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Embryotoxic effects of CKD-602, a new camptothecin anticancer agent, in rats

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Abstract

CKD-602 is a newly developed camptothecin anticancer agent. Preclinical studies suggest that it may have greater antitumor activity and lower toxicity than other camptothecin anticancer agents. The potential of CKD-602 to induce embryotoxicity was investigated in the Sprague-Dawley rat. One hundred mated females (sperm in vaginal lavage = day 0) were distributed among three treatment groups and a control group. CKD-602 was administered intravenously at dose levels of 0, 5, 20 and 80 μg/kg/d to pregnant rats from days 6 to 15 of gestation. The vehicle control rats received an equivalent volume of 1 ml distilled water with D-mannitol 50 mg and tartaric acid 0.06 mg. All dams were subjected to the caesarean section on day 20 of gestation. There were no signs of maternal toxicity or embryotoxicity at 5 μg/kg/d, but at 20 μg/kg/d, there was an increase in relative brain weight. At 80 μg/kg/d, reduced food intake, suppressed body weight and increased weight of spleen were observed in dams. An increase in the resorptions and dead fetuses, a decrease in litter size, fetal and placental weights were also found. In addition, various types of external, visceral and skeletal malformations occurred. Characteristic malformations included absent eye bulge, agnathia, dilated cerebral ventricle, anophthalmia, absent thoracic centrum, fused vertebral arch, fused rib, among others. Visceral and skeletal variations were observed. Retarded ossification of several skeletal districts and delayed ossification of sternebrae, metatarsals and sacrocaudal vertebrae were also observed. The results show that CKD-602 is embryotoxic and teratogenic at a minimally maternally toxic dose, i.e. at 80 μg/kg/d in rats. The no-observed-adverse-effect level (NOAEL) of CKD-602 for developmental toxicity was considered to be 20 μg/kg/d, however, the NOAEL for maternal toxicity was 5 μg/kg/d.

Keywords: CKD-602; Anticancer agent; Camptothecin; Teratogenicity; Rats

1. Introduction

Camptothecin (CPT) is a cytotoxic alkaloid extracted from the bark, fruit, and leaves of the Chinese tree *Camptotheca acuminata*. Although some antitumor activity was observed, development as an anticancer agent was hampered by poor solubility and unpredictable toxicities such as hemorrhagic cystitis, myelosuppression and diarrhea [1–3]. Since then, a search for structural analogues of CPT was begun with the aim of overcoming these limiting factors in development of the parent drug. This resulted in the discovery of a number of CPT analogues such as CPT-11 (irinotecan), topotecan and 9-aminocamptothecin (9-AC) [4–6]. They have been observed to show high clinical efficacy in treating human neoplasms such as colorectal cancer and ovarian cancer.

The mechanism of action of CPT derivatives lies in the inhibition of topoisomerase I which is an important nuclear enzyme for various DNA functions including transcription and replication. There is one exception to this rule: irinotecan, which is a prodrug, must first be converted to the active metabolite, SN-38, by a carboxyl esterase converting enzyme [7,8]. The CPTs all contain a terminal lactone ring that

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makes them unstable in aqueous solutions by undergoing a rapid, pH-dependent, non-enzymatic hydrolysis to form an open-ring hydroxy carboxylic acid [9], which is a much less potent inhibitor of topoisomerase I [10]. Because they cause DNA damage, the CPTs are potentially mutagenic and can induce chromosomal aberrations including sister chromatid exchanges, gene deletions, and gene rearrangements [11]. Clinical trials demonstrated that CPTs require a prolonged schedule of administration given continuously at low doses or frequently fractionated dosing schedules to be most effective [12].

CKD-602 is a new camptothecin derivative antitumor agent with a formula (7-[2-(*N*-isopropylamino)ethyl]-(20*S*)-camptothecin) developed by Chong Kun Dang Pharmaceutical Company in Korea [13,14]. Clinical trials showed that it has improved toxicity profiles and antitumor activity compared to other CPT derivatives [15,16]. CKD-602 showed significant anticancer activity against gastric and ovarian cancer. Genotoxicity studies showed that it did not induce mutagenicity in *Salmonella typhimurium* TA 98, TA 100, TA 1535, and TA 1537 and caused no chromosome aberration in Chinese Hamster Lung cells in the presence of metabolic activation system. In contrast, there was an increased incidence of micronucleated polychromatic erythrocytes in bone marrow of ddY male mice [17].

Concerning reproductive and developmental toxicity, the embryotoxicity of CPT has been reported in rats and rabbits [18,19]. The present study was performed to evaluate the potential of CKD-602 to induce fetal dysmorphogenesis, prenatal mortality and intrauterine growth retardation in the Sprague-Dawley rat. It was conducted according to the test guidelines from the Korea Food and Drug Administration (KFDA) and Organisation for Economic Cooperation and Development (OECD) guidelines for the testing of chemicals under modern Good Laboratory Practice Regulations.

2. Materials and methods

2.1. Animal maintenance and mating procedure

Sprague-Dawley rats (Korea Research Institute of Chemical Technology, Toxicology Center Breeding Facility) were kept under SPF (specific pathogen free) conditions at a constant day/night cycle as 08:00–20:00 h light. Standard laboratory rodent diet (Jeil Feed Company, Daejeon, Korea) and sterilized water were available ad libitum. For mating, two females were placed into the cage of one male overnight and the first 24 h period following the mating procedure was designated as day 0 of pregnancy if vaginal sperm were detected. This experiment was conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). All procedures were approved by our Institutional Animal Care and Use Committee (IACUC).

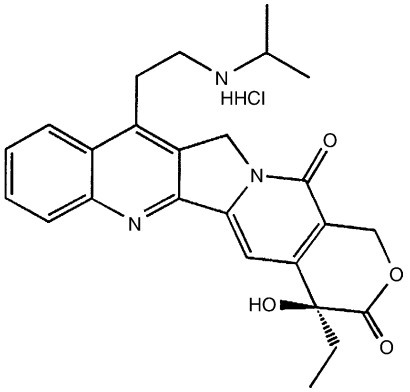

Fig. 1. Chemical structure of CKD-602.

2.2. Test substance

CKD-602 (7-[2-(*N*-isopropylamino)ethyl]-(20*S*)-camptothecin), a colorless white powder, was chemically synthesized and provided by Chong Kun Dang pharmaceuticals (Seoul, Korea). The chemical structure of CKD-602 is depicted in Fig. 1. High-dose solution was prepared before treatment twice a week because the stability of prepared test article for 4–5 days was confirmed. CKD-602 was dissolved in distilled water with D-mannitol 50 mg, tartaric acid 0.06 mg in 1 ml and adjusted to pH 3.50 with sodium hydroxide. Dosing solutions for the lower dose groups were prepared by stepwise dilution of the high-dose solution.

2.3. Drug treatment

The application volume was 3 ml/kg. The daily application volume was calculated according to the body weight on days 6, 9 and 12 of gestation. CKD-602 was administered intravenously in a tail vein at the speed of 2 ml/min to rats from days 6 to 15 of gestation.

2.4. Experimental groups

Four groups were constructed: CKD-602 5, 20, 80 μg/kg/d, and a vehicle control. Twenty-five mated females were used in each group.

2.5. Dose selection

Dosages of 10, 30, 60, 100, and 200 μg/kg/d were given in a intravenous pilot study to six pregnant dams per group. The doses of 30 μg/kg/d or less were well tolerated. At 100 and 200 μg/kg/d, embryolethality was 100% and the depression of maternal body weight was extreme. At 60 μg/kg/d, the depression of maternal body weight was slight. Based on these results, 80 μg/kg/d was selected for the highest dose in the definitive study.