Effects of the association zidovudine plus ritonavir on the liver and kidneys of pregnant rats. Morphological and biochemical aspects

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Summary

Purpose: To evaluate biochemical and morphological effects on rats submitted to three different doses of the association zidovudine and ritonavir administered throughout pregnancy. *Methods:* Forty pregnant EPM-1 Wistar rats weighing about 200 g were randomly divided into the control group (Ctr = drug vehicle control, n = 10) and three experimental ones which were treated with an oral solution of zidovudine/ritonavir (Exp1 = 10/20 mg/kg bw, n = 10; Exp2 = 30/60 mg/kg bw, n = 10; Exp3 = 90/180 mg/kg bw, n = 10) from 'day 0' up to the 20th day of pregnancy. At term (20th day) the rats were anesthetized. Blood and fetal and maternal organ samples (livers and kidneys) were taken for morphological and biochemical analyses. *Results:* Upon histological examinations fetal livers and kidneys appeared normal. In contrast the maternal samples revealed structural alterations. Maternal kidneys of the three experimental groups exhibited progressive and dose-dependent histological alterations; liver alterations were detected only in Exp3. Blood levels of AST and ALT were not significantly different from the control group but urea and creatinine levels were lower in groups Exp3 and Exp1. Conclusions: The administration of zidovudine plus ritonavir throughout rat pregnancy can cause morphological as well as functional changes in maternal kidneys.

Key words: Ritonavir; Zidovudine; Rat; Pregnancy.

Introduction

Human acquired immunodeficiency syndrome (AIDS) outbreak began around three decades ago and disseminated worldwide with increasing mortality rates, representing one of the most frightening infections of the two last centuries. In the last ten years, the introduction of highly active antiretroviral therapy (HAART) attenuated those mortality indices, controlled the opportunistic infections and increased survival rates [1-4].

By comprising a 3-drug combination, including a protease inhibitor component, HAART promoted an important and sustained suppression of viral replication, rising survival of serum-positive patients and thus rising AIDS prevalence [5, 6].

Depending on the world region, 25-57% of human immunodeficiency virus (HIV)-infected people are women in fertile age, many of them under antiretroviral therapy. Pregnancy in such groups is of high medical concern, not only due to the possibility of virus vertical transmission but also regarding the adverse maternal and fetal effects of antiretroviral drugs [7].

In developed countries the wide implementation of combined antiretroviral drugs during pregnancy not only significantly contributed to reduction of the incidence of AIDS cases among children, but also remarkably reduced the HIV vertical transmission indices from as high as 20-45% to less than 2% [4, 8, 9]. Notwithstanding, the effects of the various antiretroviral drugs in association still need to be thoroughly investigated in animal models of pregnancy, aiming to a better understanding of their pharmacokinetic and toxicological profiles in human gestation [10].

The present experiments were carried out taking into consideration that pregnancy modifies the pharmacodinamics of antiretroviral drug combinations [11]. Being so, we used zidovudine concomitantly with ritonavir during the entire rat pregnancy period and examined the main metabolic and elimination organs, namely both the maternal and fetal livers and the kidneys.

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 experiment. The experiment was approved (Report no. 1397/04) by the local Animal Care Committee, following guidelines which comply with those of the Canadian Council on Animal Care [12].

The animals were held 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 a.m), with free access to pelleted Purina® rat diet and tap water.

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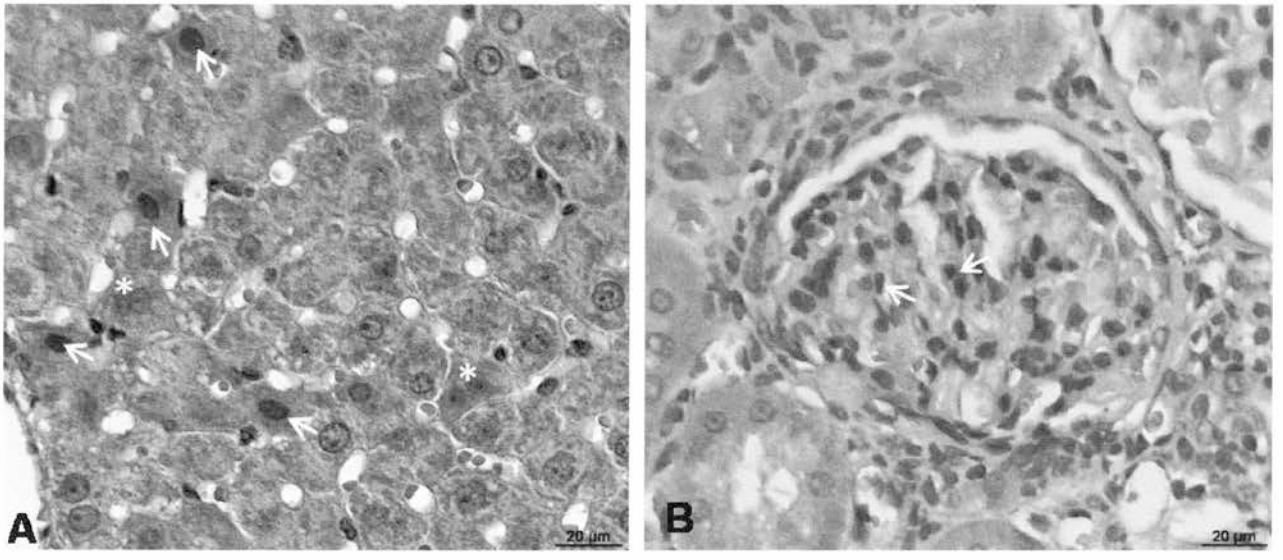

Figure 1. — Photomicrographs of a typical histological sections of a maternal liver (A) and maternal kidney (B) from the group treated with zidovudine-ritonavir combined (90 mg and 180 mg/kg, per day during the entire pregnancy) (Exp3). Observe hepatocytes with nuclei pyknosis (*) and binucleation (arrows). In the kidney glomeruli with pyknosis nuclei can be observed (arrows); H.E. staining.

After a 7-day period of adaptation, the animals were mated in the proportion of one male to three females for 2 h. The immediate 24-h period after mating was taken as day 0 of pregnancy if spermatozoids were detected in vaginal smears [13]. Forty pregnant rats were then distributed at random into four animal groups, as follows: Ctr (n = 10) was treated daily with 0.5 ml of propyleneglycol by the oral route (drug vehicle and stress controls); Exp1 (n = 10) was treated with a combination of oral zidovudine/ritonavir (zidovudine, GlaxoSmithKline Labora-tories, London, plus ritonavir, Abbott Laboratories, North Chicago, Illinois) corresponding to a daily dose of 10 mg/kg zidovudine plus 20 mg/kg of ritonavir. Exp2 (n = 10) was treated daily with 30 and 60 mg/kg of zidovudine and ritonavir, respectively. Finally, Exp3 (n = 10 was treated with 90 and 180 mg/kg of zidovudine and ritonavir, respectively. Vehicle and drugs were administered by gavage, once daily, in a final volume of 0.5 ml. The treatment was started on 'day 0' of pregnancy and extended until term (20th day), when the rats were anesthetized with a mixture of xylazine (2 mg/kg) and ketamine (100 mg/kg) by the intraperitoneal route. Upon laparotomy, 4 ml of maternal blood was taken directly from the ventricular chambers for further biochemical determinations: aspartate- (AST) and alanine- (ALT) aminotransferases [14], blood urea nitrogen (BUN) [15] and creatinine [16]. Maternal and fetal samples of livers and kidneys were taken and fixed in buffered 10% formaldehyde for further routine processing, hematoxylin-eosin staining and light microscopy study. Two different investigators blindly analyzed the specimens and the results were compared.

Statistical analysis

Results were analyzed by one-way analysis of variance (ANOVA) and the Tukey-Kramer's multiple comparison test. ASSP for Windows 13 software was used for this purpose. The differences were considered statistically significant when $p < 0.05$.

Table 1. — *Effects of treatment with a zidovudina/ritonavir combination during the entire period of rat gestation on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and on the levels of blood urea nitrogen (BUN) and creatinine in maternal blood at term.*

Groups	AST (mU/ml)	ALT (mU/ml)	BUN (mg/100 ml)	Creatinine (mg/100 ml)
Ctr (n =10)	118.0 ± 11.7	74.2 ± 3.7	65.5 ± 2.0	0.51 ± 0.01
Exp1 (n =10)	137.3 ± 6.5	74.2 ± 3.7	67.0 ± 2.0	0.41 ± 0.02*
Exp2 (n =10)	139.6 ± 11.2	67.6 ± 3.6	57.4 ± 2.7*	0.48 ± 0.02
Exp3 (n =10)	147.7 ± 25.1	75.3 ± 4.6	43.6 ± 2.3**	0.48 ± 0.02

Values are mean ± S.E.M. of determinations in duplicate. Groups of pregnant rats (n = number of animals at term) were treated once daily during the entire gestation with zidovudine+ritonavir by the oral route (gavage), as follows. Exp1 = 10+20 mg/kg; Exp2 = 30+60 mg/kg; Exp3 = 90+180 mg/kg, respectively. Control (C) rats were treated with the drugs vehicle (propyleneglycol). *$p < 0.05$ and compared with the group C. **$p < 0.01$ and compared with the group Exp2.

Results and Discussion

Essentially identical results were observed regarding the abnormalities-free morphological aspects (optical microscopy) of maternal livers in the group Exp1 (which was treated with doses of ritonavir/zidovudine comparable to those used in humans) and Exp2 (treated with 3-fold higher doses) as compared to controls. Also, no alterations of blood AST and ALT activities were detected in those groups. Conversely, in maternal livers of the group Exp3 (pregnant rats treated with 9-fold higher doses of ritonavir/zidovudine) we noticed the presence of fatty infiltration and hepatocyte nuclei alterations (pyknosis, binucleation) (Figure 1A). This, however, was not accompanied by peripheral blood AST or ALT activities alterations.

The histologic feature of nuclear hyperpigmentation in group Exp3 hepatocytes is an indirect sign of apoptosis, which may develop in the absence of overt biochemical repercussions [17]. The fatty infiltration identified in that

group is presumably more related to the action of ritonavir than to zidovudine [18]. In fact, protease inhibitors such as ritonavir can cause hepatic steatosis consequent to modification of lipoproteins metabolism, eventually leading to an accumulation of visceral fat [18, 19]. It is interesting that the definite liver injury observed in group Exp3 coexisted with normal circulating levels of AST and ALT activities, a fact which was also observed by other authors with the same antiretroviral drugs [20, 21]. In this respect, Travlos *et al.* [22] argue that blood repercussions of hepatic damage can be blunted by the high capacity of liver regeneration, although this may not be observed in all instances. The return to normality can occur in a short time, after the initial damage. Furthermore, it is known that the liver is an organ capable of maintaining its biochemical functions within proper physiological limits, even with a reduced tissue mass [22, 23]. Interestingly, the zidovudine/ritonavir combination was less deleterious to the maternal liver than when each drug was used isolately during rat pregnancy.

Dose-dependent histological changes were observed in the maternal kidneys of the three experimental groups studied. The lesions were observed only in the proximal and distal tubules and the glomeruli of the cortical region (Figure 1B). These cell-death indicative histological alterations were characterized by nuclear condensation or pyknosis in proximal tubules and the glomeruli in samples of Exp1, Exp2 and Exp3 groups.

Though less intense, the same morphological changes found in the present experimental groups have been also observed in other studies using either ritonavir alone [20] or with other combinations such as ritonavir/lopinavir [24] and ritonavir/zidovudine/lamivudine [21]. The renal histological changes caused by ritonavir isolately were exacerbated by the combination with zidovudine in our experiment, although no biochemical signs of renal impairment (namely, increase in the blood level of urea and creatinine) were noticed.

During the course of renal lesions, an early rise of blood urea is followed by a late rise of blood creatinine, and this temporal pattern is of prognostic value in the evaluation of kidney pathologies [25]. Despite the drug-induced renal damage seen in our experiment, the values of circulating urea and creatinine did not rise. In fact, we observed decreases of blood urea (in Exp3) and creatinine (in Exp1 rats). Similar results were obtained in our laboratories with other antiretroviral drugs, namely indinavir [26], ritonavir [20, 27] and ritonavir/lopinavir [24].

It is apparent from our results that the mechanisms of renal excretion of these drugs are not involved with their kidney side-effects. It is known that 63-95% of the dosage of zidovudine undergoes renal excretion, and only 11% of that of ritonavir are so excreted. Notwithstanding, harm-evoked effects of ritonavir on maternal kids were much more pronounced than with zidovudine.

As a final product of protein metabolism, urea is synthesized in the liver and subsequently excreted by the urine. Conceivably, the liver injury found only in our group Exp3 could be related to the decreased plasma levels of urea. This fact is indicative that ritonavir is involved with liver injury which in turn may have caused that reduction in protein metabolism and thus a decrease in urea production. The mechanisms responsible for these effects are at present unclarified.

Creatinine is a metabolic product of creatine, which is a molecule of major importance for energy production in muscles. Low blood levels of creatinine may occur in states of decreased muscle mass, severe liver disease, and even pregnancy [28]. It is known that therapeutic doses of ritonavir (9-12%) and zidovudine (11%), either in monotherapy or in combination (9.6%) can raise the circulating levels of creatine phosphokinase (CPK), indicating muscle damage [18]. Such effects could well explain the reduction of serum creatinine observed herein, since a decrease of creatinogenesis rate would be consequent to some restriction of protein delivery for CPK synthesis. The association of zidovudine/ritonavir was deleterious to maternal kidneys, and this effect was more intense than that observed when the drugs were used alone.

Regarding the offspring, no changes in fetal livers or kidneys were observed in any group. This fact was also observed in several other series of similar experiments at the Department of Experimental Obstetrics, which studied the action of other antiretroviral drugs (ARVs) on pregnant female rats using the same general design employed in the present study. It has been consistently observed that even at high doses, ARVs do not cause structural changes in fetal livers and kidneys [20, 24, 26, 29-33]. The reduction in transplacental transfer of ritonavir may be ascribed to the blockade possibly exerted by P-glycoprotein (P-gp), a transmembrane transport protein that exists in high concentrations in placental structures [20, 27, 34-36]. It can be also argued that the liver lesions could be consequent to the fact that, at the beginning of gestation, there was low or almost absent placental ability to express P-gp, thus allowing the transfer of important amounts of ritonavir. With the progress of pregnancy towards term, two factors could be operating to the progressive recovery of the liver tissue: first, the progressive increase of placental expression of fully functional P-gp molecules, and second, the typical capability of rapid and intense rat liver regeneration [23, 27, 34-39].

Today there is a growing importance of ARV combinations not only for their effectiveness regarding pregnant women but also for their high efficacy in drastically lowering the vertical transmission of HIV. Notwithstanding, though our experimental findings can not be directly extrapolated to humans, they may serve as an alert to the relevance of experimental researches for the planning of safe therapeutical anti-HIV regimens during pregnancy.

Conclusion

The combination of zidovudine and ritonavir administered throughout the pregnancy of the rats affected the histological structure of the kidneys of the mothers in three experimental groups: it reduced blood levels of creatinine and urea, respectively, in groups that received the

lowest equivalent therapy dose (Exp1) and the highest one (Exp3); It also amended the liver histological structure of the matrices only in the group that received nine times the therapeutic dose (Exp3) without, however, leading to changes in serum AST and ALT. On the other hand, it did not cause any structural changes in the livers and kidneys of the fetuses in any of the groups studied.

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