

Extended administration of the association of zidovudine plus ritonavir during rat pregnancy: maternal and fetal effects

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Summary

The purpose of the study was to evaluate at term, the effects of the association of zidovudine/ritonavir administered during the entire period of rat pregnancy. Forty pregnant EPM-1 Wistar rats were divided randomly into four groups: one control (drug vehicle control, n = 10) and three experimental treated with an oral solution of zidovudine/ritonavir (Exp 1 = 10/20 mg/kg bw, n = 10; Exp 2 = 30/60 mg/kg bw, n = 10; Exp 3 = 90/180 mg/kg bw, n = 10) from day 0 up to day 20 of pregnancy. Maternal body weights were recorded at the start of the experiment and at the 7th, 14th and the 20th day thereafter. At term (20th day) the rats were anesthetized and, upon laparotomy and hysterotomy, the number of implantations, resorptions, living fetuses, placentae and intrauterine deaths were recorded. The collected fetuses and placentae were weighed, and the concepts were examined under a stereoscopic microscope for external malformations. The maternal body gain and the mean fetal weight at term were both significantly lower ($p < 0.01$ and $p < 0.0001$, respectively) in the experimental groups compared to the control. The recorded resorptions were higher in Exp 2 and Exp 3 groups than in the control group. The other parameters were not affected. The exposure of pregnant rats at term to a 1:2 association of zidovudine plus ritonavir resulted in a significant reduction in maternal body weight gain and increased rate of fetal resorption.

Key words: Ritonavir; Zidovudine; Rat; Pregnancy.

Introduction

Due to its high morbidity and lethality as well as diffused worldwide dissemination, the acquired immunodeficiency syndrome (AIDS) has constituted one of the most severe public health problems for more than two decades, and currently is among the highest epidemiologically and socioeconomically impacting infections.

In the last ten years, the introduction of 'highly active antiretroviral therapy' (HAART) has brought about some blunting of that impact by diminishing vertical transmission, controlling opportunistic infections and lowering mortality rates [1-3]. The three-drug combination therapy, which includes the use of protease inhibitors (PI), has caused an important and sustained suppression of human immunodeficiency virus (HIV) replication and increased survival rates among seropositive patients [4, 5].

Notwithstanding, gestation in HIV-infected patients is still an issue of great medical concern, not only for vertical transmission of the virus but also for the possible adverse maternal-fetal effects of the antiretroviral therapy.

Without antiretroviral therapy, the rates of HIV vertical transmission are about 20%, and during breastfeeding the risk may rise to about 45%; this kind of transmission cor-

responds to about 86% of the causes of infant contamination by HIV [3, 6]. In developed countries, the adoption of an aggressive combination of antiretroviral regimens during gestation has contributed significantly to the reduction of the incidence of cases of AIDS among infants, by reducing the chances for vertical transmission to as low as 2% or even lower [3, 7].

With varying figures around the world, 25% up to 57% of the total population infected with HIV are women in reproductive age, many of which are on antiretroviral therapy [8]. Some of the antiretroviral drugs are formally contraindicated in this period. However, no well controlled studies exist about the effects of antiretroviral drugs in several association protocols on the pregnancy of experimental animals or on human gestation [9].

Accordingly, in this paper we examined the combined action of two antiretroviral drugs, zidovudine and ritonavir, in an extended administration protocol on albino rat pregnancy.

Materials and Methods

Wistar female rats (*Rattus norvegicus albinus*) of the EPM-1 variant, with approximately 200 g body weight provided by the Center for the Development of Experimental Models (CEDEME) of the Federal University of São Paulo - Escola Paulista de Medicina (UNIFESP-EPM) were used throughout the study. Experi-

ments were approved (Report No. 1397/04) by the local Animal Care Committee, following guidelines which comply with those of the Canadian Council on Animal Care [10].

The animals were kept in plastic cages under controlled room temperature set at 22°C and artificial light by fluorescent lamps with a photoperiod of 12 h (lights on at 7 am), with free access to pelleted Purina® rat diet and tap water.

After a 7-day period of adaptation, the animals were mated in the proportion of one male to three females for two hours. The immediate 24-h period after mating was taken as day 0 of pregnancy if spermatozooids were detected in vaginal smears [11]. Forty pregnant rats were then distributed at random into four animal groups as follows: control ($n = 10$) were rats treated daily with 0.5 ml of propyleneglycol by the oral route (drug vehicle and stress controls); Exp 1 ($n = 10$) were rats treated with a combination of oral zidovudine/ritonavir (zidovudine, GlaxoSmithKline Laboratories, London, plus ritonavir, Abbott Laboratories, Chicago, IL) corresponding to a daily dose of 10 mg/kg zidovudine plus 2 mg/kg of ritonavir; Exp 2 ($n = 10$) were similarly scheduled rats treated daily with 30 and 60 mg/kg of zidovudine and ritonavir, respectively; and finally, Exp 3 ($n = 10$) rats were treated with 90 and 180 mg/kg of zidovudine and ritonavir, respectively. Vehicle and drugs for Exp 2/3 groups were administered by gavage, once daily, in a final volume of 0.5 ml starting on day '0' and extending until the term of pregnancy.

Body weights were recorded at days 0, 7, 14 and 20 of pregnancy and expressed as percentages of body weight gain.

At term (20th day), the animals were weighed and anesthetized with a mixture of xylazine (20 mg/kg) and ketamine (100 mg/kg) by the intraperitoneal route. Upon wide open laparotomy and hysterotomy, the following parameters were recorded: fetal and placental weights, number of implantations, number of resorptions, and number of living and dead fetuses. The fetuses were closely examined under a stereoscope microscope for gross external malformations (limb shortening, bifid spine, cleft lip, cleft palate or hypospadias).

Whenever appropriate the data are expressed as mean \pm SEM; the results were subjected to ANOVA and further analyzed by the Kruskal-Wallis multiple comparisons test. Contingency tables and chi-square tests were used to analyze the death rates. The significance level was set at 0.05%.

Results and Discussion

The profiles of body weight gain during pregnancy for the groups studied are shown in Table 1 and Figure 1.

From the beginning up to the 14th of pregnancy all the rats in the drug-treated groups (Exp 1, Exp 2 and Exp 3) had body weight gains slightly higher than the control rats, but this was significant ($p < 0.05$) only for the Exp 1 animals at the 14th day. It is conceivable that this is related to the maternal hyperlipidemia, hyperglycemia and lipodystrophy caused by ritonavir at a time when fetal and placental weights have little involvement in the total maternal weight gain [12–16]. There was a reduction in the rate of body weight gain from the 14th day of gestation in all the experimental groups, thus causing significantly less body weight gain ($p < 0.01$) with regard to the group control at term (Figure 1). This result might be related to the fact that in this gestational period (14th–21st day), the so-called 'fetal period', the maternal rat body is significantly

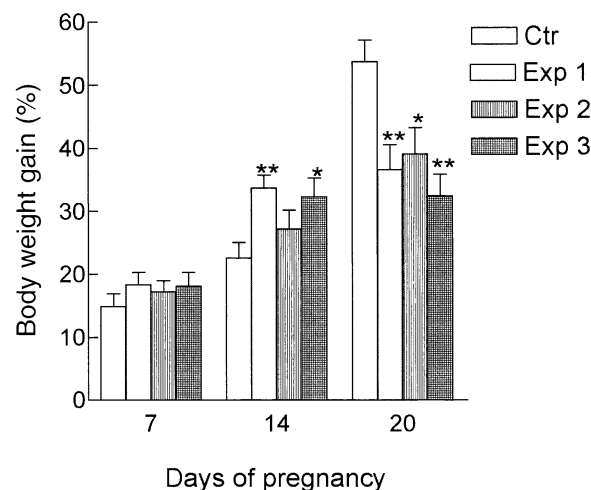


Figure 1. — Profiles of body weight gain of pregnant rats expressed as % of the starting weight (at day zero of pregnancy). The rats ($n = 10$ throughout) were treated as follows: Control (Ctr) = drug vehicle (propyleneglycol); experimental animals were treated once a day with zidovudine/ritonavir by gavage during the entire period of gestation: Exp 1 = 10/20 mg/kg, Exp 2 = 30/60 mg/kg, and Exp 3 = 90/180 mg/kg bw respectively. Within every gestational day, the significant differences regarding the corresponding Ctr values are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$.

influenced by the weight of the concepts and their placental compartment. Since in the experimental groups there were significant reductions of placental and fetal weights at term (Table 1), the final maternal weights of Exp 1, Exp 2 and Exp 3 rats were significantly lower than those of the control group.

When even high doses of zidovudine (60 or 100 mg/kg per day) were administered in a single regimen to similarly scheduled pregnant rats no alterations in body weight gain were observed [17, 18]. Also, the administration of ritonavir to pregnant rats was devoid of deleterious effects on body weight gain, except in doses 9-fold over the human therapeutic range; this was related to dose-dependent gastrointestinal adverse effects and time of exposure to ritonavir [19].

The number of implantations was similar in the four groups studied, but the number of resorptions (about 18%) was significantly higher in the Exp 2 and Exp 3 groups ($p < 0.01$) with regard to the control group (Table 1). This is relevant since in Wistar rats at the 20th day of pregnancy the normal resorption index is expected to be below 5% [17, 19]. This finding is in accordance with observations by Carvalho *et al.* [19] that ritonavir caused a significant reduction of implantations in their animals. In addition, the resorption index was about 36% in the group treated with 180 mg/kg ritonavir per day (9-fold higher than the human therapeutic dose) with regard to controls. Figueiró *et al.* [18] observed that 100 mg/day zidovudine (10-fold higher than the human dose) increased the rate of fetal resorption (about 27%). On the

Table 1. – Effects of extended administration of the association zidovudine/ritonavir on several indicators of rat pregnancy. Groups are as in the legend to Figure 1. Data are expressed as mean \pm SEM. No external fetal malformations were observed.

	GROUPS			
	Ctrl (n = 10)	Exp 1 (n = 10)	Exp 2 (n = 10)	Exp 3 (n = 10)
No. of fetuses	11.20 \pm 0.87	10.40 \pm 0.78	9.90 \pm 0.94	10.30 \pm 0.88
No. of placentae	11.20 \pm 0.87	10.40 \pm 0.78	9.90 \pm 0.94	10.30 \pm 0.88
Fetal weight (g)	3.85 \pm 0.07	2.44 \pm 0.06*	2.36 \pm 0.05*	3.13 \pm 0.14*
Placental weight (g)	0.65 \pm 0.02	0.56 \pm 0.02**	0.54 \pm 0.01**	0.73 \pm 0.04
No. of implantations	11.20 \pm 0.87	11.40 \pm 0.65	12.1 \pm 0.57	12.4 \pm 0.48
No. of reabsorptions	0	1.0 \pm 0.68	2.2 \pm 0.62***	2.1 \pm 0.59***
Maternal deaths	0	0	0	0
Fetal deaths	0	0	0	0

* $p \leq 0.01$ with regard to controls (Ctrl); ** $p \leq 0.05$ with regard to the Ctrl and Exp 3 groups; *** $p \leq 0.01$ with regard to Ctrl.

other hand, Mamede *et al.* [17] found that the treatment of pregnant rats with lower doses of zidovudine (6-fold higher than the human dose) did not increase the fetal resorption rate.

Overall, these data show that zidovudine and ritonavir administered to pregnant rats isolated or in combination protocols cause a rise in fetal resorption rates; the effects are dose-dependent and seem to be more intense when the drugs are used isolatedly. The effect on resorption may be more importantly related to the toxicity of ritonavir [19] and to rat placental development with regard to the blockade of xenobiotics which travel into the embryonic circulation [20-24].

We observed that the mean number of living fetuses was equal to that of the placentae in all litters of the four groups studied. However, the mean fetal weights of all the experimental groups were significantly lower ($p < 0.001$) than that of the control group. In contrast, the mean placental weight was significantly lower than the controls ($p < 0.001$) only for the Exp 1 and Exp 2 groups (Table 1). This effect on fetal growth was not observed by Carvalho *et al.* [19] after treatment of pregnant rats with similar doses of ritonavir alone, nor was it observed in experimental or observational human studies with zidovudine alone [16, 25-27]. These facts prompted us to infer that some kind of a hitherto poorly understood drug interaction involving zidovudine and ritonavir may influence fetal rat development.

Though ritonavir is a low-molecular-weight drug (720 D), its transplacental passage is extremely reduced, probably due to the effective transfer blockade exerted by the P-glycoprotein or multidrug resistance proteins which are highly expressed in placental tissue [23-25, 28, 29]. The function of such proteins most conceivably explains the absence of any ritonavir effect on the intrauterine contents – implantations, resorptions, weight and neonatal mortality – observed by Carvalho *et al.* [19] with ritonavir alone administered over the entire period of rat pregnancy.

Overall, we could put forward the hypothesis that either zidovudine might have inhibited to some degree the selective action of placental P-glycoprotein, thereby enhancing the passage of ritonavir, or the combination of the drugs might have interfered with the mechanisms of

transport of aminoacids and ions needed for adequate fetal growth [30].

It was noticeable that the deleterious effects of the zidovudine-ritonavir combination used here on the rat concepts coexisted with an absence of fetal malformations or maternal or fetal deaths (Table 1). This lack of toxicity was also observed in similar experiments using these drugs alone during the entire rat pregnancy [17, 19, 31]. Notwithstanding, previous data from our own studies [19] indicated that ritonavir induced about 40% of maternal mortality when used in doses 9-fold higher than the human therapeutic dose, mostly due to hepatorenal toxicity.

In the present study, the absence of maternal deaths may be explained by the putative interference of zidovudine on the oral bioavailability of ritonavir, or by some activation of the liver CYP3A enzyme system, and/or by the stimulation of the activity of the intestinal P-glycoprotein, thereby reducing the circulating levels of ritonavir and consequently reducing its toxic effects [19, 32-34]. This explanation is a sound one, since proteins such as P-glycoprotein and multidrug resistance-associated proteins located in various sites besides the placenta (namely, in the blood-brain barrier, genital apparatus, gut, lymphocytes, kidneys and biliary canaliculi) and consequently can interfere with the mechanisms of absorption, distribution and elimination of those drugs [23].

Taking into account that the current therapeutic approaches with antiretrovirals for seropositive female patients involves more than two drugs, the investigation of potential drug interactions is warranted. The scarcity of human data on this issue can be initially overcome by kinetic drug experiments in pregnant animals.

Conclusion

The zidovudine-ritonavir combination used throughout rat pregnancy may reduce maternal body weight gain without maternal or fetal deaths, or impairment of the implantation rates. No gross fetal malformations were observed in any group. Complex mutual kinetic drug interference may have been involved which explains, at least in part, these results.

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