Effect of Maternal Use of Antiretroviral Agents on Serum Insulin Levels of the Newborn Infant

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OBJECTIVE — The aim of this study was to investigate the effect of antiretroviral drugs on neonatal serum insulin levels.

RESEARCH DESIGN AND METHODS — A prospective study was conducted on 57 pregnant women divided into three groups: the zidovudine (ZDV) group, HIV-infected women taking ZDV ($n = 20$); the triple treatment group, HIV-infected women taking triple antiretroviral agents ZDV + lamivudine + nelfinavir ($n = 25$); and the control group, pregnant women considered normal from a clinical and laboratory standpoint ($n = 12$). Blood was collected from the umbilical cord of newborn infants upon delivery for measurement of insulin level. The insulin measurements were performed in duplicate by radioimmunoassay.

RESULTS — Demographic and anthropometric data were homogeneous, and pregnant women with a personal and family history of diabetes were excluded. There was no difference between groups regarding glycemia in the newborn. Median newborn insulin doses were 2.9, 4.8, and 6.5 μU/ml for the triple treatment, ZDV, and control groups, respectively ($P < 0.05$).

CONCLUSIONS — Use of triple therapy during pregnancy induced a significant decrease in serum levels of neonatal insulin compared with the control group. Active surveillance of short- and long-term adverse events is imperative to issue a definitive statement regarding the impact that use of protease inhibitors during pregnancy will have on infant metabolism.

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The objective reduction of vertical transmission of HIV type 1 (HIV-1) with the use of antiretroviral drugs represents one of the most notable advances in the fight against the infection because it permits a reduction of >70% of this form of transmission. However, some adverse effects are associated with the use of these medications. Among these possible adverse effects are changes in glucose metabolism, especially with the use of protease inhibitors (1,2).

Despite the association between the use of protease inhibitors and adverse glycemic metabolic effects, little evidence is available regarding unequivocal proof of the safety on uninfected infants born to HIV-infected mothers taking antiretroviral drugs (3). In the present series, we evaluated the effect of antiretroviral drugs during gestation on the glucose and insulin levels from the newborn infant.

RESEARCH DESIGN AND METHODS

— A prospective cohort study was conducted from September 2001 to March 2003 on 57 women aged 16–43 years with singleton gestations. A total of 45 of these women were infected with HIV-1; the remaining 12 women were normal in both clinical and laboratory terms and were selected at the time they started prenatal care. The present study was approved by the research ethics committee of the institution, and written informed consent to participate was obtained from each subject.

Women were considered infected with HIV-1 when two different serum samples were found to be positive for HIV-1 antibodies by enzyme-linked immunoassay and confirmed by Western blot. Only HIV-infected patients who had not been treated previously with antiretroviral drugs were selected for the study.

The HIV-1–infected women were divided into two groups: the zidovudine (ZDV) group and the triple treatment group. The ZDV group consisted of 20 pregnant women who fulfilled the requirements for the prophylactic use of ZDV (CD4 >500 cells/mm^3 and viral load <1.000 copies/ml). The triple treatment group consisted of 25 pregnant women with a clinical and laboratory indication (CD4 <500 cells/mm^3) for triple antiretroviral treatment (ZDV + lamivudine + nelfinavir) according to the criteria established by the Perinatal HIV Guidelines Working Group Members regarding antiretroviral treatment of pregnant women. The control group consisted of 12 pregnant women considered normal from a clinical and laboratory standpoint.

Antiretroviral agents recommended, since the 14th week of pregnancy, were 300 mg twice daily for the ZDV group and 300 mg ZDV, 150 mg lamivudine, and

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Abbreviations: AUCI, area under the curve for insulin; HIV-1, HIV type 1; ZDV, zidovudine.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*AUCI among three groups obtained in four equidistant periods during pregnancy*

	Control group	ZDV group	TT group	*P*
n	12	20	25	
AUCI 1: 14–20 weeks	5,368 (4,963–6,032)	4,314 (3,449–5,336)	3,450 (2,338–5,194)	0.008*
AUCI 2: 21–26 weeks	6,898 (5,875–8,175)	5,628 (3,971–6,687)	4,133 (2,927–6,203)	0.012*
AUCI 3: 27–32 weeks	9,873 (9,196–10,680)	7,060 (5,326–7,688)	5,493 (4,018–7,964)	0.0004†
AUCI 4: 33–38 weeks	10,920 (10,210–11,960)	7,523 (5,649–9,060)	7,821 (4,692–9,315)	0.0009†
P	0.0001‡	0.0001‡	0.0001*‡	

Data are median (range) and 1st and 3rd quartiles. *Triple treatment (TT) group × control group: $P \leq 0.01$ (Kruskal-Wallis test followed by the post hoc Dunn test); †control group × TT group and ZDC group: $P \leq 0.001$ (Kruskal-Wallis test followed by the post hoc Dunn test); ‡AUCI 1 × AUCI 3 and AUCI 4: $P \leq 0.0001$ (Friedman test followed by the post hoc Dunn test).

1,250 mg nelfinavir in two daily doses for the triple treatment group.

Exclusion criteria were as follows: renal and hepatic insufficiency; personal or first-degree relative history of diabetes; initial BMI >30 kg/m^2; pregnancy with predictors of recurrent gestational diabetes such as history of spontaneous abortions, major congenital malformations, stillbirth, and/or macrosomia in previous pregnancies; noncompliance with or irregular use of antiretroviral drugs; and use of other medications of known diabetogenic effect.

Blood samples for measurement of fasting plasma glucose and serum insulin levels and for the 75-g oral glucose tolerance test were collected at the first visit and three more times during pregnancy at equal time intervals, i.e., between weeks 14 and 20, weeks 21 and 26, weeks 27 and 32, and weeks 33 and 38. The area under the curve for glucose and the area under the curve for insulin (AUCI) were obtained on the basis of fasting glucose values and the values obtained 30, 60, 90, and 120 min after ingestion of 75 g dextrose.

Three pregnant women and their neonates (two in the triple treatment group and one in the ZDV group) were excluded because glucose intolerance had developed during pregnancy, and two other neonates (one in each group) were excluded due to technical problems; the total number of newborns included in the study was 52. Each selected child was defined as an HIV-1–uninfected infant if results of HIV-1 RNA quantitative analysis were negative at 2 days of age and at 7–10 weeks of age according to the criteria demonstrated by Cunningham et al. (4) to indicate early diagnosis of HIV-1 infection in infants.

Neonatal insulin and glucose concentrations were determined in umbilical cord blood. Plasma was collected into a Vacutainer tube containing sodium fluoride and EDTA, and glucose levels were determined by the hexokinase method using the Cobas Mira-S (Roche, Montclair, NJ). Serum was collected into a Vacutainer containing no anticoagulant, and insulin levels were determined in duplicate by radioimmunoassay using Abbott-Kit DPC Medlab ANSR equipment (Abbott, North Chicago, IL). The intra-assay coefficient of variation for this analysis was 4.6%.

The variability of the plasma glucose and serum insulin concentrations was calculated on the basis of the median and interquartile variation (1st and 3rd quartiles, respectively). The nonparametric χ^2, Mann-Whitney *U*, and Kruskal-Wallis tests were used; $P < 0.05$ was considered significant. All analyses were performed using the SPSS 10.0 software (SPSS, Chicago, IL).

RESULTS — The median maternal age in this study was 22.5 years. The interquartile variation was 6 years for the control group, 24 years (7 years) for the ZDV group, and 27 years (6 years) for the triple treatment group. No statistical difference was noted between groups ($P = 0.13$, Kruskal-Wallis test). Race distribution (white and nonwhite) was also uniform in the three groups ($P = 0.14$, χ^2 test). Data on smoking habits also did not differ significantly between groups ($P = 0.10$, χ^2 test). Initial BMI, BMI at the end of pregnancy, and maternal weight gain from the beginning of prenatal care to delivery showed median values of 21.95, 25.57, and 10.5, respectively, for the pregnant women in the control group. No differences were observed between the three groups ($P = 0.10$, Kruskal-Wallis test).

The AUCI results for the three groups are presented in Table 1. Increased AUCI values were observed for the three groups studied during gestation, regardless of the treatment used. The median AUCI values were 5,368 μU/ml at 2 h between weeks 14 and 20 for the control group, which is significantly higher than that observed for the triple treatment group ($P = 0.008$). Values of 10,920 μU/ml at 2 h were obtained between weeks 33 and 38, which was significantly different from the ZDV and triple treatment groups ($P = 0.0009$).

In the present series, median gestational age at delivery was 39 weeks for the control group, 38.1 weeks for the ZDV group, and 38.5 weeks for the triple treatment group (Kruskal-Wallis test, $P = 0.57$). Median weight was 3,250 g for the control group, 3,080 g for the ZDV group, and 3,100 g for the triple treatment group (Kruskal-Wallis test, $P = 0.447$). Analysis of the Apgar score and of adequacy of anthropometric classification did not show any significant differences among the newborns of the various groups (χ^2 test, $P = 0.59$). Cesarean section was performed in 16.7, 45.0, and 36.0% of women in the control, ZDV, and triple treatment groups, respectively (χ^2 test, $P = 0.26$).

Median glucose level in the triple treatment group was 78 mg/dl; no difference was observed among the newborns for the control and ZDV groups (Kruskal-Wallis test, $P = 0.38$). The values of neonatal insulin and glucose levels are shown in Table 2. It can be seen that neonates in the triple treatment group had umbilical cord blood insulin levels of 2.9 μU/ml, which were significantly lower than those in neonates in the control group ($P = 0.047$).

There were no cases of vertical HIV-1 transmission.

CONCLUSIONS — A higher frequency of association between diabetes and use of antiretroviral agents has been reported over the last decade for HIV-1–infected adults. The spectrum of abnor-

Table 2—*Insulin and glucose in umbilical cord blood from the newborn at the time of delivery*

		Insulin (μU/ml)		Glucose (mg/dl)	
Group	*n*	Median (range)	*P*	Median (range)	*P*
Control	12	6.5 (3.0–9.5)	—	73.5 (69.0–81.0)	—
ZDV	18	4.8 (0.8–9.0)	0.35	77.0 (69.5–83.7)	0.54
TT	22	2.9 (1.8–4.8)	0.047*	78.0 (71.7–85.0)	0.18

Data are 1st and 3rd quartiles. *Control group × triple treatment (TT) group: $P < 0.05$ (Mann-Whitney *U* test).

mality of glucose metabolism has been demonstrated to involve parameters such as insulin resistance, hyperglycemia, exacerbation of diabetes, and diabetic ketoacidosis, although wide variations have been noted among the results reported (1,2,5).

One of the positive points of the present study is the homogeneous sampling of the patients, particularly in light of the many risks for development of gestational diabetes. The most important of these risks are personal history of gestational diabetes, history of first-degree relatives with diabetes, presence of a smoking habit, and nonwhite ethnicity (6,7).

The AUCI was persistently significantly lower in pregnant women taking antiretroviral drugs, especially in those taking combined schemes, compared with control women, even before the introduction of their use during pregnancy. These results are consistent with an effect primarily related to HIV infection itself. This hypothesis is supported by reports of pancreatic vulnerability to opportunistic infections, cancer, drug toxicity, and possibly direct viral toxicity in patients with AIDS. Some studies have demonstrated that HIV-1 infection, regardless of use of any antiretroviral agent, is associated with pancreatic involvement in ~50% of patients, as detected at autopsy (8–10).

Little is known about the effects of antiretroviral schemes including protease inhibitors on the fetus. This fact is particularly evident when we consider the reduced number of studies analyzing the blood biochemistry of newborn infants during the immediate postnatal period (3). No studies are currently available regarding the effect of the use of antiretroviral drugs during pregnancy and the potential risk of their interference on insulin levels in newborns of HIV-1–infected pregnant women taking at least two different combinations of antiretroviral drugs. In this respect, the series reported herein can be considered to be original.

Regardless of the maternal results of the AUCI, which indicate a possible effect of HIV-1 infection, the differences in the results of these analyses for the AUCI values among the groups studied in the present series became more marked over the time of use of antiretroviral drugs, demonstrating a possible time-dependent effect. Experimental studies have shown that most antiretroviral agents reduce glucose tolerance to a greater or lesser extent, either because of compromised secretion and action of insulin or even because of the direct toxic effect of these agents on pancreatic β-cells (11). Protease inhibitors have elicited greater interest because of the larger number of cases of glucose intolerance and even of overt diabetes associated with, or triggered by, their use (12).

Biochemical analysis of umbilical cord blood at birth of infants exposed to these agents demonstrated a damaging effect, although at a subclinical level, of the antiretroviral drugs taken by the mother during pregnancy on the endocrine portion of the fetal pancreas. No difference in umbilical cord blood glucose levels was detected at birth among the neonates of the three groups. Pharmacokinetic studies have definitely demonstrated that the placental barrier is permeable to these agents; fetal-maternal ratios were 0.85 for ZDV, close to 1.0 for lamivudine, and minimal for nelfinavir (12). An experimental study (11) has already shown a reduction in maternal insulinemia generally observed with the use of antiretroviral drugs, regardless of the use of ZDV, lamivudine, and nelfinavir separately or in combination. In addition, in the present series, there was no case of perinatal transmission, perhaps owing to the care taken regarding compliance with the prophylactic measures or perhaps owing to the limited number of cases for this type of evaluation.

Several hypotheses have been considered as possible mechanisms triggering the adverse metabolic effect of antiretroviral drugs. As observed in our study, the combined use of antiretroviral drugs showed reduction of neonatal insulin in uninfected newborn infants exposed to combined schemes of antiretroviral drugs in utero. These data may indicate an early damaging effect on fetal pancreas that is supported by reports of pancreatic vulnerability to drug toxicity as a consequence of insulin hyposecretion, in agreement with studies showing that protease inhibitors cause insulin deficiency similar to that observed in type 1 diabetes, with the possible contribution of events such as inhibition of proinsulin conversion to insulin due to the activity of a protease and to other factors such as reduced insulin secretion and increased insulin clearance. Cumulative damaging effects on pancreatic β-cells may culminate in the death of these cells, with consequent insulinopenia followed by clinical diabetes. Therefore, it is reasonable to assume that these findings eventually detected in humans may indicate a still early diabetogenic effect secondary to the use of antiretroviral drugs during the limited period of pregnancy. However, few conclusive studies are available about the physiopathology of the changes induced by these drugs from the viewpoint of glucose intolerance (5,11,13,14).

We conclude that combined schemes of antiretroviral drugs during pregnancy result in the reduced serum insulin levels in newborn infants exposed to the drugs in utero but who are not infected. These data may indicate that there is an early damaging effect of the antiretroviral drugs on fetal pancreatic function.

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