Mello G, Parretti E, Mecacci F, La Torre P, Cioni R, Cianciulli D, Scarselli G. What degree of maternal metabolic control in women with type 1 diabetes is associated with normal body size and proportions in full-term infants? Diabetes Care 2000;23:1494–1498
9. Jovanovic-Peterson L, Peterson CM, Reed GF, Metzger BE, Mills JL, Knopp RH, Aarons JH. Maternal postprandial glucose levels and infant birth weight: the Diabetes in Early Pregnancy Study. The National Institute of Child Health and Human Development–Diabetes in Early Pregnancy Study. Am J Obstet Gynecol 1991;164:103–111
10. Kitzmiller JL, Blount JW, Brown FM, Catalano PM, Conway DL, Coustan DR, Gun-
11. Rosenn BM, Miodovnik M, Holberg G, Khoury JC, Siddiqi TA. Hypoglycemia: the price of intensive insulin therapy for pregnant women with insulin-dependent diabetes mellitus. Obstet Gynecol 1995; 85:417–422

12. Campbell RK, White JR, Levien T, Baker D. Insulin glargine. Clin Ther 2001; 23:1938–1957; discussion 1923

13. Menon RK, Cohen RM, Sperling MA, Cutfield WS, Mimouni F, Khoury JC. Transplacental passage of insulin in pregnant women with insulin-dependent diabetes mellitus: its role in fetal macrosomia. N Engl J Med 1990; 323:309–315

14. Duckworth WC, Bennett RG, Hamel FG. Insulin degradation: progress and potential. Endocr Rev 1998; 19:608–624

15. Kurtzhals P, Schaffer L, Sorensen A, Kristensen C, Jonassen I, Schmid C, Trub T. Correlations of receptor binding and metabolic and mitogenic potencies of insulin analogs designed for clinical use. Diabetes 2000;49:999–1005

16. Ciardelli TP, Phillips SA, Carter L, Arod V, Mudalilar S, Henry RR. Effects of the rapid-acting insulin analogue insulin glargine on cultured human skeletal muscle cells: comparisons to insulin and insulin-like growth factor-1. J Clin Endocrinol Metab 2005;86:5838–5847

17. Gallen IW, Jaap A, Roland JM, Chirayath HH. Survey of glargine use in 115 pregnant women with type 1 diabetes. Diabet Med 2008;25:165–169

18. Imbergamo MP, Amato MC, Sciortino G, Gambina M, Accidenti M, Criscimanna A, Giordano C, Galluzzo A. Use of glargine in pregnant women with type 1 diabetes mellitus: a case-control study. Clin Ther 2008;30:1476–1484

19. Pöyhönen-Alho M, Rönnefors M, Saltevo J, Ekblad U, Kaaja RJ. Use of insulin glargine during pregnancy. Acta Obstet Gynecol Scand 2007;86:1171–1174

20. Price N, Bartlett C, Gillmer M. Use of insulin glargine during pregnancy: a case-control pilot study. BJOG 2007;114:453–457

21. Derewlany LO, Leeder JS, Kumar R, Radde IC, Knie B, Koren G. The transport of digoxin across the perfused human placental lobule. J Pharmacol Exp Ther 1991;256:1107–1111

22. Leppäniemi M, Panamalai S, Fanelli C, Porcellati F, Bartocci I, Di Vincenzo A, Cordoni C, Costa E, Brunetti P, Bolli GB. Pharmacokinetics and pharmacodynamics of subcutaneous injection of long-acting human insulin analog glargine, NPH insulin, and ultralente human insulin and continuous subcutaneous infusion of insulin lispro. Diabetes 2000;49:2142–2148

23. Moriyama M, Hayashi N, Ohyabu C, Mukai M, Kawano S, Kumagai S. Performance evaluation and cross-reactivity from insulin analogs with the ARCHITECT insulin assay. Clin Chem 2006;52:1423–1426

24. Steel RB, Mosley JD, Smith CH. Insulin and placenta: degradation and stabilization, binding to microvillous membrane receptors, and amino acid uptake. Am J Obstet Gynecol 1979;135:522–529

25. Kuerzel GU, Shukla U, Scholtz HE, Pretorius SG, Wessels DH, Venter C, Potgieter MA, Lang AM, Koose T, Bernhardt E. Biotransformation of insulin glargine after subcutaneous injection in healthy subjects. Curr Med Res Opin 2003;19:34–40