A morphological and biochemical appraisal of the liver and renal effects of lamivudine on rat pregnancy

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Summary

No data exist on the perinatal safety of lamivudine alone, as it is used in combination with other antiretroviral agents. Until now, only preliminary data on the lamivudine-zidovudine combination have been available, thus we decided to examine the gross maternal and fetal effects of lamivudine administered alone during the entire period of rat pregnancy. Forty pregnant animals were assigned at random to four groups (C1= control; E1 = 5 mg/kg; E2 = 15 mg/kg; E3 = 45 mg/kg) from day 0 up to the 20th day of gestation. These doses were divided into two daily administrations by gavage. Controls (n = 10) received distilled water in the same schedule. At term-pregnancy, the rats were deeply anesthetized and blood samples were collected for alanine and aspartate aminotransferases, creatinine and urea determinations. Fragments of maternal and fetal livers and kidneys were taken and processed for histopathological study. In all groups blood transaminases were within the normal limits, as were the levels of creatinine and urea, thus indicating that treatment with lamivudine during the entire gestation was essentially devoid of liver or kidney effects which could result in altered metabolic parameters. Morphological (light microscopy) studies revealed that no significant effects of the drug could be detected regarding either maternal or fetal organs of the E1 and E2 groups. However, the maternal hepatocytes in the E3 group showed heterochromatic nuclei. In addition, there was some fatty infiltration, congested sinusoids and portal dilatation. Maternal kidneys in the E3 group revealed vascular dilation around the convoluted tubules. It is concluded that only doses of lamivudine used during the entire gestation in doses well above the usual human doses could be considered to be potentially hepatotoxic for the pregnant rat.

Key words: Lamivudine; Toxicology; Rat; Pregnancy.

Introduction

The human acquired immunodeficiency syndrome (AIDS) was initially restricted to a small group of homosexual men [1-3]. Within months, the disease became recognized in other human groups (drug users, hemophiliacs) and, despite continuing medical and scientific efforts, it is now evident that virtually every practicing physician in the world will be required to have some degree of familiarity with the workup, diagnosis, and treatment of HIV-infected individuals [4].

In the last two decades there have been about 20 million victims of AIDS; there are cur-rently 42 million infected people, from which 19.7 million are women in reproductive age [5]. This fact implies the occurrence of maternal-fetal transmission (MFT) of HIV [6, 7] in 20% of untreated pregnancies [8]. Among the management protocols for attenuating MFT (elective cesarian section, breast-feeding elimination, anti-HIV therapy), the most important one is the use of antiretroviral drug therapy during the pregnant-puerperal cycle [9], because it reduces the viral load and alleviates maternal immunodepression [10].

The AIDS Clinical Trials Group (ACTG) 076 study concluded that in pregnant women with mildly symptomatic HIV disease, zidovudine reduced the risk of maternal-infant HIV transmission by approximately two thirds, with minimal short-term toxic effects [11]. Since then, not only has the use of antiretroviral drugs during pregnancy been highly recommended but also the maintenance of the therapeutic protocols of HIV-positive women starting at the 14th week of gestation [12]. The use of two antiretroviral drugs has reduced MFT down to 3.4% and, more recently, the use of highly active antiretroviral therapy (HAART) with three or more drugs, one of them being a protease inhibitor agent, produced reductions of MFT to about 1.2% of the cases [13].

A cohort study comprising 2,123 women who received antiretroviral therapy during pregnancy (monotherapy in 1,590, combination therapy without protease inhibitors in 396, and combination therapy with protease inhibitors in 137) and 1,143 women who did not re-ceive antiretroviral therapy revealed that, as compared with no antiretroviral therapy or monotherapy, combination therapy for HIV-1 infection in pregnant women is not associated with increased rates of premature delivery or with low birth weight, low Apgar scores, or stillbirth in their infants [14]. In this context, however, care must be taken when balancing the risks and benefits of such treatments for both mothers and their children [9].

In a previous paper from our laboratory, pregnant albino rats treated during the entire pregnancy with lamivudine did not show any adverse effects concerning maternal body weight gain or fetal and placental weights

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Fig. 1

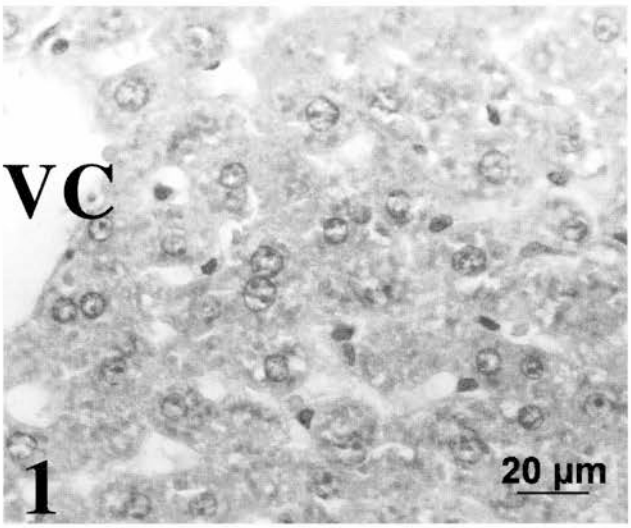

Fig. 2

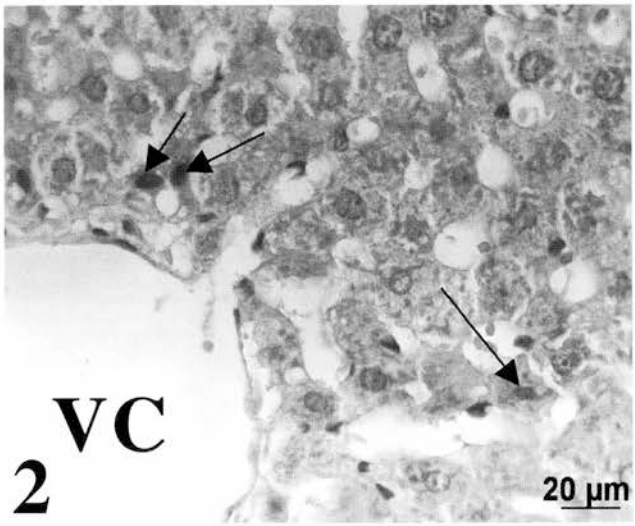

Fig. 3

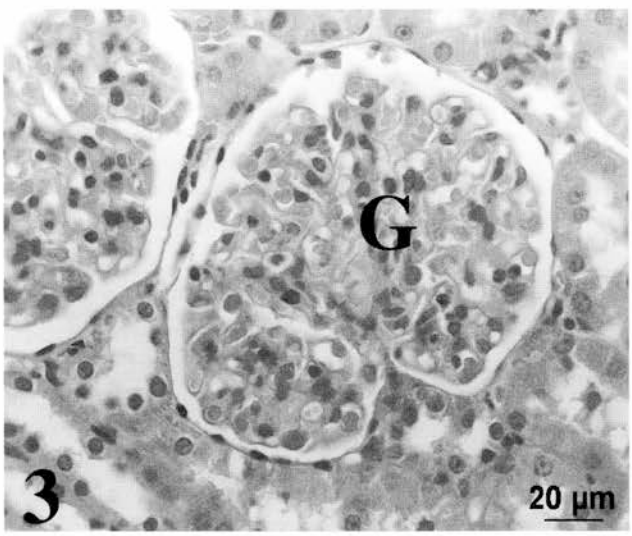

Fig. 4

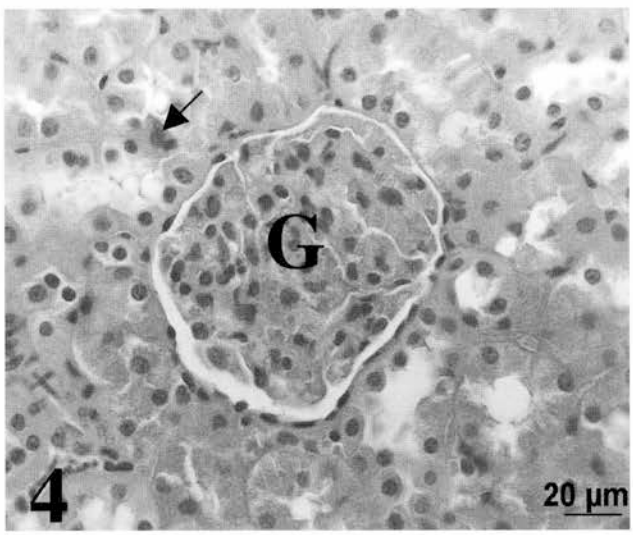

Figure 1. — Photomicrograph showing part of a hepatic lobule of a rat that had been treated with 15 mg/kg bw of lamivudine during the entire period of pregnancy (E2). Vc = lobular central vein; arrow = conserved hepatocyte.

Figure 2. — Photomicrograph showing part of a hepatic lobule of a rat that had been treated with 45 mg/kg of lamivudine during the entire period of pregnancy (E3). Vc = lobular central vein; arrows = hepatocytes with irregular and heterochromatic nuclei showing signs of apoptosis.

Figure 3. — Photomicrograph showing part of a glomerulus (G) of a pregnant rat in group E2 (25 mg/kg) showing a normal glomerulus.

Figure 4. — Photomicrograph showing part of a glomerulus of a pregnant rat in group E3 (45 mg/kg) showing glomerulus (G) and convoluted tubules containing heterochromatic nuclei (arrow).

at term; also, no increase of fetal reabsorptions, gross malformations or intrauterine deaths were observed [15]. However, we could not discard the occurrence of more subtle, cellular-level toxic effects of lamivudine when administered during the entire pregnancy period, especially taking into account its hepatic metabolism and renal excretion [16, 17]. Particularly the possibility of hepatitis flares, hepatic decompensation and liver-disease-related adverse effects during long-term lamivudine treatment should be taken into account [18]. Thus, the present paper was undertaken to examine directly these compartments.

Material and Methods

Animals and treatments

The local Institution's guidelines for the care and use of the animals are similar to those of the Canadian Council on Animal Care (CCAC) and the NIH's Institutional Animal Care and Use Committee Guidebook. The experimental protocol was approved by the local Ethics Committee on Animal Experimentation. Female adult virgin, EPM-1 Wistar rats were selected after three regular consecutive estrous cycles and kept under specific pathogen-free conditions at a constant day/night cycle (lights on 07:00-19:00). Animals were fed Purina® pelleted rat food and tap water *ad libitum*. The animals were mated

in the proportion of one healthy male to two females overnight. The immediate 24-hour period after mating was taken as day 0 of pregnancy if spermatozoids were detected in vaginal smears [19]. Forty pregnant rats were then randomly divided into four groups with ten animals each, one control (C, treated with the drug vehicle) and three experimental drug-treated groups: E1, E2 and E3 (treated daily with 5, 15 or 45 mg/kg of nelfinavir dissolved in distilled water, respectively). Drug or vehicle was given in one daily administration. The treatment started on day 0 and continued until the 20th day of pregnancy.

Sampling

At term, the animals were anesthesized with 20 mg/kg of sodium thiopental IP and 20 mg/kg of xylazine IP. Upon thoraco-laparotomy, 4 ml of maternal blood were taken directly from a ventricular chamber for further biochemical determinations: aspartate (AST) and alanine (ALT) aminotransferases [20], creatinine [21] and urea [22]. Maternal and fetal samples of livers and kidneys were taken and fixed in buffered 10% formaldehyde for fur-ther routine processing, hematoxylin-eosin staining and light microscopy study.

Statistical analyses

The Kruskal-Wallis test for independent samples was used. The statistically significant differences were further analyzed by Dunn's multiple comparisons test [23].

Results and Discussion

Though less than 10% of an oral dose of lamivudine is metabolized at the hepatic level [17], some definite adverse effects on the liver, although controversial, have been reported. Interestingly, apart from being well tolerated, it not only reduced the necrosis and inflammation due to hepatitis B but also reverted liver fibrosis after three years of treatment [24].

The rate of hepatic metabolization of lamivudine is lower than 10% [17], and no dosing adjustments are needed in patients with liver disease; moreover, it can be administered after liver transplantation [25]. In contrast, there have been reports of hepatomegalia followed by liver steatosis [26-28], in addition to an about 5.7% incidence of severe hepatic cytolisis in 249 cases treated with this drug [29].

Among the groups of rats used in our study, only the livers of the animals which had been treated with the highest dose of lamivudine (45 mg/kg body weight) during the entire period of pregnancy showed hepatocytes with irregular and heterochromatic nuclei around dilated portal spaces, vascular sinusoid congestion and signs of apoptosis (Figures 1 and 2). With this dosing there were also significant increases of AST and ALT activities (Table 1) which may be viewed as predictive of 75-100% of the cases of histopathological liver changes [30].

There are less conflicting data regarding the effects of lamivudine on maternal kidneys, a route by which 70% of the unaltered drug is excreted. Though it can be administered to patients with kidney transplants [25], the doses of lamivudine must be adjusted in cases of renal failure [31]. Notwithstanding, the occurrence of tubular acidosis with hypophos-phatemia described by Morris *et al.* [32] may be rather consequent to AIDS itself, as has been pointed out by Glassok *et al.* [33].

As occurred with the liver, renal alterations were observed only in the group treated with the highest dose of lamivudine (45 mg/kg body weight, group E_3). An intense vascular dilation could be seen around the convoluted tubules as well as altered tubules (proximal convoluted tubules bearing heterochromatic nuclei and eosinophilic cytoplasm) (Figures 3 and 4). These findings were paralleled by significantly raised blood levels of creatinine and urea (Table 1).

Table 1. — *Effects of treatment with lamivudine during the entire period of rat gestation on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and on the levels of urea and creatinine in maternal blood at term. Values are mean ± SEM of determinations in duplicate.*

Groups	AST mU/ml	ALT mU/ml	Urea mg/100 ml	Creatinine mg/100 ml
C	71.4 ± 3.9	119.6 ± 10.5	51.3 ± 1.9	0.50 ± 0.01
E1	73.2 ± 4.3	154.0 ± 24.6	56.1 ± 2.1	0.52 ± 0.05
E2	71.5 ± 3.3	155.0 ± 14.6	52.3 ± 2.7	0.51 ± 0.02
E3	101.7 ± 6.6*	223.1 ± 20.3*	63.2 ± 2.1*	0.73 ± 0.05*

Groups of pregnant rats (n = 10 animals in every group) were treated once daily during the entire gestation with lamivudine dissolved in distilled water as follows. E_1 = 5 mg/Kg; E_2 = 15 mg/Kg; E_3 = 45 mg/Kg. Control (C) rats were treated with the drug vehicle. *$p < 0.05$ with regard to the group C.

Lamivudine is a low molecular weight drug (229 D) and crosses the placenta by simple diffusion [34] without causing adverse effects to the concept with doses 130 times those used in regular therapeutic protocols [35]. Accordingly, we could not notice any alteration either in fetal livers or kidneys of the animal groups studied.

In conclusion, although lamivudine can be viewed as a relatively safe alternative for the control of HIV infection during pregnancy, there seems to be a link between the blood bio-chemical alterations and the cellular changes elicited by the drug on the liver and kidney maternal compartments when administered to rats during the entire period of pregnancy.

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