Reproductive toxicity study with a novel deoxyguanosine analogue (Metacavir) in pregnant SD rats

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Abstract Our preliminary studies demonstrated that Metacavir has potential to become a new anti-HBV agent. The main targets of the toxic effects of Metacavir, in rhesus monkeys, were gastrointestinal tracts, liver, blood, and kidneys, which were not related to mitochondrial effects. In this study, the maternal toxicity, embryo-fetal developmental toxicity and teratogenicity were studied in pregnant Sprague-Dawley rats after intragastric administration of Metacavir (200, 100, 50, 0 mg/kg body weight) during the first 6–15 days of pregnancy. Slower weight gain was observed in 5 out of 21 rats subjected to a 200 mg/kg dose, as well as 2 out of 20 subjected to a 100 mg/kg dose. Compared with the solvent control group, the calibration weight gain in the 200 mg/kg and 100 mg/kg dosage groups respectively, during first 6–20 pregnant days were significantly different (P < 0.01, P < 0.05). Significant dose related adverse effects to other reproductive parameters were not seen in F0 and F1, but the number of stillbirths in high dose group showed notably difference compared with the control group (P < 0.05), while the litter incidence showed no difference. No Metacavir-associated pathological changes were observed. The present research indicated that at a dose of 200 mg/(kg·d) (i.e., 40 times the effective dose in rats), Metacavir shows some maternal toxicity to SD rats. The embryotoxicity in the 200 mg/kg group encompass decreased fetal body weight, and higher fetal mortality rates, compared with the control group. However, the litter incidence showed no statistical difference. All the treated rats displayed normal bone development, no teratogenicity and without adverse effects on fetal development, thus indicating that below a dose of 200 mg/(kg·d) there is no teratogenic side effects.

Keywords deoxyguanosine analogue; Metacavir; pregnancy; maternal toxicity; embryo toxicity; teratogenicity

Introduction

Viral hepatitis B, caused by hepatitis B virus (HBV), remains a global health concern due to its high morbidity and mortality. With clear action mechanisms and efficacies, nucleoside analogs have become the main first-line drugs for combating viral hepatitis B [1,2]. Some newly licensed nucleoside analogs have showed strong inhibitory effects on HBV; however, high chance of recurrence after drug withdrawal and high resistance rate also restrict their application. Therefore, it is a high priority to develop a new nucleoside analog with high efficiency, low toxicity, high bioavailability, and low resistance to combat HBV [3].

Antiretroviral drugs function by reducing the residual rate of HIV vertical transmission [4], and to improve maternal health [5,6]. Some studies suggest that the combination of antiretroviral therapy in pregnant women with human immunodeficiency virus type 1 (HIV-1) infections, increases the risk of premature birth and other adverse outcomes of pregnancy. However, data on complications of pregnancy, associated with monotherapy or combination therapy with antiretroviral agents, are limited. In 1998, a retrospective Swiss study consisting of 30 women with HIV-1 who had received a combination of different antiretroviral therapy during pregnancy (with protease inhibitors in 13 women and without protease inhibitors in 17) showed that such treatment was associated with a 33% risk of premature delivery [7]. Contempor
Metacavir, a prodrug of 2′,3′-dideoxyguanosine (ddG), is a novel synthetic nucleoside analogue for the treatment of hepatitis B virus (HBV) [9]. Many preliminary studies have shown that Metacavir displays good anti-HBV activities, both in vitro and in vivo [10–13]. It can remarkably inhibit the Hbs Ag and Hbe Ag secreted by 2215 cells and cell total HBV DNA. In ducks infected by duck hepatitis B virus (DHBV), Metacavir at the dosages of 40 and 80 mg/kg showed anti-DHBV activities, and significantly decreased the serum DHBV-DNA and intra-hepatic DHBV-DNA levels [10]. Monkey PK/PD results showed that Metacavir is highly concentrated in liver (or liver-enriched), and thus can effectively combat HBV in the target organ with fewer side effects [11]. The LD_{50} for the orally, intravenously, and intraperitoneally administered Metacavir were 1500, 800, and 700 mg/kg, respectively, in rats. In a monkey acute toxicity experiment, a dosage of 800 mg/kg was associated with gastrointestinal toxicities and liver dysfunction; however, no death was noted. All these preliminary studies have demonstrated that Metacavir has a potential to become a new anti-HBV agent. Furthermore, we found the main target organs of the toxic effects of Metacavir were the gastrointestinal tract, liver, blood, and kidneys, and the no-observed-adverse-effect-level (NOAEL) of Metacavir for rhesus monkey was considered to be 50 mg/(kg·d) [12]. Another of our studies found that mitochondrial injuries in a 40 mg/kg Metacavir-treated group were mild in each type of tissue, without any obvious change in mitochondrial function. At week 4 in the recovery phase, results showed that all these injuries were reversible after drug withdrawal [13]. Nowadays, drugs like nucleoside analogues are used in pregnant women according to hazard rating classifications (A, B, C, D, X) based on risk caused by drugs in fetal rat (according to the U.S. Food and Drug Administration, FDA). It is necessary to evaluate the level of their reproductive toxicity, so we did a reproductive toxicity experiment to evaluate some related indicators. In the present study, the potential reproductive toxicity of Metacavir, including maternal toxicity, embryo-fetal developmental toxicity and teratogenicity, was assessed during gestation.

**Materials and methods**

**Drugs**

Metacavir (100% in powder form) were provided by Nanjing Chang’ao Company (Lot No. 20080412). This product is highly hydroscopic and should be stored at 4°C under dry conditions.

**Animal grouping and treatment**

Sprague-Dawley (SD) rats (160 females and 80 males, 11 weeks old) were obtained from the Institute of Laboratory Animal, Sichuan Academy of Medical Sciences (SCXK: Chuan 2004-16), and animals were raised in a 12-h light/12-h dark cycle with free access to food and water. During the quarantine stage, each cage contained 5 rats. Seven days later, the quarantine period ended. One male and two females were housed together in each cage for mating. Vaginal plugs and sperms were monitored by vaginal smears every morning. Based on this, the start of pregnancy was defined as the day when sperms were discovered. Trinitrophenol was used to do body surface symbol during mating period and administration period. Finally, we obtained 81 female rats that mated successfully. These rats got divided into 4 groups randomly: a low dose group (20), a middle dose group (20), a high dose group (21), and a solvent control group (20). Based on previous results [12] as well as the clinically recommended dose, we prepared 5, 10, and 20 mg/ml Metacavir solutions respectively, with double distilled water (according to the manufacturers recommendations), which were intragastrically administered as with10 mg/kg dose, that was 50, 100, 200 mg/kg body weight respectively, corresponding to 10, 20, and 40 times the recommended clinical dosage for rat. In the solvent control group, the drug had been substituted with double distilled water. During the 6–15 days of pregnancy, Metacavir was administered intragastrically once every day. Females were sacrificed in the afternoon when reaching 20 days of pregnancy, and subsequently gross examination and dissection was carried out. The experimental procedures for the care and use of laboratory animals were in accordance with the guidelines of the Ministry of Science and Technology of China [14].

**F0 female observations**

During the administration period (6–15 pregnant days), rats were observed once every day prior to administering the drug, as well as after the administration. The body weight at the 0 and 3 days of pregnancy, during the administration period (6, 10, 13, 16 days of pregnancy, and at 20 days of pregnancy (dissection day) were recorded.