Effect of chronic ritonavir administration on pregnant rats and their fetuses

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Summary

In view of the very important role played by ritonavir in the prevention of maternal-fetal HIV-vertical transmission, the aim of this experimental study was to evaluate its possible effects on several important obstetric parameters. Ritonavir was administered daily to three groups of pregnant rats (E₁ = 20 mg/kg; E₂ = 60 mg/kg; E₃ = 180 mg/kg; n = 10 in every group) from ‘zero’ up to the 20th day of pregnancy. Controls (n = 10) were injected with the drug vehicle (propylene glycol) in the same schedule. We evaluated the effects on fetal and maternal weight gain, placental weight, number of implantations and resorptions, malformations, fertility rate, and maternal and fetal death rates. Body weight gain of the E₁ group was significantly lower than that of the other groups, most likely due to a toxic effect of the highest dose of ritonavir. Ritonavir did not affect the number of implantations. Group E₂ had five resorptions and some reduction in fertility. The mortality rate was significantly affected by ritonavir (2/10 maternal deaths in E₁ and 4/10 in E₂). On the other hand, no alterations were observed in the fetuses, a finding which could be due at least in part to the protective action of placental P-glycoprotein.

Key words: Ritonavir; Toxicology; Pregnancy; Rat.

Introduction

Protease inhibitors markedly reduced morbidity and mortality due to AIDS [1–4]. Among them, ritonavir, a p-toluenesulfonic acid salt, is thought to be the most potent inhibitor of the 3A4 isoform of the cytochrome P450 enzymes [5–8]. In fact, it has been demonstrated in dogs and rats that the blood levels of other antiretrovirals were increased 8 to 46 times when co-administered with ritonavir [9]. This feature makes the drug a “pharmacokinetic enhancer” to increase the plasma concentrations of other HIV-protease inhibitors, and this use has become common in clinical practice [10].

In humans, the recommended dose of ritonavir is 600 mg twice a day, which is considered to be an adequate treatment for AIDS but related to potentially serious adverse reactions [11–13]. When associated with other drugs for the treatment of AIDS, ritonavir can be used in lower doses (100 or 200 mg twice a day). Such doses are well tolerated, safe and efficient [10].

Ritonavir is classified as a pregnancy category B drug [13], and only a few papers deal with its effects on rat pregnancy. For instance, Kumat et al. [5], Denissen et al. [14] and Yamaji et al. [15] studied the effects of ritonavir on non-pregnant rats only. On the other hand, Minkoff & Augenbraun [13] reported that ritonavir produced no effects on fertility in female rats at drug exposures of approximately 60% of those achieved with therapeutic dosages; higher dosages were related to hepatic toxicity, but malformations were not observed in pregnant rats.

Developmental toxicity was observed (early resorptions, decreased fetal body weight, ossification delays and developmental changes) with blood concentrations approximately 30% of those achieved with the therapeutic dose. A slight increase was noticed in the incidence of cryptorchidism in rats at an exposure approximately 22% of that achieved with the proposed therapeutic dose [13]. In accordance with Mirochnick et al. [16], very small quantities of ritonavir cross the placental barrier.

The high metabolic activity of ritonavir, mainly at the level of heme-thiolate proteins as the cytochrome P450 enzymes and its possible consequences on embryo development, prompted us to investigate the effects of this drug on rat pregnancy.

Materials and Methods

Animals and treatments

Adult virgin female EPM-1 Wistar rats, weighing about 200 g, under routine laboratory conditions, were mated at random in the proportion of one male for three female rats. The finding of spermatozoa in the vaginal smear was labeled as the ‘zero’ day of pregnancy [17]. Forty pregnant rats were then randomly divided into four groups with ten rats each, one control (C) and three experimental drug-treated groups (E₁, E₂ and E₃). Control animals were given the drug vehicle (10% ethanol and 90% propylene glycol) from day zero up to the 20th day of pregnancy. The experimental groups were given 20 mg/kg (E₁), 60 mg/kg (E₂) or 180 mg/kg (E₃) per day of ritonavir, respectively, during the entire period of pregnancy. Drug and vehicle administrations were done by gavage. Body weights were taken on day zero and the 7th, 14th and 20th days of pregnancy.
On the 20th day of pregnancy the rats were killed under excess anaesthesia. On laparotomy and opening of the uterine horns, the following items were recorded: number of implantations and resorptions, number of placentae, placental and fetal weights and occurrence of malformations.

**Statistical analysis**

Data were analysed by ANOVA and the Kruskal-Wallis test. When the p value reached significance (p ≤ 0.05), the Dunn’s multiple comparisons test was employed [18, 19].

**Results and discussion**

One of the aspects of concern during antiretroviral therapy with protease inhibitors in humans is body weight follow-up. With regard to body weight gain during pregnancy, the issue is highly relevant in view of the potentially serious consequences of impaired nutrient intake on both maternal and fetal compartments [20]. Since no reports in the literature were found about the effects of ritonavir on the body weight gain of pregnant rats, this aspect was examined in our animals.

Figure 1 shows the body weight increase of pregnant rats throughout the experimental period. Similarly to what occurs in normal rats, it should be noticed that all groups reached their fastest weight gain in the last third of the pregnancy. However, with the highest dose of ritonavir (E3 group) the body weight gain was severely impaired as the incremental curve slope was significantly lower than those of the other groups. Part of this result could be due to the gastrointestinal side-effects of ritonavir. In fact, in humans the drug can cause dose-dependent nausea, diarrhea, anorexia, abdominal pain and taste perversion. However, it is known that in humans a significant weight gain is common during antiretroviral therapy with protease blockers [21-25] regardless of pregnancy.

It has been reported that the weight increment is mainly in adipose tissue, with no changes in the lean body mass (skeletal muscle) [26], though no significant correlation was found between the use of protease inhibitors and lipodystrophy or hyperlipidemia [27]. With regard to our experimental groups, it can be seen in Figure 1 that at mid-pregnancy (14 days) E1 and E2 had mean incremental body weights somewhat higher than the controls, a result which could reflect the action of ritonavir. Nonetheless, in the E3 group, in addition to the gastrointestinal effects, our results regarding weight loss at term could also be attributable to the metabolic disturbances caused by ritonavir [24].

Although reduced by some 12% in the E3 group, the number of implantations recorded was not significantly affected by ritonavir (Table 1). However, the highest dose of ritonavir did increase the number of resorptions, an effect which may relate to its passage through the cell membrane and its putative effect on intracellular targets, similarly to what was proposed to occur in preadipocytes and adipocytes [28]. Since the observation that ritonavir is a relatively poor P-glycoprotein inhibitor in vivo [29], a self-limited cell entrance would not be exerted and then specific metabolic disturbances are likely to occur as a result of the ensuing critical intracellular concentrations of the drug.

**Table 1. — Effects of ritonavir administration on rat embryo implantations and resorptions, resorption/implantation ratios, mortality data and on mean placental weights. Mortality data are in absolute figures. Other values are mean ± SEM (n = 10 pregnant rats for every group).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Implantations</th>
<th>Resorptions</th>
<th>Resorption/Implantation Ratio</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>10.0 ± 0.59</td>
<td>0</td>
<td>0</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>E1</td>
<td>6.4 ± 0.52</td>
<td>0</td>
<td>0</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>E2</td>
<td>9.5 ± 0.53</td>
<td>0</td>
<td>2</td>
<td>0.61 ± 0.02</td>
</tr>
<tr>
<td>E3</td>
<td>8.8 ± 2.18</td>
<td>1.7 ± 1.2*</td>
<td>0.4 ± 0.3*</td>
<td>4</td>
</tr>
</tbody>
</table>

Rats were control (C) or treated during the entire period of pregnancy with ritonavir (E, 20 mg/kg; E, 60 mg/kg; E, 180 mg/kg once a day, by gavage). *p < 0.001 with regard to C, E, and E; p < 0.05 with regard to C and E.

The high-dose regimen of ritonavir (E3 group) had a severe effect on fertility, since only 50% of the surviving rats in that group got pregnant, and only two among them had term concepts. In fact, ritonavir at 180 mg/kg per day was related to a 40% mortality rate and severe toxicity such as necrosis in the proximal convoluted tubules of the kidney and severe hepatic congestion, which could be confirmed by necropsy as well. It was noticed that a high mortality rate (40%) in the E3 group coexisted with a 100% survival rate of fetuses (Table 1), suggesting that there may be an important compartmentation of the drug which shifts its toxicity from the fetal to the maternal side.

There was no evidence of malformation. Lack of malformations in rats under the use of ritonavir has already been reported by Minkoff & Augenbraun [13]. This could be explained by a protective factor that does not allow ritonavir to cross the placental barrier. In fact, an effec-
tively functioning placental drug transporter, P-glycoprotein, might significantly limit the access of ritonavir to the fetus [16, 30] and thus might explain why no fetal damage was observed. On the other hand, this limited passage would represent a handicap, if one of the targets of the treatment were to protect the fetus from mother-to-child HIV transmission.

References

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