CEOG Clinical and Experimental Obstetrics & Gynecology

Experimental Research

Chronic action of lamivudine and ritonavir on maternal and fetal liver and kidney of albino pregnant rats (Rattus norvegicus albinus, Rodentia, Mammalia): morphological and biochemical aspects

A.d.M. Vangelotti[1], E. Araujo Júnior[1], M.d.J. Simões[2], A.M. Amed[1], M.U. Nakamura[1], L. Kulay Júnior[1]

[1] Department of Obstetrics, Federal University of São Paulo (UNIFESP), São Paulo, SP
[2] Department of Morphology, Federal University of São Paulo (UNIFESP), São Paulo, SP (Brazil)

Summary

Purpose: To investigate the morphological and biochemical effects of lamivudine associated with ritonavir on maternal and fetal livers and kidneys throughout the pregnancy of albino rats. *Materials and Methods:* Forty pregnant rats were divided into four numerically equal groups: control (C), experiment 1 (E1), experiment 2 (E2), and experiment 3 (E3). Only distilled water was given to the control group, while groups E1, E2, and E3 received, respectively, 5, 15 and 45 mg/kg of lamivudine associated with 20, 60, and 180 mg/kg of ritonavir, per day, throughout the pregnancy. On the 20th day of the pregnancy, the histological structure of the maternal and fetal livers and kidneys was analyzed by means of optical microscopy, along with the blood concentrations of AST, ALT, urea, and matrix creatinine. The numerical variables were analyzed using the Kruskal-Wallis test and Dunn's multiple comparison test. *Results:* The histological alterations occurred in both the maternal livers and the maternal kidneys, particularly in group E3, which received the greatest therapeutic dosage (nine times). The blood levels of ALT in group E3 were significantly lower than in the other groups ($p = 0.0037$). The urea and creatinine levels in the blood were significantly lower in group E1 ($p = 0.0420$ and $p = 0.0108$, respectively). *Conclusions:* The association of lamivudine and ritonavir affected the histological structure of the kidneys of the matrices of group E3. There was a significant decrease in the blood values of urea e creatinine in group E1.

Key words: Lamividine; Ritonavir; Histology; Biochemical; Teratology; Pregnant rat.

Introduction

The acquired immunodeficiency syndrome (AIDS) is a severe multisystemic disorder that affects the immune system, causing severe immunosuppression and, consequently, the appearance of opportunistic infections and neoplasia [1]. The liver and the kidneys can be affected in this syndrome by opportunistic infections, neoplasia, use of drugs for treatment of infections and, particularly, by use of antiretroviral drugs [2, 3]. Drug-induced hepatitis associated with use of antiretroviral drugs as protease inhibitors, particularly ritonavir, seems to be more frequent among patients with previous infection by the hepatitis B or C viruses [4, 5].

Although monotherapy using zidovudine was initially recommended, pregnant women should receive combined regimens of more potent drugs, because this measure has been correlated with a smaller rate of vertical transmission [6]. The current recommendation for use of antiretroviral drugs is an aggressive combination of drugs that suppresses viral replication as much as possible, preserves immunological function, and minimizes development of viral resistance [7].

Antiretroviral therapy during pregnancy using multiple antiretroviral medications, in comparison with absence of treatment or with treatment using only one drug, has not been associated with increased rates of premature labor, low birth weight, low Apgar scores or stillbirth [8]. A combination of three or more antiretroviral agents is recommended during pregnancy, but it is worth emphasizing that studies evaluating the safety of these drugs during pregnancy, regarding their toxicity and teratogenic potential, are necessary [9, 10].

The antiretroviral drugs administered to pregnant women generally cross the placenta, with consequent exposure of

Revised manuscript accepted for publication February 17, 2014

the developing embryo and fetus to their pharmacological and teratogenic effects. The critical factors that affect placental transfer of drugs and their effects on the fetus include: the physical-chemical properties of the drug, the speed at which it crosses the placenta, and the amount that reaches the fetus; the duration of exposure to the drug, the distribution characteristics of the drug in different tissues of the fetus, the development stage of the placenta and fetus at the time of exposure to the drug, and the effects of drugs used in association [9].

Following the notable success in reducing vertical transmission, through combined antiretroviral therapy, attention is now focused on the safety of these drugs for the mother-fetus pair. Not always have studies shown agreement regarding the pharmacokinetics and pharmacodynamics of antiretroviral drugs during pregnancy. Placental transference to the fetus is variable, and the characteristics of the receptors and the responses produced from the associations of drugs are not always elucidated. The potential for toxicity in the mother and in the fetus is always a concern. Although the benefits surpass the potential risks for the fetus, with regard to prevention of vertical transmission, there are legitimate concerns about the use of antiretroviral drugs during pregnancy [10].

With the aim of better observing the effects of associations of antiretroviral drugs during pregnancy, the authors decided to carry out this experiment among pregnant albino rats, with the objective of evaluating the morphology and histology of the maternal and fetal livers and kidneys and the maternal hepatic and renal biochemical parameters, with use of lamivudine in association with ritonavir throughout the pregnancy.

Materials and Methods

Albino Wistar rats (*Rattus norvegicus albinus; Rodentia; Mammalia*) of the EPM–1 lineage were used. They were virgin adults of approximately 90 days of age, weighing close to 200 g, from the Central Vivarium of the Federal University of São Paulo (UNIFESP). This experiment was approved by the Institution's Research Ethics Committee, under the number 1078/05. The animals were raised and maintained at the Central Vivarium of UNIFESP, where they remained confined in metal cages of dimensions 45 x 30 x 15 cm (length, width, and height, respectively). Five animals were kept in each cage, with *ad libitum* food and water acidified at pH 2.7-3.1, for a 15-day adaptation period. The environmental temperature was 22ºC ± 2ºC, and artificial lighting produced by fluorescent 40-watt lamps was used, with photoperiods of 12 hours of light and 12 hours of dark. The cages were cleaned and the sawdust was changed daily. The air was filtered with 95% efficiency for five μm particles.

After the adaptation period of approximately 15 days, the animals were mated in the proportions of one male for every two females, and the beginning of the pregnancy was determined through the Hamilton and Wolf technique [11], i.e. detection of the presence of spermatozoids in the vagina of the rat on the morning after mating. This was considered to be day zero of the pregnancy. The pregnant rats were confined in individual cages measuring 30 x 15 x 12 cm (length, width, and height, respectively), and underwent random distribution into four groups of ten animals each.

The animals were divided into four groups: control (C), experiment 1 (E1), experiment 2 (E2), and experiment 3 (E3). Group C did not receive any drug, but received two ml of distilled water, by means of gavage, in a single daily dose, from day zero to the 20th day of the pregnancy. Group E1 received five mg/kg/day of lamivudine in association with 20 mg/kg/day of ritonavir, in a single daily dose, orally means of gavage, from day zero to the 20th day of the pregnancy. Group E2 received 15 mg/kg/day of lamivudine in association with 60 mg/kg/day of ritonavir, in a single daily dose, orally by means of gavage, from day zero to the 20th day of the pregnancy. Group E3 received 45 mg/kg/day of lamivudine in association with 180 mg/kg/day of ritonavir, in a single daily dose, orally by means of gavage, from day zero to the 20th day of the pregnancy.

On the 20th day of the pregnancy, all the rats were anesthetized with xylazine and ketamine, at dosages of two mg/kg and 100 mg/kg, respectively, intraperitoneally. Immediately after anesthesia, a longitudinal thoracic-abdominal incision was made on the median line, thereby exposing the internal organs (liver, kidneys, and heart). Then, three ml of blood was collected using a disposable syringe of three ml in total volume, with a needle of 25 x 07 mm (22GI), to aspirate the ventricular cavity. The blood volume obtained was transferred to dry tubes, which were non-heparinized. This material was sent to a laboratory in order to determine the values of the transaminases aspartate aminotransferase (AST) and alanine aminotransferase (ALT), in order to evaluate hepatic function, and to determine the urea and creatinine values, for renal function. These were done respectively by means of the kinetic colorimetric and enzymatic colorimetric methods, analyzed using an automated chemistry system equipment. Following this, hysterectomy was carried out to remove the fetuses. Rapidly, from each rat and its offspring, the livers and kidneys were extracted for macroscopic analysis. This material was immersed in 10% formol, buffered using phosphate buffer and was then processed for embedding in paraffin, to enable analysis under an optical microscope.

The mothers, still under anesthesia, were sacrificed by using scissors to perforate the myocardium, and the offspring were decapitated. After these specimens had been obtained for optical microscopy, they were rapidly dissected and, using a steel blade, fragments of thickness three to four mm were removed from the mothers' and their offspring's livers and kidneys, by means of a sagittal cut. The fragments for use in the optical microscopy evaluation were immersed in a 10% formol solution for a period of 12 hours. Subsequently, they were subjected to dehydration using ethyl alcohol at progressively greater concentrations until reaching absolute alcohol. After dehydration, the specimens were cleared using xylene and impregnated with liquid paraffin in a laboratory oven-regulated at a temperature of 60ºC. The blocks were then cut using a microtome, adjusted to a thickness of five μm. The sections were placed on slides that had previously been greased with Mayer's albumin and were then kept in an oven regulated at a temperature of 37ºC for 24 hours, for the sections to dry and glue on. Afterwards, hematoxylin-eosin (HE) staining was performed. The optical microscopy evaluation was done using a microscope, with an eyepiece lens of 10x magnification and objective lenses of between 4x and 100x magnification. The histological results were demonstrated through photomicrographs of the maternal and fetal hepatic lobes, and of the cortical and medullary regions of the maternal and fetal kidneys, and a comparative analysis was carried out between group C and the E groups.

The mean quantitative AST, ALT, urea and creatinine values measured in the different groups on the 20th day of pregnancy were analyzed statistically using the Kruskal-Wallis test, which was complemented by Dunn's multiple comparison test when there was statistical significance, in order to locate the groups in which these differences occurred [12]. The histological results

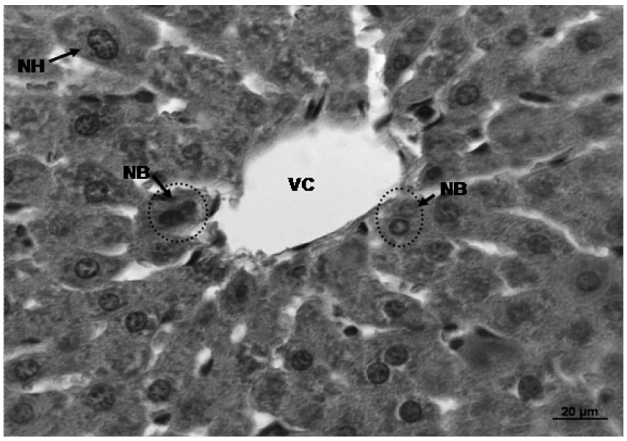

Figure 1. — Photomicrograph showing the central portion of the hepatic lobe of a rat in group E3. Note the alteration of some hepatocytes (H) that present a hyperchromic nucleus (NH) and other binucleated cells (NB), closer to the central-lobular vein (VC) (HE x 320).

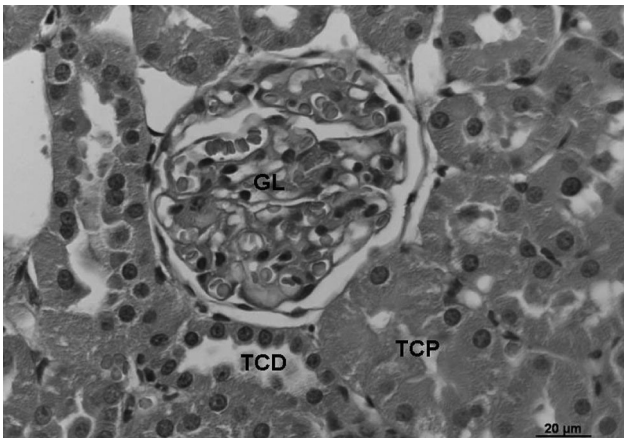

Figure 2. — Photomicrograph showing the maternal kidney with renal glomeruli (GL), proximal convoluted tubules (TCP), and distal convoluted tubules (TCD). The maternal kidney presented a narrower cortex and a reduction in the number of glomeruli, in which the endothelial cells showed pyknotic nuclei (HE x 320).

from the maternal and fetal livers and kidneys were evaluated by means of comparative analysis between group C and the E groups. In all the analyses, the significance level used was $p < 0.05$.

Results

Morphological analysis

1) Morphological analysis on the maternal liver

There were no differences among groups C, E1, and E2. The hepatic parenchyma consisted of a great concentration of hepatocytes, which were organized forming lines, which, in turn, converged to the central-lobular vein. Between the lines of hepatocytes, there were hepatic sinusoids and nuclei of several shapes, which were generally heterochromatic. The hepatocytes were polyhedral voluminous cells, with one or two centrally positioned spherical nuclei that were rich in chromatin, with evident nucleoli. The cytoplasm was not homogeneous, and basophilic and eosinophilic areas were seen to be present. For group E3, the only difference was the alteration of some hepatocytes, which presented hyperchromic nuclei, while others were binucleated and closer to the central-lobular vein (Figure 1).

2) Morphological analysis on the fetal liver

There were no differences among the C, E1, E2, and E3 groups. The fetal liver consisted of a great concentration of hepatocytes that were organized resembling lines. These cells were small and polyhedral, with spherical nuclei that occupied most of the cytoplasm. A nucleolus could clearly be seen in the nucleus and, in the cytoplasm, there were areas with intense acidophilia. In addition, many mitosis figures were observed, indicating cell proliferation. Among the hepatocytes there were capillary blood vessels, a large concentration of nucleated red blood cells, and megakaryocytes.

3) Morphological analysis on the maternal kidney

There were no differences between groups C and E2. The kidneys were well preserved, with the presence of renal corpuscles and proximal and distal convoluted tubules. The glomeruli were composed of capillaries, podocytes, endothelial cells, and mesangial cells. Capsular space and a parietal layer were observed in Bowman's capsule. It should also be noted that most of the parenchyma and cortical region consisted of proximal tubules, which were formed by cubic or polyhedral cells, containing eosinophilic cytoplasm and a rounded nucleus. For group E2, the morphology was similar, with the exception of some areas with capillary dilation. On the other hand, group E3 presented a narrower cortex, with a reduction of the area of glomeruli, considering that among these the endothelial cells showed pyknotic nuclei (Figure 2).

4) Morphological analysis on the fetal kidney

There were no differences among groups C, E1, E2, and E3. On the 20th day of pregnancy, the kidneys were not completely formed, although the cortical and medullary regions were identifiable. In the cortical region, there were some glomeruli and convoluted tubules, of which the proximal ones were more eosinophilic. In the medullary region there were collector ducts and some loops of Henle.

Biochemical analysis

The mean quantitative values measured for AST in the blood of the rats of each study group on the 20th day of pregnancy are reported in Table 1. There were no statistically significant differences among the groups studied regarding AST values ($p = 0.5341$).

The mean quantitative values measured for ALT in the blood of the rats of each study group on the 20th day of

Table 1. — *Descriptive statistics on AST in the samples studied.*

Groups	AST (U/L)					
	N	Mean	SD	Median	Minimum	Maximum
C	10	118.00	37.19	109.50	75.00	184.00
E1	10	126.60	31.45	125.00	75.00	164.00
E2	09	134.22	31.94	128.00	92.00	195.00
E3	09	125.44	19.86	122.00	96.00	158.00

Kruskal-Wallis test: $p = 0.5341$ (C = E1 = E2 = E3).

Table 2. — *Descriptive statistics on ALT in the samples studied.*

Groups	ALT (U/L)					
	N	Mean	SD	Median	Minimum	Maximum
C	10	74.20	11.76	76.00	49.00	90.00
E1	10	61.40	7.32	61.50	51.00	74.00
E2	09	67.88	9.13	70.00	54.00	80.00
E3	09	54.77	10.95	53.00	36.00	67.00

Kruskal-Wallis test: $p = 0.0037$ * E3 < (C = E1 = E2).

Table 3. — *Descriptive statistics on the urea in the samples studied.*

Groups	UREA (mg/dL)					
	N	Mean	SD	Median	Minimum	Maximum
C	10	65.50	6.48	66.50	58.00	74.00
E1	10	53.90	6.85	54.00	41.00	62.00
E2	09	61.00	9.87	61.00	46.00	77.00
E3	09	60.44	7.82	64.00	43.00	66.00

Kruskal-Wallis test: $p = 0.0420$ * E1 < (C = E2 = E3).

Table 4. — *Descriptive statistics on the creatinine in the samples studied.*

Groups	CREATININE (mg/dL)					
	N	Mean	SD	Median	Minimum	Maximum
C	10	0.51	0.03	0.50	0.50	0.60
E1	10	0.43	0.04	0.40	0.40	0.50
E2	09	0.47	0.04	0.50	0.40	0.50
E3	09	0.50	0.19	0.40	0.40	1.00

Kruskal-Wallis test: $p = 0.0108$ * E1 < (C = E2 = E3).

pregnancy are reported in Table 2. There was a statistically significant difference among the groups studied regarding ALT values ($p = 0.0037$). Dunn's multiple comparison test showed that group E3 presented significantly lower ALT values than those of group C, and that group C did not differ from groups E1 and E2.

The mean quantitative values measured for urea in the blood of the rats of each study group on the 20th day of pregnancy are reported in Table 3. There was a statistically significant difference in urea values ($p = 0.042$) among the groups studied. Dunn's multiple comparison test showed that group E1 presented urea values that were significantly lower than those of group C, and that group C did not differ from groups E2 and E3.

The mean quantitative values measured for creatinine in the blood of the rats of each study group on the 20th day of pregnancy are reported in Table 4. There was a statistically significant difference in creatinine values ($p = 0.0108$) among the groups studied. Dunn's multiple comparison test showed that group E1 presented significantly lower creatinine values than those of group C, and that group C did not differ from groups E2 and E3.

Discussion

The doses of lamivudine and ritonavir that were applied to group E1 were determined based on calculations of equivalence to the therapeutic dose for humans, according to the mean weights of the rats on day zero of the pregnancy. The doses calculated for groups E2 and E3 were, respectively, equivalent to three and nine times the therapeutic dose for humans, following the research protocol used in the present service, which allowed greater veracity in the comparative analysis on the results. The objective of studying this association in high dosages was to evaluate maternal-fetal toxicity, considering that the drugs are metabolized much faster in rats than in humans [13], and that their bioavailability may be 50% to 90% lower [14].

The period over which the drugs were administered, from day zero until the 20th day of pregnancy, had the objective of covering the implantation phase, embryogenesis (until the 15th day), and fetal development (beginning on the 15th day). Therefore, a wide-ranging period was covered in order to analyze possible deleterious effects on the fetuses [15].

It was decided to study the hepatic and renal morphology and biochemistry of the pregnant rats because of the way in which lamivudine and ritonavir are metabolized and eliminated. At the therapeutic dose, less than 10% of lamivudine is metabolized by the liver, and it is predominantly eliminated through the renal system, by means of glomerular filtration and active tubule secretion [16]. Ritonavir is metabolized by the hepatic system and up to 86% is eliminated by the hepatobiliary system and, to a smaller extent, by the renal system. At high dosages, use of these drugs can lead not only to hepatotoxicity but, in some cases, to nephrotoxicity [17]. In previous studies carried out by the present group, lamivudine and ritonavir were used, at the same doses used in the present study, but separately [18, 19].

In the control group of the present study, the hepatic and renal morphological and biochemical analysis of the mothers and their offspring did not reveal any alteration, thus demonstrating that administering the drugs and feeding and handling the animals did not interfere with the re-

sults from the experiment. This result was also observed with other antiretroviral drugs researched by the present group [18-22].

Regarding the maternal livers, the present histological examinations demonstrated that when the combination of lamivudine and ritonavir was given to the pregnant rats at a dose equivalent to the dose used for humans or equivalent to three times the therapeutic dose, there were no morphological and biochemical alterations. However, alterations were present when the equivalent of nine times the therapeutic dose (E3) was given. The condensation of the cell nucleus or pyknosis that was demonstrated by the presence of hyperchromic nuclei in the hepatocytes of group E3 is an indirect sign of apoptosis, which may indicate the presence of a histological lesion with no biochemical repercussions. The finding of binucleated nuclei is a probable sign of cell multiplication. Thus, the association of lamivudine and ritonavir (at nine times the therapeutic dose) caused slight hepatic cytotoxicity, shown by signs of cell reactivity, in accordance with the observations of Goldman *et al.* [23].

A statistically significant decrease in the levels of ALT in group E3 was observed, in comparison with the other groups evaluated. Alteration of the ALT levels would be expected, because if the liver has been injured, this enzyme would increase at an earlier stage. One hypothesis, although not very likely, would be that the aggression may initially have occurred more intensely, thereby causing an increase in the ALT levels. Following this, with exhaustion of ALT, a subsequent fall in its levels would have occurred in group E3. Since exhaustion of ALT only occurs in the presence of intense hepatic alterations, this hypothesis would have very little basis for explaining this decrease [24]. The most likely explanation is that although the decrease in the level of this enzyme in group E3 was statistically significant, it did not have any biological significance. The injury to the maternal livers of group E3 was probably induced by the action of ritonavir, due to its high rate of hepatic metabolization and hepatobiliary elimination. This may, to some extent, suggest that hepatotoxicity occurs when the drugs are administered at high doses, either as monotherapy or in associations, for humans [5] or rodents [19].

Regarding lamivudine, some studies have shown that it is safe when administered during pregnancy [25, 26]. In a study carried out by the present group, Pontes *et al.* [18] used lamivudine in albino rats, separately, at dosages of 5, 15, and 45 mg/kg/day, from day zero until the 20th day of pregnancy, and demonstrated that the drug had no effect that could cause fetal malformations, at those dosages. Unlike other analogous nucleoside reverse-transcriptase inhibitors (zidovudine, didanosine, and stavudine), lamivudine has little action on the DNA polymerase of mammals and is not incorporated into mitochondrial DNA. Incorporation of the nucleoside analogue into the mitochondrial DNA is implied in several toxic effects, among which is liver failure. Clinically, lamivudine produces minimal adverse effects, in comparison with other nucleoside analogues, and it does not show any capacity to cause mitochondrial toxicity [26]. It is believed that it does not induce any important adverse hepatic affects [25] and, because of this, if used in combination with ritonavir, it might attenuate the hepatotoxicity frequently observed with the use of the latter drug.

In the present study, the hepatic histological alterations caused by the association of these two drugs were less pronounced than in studies that used lamivudine and ritonavir separately [18, 19]. The present authors observed that hepatic alterations could already be demonstrated at smaller doses of ritonavir [19], which reinforces the idea that lamivudine may attenuate the maternal toxic effects caused by ritonavir [19]. These alterations to the maternal liver were also observed when these drugs were administered separately, starting at 45 mg/kg for lamivudine (nine times the therapeutic dose) and starting at 60 mg/kg for ritonavir (three times the therapeutic dose). However, when these two drugs were used in association, these alterations were only observed beginning at higher doses of ritonavir (45 mg/kg of lamivudine in association with 180 mg/kg of ritonavir) and at lower intensity. In another study, the adverse effects of ritonavir were 37% more intense when used separately than when used in association with another protease inhibitor, in this case saquinavir (16%) [27]. Thus, this demonstrated that the effect of ritonavir was attenuated by another antiretroviral.

To evaluate renal function, serum urea and creatinine were assayed. Considering the aggression towards the kidneys in group E3, it was expected that these parameters would increase, but there was no increase in the urea levels, which became higher in the more initial phases of aggression. Moreover, there was no increase in creatinine level, which became higher later on. These findings suggest that, despite the occurrence of some degree of structural injury in this group, the reserve functional capacity of the kidneys was sufficient to tolerate the aggression promoted by the combination of these drugs.

In the same way that in other studies carried out by the present group, using separate or combined antiretroviral drugs, even at high dosages, i.e. nine times greater than the equivalent therapeutic dose for humans, there were no structural alterations in the fetal livers and kidneys, thus corroborating the present findings [18-22]. The most adequate scientific foundation for this condition probably relates to the protection that the placental P-glycoprotein (a transmembrane transport protein that exists in great concentration on the maternal surface of the placenta) exerted when stopping appreciable amounts of ritonavir and lamivudine from going through the placental barrier [28].

Conclusion

In summary, the present study showed that the association of lamivudine and ritonavir did not cause fetal liver and kidney alterations, although it was harmful to the maternal livers and kidneys. The lesions observed were less intense than when these drugs were used separately during the pregnancy of albino rats. Although no direct correlation can be made regarding the toxicity of a drug between rats and humans, the findings from the present study suggest that the association of lamivudine and ritonavir promoted alterations to the pharmacokinetics of the drugs. Finally, it needs to be emphasized that with the various antiretroviral drugs available, especially considering their use in associations, research needs to be accelerated with the aim of improving anti-HIV therapy, so as to promote maximum effectiveness in impeding vertical transmission, with minimal deleterious effects to the mother and fetus.

References

[1] Grant A.D., Djomand G., De Cock K.M.: "Natural history and spectrum of disease in adults with HIV/AIDS in Africa". *AIDS*, 1997, *11*, S43.

[2] Duong M., Sgro C., Grappin M.: "Renal failure after treatment with ritonavir". *Lancet*, 1996, *348*, 693.

[3] Chugh S., Bird R., Alexander E.A.: "Ritonavir and renal failure". *N. Engl. J. Med.*, 1997, *336*, 138.

[4] Markowitz M., Saag M., Powderly W.G., Hurley A.M., Hsu A., Valdes J.M., *et al.*: "A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection". *N. Engl. J. Med.*, 1995, *333*, 1534.

[5] Sulkowski M.S., Thomas D.L., Chaisson R.E., Moore R.D.: "Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection". *JAMA*, 2000, *283*, 74.

[6] CDC – Public Health Service Task Force: "Recommendations for Use of Antiretroviral Drugs in Pregnant HIV-1-Infected Women for Maternal Health and Interventions to Reduce Perinatal HIV-1 Transmission in the United States. *MMWR*, 2008, 1. Available at: http://aidsinfo.nih.gov/contentfiles/lvguidelines/Peri_Recommendations.pdf.

[7] Bartlett J.A., DeMasi R.A., Quinn J.B., Moxham C., Rousseau F.: "Overview of the effectiveness of triple combination therapy in antiretroviral-naive HIV-1 infected adults". *AIDS*, 2001, *15*, 1369.

[8] Tuomala R.E., Shapiro D.E., Mofenson L.M., Bryson Y., Culnane M., Hughes M.D., *et al.*: "Antiretroviral therapy during pregnancy and the risk of an adverse outcome". *N. Engl. J. Med.*, 2002, *346*, 1863.

[9] Gedeon C., Koren G.: "Designing pregnancy centered medications: drugs which do not cross the human placenta". *Placenta*, 2006, *27*, 861.

[10] Stek A.M.: "Antiretroviral treatment in pregnancy". *Curr. Opin. HIV AIDS*, 2008, *3*, 155.

[11] 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.

[12] Hollander M., Wolfe D.A.: "Nonparametric statistical methods". New York: John Wiley & Sons, 1973.

[13] Fagundes D.J., Taha O.: "Modelo animal de doença: critérios de escolha e espécies de animais de uso corrente". *Acta Cir. Bras.*, 2004, *19*, 59.

[14] Kaul D.R., Cinti S.K., Carver P.L., Kazanjian P.H.: "HIV protease inhibitors: advances in therapy and adverse reactions, including metabolic complications". *Pharmacotherapy*, 1999, *19*, 281.

[15] Lewi P.J., Marsboom R.P.: "Toxicology reference data for the Wistar rat". *Arch. Toxicol. Suppl.*, 1982, *5*, 271.

[16] Johnson M.A., Moore K.H., Yuen G.J., Bye A., Pakes G.E.: "Clinical pharmacokinetics of lamivudine". *Clin. Pharmacokinet.*, 1999, *36*, 41.

[17] Bochet M.V., Jaquiaud C., Valantin M.A., Katlama C., Deray G.: "Renal insufficiency induced by ritonavir in HIV-infected patients". *Am. J. Med.*, 1998, *105*, 457.

[18] Pontes R.D., Amed A.M., Simões R.S., Oliveira-Filho R.M., Simões M.J., Kulay L.J.: "A morphological and biochemical appraisal of the liver and renal effects of lamivudine on rat pregnancy". *Clin. Exp. Obstet. Gynecol.*, 2006, *33*, 209.

[19] Wagner A., de Carvalho A.M., Nakamura M.U., Simões R.S., Fontes T.M., Espiridião S., Kulay Júnior L.: "Morphological and physiological analysis of livers and kidneys of pregnant rats and their fetuses treated by the association of zidovudine, lamivudine and ritonavir for the whole period of pregnancy". *Rev. Bras. Ginecol. Obstet.*, 2010, *32*, 556. [Article in Portuguese].

[20] Mamede J.A., Oliveira-Filho R.M., Simões M.J., Mora O.A., Espiridião S., Kulay L. Jr.: "Hepatic and renal effects of azidothymidine and acyclovir on pregnant rats". *Clin. Exp. Obst. Gyn.*, 2000, *27*, 227.

[21] Amed A.M., Simões R.S., Oliveira-Filho R.M., Kulay L. Jr.: "Safety of nelfinavir use during pregnancy. An experimental approach in rats". *Clin. Exp. Obst. Gyn.*, 2005, *32*, 163.

[22] Quintino M.P., Simões R.S., Oliveira F.H., Oliveira-Filho R.M., Simões M.J., Nakamura M.U., Kulay L. Jr.: "Morphological and biochemical appraisal of the liver and renal effects of indinavir on rat pregnancy". *Clin. Exp. Obst. Gyn.*, 2007, *34*, 232.

[23] Goldman R., Gruenbaum Y., Moir R., Shumaker D., Spann T.: "Nuclear lamins: building blocks of nuclear architecture". *Genes Dev.*, 2002, *16*, 533.

[24] Kondo M., Toledo C.F., Silva A.E.: "Insuficiência hepática aguda e crônica". *In:* Prado F.C., Ramos J., Valle J.R., (eds). *Atualização terapêutica.* 21st ed. São Paulo: Artes Médicas, 2003, 495.

[25] Staszewski S., Miller V., Renmet S., Stark T., De Crée J., De Brabander M., *et al.*: "Virological and immunological analysis of a triple combination pilot study with loviride, lamivudine and zidovudine in HIV-1 infected patients". *AIDS*, 1996, *10*, F1.

[26] Lee H., Hanes J., Johnson K.A.: "Toxicity of nucleoside analogues used to treat AIDS and the selectivity of the mitochondrial DNA polymerase". *Biochemistry*, 2003, *42*, 14711.

[27] Kirk O., Katzenstein T.L., Gerstoft J., Mathiesen L., Nielsen H., Pedersen C., Lundgren J.D.: "Combination therapy containing ritonavir plus saquinavir has superior short-term antiretroviral efficacy: a randomized trial". AIDS, 1999, *13*, F9.

[28] Marzolini C., Rudin C., Decosrterd L.A., Telenti A., Schreyer A., Biollaz J., et al.: "Transplacental passage of protease inhibitors at delivery". *AIDS*, 2002, *16*, 889.

Address reprint requests to:
E. ARAUJO JÚNIOR, PhD
Department of Obstetrics,
Federal University of São Paulo (UNIFESP)
Rua Carlos Weber, 956 apt. 113 Visage
Vila Leopoldina – São Paulo
CEP 05303-000 (Brazil)
e-mail: araujojred@terra.com.br