Administration of lopinavir/ritonavir association during rat pregnancy: maternal and fetal effects

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Summary

Purpose: To evaluate the effects of the association of lopinavir and ritonavir administered during the whole period of rat pregnancy. *Methods:* 62 Wistar rats of the EPM-1 variant weighing about 200 g were randomly divided into five groups: two controls (Ctr1 = stress control, n = 10; and Ctr2 = drug vehicle control, n = 10) and three experimental ones which were treated with an oral solution of lopinavir/ritonavir (Exp1 = 12.8/3.2 mg/kg b.w., n = 14; Exp2 = 38.4/9.6 mg/kg b.w., n = 14; Exp3 = 115.2/28.8 mg/kg b.w., n = 14) from 'day 0' up to the 20th day of pregnancy. Maternal body weight was recorded at the start of the experiment and on the 7th, 14th and 20th day thereafter. At term (20th day), upon laparotomy and hysterotomy, the rats were anesthetized and the amount of implantations, reabsorptions, living fetuses, placentae and intrauterine deaths were recorded. The collected fetuses and placentae were weighed and the concepts were examined under a stereoscope microscope for external malformations. *Results:* An apparent dose-unrelated lethal effect of the antiviral association on the pregnant rats was observed; notwithstanding, the body weight gain of the surviving rats had no changes, independent of the considered group. It was noted that the quantitative and qualitative intrauterine content of living term rats was indistinguishable from that of the controls. *Conclusion:* There was some degree of deleterious effects of the administration of the lopinavir/ritonavir association on pregnant rats; such effects eventually led to maternal death. However, neither the surviving rats showed toxicity nor did their concepts present any detectable change which could be related to the drug association.

Key words: Rat; Pregnancy; Lopinavir; Ritonavir.

Introduction

After its initial description in two small groups of previously healthy homosexual men [1, 2], the acquired immunodefficiency syndrome, so-called AIDS [3], evolved to constitute a pandemic.

Currently, in addition to the 20 million deaths registered till now, it is estimated that more than 40 million people worldwide are infected by the human immunodeficiency virus (HIV). The incidence distribution among men and women [4], mainly during the reproductive years is similar. It is thought that infected women are responsible for 20% of intrauterine transmissions [5, 6].

The most effective approach to the prophylaxis of such a vertical transmission is reduction of the viral load of pregnant women down to as low as 1,000 viral particle copies per milliliter [7].

The use of zidovudine during pregnancy and delivery has reduced HIV infection in one-third of the concepts [5]. Accordingly, the US Public Health Service has recommended that HIV-infected women continue with the same treatment protocol during gestation adopted in the pre-conception period [8].

Among the several treatment protocols, the highly active antiretroviral therapy (HAART) usually includes one nucleoside analog (DNA chain terminator), one protease inhibitor and either a second nucleoside analog ("nuke") or a non-nucleoside reverse transcription inhibitor [9]. Though the association of antiretroviral drugs has been shown to be largely safe for the concept [10], it is supposed that association of multiple drugs may introduce changes in their pharmacokinetics [11] and bring about unpredictable results for maternal and/or fetal compartments.

It is well established that HIV antiretroviral drugs, particularly protease inhibitors (e.g., ritonavir and lopinavir) frequently elicit a metabolic syndrome that may include hyperlipidemia, lipodystrophy and insulin resistance. Cao *et al*. [12] in studies *in vitro* have demonstrated that most protease inhibitors not only induce the accumulation of intracellular free cholesterol and lipids, activating the urokinase-type plasminogen activator (UPR) in hepatocytes and macrophages but also increase the release of inflammatory cytokines promoting foam cell formation in macrophages [13, 14].

Pistell *et al*. [15] showed that lopinavir/ritonavir administration in mice caused significant metabolic derangement, including changes in body weight and fat mass as well as dose-dependent patterns of hyperlipidemia, hypoadiponectinemia, hypoleptinemia, and hyperinsulinemia.

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Fig. 1a

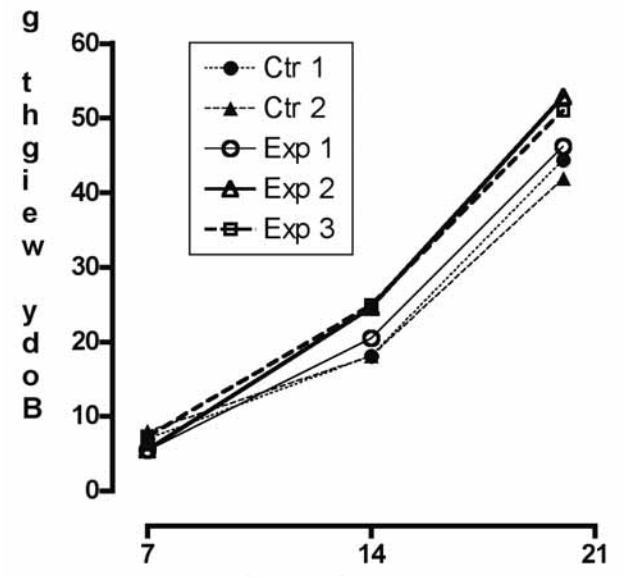

Figure 1. — Percentual profiles of body weight gain among pregnant rats as a function of the starting weight (at the zero day of pregnancy). Groups comprised: Ctr 1 = intact, stress control, n = 10; Ctr 2 = drug vehicle (propyleneglycol) control, n = 10; Exp 1, 2 and 3 = treated with lopinavir/ritonavir association, 12.8/3.2 mg/kg (n = 14), 38.4/9.6 mg/kg (n = 14) and 115.2/28.8 mg/kg b.w. (n = 14) respectively, from day 0 up to the 20th day of pregnancy. No significant differences were observed.

Since preliminary results in our laboratory [16] have indicated some degree of toxicity of the lopinavir/ritonavir association on the rat pregnancy, this point has been specifically studied in this paper.

Materials and Methods

Wistar female rats *(Rattus norvegicus albinus)* of the EPM-1 variant, provided by the Center for the Development of Experimental Models (CEDEME) of Sao Paulo Federal University – School of Medicine (UNIFESP-EPM), weighing approximately 200 g, were used throughout the experiment. The experiment was approved by the local Animal Care Committee (Report no. 1397/04), in accordance with the guidelines which comply with those of the Canadian Council on Animal Care [17].

The animals were held in plastic cages under controlled room temperature (22ºC) and artificial light by fluorescent lamps with a photoperiod of 12 h (lights on at 7 a.m.) 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-hour period after mating was taken as day 0 of pregnancy if spermatozoids were detected in vaginal smears [18]. Sixty-two pregnant rats were randomly divided into five animal groups, as follows: Ctr 1 (n = 10) was represented by rats which received no drugs, that is, the stress control; Ctr 2 (n = 10) was a group daily treated with 0.5 ml of propyleneglycol by oral route (drug vehicle control); Exp 1 (n = 14) was a group treated with the association of lopinavir/ritonavir (Kaletra, Abbott Laboratories, IL, USA) by oral route corresponding to a daily dose of 12.8 mg/kg lopinavir/3.2 mg/kg of ritonavir; Exp 2 (n = 14) was a group treated daily with 34.4 and 9.6 mg/kg of lopinavir and ritonavir, respectively; Exp 3 (n = 14) was a group treated daily with 115.2 and 28.8 mg/kg of lopinavir and ritonavir, respectively. Vehicle and drugs were administered by gavage, once daily, in a final volume of 0.5 ml, starting at day '0' and extending until the term of pregnancy.

Table 1. — *Effects of the daily administration of the association lopinavir/ritonavir on several indicators of the pregnant rats for the whole period of pregnancy. Dispersion of data is given as SEM. No fetal external malformations were observed.*

	Group[b]				
	Ctr 1 (n = 10)	Ctr 2 (n = 10)	Exp 1 (n = 14)	Exp 2 (n = 14)	Exp 3
No. of fetuses	10.0 ± 1.8	10.3 ± 2.3	9.3 ± 4.6	10.3 ± 2.0	11.0 ± 1.9
No. of placentae	10.0 ± 1.8	10.3 ± 2.3	9.3 ± 4.6	10.3 ± 2.0	11.0 ± 1.9
Fetal weight (g)	4.13 ± 0.34	4.11± 0.25	4.05 ± 0.42	4.04 ± 0.58	4.17 ± 0.24
Placental weight (g)	0.64± 0.05	0.63 ± 0.05	0.73 ± 0.14	0.71 ± 0.09	0.71 ± 0.08
No. of reabsorptions	0.30 ± 0.67	0	0.30 ± 0.95	0	0.17 ± 0.41
Maternal deaths	0	0	4*	8*	8*

*$p < 0.05$ with regard to the controls.

Body weights were recorded for all animals on day 0, and the 7th, 14th and 20th day of pregnancy and expressed as percentuals 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, reabsorptions, living and dead fetuses. The fetuses were closely examined under a stereoscope microscope for gross external malformations (limb shortening, spina bifida, cleft lip, cleft palate and hypospadia).

Whenever appropriate the data were expressed as mean ± SEM. The results were submitted to ANOVA analysis and analyzed further by the Kruskal-Wallis' multiple comparison test. Contingency tables and chi-square tests were used to analyze the death rates; the significance level was set at 5%.

Results and Discussion

At advanced stages of HIV disease, patients used to show constitutional symptoms and severe cachexia. However, upon the development of HAART protocols [19], metabolic abnormalities (hyperlipidemia and insulin resistance) and morphological changes (central fat accumulation and peripheral fat atrophy) have been reported in HIV-1 patients mostly among those receiving therapies containing HIV-1 protease inhibitors, particularly ritonavir [20]. The so-called lipohypertrophic syndrome [21] includes clinical features as increased abdominal girth [22], fat accumulation, breast hypertrophy and buffalo hump [23].

In our study, pregnant rats treated with lopinavir/ritonavir showed no significant alteration in body weight gain, nor did the controls or experimental groups (Exp 1-3) (Table 1). This finding was presumably due to the fact that rats lack some of the multiple factors involved in the pathogenetic mechanisms proposed for humans, which

include the interference of several regulatory proteins such as sterol regulatory enhancer binding protein-1, the proteasome, mitochondrial DNA polymerase gamma and GLUT-4 [24]. Otherwise, the short time of exposition to the drugs (3 weeks) could have played some role in this result [25].

We observed a lethal effect of the drug treatment, that is, 4/14 deaths were recorded in the Exp 1 group (treated with a dose of lopinavir/ritonavir proportionally similar to that used in humans), and a two-fold higher figure was observed in the Exp 1 and 2 groups (treated with 3- and 9-fold higher doses of the drugs) (Table 1).

This finding addresses two relevant issues. First, no clear-cut dose-dependent effect can be inferred for this result. On the other hand, it may instead be related to the individual sensitivity of the animals to the drugs. Such individual variation could be linked to the functional levels of P glycoprotein (P-gp) [26], a membrane efflux pump pertaining to the superfamily of ATP binding cassette proteins [27]. As is known, this protein is encoded by the 'multidrug resistence' genes Mdr 1a and Mdr 1b [28] and expressed in tissues involved with drug absorption, metabolism and excretion [29]. P-gp limits penetration of potentially harmful or therapeutic hydrophobic compounds, thus providing protection of an organism against potentially toxic compounds of the environment. Accordingly, a placental P-gp may play an important role in the protection of the developing fetus [30]. Other barriers in which P-gp is importantly involved are the blood-brain, the blood-nerve, the blood-testis and the intestinal barrier [31]. Predictably, the absence of P-gp may lead to adverse effects when an organism is exposed to drugs such as antineoplastic agents, cardiac glycosides, beta-blockers, calcium channel blockers and HIV-protease inhibitors [32].

It is conceivable that the maternal deaths observed herein were due to some degree of individual variation of the intestinal content of P-gp, leading to defective drug protection [30]. Such 'drug inward leakage' at the intestinal, absorptive level, could have resulted in blockade of the metabolic degradation of lopinavir as a consequence of CYP3A isoenzyme inhibition caused by ritonavir [33-35]. This drug accumulation could be accounted for by the maternal deaths observed in our groups Exp 2 and 3.

Our results are supported in part by previous data obtained in our laboratory with ritonavir alone [36]. This drug was administered (60 and 180 mg/kg b.w.) during the entire period of rat pregnancy and caused 2/10 and 4/10 deaths, respectively. In the present paper, much lower ritonavir doses in association with lopinavir were able to cause a higher lethal effect, most presumably due to the association-induced, altered drug pharmacokinetics [11].

Regarding the fetal compartment, we may infer that the placental expression of P-gp [32] was sufficient as to properly hinder the drug transfer to the uterine space. In fact, no alterations were observed regarding the numbers of implantations and fetuses, and the fetal and placental weights between the control and the treated groups of animals. Similarly, no reabsorptions, intrauterine deaths or fetal malformations were observed.

To conclude, though maternal deaths have been recorded in the animal groups treated with the two highest doses of lopinavir/ritonavir, no definite dose-related effects could be established. On the other hand, the fetuses from surviving rats showed no detectable alterations, suggesting the efficacious operation of protective drug systems at the placental level.

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