Morphological and biochemical appraisal of the liver and renal effects of indinavir on rat pregnancy

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Summary

Since indinavir is currently used in combination with other antiretroviral agents, there is a scarcity of studies in the literature on its single-drug perinatal safety. Thus, we decided to examine the gross maternal and fetal effects of indinavir administered alone during the entire period of rat pregnancy. Forty pregnant animals were assigned at random to four groups (C = control) treated with the drug vehicle (distilled water); the experimental groups were treated with indinavir as follows: E1 = 40 mg/kg; E2 = 120 mg/kg; E3 = 360 mg/kg from "zero" up to the 20th day of gestation. Drug or vehicle were administered daily by gavage. Each group consisted of ten animals. At term-pregnancy, the rats were deeply anesthetized and blood samples were collected for alanine aminotransferase (ALT) and aspartate aminotransferase (AST), creatinine and urea determinations. Fragments of maternal and fetal livers and kidneys were taken and routinely processed for histopathological study. Serum ALT activity in the E2 group was significantly higher ($p < 0.01$) than that of the other groups. The concentration of creatinine in blood was lower in the E2 and E3 groups than in group E1 ($p < 0.01$), whereas blood urea in group E3 was significantly lower than in the other groups ($p < 0.01$). 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 dilation. Maternal kidneys in the E2 and E3 groups revealed vascular dilation around the convoluted tubules. Regarding the biochemical determinations, the alterations observed were mild, without biological relevance, thus indicating that the treatment with indinavir during the entire gestation was essentially devoid of hepatic or renal effects which could result in altered metabolic parameters. It is concluded that indinavir was well tolerated in therapeutic and even in 9-fold higher doses. Notwithstanding, discrete morphological alterations occurred in the maternal compartment, but with no functional expression that could indicate deleterious effects on mothers and/or fetuses.

Key words: Indinavir; Toxicology; Rat; Pregnancy.

Introduction

About 25 years ago, when the acquired immunodeficiency symdrome (AIDS) was described [1] to be caused by the human immunodeficiency virus HIV [2, 3], just a few number of small homosexual groups were involved [4, 5].

Upon contact with mucous membranes, blood or hemoderivatives from HIV-positive individuals, there was a widespread contamination of both hetero- and bisexual people, thence ensuing a true pandemic. There are currently about 40 million people infected around the world, and 20 million deaths have been recorded [5]. As a consequence, the number of women with AIDS rapidly increased. Particularly in Brazil, the infected men:women ratio increased from 82:1 in 1984 to 4:1 in 1993; currently this ratio is presumedly close to 1:1 [6].

Most women are infected during the reproductive period of life [7], thus favoring maternal-fetal transmission in about 20% of pregnancies [8]; about 50 up to 70% of the cases occur close to or during delivery, either through the placenta [9] or the genital tract [10]. Infection also occurs during breast-feeding [11]. In addition to the recommended procedures to avoid MFT such as elective cesarean delivery and breast-feeding interruption [12], the use of highly effective antiretroviral (ARV) therapy starting at the 12th week of gestation is extremely important [13]. The correctness of such scheme has been well evidenced starting with the use of zidovudine during pregnancy, thereby reducing the incidence of AIDS among newborns to HIV-infected mothers by about 50% [14].

There is currently a consensus about the advantages of associating several antiretroviral drugs (ARV) drugs (one of them being necessarily a protease inhibitor) in the so-called highly active antiretroviral treatment (HAART) [15]. As compared with no antiretroviral therapy or monotherapy, combination therapy for HIV-1 infection in pregnant women was not found to be associated with increased rates of premature delivery or with low birth weight, low Apgar scores, or stillbirth in their infants [16]. On the other hand, it must not be ruled out that drug pharmacokinetics can be importantly affected when two or more specific agents are concomitantly administered [17].

Accordingly, due to the scarcity of experimental data in the literature on the toxicity of ARV protease inhibitors, we were prone to evaluate the chronic effects of indinavir administered as monotherapy during the entire length of the rat pregnancy.

Revised manuscript accepted for publication March 26, 2007

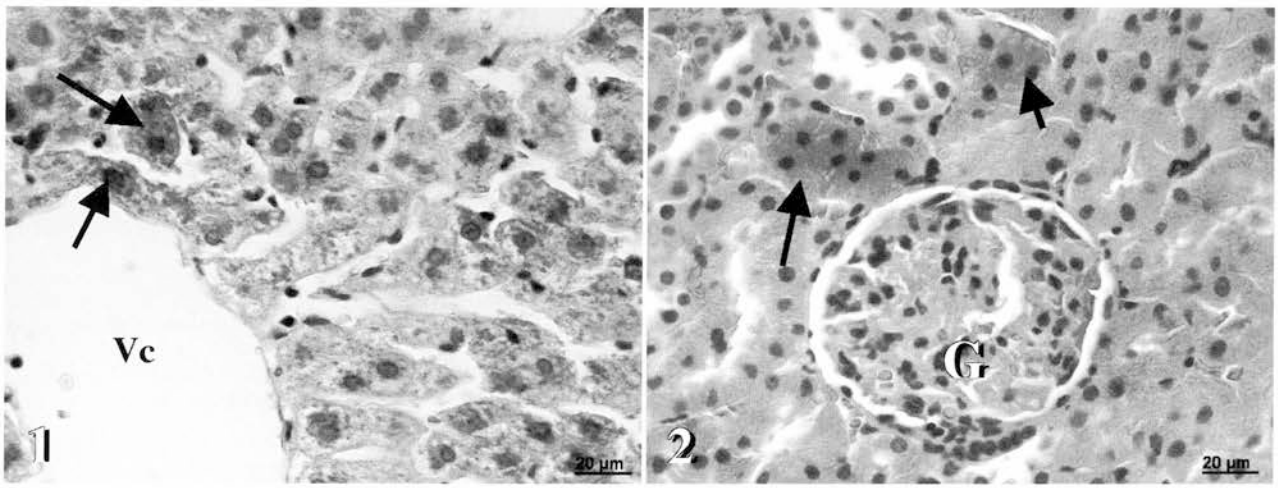

Figure 1. — Photomicrograph showing part of the hepatic lobule of a rat treated with 360 mg/kg BW of lamivudine during the entire period of pregnancy (group E3). Vc = lobular central vein; arrow = hepatocytes with irregular and heterocromatic nuclei.
Figure 2. — Photomicrograph showing part of the kidney stroma of a pregnant rat in group E3 showing glomerulus (**G**) and a proximal convoluted tubulus containing eosinophilic areas (arrow) with heterochromatic nuclei.

Material and Methods

Animals and treatments

The local Institution's guidelines for the care and use of animals were followed; these guidelines comply with those of the Canadian Council on Animal Care [18] 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 from 07:00–19:00). Animals were fed Purina® pelleted rat food and tap water *ad libitum*. The animals were mated overnight in the proportion one healthy male to three females. The immediate 24-h period after mating was taken as 'day zero' of pregnancy provided 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: E_1, E_2 and E_3 (treated daily with 40, 120 or 360 mg/kg of indinavir dissolved in distilled water, respectively). Drug or vehicle were given once a day by gavage. The treatment was started on day 'zero' and extended until the 20th day of pregnancy.

Sampling

At term the animals were deeply anesthesized with ketamine (100 mg/kg, IP) and xylazine (20 mg/kg, IP). Upon thoracolaparotomy, 4 ml of maternal blood was taken directly from a ventricular chamber for further biochemical determinations: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [20], creatinine [21] and urea [22]. 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.

Statistical analysis

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

Results and Discussion

Regarding the maternal livers, we observed that there were discrete foci of hepatic steatosis, eosinophilic cytoplasm bearing pyknotic nuclei, as well as a great number of mitotic figures and vascular dilation only among the rats treated daily with 360 mg/kg BW indinavir (group E3) (Figure 1). However, these lesions were not followed by any alterations of the plasma levels of ALT activity (Table 1). On the other hand, ALT was significantly increased in group E2, which was treated daily with 120 mg/kg BW indinavir (Table 1), and this coexisted with absence of any detectable hepatic lesion on light microscope examination.

Table 1. — *Effects of treatment with indinavir 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 determination in duplicate.*

	Group			
	C	E1	E2	E3
AST (mU/ml)	71.4 ± 3.9	70.3 ± 4.3	74.3 ± 3.3	73.4 ± 4.3
ALT (mU/ml)	119.6 ± 15.3	129.8 ± 14.6	164.2 ± 19.1*	134.5 ± 18.2
Urea (mg/100 ml)	51.3 ± 1.9	53.2 ± 2.7	52.3 ± 2.1	45.8 ± 3.2*
Creatinine (mg/100 ml)	0.50 ± 0.01	0.51 ± 0.03	0.46 ± 0.02*	0.44 ± 0.04*

Groups of pregnant rats (n = 10 in every group) were treated once daily during the entire gestation with indinavir dissolved in distilled water as follows. E1 = 40 mg/Kg; E2 = 120 mg/Kg; E3 = 360 mg/Kg. Control (C) rats were treated with the drug vehicle (* $p < 0.01$ with regard to group C).

As a hepatocyte-specific enzyme, ALT activity can be regarded as predictive of a hepatic lesion [24] only when its levels rise 2-fold higher than the upper normal limits [25], and this was found herein. Therefore, it is conceivable that the observed cell lesions were not severe enough to interfere with the liver function. This assumption is further supported by the low levels of AST activity detected in the serum. Alterations of this enzyme may

reflect cell lesions in many organs and tissues, including the liver, but AST remained within the normal values in all the studied groups (Table 1).

The rate of renal excretion of unmetabolized indinavir is about 20% [26]. It should be noted that the kidneys of the rats treated with indinavir at doses equivalent to the human daily therapeutic dose (40 mg/kg BW, group E1) did not show any structural alterations. The kidneys of the rats treated with 3-fold higher doses of indinavir (group E2) showed focal alterations of the convoluted tubules, eosinophilic areas and hyperchromatic nuclei; discrete areas of vasodilation were also observed. In the E3 group (treated daily with 360 mg/kg BW indinavir) the histological appearance was similar to that of group E2 but even more intense. Moreover, there was connective tissue proliferation in the glomeruli along with difuse vasodilation (Figure 2).

With severe renal lesions, it is known that the plasma levels of urea are altered early, and those of creatinine later [27]. Notwithstanding, we did not find any alteration of these metabolites in any of the studied groups (Table 1). Interestingly, the animals bearing more conspicuous renal alterations at the histological level showed lowered levels of urea and creatinine (Table 1). It can be presumed that the observed dilation of intrinsic renal vasculature could be accounted for by the observed normal or increase of metabolite excretion.

Regarding the effects on the intrauterine compartment, a 712-dalton weight molecule of indinavir [26] can cross the placental barrier. However, this seemed not to be the case in our experiments, most conceivably due to the operation of glycoprotein P (PGp) [28], which is expressed in rat syncytiotrophoblasts. PGp is one of a family of multidrug resistence encoded genes (mdz1, mdz2, mdz3) [29], pertaining to the ATP protein superfamily binding cassette [30], that acts to block the travel of protease inhibitor ARV drugs onto the conceptus, including indinavir [31]. Accordingly, this fact may be responsible for the observed structural integrity of the livers and kidneys of the fetuses from rats in our experimental groups.

In conclusion, the treatment of rats with indinavir during the entire length of pregnancy with daily doses as high as 9-fold those recommended for human use produced some lesions at the hepatic level, but was not able to interfere with the normal development of fetal organs.

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