

Safety of nelfinavir use during pregnancy. An experimental approach in rats

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Summary

This experimental study aimed to evaluate the safety of nelfinavir when administered in normal up to high doses during the entire period of rat pregnancy. The renal and liver compartments of both mothers and fetuses were studied. For this purpose, three groups of pregnant rats were treated with nelfinavir ($E_1 = 40$ mg/kg; $E_2 = 120$ mg/kg; $E_3 = 360$ mg/kg; no. = 10 in every group) from "zero" up to the 20th day of gestation. These doses were divided into two daily administrations by gavage. Controls (no. = 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 the treatment with nelfinavir 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 E_1 and E_2 groups. However, the maternal hepatocytes in the E_3 group showed heterochromatic nuclei. In addition, there was some fatty infiltration, congested sinusoids and portal dilatation. It is concluded that only doses of nelfinavir 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: Nelfinavir; Toxicology; Pregnancy; Rat.

Introduction

By December 2003 the World Health Organization estimated that 40 million people were contaminated with HIV - 37 million adults and 2.5 million subjects under 15 years of age. In the last 25 years acquired immunodeficiency syndrome (AIDS) has become the main cause of death in the age range of 15-59 years around the world. In the early 1980s, the infection occurred in about 100 men per one woman. Later, this proportion was progressively altered; currently a 3:1 and even a 1:1 man: woman contamination rate in certain Brazilian cities can be found. Among the 13-19 age group, however, that relationship may be inverted to be 1.2 females per 1 contaminated male [1]. When HIV infection occurs during the reproductive years there is great concern about vertical transmission, which may be responsible for as much as 80% of the HIV-positive cases in Brazil [2]. Thus, an enormous effort has been directed towards finding effective antiviral agents; the importance of their safe use during gestation should not be minimized [3].

By 1994 the results of the AIDS Clinical Trials Group Protocol 076 pointed to a 67.5% reduction of HIV-vertical transmission with the use of zidovudine during gestation and delivery [4]. The current consensus on this issue is that the combined antiretroviral schedules must contain, as much as possible, zidovudine and lamivudine associated with nelfinavir or nevirapine. The choice for each of these afore-mentioned drugs is based on gesta-

tional age, degree of maternal immunodeficiency, viral load, patient compliance and the use of other drugs. In this context, nelfinavir has been proven to be effective as an antiviral agent for gestational ages under 28 weeks and for more severely immunodepressed women [5, 6].

Even if doses of nelfinavir as high as 1,000 mg/kg daily from the 6th up the 17th day of rat gestation did not result in embryo-fetal toxicity [7], the possibility of glucose metabolism disturbances has been suggested [8, 9]. Thus, the effects of nelfinavir when administered for the entire gestation are a matter of concern. Accordingly, in the present paper we evaluated the possible anatomo-functional repercussions of nelfinavir administration during the entire period of rat gestation on the livers and kidneys of both maternal and conceptual compartments.

Material and Methods

Animals and treatments

The guidelines of the local Institution for the care and use of animals were followed; these guidelines 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 one healthy male to three females overnight. The immediate 24-hr period after mating was taken as day 0 of pregnancy if spermatozooids were detected in vaginal smears [10]. 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 40, 120 or 360 mg/kg of nelfinavir dissolved in distilled water, respectively). Drug or vehicle were given in two daily divided administrations. The treatment started on day 0 and continued until the 20th day of pregnancy.

Sampling

At term the animals were deeply anesthetized with ketamine (100 mg/kg, IP) and xylazine (20 mg/kg, IP). At laparotomy, 4 ml of maternal blood was taken directly from the ventricular chamber for further biochemical determinations: aspartate (AST) and alanine (ALT) aminotransferases [11], creatinine [12] and urea [13]. 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 analyses

The Kruskal-Wallis test for independent samples was used. The statistically significant differences were further analyzed by multiple comparisons (Dunn's test) [14].

Results and discussion

With doses of nelfinavir similar to those used in humans (40 mg/kg, group E₁), the histological appearance of maternal livers were indistinguishable from that of the control group. In group E₂ some hepatocytes with slight heterochromatic nuclei and low degree of fatty infiltration were observed. In the group treated with the highest dose (E₃) signs of toxicity were evidenced by many hepatocytes with heterochromatic nuclei and well-established fatty infiltration (Figure 1); there was also sinusoid congestion and dilatation of the portal spaces. These findings indicate that nelfinavir displays a definite dose-dependent hepatotoxicity as pointed out by Minkoff

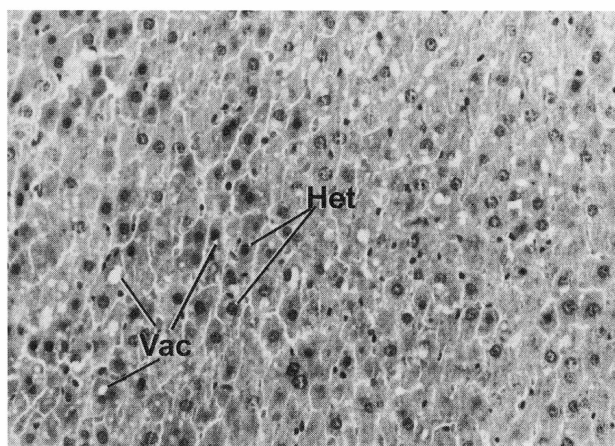


Figure 1. — Photomicrograph of a typical histological section of a rat liver from the group treated with nelfinavir (360 mg/kg per day during the entire pregnancy) (group E₃). Observe that a large number of hepatocytes bear heterochromatic nuclei ("Het"); also, a well-established fatty infiltration can be diagnosed in view of the high concentration of lipid vacuolization ("Vac"). H&E staining (140 x).

& Hugenbraun [15]. Similar findings were described by Sulkowski *et al.* [16] in patients treated with that class of drugs.

The histopathological pictures were only barely paralleled by the alterations of transaminases (Table 1). In fact, group E₃ showed some, but not significant, increase of hepatic ALT activity; in other words, it is presumable that the hepatic alterations were maintained below the levels needed to exceed the liver reserve capacity as it occurs, for example, in hepatitis or hepatic cirrhosis [17]. On the other hand, since AST is highly represented in extrahepatic tissues as well, the significantly raised levels of blood AST activity (Table 1) – even in the group treated with the lowest dose of nelfinavir (group E₁) – correlate well with the known adverse effects of this drug at the muscle compartment level [18].

Table 1. — Transaminases (AST and ALT), urea and creatinine in the blood at term (20th day) pregnant rats. Values are mean \pm SEM of 10 determinations in duplicate.

Group	AST U/L	ALT U/L	Urea mg/dL	Creatinine mg/dL
C	175.7 \pm 16.5	82.0 \pm 5.0	57.8 \pm 1.5	0.67 \pm 0.02
E ₁	255.2 \pm 25.9*	77.8 \pm 2.8	59.9 \pm 2.5	0.80 \pm 0.03**
E ₂	253.6 \pm 59.3*	89.1 \pm 9.8	58.3 \pm 2.1	0.69 \pm 0.03
E ₃	371.7 \pm 143.7*	101.3 \pm 12.3	53.1 \pm 1.5	0.62 \pm 0.02†

C (control) group animals were treated with drug vehicle (distilled water); the experimental groups (E₁, E₂, and E₃) were rats treated throughout the entire gestation with nelfinavir by gavage (40, 120 or 360 mg/kg per day, respectively, divided in two daily administrations). In every group, no. = 10. * $p < 0.05$ for controls; ** $p < 0.05$ for C and E₂; † $p < 0.05$ for E₁.

AST = aspartate aminotransferase; ALT = alanine-aminotransferase.

Regarding the maternal kidneys, the absence of alterations of cortical and medullary structures for all the experimental groups is paralleled by unaltered renal function indices (Table 1). Blood creatinine values in the E₁ group were slightly increased with regard to the other groups, but the pharmacological meaning of this finding is at present uncertain. It is conceivable that the relative absence of nephrotoxicity may be due, at least in part, to the low level of renal excretion of the drug. In fact, there is no recommendation for dosing adjustments in patients with renal insufficiency [19].

Although nelfinavir can cross the placental barrier, we did not observe any architectonic alterations in fetal livers or kidneys of the experimental groups. This can be ascribed to the action of P-glycoprotein [20], a family of transporter proteins with three members in rodents (mdr1, mdr2 and mdr3) and two members in humans (MDR1 and MDR2). In rat placenta, P-glycoproteins are expressed in the syncytial trophoblast [21], and act to exclude a series of chemically unrelated toxins and drugs, including antineoplastic and protease inhibitors antiretroviral agents from the organism [22] by means of the ABC family of transporters [23]. Expression of the protein is proportional to duration of treatment and fall in intracellular drug concentrations [24] in order to protect tissues and systems from xenobiotics.

In conclusion, the administration of nelfinavir even in high doses to rats during the entire pregnancy was remarkably devoid of severe toxic effects on the blood,

liver or kidney compartments of both pregnant rats and their concepts.

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