Effects of daily intake of zidovudine-stavudine on rat pregnancy outcome: biological essay

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Summary

Purpose: To evaluate the effects at term of a highly active antiretroviral drug association when administered for the whole period of rat pregnancy. *Methods:* Forty pregnant rats weighing about 200 g were randomly divided into four groups: a control group (Ctr = drug vehicle control, n = 10) and three experimental groups, which were treated with an oral solution of zidovudine-stavudine (Exp1x = 10/1 mg/kg b.w., n = 10; Exp3x = 30/3 mg/kg b.w., n = 10; Exp9x = 90/9 mg/kg b.w., n = 10) from "day 0" up to the 20th day of pregnancy. Maternal body weights were recorded at the start of the experiment and on the 7th, 14th and 20th day thereafter. At term (20th day) the rats were anesthetized and submitted to hysterotomy. Implantations, reabsorptions, living fetuses, placentae and intrauterine deaths were looked for and recorded. The collected fetuses and placentae were weighed and the concepts were examined by a stereoscopic microscope looking for external malformations. *Results:* No significant alterations due to the antiretroviral drug treatment could be detected regarding the number of implantations, fetuses, placentae, absorptions and malformations nor regarding maternal and fetal mortality. *Conclusions:* Administration of the association zidovudine/stavudine for the whole period of rat pregnancy did not interfere with the maternal, fetal and placental weight gain as well as abnormalities detectable by the employed methodology.

Key words: Rat; Pregnancy; Zidovudine; Stavudine; Adverse effects.

Introduction

Due to the worldwide situation of the HIV infection, taking into consideration epidemiological and clinical aspects, there is urgent necessity to know the obstetrical challenges related to such condition in HIV-infected women [1, 2].

In September 2009 the United Nation Organization published a report estimating that HIV had infected 33.4 million people. About two-thirds of such population lived in the sub-Saharian region of Africa [3, 4].

In 1994, the *Aids Clinical Trial Group* (ACTG) 076, under subvention of the *National Institute of Allergy and Infectious Diseases* of the United States of America, pointed out that zidovudine (AZT) had reduced the perinatal transmission of HIV to newborns of infected women in two-thirds [5, 6]. From this outcome, zidovudine came to be the ideal monotherapy drug for prevention of perinatal HIV transmission. Its use in different countries resulted in lower levels of vertical transmission. Later on another experiment (ACTG 175) showed that there was a desirable effect of zidovudine on pregnant women at a level of CD4 < 200 cells/mm^3 (very immunodepressive) as well as on women under use of AZT before the pregnancy period [7]. Between 1994 and 1997, other reverse transcriptase inhibitors were discovered. Among them is stavudine (d4T) which is analagous to nucleoside. Later on other classes of antiretrovirals were discovered. From that point on an associated therapeutic treatment was proposed that proved to be superior in relation to administrating one drug alone. The association resulted in a significant reduction of viral load in plasma. Sometimes the reduction of the viral load carried to undetectable levels of the virus [8, 9].

The associated therapy for women infected by HIV in fertile age has been used more and more. Due to this it is urgent to know the possible effects of such drugs on pregnancy as well as on the fetus. Because stavudine is very cheap, in spite of not being an eligible drug alone for AIDS treatment, it is largely used in some regions where such disease is prevalent and the population is very poor. Certainly one can not extrapolate from animal effects of drugs to human beings. On the other side, probably studying the effect of drugs on animal pregnancy is the best model to comprehend the effect of such drugs on humans. This experimental way of studying the effect of drugs on pregnancy was adopted by the Department of Experimental Obstetrics at the Federal University of Sao Paulo, Brazil. Drugs have been studied solely or in association with others. The aim of such studies is to verify any pharmacokinetic change of the antiretroviral drugs that can provoke adverse effects. It is well known that there are not a lot of trials of drug effects on rat pregnancy. This is one of the reasons that led us to such experiment. The choice for stavudine is related to it being an inexpensive drug and easy to use, in accordance with the WHO [10]. On the other hand, zidovudine has been systematically recommended for use in pregnancy since the ACTG 076 [11].

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Table 1. — *Effects of administration of the association zidovudine/stavudine on several indicators of rat pregnancy. Data are expressed as mean ± SEM. No fetal external malformations were observed (n = number of animals).*

	GROUPS				
	Ctrl (n = 10)	Exp1x (n = 10)	Exp3x (n = 10)	Exp9x (n = 10)	Kruskal-Wallis Test
No. of fetuses	11.30 ± 2.36	10.60 ± 2.63	10.10 ± 3.11	10.00 ± 1.63	0.330 (NS)
No. of placentae	11.30 ± 2.36	10.50 ± 2,640	10.10 ± 3.11	10.00 ± 1.63	0.362 (NS)
Litter weight (g)	43.69 ± 10.49	42.20 ± 9.22	41.27 ± 12.91	41.09 ± 6.68	0.924 (NS)
Placenta weights (g)	6,72 ± 1.70	5.74 ± 1.47	5.81 ± 1.55	6.15 ± 1.1.2	0.436 (NS)
No. of implantations	11.40 ± 2.12	10.50 ± 2.64	10.10 ± 3.11	9.70 ±1.83	0.266 (NS)
No. of reabsorptions	0	0	0	0	
Maternal deaths	0	0	0	0	
Fetal deaths	0	0	0	0	

The rats (n = 10 throughout) were treated as follows: Control (Ctrl) = drug vehicle (propyleneglycol); experimental animals were treated daily with the association zidovudine/stavudine by gavage for the whole period of pregnancy: Exp1x = 10/1 mg/kg, Exp3x = 30/3 mg/kg, and Exp9x = 90/9 mg/kg b.w. respectively.

Materials and Methods

Wistar female rats (*Rattus norvegicus albinus*) of the EPM-1 variant, with approximately 250 g of body weight, provided by the Center for the Development of Experimental Models (CEDEME) of the Federal University of Sao Paulo (UNIFESP-EPM) were used throughout. Experiments were approved (Report N°. 1516/06) by the local Animal Care Committee, following guidelines which comply with those of the Canadian Council on Animal Care [11].

The animals were kept in plastic cages under controlled room temperature set at 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 2 h. The immediate 24-h period after mating was taken as day 0 of pregnancy if spermatozoids were detected in vaginal smears [12]. Forty pregnant rats were then distributed at random into four animal groups, as follows: Ctr1 (n = 10) were rats treated daily with 0.5 ml of propyleneglycol by the oral route (drug vehicle controls); Exp1x (n = 10) were rats treated with an association of oral dose of zidovudine/stavudine corresponding to a daily dose of 10 mg/kg zidovudine plus 1 mg/kg of stavudine (zidovudine GlaxoSmithKline Laboratories, London, plus stavudine Bristol-Myers Squibb, Canada Inc); Exp3x (n = 10) were similarly scheduled rats treated daily with 30 and 3 mg/kg of stavudine, respectively. Finally, Exp9x (n = 10) rats were treated with 90 and 9 mg/kg of stavudine 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.

Body weights were recorded for all animals on day 0, 7, 14 and 20 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, number of reabsorptions, number of living and dead fetuses. The fetuses were also evaluated under a stereomicroscope for gross external abnormalities, such as congenital thoracic abnormalities, spina bifida, cleft lip/palate, micrognathia, microglossia, hydrocephalus axial skeletal deviations polydactyly, rib malformations or member abnormalities.

Whenever appropriate the data are expressed as mean ± SEM. The results were subjected to ANOVA and further analyzed 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% ($p < 0.05$).

Results and Discussion

The zidovudine/stavudine association did not interfer with the maternal body weight directly. In fact, there was maternal body gain for the whole period of pregnancy. The control and experimental groups showed a normal curve represented by Figure 1. The matrix weight gain increased gradually up to the 14th day. The weight increase continued until the 20th day of pregnancy which means that the association of drugs did not interfere with such variable. Even high-dose zidovudine did not provoke changes in maternal weight gain when used solely (60 and 100 mg/kg daily). Such result is in accordance with Mamede *et al*. [13, 14] and Figueiró Filho *et al*. [15]. Similarly to stavudine used in 1-3 mg/kg daily and solely, it did not interfere with the matrix weight gain for the whole period of pregnancy [16].

The zidovudine/stavudine association did not interfere with the number of implantations and reabsorptions (Figure 1). This outcome is similar to the research of Mamede *et al*. [13] who used zidovudine alone. On the other side, the research of Barreto *et al*. [16] that used stavudine solely showed increased reabsorptions when the administered dose was nine times the control one. This can be understood taking into consideration that zidovudine inhibits phosphorilation of stavudine. It mainly competes with stavudine in a phosphorylated way which provokes intracellular diminishing of stavudine triphosphate (active form) [17, 18]. *In vitro* studies of zidovudine/stavudine showed antagonistic results as well [19].

In relation to antiretroviral drugs it is known that reverse transcriptase inhibitors cross the syncytiovascular membrane quite well [20]. Through facilitated diffusion drugs can get to intervillous spaces, since they fulfill some conditions: molecular weight less than 800, soluble in lipids, and not conjugable. Stavudine has a molecular weight of 224.2 Da [21]. The association AZT + dT4, due to the related antagonism, diminishes the mean effective

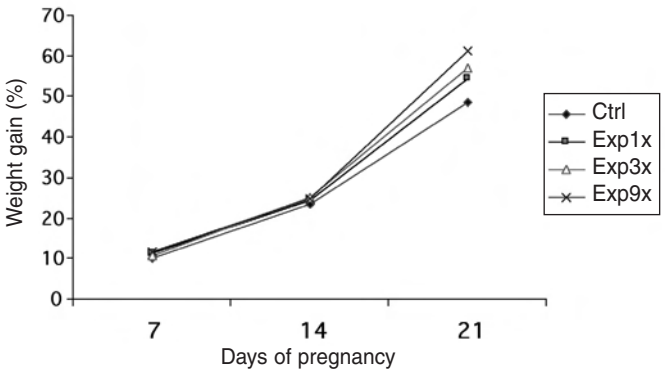

Figure 1. — Mean weight gain (%) evolution of the matrices during the whole period of pregnancy.

biodisponibility of stavudine which is 86.4% [22]. For this reason, it has a lower chance of promoting reabsorptions.

Our results showed that the association of zidovudine/stavudine had no effect on the weight, number of fetuses and/or placentae taking into consideration the three experimental groups (GExp1x, GExp3x e GExp9x) in relation to the control (Figure 1).

Zidovudine crosses the placenta barrier by simple diffusion at plasmatic levels similar to those observed in the maternal [23]. Crossing through the placenta is by passive diffusion. It is known that zidovudine is not able to change the placenta and fetus weight nor the fetus and placenta number, except in Figueiró *et al.'s* [15] research. In accordance with such authors diminishing of the number of fetuses was observed when zidovudine was used in a higher dose, i.e., ten times the therapeutic dose [13, 24, 25]. Research on the effect of zidovudine used solely on Wistar pregnant rats showed that it does not interfere with mean fetal and placenta weight and the number of fetuses and placentae even in high doses (9 times higher than the therapeutic dose). Only the group which had an intake of ten times the therapeutic dose showed diminishing of fetal weight and the number of fetuses [13, 15]. In our research stavudine alone taken at 1, 3 and 9 mg/kg daily had no effect on the fetus and placenta weight. In short, it had no adverse effect on albino rat pregnancy when taken in 1 and 3 mg/kg daily. But at 9 mg/kg daily there was increase in implantations and reabsorptions [16].

At the same time such association of drugs neither caused any malformation nor maternal and fetal deaths (Table 1). Other researchers administered zidovudine or stavudine alone for the whole period of albino rat pregnancy but observed no malformation or fetal deaths [13, 16].

Expansion of the use of associated antiretroviral drugs in human pregnancy diminished the occurrence of perinatal HIV infection leading to levels lower than 2%. On the other hand, this has brought into discussion the possibility of adverse effects of such associations on pregnancy immediately, and the impact on women and their children in the future [26].

Taking into consideration the modern antiretroviral triple therapy for HIV infection, it is often difficult to visualize the sort of interactions among the used drugs and which drugs are responsible for this or that effect. This challenge may be overcome by future biological assays using animal models when associated antiretroviral drugs can be used for a better comprehension of their effect on maternal and fetal bodies.

References

[1] Chirenje Z.M., Marrazzo J., Parikh U.M.: "Antiretroviral-based HIV prevention strategies for women". *Expert. Rev. Anti. Infect. Ther*., 2010, *8*, 1177.

[2] Mirochnick M., Best B.M., Clarke D.F.: "Antiretroviral pharmacology: special issues regarding pregnant women and neonates". *Clin. Perinatol*., 2010, *37*, 907.

[3] Negin J., Cumming R.G.: "HIV infection in older adults in sub-Saharan Africa: extrapolating prevalence from existing data". *Bull. World. Health. Organ.,* 2010, *88*, 847.

[4] Anema A., Au-Yeung C.G., Joffres M., Kaida A., Vasarhelyi K., Kanters S. *et al*.: "Estimating the impact of expanded access to antiretroviral therapy on maternal, paternal and double orphans in sub-Saharan Africa, 2009-2020". *AIDS. Re.s Ther*., 2011, *8*, 13.

[5] Connor E.M., Sperling R.S., Gelber R., Kiselev P., Scott G., O'Sullivan M.J. *et al*.: "Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group". *N. Engl. J. Med*., 1994, *331*, 1173.

[6] Khandazhinskaya A., Matyugina E., Shirokova E.: "Anti-HIV therapy with AZT prodrugs: AZT phosphonate derivatives, current state and prospects". *Expert. Opin. Drug. Metab. Toxicol*., 2010, *6*, 701.

[7] Lambert J.S., Nogueira S.A.: "Manual para o acompanhamento clínico da gestante infectada pelo HIV"**.** Programa de Assistência Integral à Gestante HIV. 4ª edição. Rio de Janeiro: Positiva, World Aids Foundation/UFRJ, 2000.

[8] Cooper A.J., Johnson C.D.: "Animal experimentation". *Br. J. Surg*., 1991, *78*, 1409.

[9] De Clercq E. "Antiretroviral drugs". *Curr. Opin. Pharmacol*., 2010, *10*, 507.

[10] Tam L.W., Hogg R.S., Yip B., Montaner J.S., Harrigan P.R., Brumme C.J.: "Performance of a World Health Organization first-line regimen (stavudine/lamivudine/nevirapine) in antiretroviral-naïve individuals in a Western setting". *HIV. Med*., 2007, *8*, 267.

[11] Olfert E.D., Cross B.M., McWilliam A.A. (eds.): Canadian Council on Animal Care Guide to the Care and Use of Experimental Animals". Ottawa, Canada: Bradda, 1993, Vol. 1.

[12] Wagner A., Carvalho A.M., Nakamura M.U., Simões R.S., Fontes T.M., Espiridião S. *et al*.: "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.

[13] Mamede J.A.V., Simões M.J., Novo N.F., Juliano Y., Oliveira R.M., Kulay L. Jr.: "Cronic effects of azidothymide and acyclovir on pregnant rats". *Gen. Pharm*., 1995, *26,* 523.

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

[15] Figueiró-Filho E.A., Duarte G., Silva A.A.M.R., Fonseca B.A.L., Mussi-Pinhata M.M., Quintana A.S. *et al*.: "Efeitos de drogas antiretrovirais sobre taxas de fertilidade de ratas Wistar". *Rev. Bras. Ginecol. Obstet*., 2002, *24*, 647.

[16] Barreto R.L., Jesus Simões M., Amed A.M., Soares J. Jr., Oliveira-Filho R.M., Kulay L. Jr.: "Stavudine effects on rat pregnancy outcome". *J. Obstet. Gynecology. Res.,* 2004, *30*, 242.

[17] Balzarini J., Herdewijn P., De Clercq E.: "Differential patterns of intracellular metabolism of 2',3'-didehydro-2',3'-dideoxythymidine and 3'-azido-2', 3'-dideoxythimidine, two potent anti-human imunodeficiency vírus compounds". *J. Biol. Chem*., 1989, *264*, 6127.

[18] Hitchcock M.J.M.: "2',3'-didehydro-2',3'-dideoxythymidine (dT4), an anti-HIV agent". *Antiviral. Chem. Chemother.,* 1991, *2*, 125.
[19] Sorensen A.M., Nielsen C., Mathiesen L.R., Nielsen J.O., Hansen J.E.: "Evaluation of the combination effect of different antiviral compounds against HIV *in vitro"*. *Scand. J. Infect. Dis.,* 1993, *25*, 365.
[20] Pavek P., Staud F., Fendrich Z., Sklenarova H., Libra A., Novotna M. *et al*.: "Examination of the functional activity of P-glycoprotein in the rat placental barrier using rhodamine 123". *J. Pharmacol. Exp. Ther*., 2003, *305*, 1239.
[21] Grasela D.M., Stoltz R.R., Barry M., Bone M., Mangold B., O'Grady P. *et al*.: "Pharmacokinetics of single-dose oral stavudine in subjects with renal impariment and in subjects requiring hemodialysis". *Antimicrob. Agents. Chemother*., 2000, *44*, 2149.
[22] Kaul S., Mummaneni V., Barbhaiya R.H.: "Dose proportionality of stavudine in HIV seropositive asymptomatic subjects: application to bioequivalence assessment of various capsule formulations". *Biopharm. Drug. Dispos*., 1995, *16*, 125.
[23] Pizzo P.A.: "Considerations for the evalution of antiretroviral agents in infants and children infected with HIV: a perspective from the National Cancer Institute". *Infect. Dis*., 1990, *12*, 5561.
[24] Litle B.B., Bawdon R.E., Christmas J.T., Sobhi S., Gilstrap L.C.: "Pharmacokinetics of azidothymidine during late pregnancy in long Evans rats". *Am. J. Obstet. Gynecol*., 1989, *161*, 732.
[25] Greene J.A., Ayers K.M., De Miranda P., Tucker Jr. WE.: "Postnatal survival in Wistar rats following oral dosage with zidovudine on gestation day 10". *Fundam. Appl. Toxicol*., 1990, *15*, 201.
[26] Watts D.H.: "Treating HIV during pregnancy: na update on safety issues". *Drug. Safe*., 2006, *29*, 467.

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