Effect of chronic ritonavir administration on pregnant rats and their fetuses

A.M. Carvalho[1], *M.D., Ph.D.*; **R.M. Oliveira-Filho**[2], *Ph.D.*; **M.J. Simões**[3], *Ph.D.*; **A.A. Amed**[3], *M.D., PhD.*; **L. Kulay Jr.**[3], *M.D., PhD.*

[1]*University of José do Rosário Vellano School of Medicine, Alfenas (MG);*
[2]*Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo (SP);*
[3]*Federal University of São Paulo School of Medicine (SP) (Brazil)*

Summary

In view of the very important role played by ritonavir in the prevention of maternal-fetal HIV-vertical transmission, the aim of this experimental study was to evaluate its possible effects on several important obstetric parameters. Ritonavir was administered daily to three groups of pregnant rats (E_1 = 20 mg/kg; E_2 = 60 mg/kg; E_3 = 180 mg/kg; n = 10 in every group) from 'zero' up to the 20th day of pregnancy. Controls (n = 10) were injected with the drug vehicle (propyleneglycol) in the same schedule. We evaluated the effects on fetal and maternal weight gain, placental weight, number of implantations and resorptions, malformations, fertility rate, and maternal and fetal death rates. Body weight gain of the E_3 group was significantly lower than that of the other groups, most likely due to a toxic effect of the highest dose of ritonavir. Ritonavir did not affect the number of implantations. Group E_3 had five resorptions and some reduction in fertility. The mortality rate was significantly affected by ritonavir (2/10 maternal deaths in E_2 and 4/10 in E_3). On the other hand, no alterations were observed in the fetuses, a finding which could be due at least in part to the protective action of placental P-glycoprotein.

Key words: Ritonavir; Toxicology; Pregnancy; Rat.

Introduction

Protease inhibitors markedly reduced morbidity and mortality due to AIDS [1-4]. Among them, ritonavir, a p-toluenesulfonic acid salt, is thought to be the most potent inhibitor of the 3A4 isoform of the cytochrome P450 enzymes [5-8]. In fact, it has been demonstrated in dogs and rats that the blood levels of other antiretrovirals were increased 8 to 46 times when co-administered with ritonavir [9]. This feature makes the drug a "pharmacokinetic enhancer" to increase the plasma concentrations of other HIV-protease inhibitors, and this use has become common in clinical practice [10].

In humans, the recommended dose of ritonavir is 600 mg twice a day, which is considered to be an adequate treatment for AIDS but related to potentially serious adverse reactions [11-13]. When associated with other drugs for the treatment of AIDS, ritonavir can be used in lower doses (100 or 200 mg twice a day). Such doses are well tolerated, safe and efficient [10].

Ritonavir is classified as a pregnancy category B drug [13], and only a few papers deal with its effects on rat pregnancy. For instance, Kumar *et al.* [5], Denissen *et al.* [14] and Yamaji *et al.* [15] studied the effects of ritonavir on non-pregnant rats only. On the other hand, Minkoff & Augenbraun [13] reported that ritonavir produced no effects on fertility in female rats at drug exposures of approximately 60% of those achieved with therapeutic dosages; higher dosages were related to hepatic toxicity, but malformations were not observed in pregnant rats. Developmental toxicity was observed (early resorptions, decreased fetal body weight, ossification delays and developmental changes) with blood concentrations approximately 30% of those achieved with the therapeutic dose. A slight increase was noticed in the incidence of cryptorchidism in rats at an exposure approximately 22% of that achieved with the proposed therapeutic dose [13]. In accordance with Mirochnick *et al.* [16], very small quantities of ritonavir cross the placental barrier.

The high metabolic activity of ritonavir, mainly at the level of heme-thiolate proteins as the cytochrome P450 enzymes and its possible consequences on embryo development, prompted us to investigate the effects of this drug on rat pregnancy.

Materials and Methods

Animals and treatments

Adult virgin female EPM-1 Wistar rats, weighing about 200 g, under routine laboratory conditions, were mated at random in the proportion of one male for three female rats. The finding of spermatozoids in the vaginal smear was labeled as the 'zero' day of pregnancy [17]. Forty pregnant rats were then randomly divided into four groups with ten rats each, one control (C) and three experimental drug-treated groups (E_1, E_2 and E_3). Control animals were given the drug vehicle (10% ethanol and 90% propyleneglycol) from day zero up to the 20th day of pregnancy. The experimental groups were given 20 mg/kg (E_1), 60 mg/kg (E_2) or 180 mg/kg (E_3) per day of ritonavir, respectively, during the entire period of pregnancy. Drug and vehicle administrations were done by gavage. Body weights were taken on day zero and the 7th, 14th and 20th days of pregnancy.

Revised manuscript accepted for publication February 27, 2004

On the 20th day of pregnancy the rats were killed under excess anaesthesia. On laparotomy and opening of the uterine horns, the following items were recorded: number of implantations and resorptions, number of placentae, placental and fetal weights and occurrence of malformations.

Statistical analysis

Data were analysed by ANOVA and the Kruskal-Wallis test. When the p value reached significance ($p \leq 0.05$), the Dunn's multiple comparisons test was employed [18, 19].

Results and discussion

One of the aspects of concern during antiretroviral therapy with protease inhibitors in humans is body weight follow-up. With regard to body weight gain during pregnancy, the issue is highly relevant in view of the potentially serious consequences of impaired nutrient intake on both maternal and fetal compartments [20]. Since no reports in the literature were found about the effects of ritonavir on the body weight gain of pregnant rats, this aspect was examined in our animals.

Figure 1 shows the body weight increase of pregnant rats throughout the experimental period. Similarly to what occurs in normal rats, it should be noticed that all groups reached their fastest weight gain in the last third of the pregnancy. However, with the highest dose of ritonavir (E_3 group) the body weight gain was severely impaired as the incremental curve slope was significantly lower than those of the other groups. Part of this result could be due to the gastrointestinal side-effects of ritonavir. In fact, in humans the drug can cause dose-dependent nausea, diarrhea, anorexia, abdominal pain and taste perversion. However, it is known that in humans a significant weight gain is common during antiretroviral therapy with protease blockers [21-25] regardless of pregnancy.

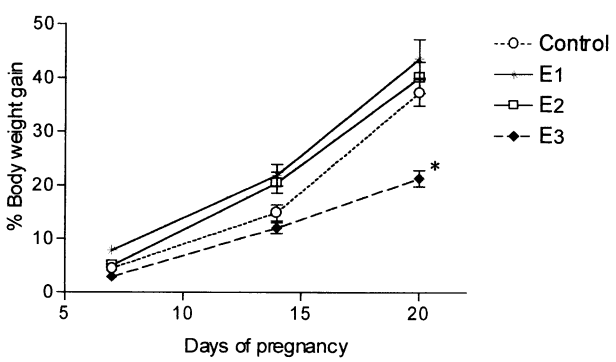

Figure 1. — Evolution of body weight gain during pregnancy in rats, control (C) or treated during the entire period of pregnancy with ritonavir (E_1 = 20 mg/kg; E_2 = 60 mg/kg; E_3 = 180 mg/kg once a day, by gavage). Values are mean ± SEM (n = 10 for every group) of percentual increments with regard to the initial weight (pregnancy day zero). Asterisks indicate significant differences with regard to the corresponding control at the same gestational age.

It has been reported that the weight increment is mainly in adipose tissue, with no changes in the lean body mass (skeletal muscle) [26], though no significant correlation was found between the use of protease inhibitors and lipodystrophy or hyperlipidemia [27]. With regard to our experimental groups, it can be seen in Figure 1 that at mid-pregnancy (14 days) E_1 and E_2 had mean incremental body weights somewhat higher than the controls, a result which could reflect the action of ritonavir. Nonetheless, in the E_3 group, in addition to the gastrointestinal effects, our results regarding weight loss at term could also be attributable to the metabolic disturbances caused by ritonavir [24].

Although reduced by some 12% in the E_3 group, the number of implantations recorded was not significantly affected by ritonavir (Table 1). However, the highest dose of ritonavir did increase the number of resorptions, an effect which may relate to its passage through the cell membrane and its putative effect on intracellular targets, similarly to what was proposed to occur in preadipocytes and adipocytes [28]. Since the observation that ritonavir is a relatively poor P-glycoprotein inhibitor *in vivo* [29], a self-limited cell entrance would not be exerted and then specific metabolic disturbances are likely to occur as a result of the ensuing critical intracellular concentrations of the drug.

Table 1. — *Effects of ritonavir administration on rat embryo implantations and resorptions, resorption/implantation ratios, mortality data and on mean placental weights. Mortality data are in absolute figures. Other values are mean ± SEM (n = 10 pregnant rats for every group).*

				Mortality		
Group	Implantations	Resorptions	Resorption Implantation Ratio	Maternal	Fetal	Placental weight (mg)
C	10.0 ± 0.59	0	0	0	0	0.51 ± 0.03
E_1	9.6 ± 0.52	0	0	0	0	0.64 ± 0.01
E_2	9.5 ± 0.53	0	0	2	0	0.61 ± 0.02
E_3	8.8 ± 2.18	1.7 ± 1.2*	0.4 ± 0.3*	4	0	0.55 ± 0.05

Rats were control (C) or treated during the entire period of pregnancy with ritonavir (E_1 = 20 mg/kg; E_2 = 60 mg/kg; E_3 = 180 mg/kg once a day, by gavage). *p < 0.001 with regard to C, E_1 and E_2; p < 0.05 with regard to C and E_1.

The high-dose regimen of ritonavir (E_3 group) had a severe effect on fertility, since only 50% of the surviving rats in that group got pregnant, and only two among them had term concepts. In fact, ritonavir at 180 mg/kg per day was related to a 40% mortality rate and severe toxicity such as necrosis in the proximal convoluted tubules of the kidney and severe hepatic congestion, which could be confirmed by necropsy as well. It was noticed that a high mortality rate (40%) in the E_3 group coexisted with a 100% survival rate of fetuses (Table 1), suggesting that there may be an important compartmentation of the drug which shifts its toxicity from the fetal to the maternal side.

There was no evidence of malformation. Lack of malformations in rats under the use of ritonavir has already been reported by Minkoff & Augenbraun [13]. This could be explained by a protective factor that does not allow ritonavir to cross the placental barrier. In fact, an effec-

tively functioning placental drug transporter, P-glycoprotein, might significantly limit the access of ritonavir to the fetus [16, 30] and thus might explain why no fetal damage was observed. On the other hand, this limited passage would represent a handicap, if one of the targets of the treatment were to protect the fetus from mother-to-child HIV transmission.

References

[1] Hogg R.S., O'Shaughnessy M.V., Gataric N., Yip B., Craib K., Schechter M.T., Montaner J.S.: "Decline in deaths from AIDS due to new antiretrovirals". *Lancet,* 1997, *349,* 1294.

[2] Selik R.M., Karon J.M., Ward J.W.: "Effect of the human immunodeficiency virus epidemic on mortality from opportunistic infections in the United States in 1993". *J. Infect. Dis.*, 1997, *176,* 632.

[3] Palella F.J. Jr., Delaney K.M., Moorman A.C., Loveless M.O., Fuhrer J., Satten G.A. *et al.:* "Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection". *N. Engl. J. Med.*, 1998, *338,* 853.

[4] Murphy S.L.: "Deaths: final data for 1998". *Natl. Vital. Stat. Rep.*, 2000, *48*, 1.

[5] Kumar G.N., Rodríguez A.D., Buko A.M., Denissen J.F.: "Cytochrome P450-mediated metabolism of the HIV-1 protease inhibitor ritonavir (ABT-538) in human liver microsomes". *J. Pharmacol. Exp. Ther.*, 1996, *277*, 423.

[6] Koudriakova T., Iatsimirskaia E., Utkin I., Gang E., Vouros P., Storozhuk E. *et al.*: "Metabolism of the human immunodeficiency virus protease inhibitors indinavir and ritonavir by human intestinal microsomes and expressed cytochrome P450 3A4/3A5: mechanism based inactivation of cytochrome P450 by ritonavir". *Drug. Metab. Dispos.,* 1998, *26,* 552.

[7] Von Moltke L.L., Greenblatt D.J., Grassi J.M., Granda B.W., Duan S.X., Fogelman, S.M. *et al.*: "Protease inhibitors as inhibitors of human cytochromes P 450: high risk associated with ritonavir". *J. Clin. Pharmacol.*, 1998, *38*, 106.

[8] Kumar G.N., Jennifer D., Roberts E.M., Jayanti V.K., Hickman D., Uchic J. *et al.*: "Potent inhibition of the cytochrome P-450 3A4 mediated human liver microsomal metabolism of novel HIV protease inhibitor by ritonavir: a positive drug-drug interaction". *Drug. Metab. Dispos.*, 1999, *27*, 902.

[9] Kempf D.J., Marsh K.C., Kumar G., Rodrigues A.D., Denissen J.F., McDonald E.M. *et al.*: "Pharmacokinetic enhancement of inhibitors of the human immunodeficiency virus protease by coadministration with ritonavir". *Antimicrob. Agents. Chemother.,* 1997, *41,* 654.

[10] Walmsley S., Berstein B,, King M., Arribas J., Beall G., Ruane P. *et al.*: "Lopinavir-ritonavir versus nelfinavir for the initial treatment of HIV infection". *N. Engl. J. Med.,* 2002, *346*, 2039.

[11 Danner S.A., Carr A., Leonard J.M., Lehman L.M., Gudiol F., Gonzales J. *et al.*: "A short-term study of the safety, pharmacokinetics, and efficacy of ritonavir, an inhibitor of HIV-1 protease". *N. Engl. J. Med.*, 1995, *333*, 1528.

[12] Lewis J.S., Terriff C.M., Coulston D.R., Garrison M. W.: "Protease inhibitors: a therapeutic breakthrough for the treatment of patients with human immunodeficiency virus". *Clin. Ther.,* 1997, *19,* 187.

[13] Minkoff H., Augenbraun M.: "Antiretroviral therapy for pregnant women". *Am. J. Obstet. Gynecol.,* 1997, *176,* 478.

[14] Denissen J.F., Grabowski B.A., Johnson M.K., Buko A.M., Krempf D.J., Thomas S.B., Surber B.W.: "Metabolism and disposition of the HIV-1 protease inhibitor ritonavir (ABT-538) in rats, dogs, and humans". *Drug. Metab. Dispos.,* 1997, *25*, 489.

[15] Yamaji H., Matsumura Y., Yoshikawa Y., Takada K.: "Pharmacokinetic interactions between HIV-protease inhibitors in rats". *Bioph. Drug. Disp.,* 1999, *20*, 241.

[16] Mirochnick M., Dorenbaum A., Holland D., Cunningham-Schrader B., Cunningham C., Gelber R. *et al.*: "Concentrations of protease inhibitors in cord blood after in utero exposure". *Pediatr. Infect. Dis. J.*, 2002, *21,* 835.

[17] Hamilton J.B., Wolfe J.M.: "The effect of male hormone substance upon birth and prenatal development in the rat". *Anat. Rec.*, 1938, *70,* 433.

[18] Sokal R.R., Rohlf F.J.: "Biometry". San Francisco, W.H. Freeman, ed., 1969, 776.

[19] Siegel S.: "Estadística no paramétrica". México, Ed. Trillas, 1975, 346.

[20] Jackson A.A., Robinson S.M.: "Dietary guidelines for pregnancy: a review of current evidence". *Public. Health. Nutr.,* 2001, *4*, 625.

[21] Carr A., Samaras K., Chisholm D.J., Cooper D.A.: "Pathogenesis of HIV-1 protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance". *Lancet,* 1998, *352,* 1881.

[22] Gagnon A., Angel J.B., Sorisky A.: "Protease inhibitors and adipocyte differentiation in cell culture". *Lancet*, 1998, *352*, 1032.

[23] Stricker R.B., Goldberg B.: "Fat accumulation and HIV-1 protease inhibitors". *Lancet*, 1998, *35*, 1392.

[24] Martinez E., Gatelli J.M.: "Metabolic abnormalities and body fat redistribution in HIV-1infected patients: the lipodystrophy syndrome". *Curr. Op. Infec. Dis.*, 1999, *12*, 13.

[25] Batterham M., Garsia R., Greenop P.: "Prevalence and predictors of HIV-associated weight loss in the era of highly active antiretroviral therapy". *Int. J. STD. AIDS*, 2002, *13* (11), 744.

[26] Silva M., Skolnik P.R., Gorbach S.L., Spiegelman D., Wilson I.B., Fernández-DiFranco M.G., Knox T.A.: "The effect of protease inhibitors on weight and body composition in HIV-infected patients". *AIDS*, 1998, *12* (11), 1645.

[27] Tsiodras S., Mantzoros C., Hammer S., Samore M.: "Effects of protease inhibitors on hyperglycemia, hyperlipidemia, and lipodystrophy: a 5-year cohort study". *Arch. Intern. Med.*, 2000, *160*, 2050.

[28] Vernochet C., Azoulay S., Duval D., Guedj R., Ailhaud G., Dani C.: "Differential effect of HIV protease inhibitors on adipogenesis: intracellular ritonavir is not sufficient to inhibit differentiation". *AIDS,* 2003, *17* (15), 2177.

[29] Huisman M.T., Smit J.W., Wiltshire H.R., Hoetelmans R.M., Beijnen J.H., Schinkel A.H.: "P-glycoprotein limits oral availability, brain, and fetal penetration of saquinavir even with high doses of ritonavir". *Mol. Pharmacol.,* 2001, *59*, 806.

[30] Mirochnick M.: "Antiretroviral pharmacology in pregnant women and their newborns". *Ann. NY. Acad. Sci.*, 2000, *918*, 287.

Address reprint requests to:
A.M. DE CARVALHO, M.D.
Dept. of Clinical Medicine,
School of Medicine
Avenida Dona Conceicão, 320
Jardim Panorama - CEP 37130-000
Alfenas-MG (Brazil)