Single dose toxicity study of CKD-602, a new camptotheacin anticancer agent, in Beagle dogs

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Abstract: The present study was carried out to investigate the potential acute toxicity of CKD-602 by a single intravenous dose in Beagle dogs. The test chemical was administered intravenously to male and female Beagle dogs at dose levels of 0.3, 0.5, or 2.5 mg/kg. Mortalities, clinical findings, and body weight changes were monitored for the 14-day period following the administration. At the end of 14-day observation period, all animals were sacrificed and complete gross postmortem examinations were performed. All males and females of the 2.5 mg/kg dose group were found dead between the fourth and seventh day after the injection. Treatment related clinical signs, including vomiting, anorexia, mucous stool, diarrhea, and no stool were observed. Decrease or suppression of body weight was observed in a dose-dependent manner. In autopsy, dark red discoloration of the gastrointestinal tract, atrophy of the thymus, paleness of the spleen, sporadic dark red spots of the lung and petechia of the heart were observed in dead animals of the 2.5 mg/kg dose group. There were no specific adverse effects on males and females of the 0.3 and 0.5 mg/kg dose groups, except for the transient clinical signs such as anorexia, vomiting, and mucus/no stool. On the basis of the results, it was concluded that a single intravenous injection of CKD-602 to Beagle dogs resulted in increased incidence of abnormal clinical signs and death, decreased body weight, and increased incidence of abnormal gross findings. The absolute toxic dose of this chemical was 2.5 mg/kg for both genders. The LD₅₀ value was 1.1 mg/kg (95% confidence limit not specified) for both genders. The no-observed-effect level (NOEL) was considered to be below 0.3 mg/kg for both genders.

Key words: Anticancer agent, CKD-602, camptotheacin, acute toxicity, LD₅₀ value, dogs

Introduction

Camptothecin (CPT) is a cytotoxic alkaloid extracted from the bark, fruit, and leaves of the Chinese tree Camptotheca acuminata [18, 19]. Although some antitumor activity was observed, its development was hampered by poor solubility and unpredictable toxicities such as hemorrhagic cystitis, myelosuppression, and

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diarrhea [3, 15, 21, 22]. Since then, extensive efforts to develop structural analogues of CPT were begun with the aim of overcoming the two key 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) [1, 2, 12]. 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 [5, 6]. Because they cause DNA damage, the CPTs are potentially mutagenic and can induce chromosomal aberrations including increased sister chromatid exchanges, gene deletions, and gene rearrangements [4]. DNA synthesis inhibiting agents and DNA damaging agents are well known to produce toxic side effects on multiple organ systems [9, 10]. The most common adverse effects associated with CPTs are diarrhea and myelosuppression [19].

CKD-602 is a new camptothecin derivative antitumor agent with a formula (7-[2-(N-isopropylamino)ethyl]- (20S)-camptothecin) developed by Chong Kun Dang Pharmaceutical Company in Korea [11, 13]. Like other camptothecin derivatives, CKD-602 is a potent inhibitor of topoisomerase I, and successfully overcomes the poor water solubility and toxicity of the parent drug. Preclinical studies of CKD-602 demonstrated broad antitumor activity against various human tumor cell lines, and the results were equal or superior to those of camptothecin and topotecan, a clinically active antitumor drug [8, 11, 14]. CKD-602 showed significant anticancer activity against gastric and ovarian cancer.

As a part of safety evaluation studies of the test article, CKD-602, a single intravenous dose toxicity study was performed in Beagle dogs. The present study was conducted according to the test guidelines from the Korea Food and Drug Administration [7] and Organisation for Economic Cooperation and Development [16] guidelines for the testing of chemicals under modern Good Laboratory Practice Regulations.

Materials and Methods

Animal husbandry and maintenance
Six Beagle dogs (Canis familiaris) of each sexes aged 4 months were purchased from Covance Research Product Inc. (Cumberland, VA, USA). During quarantine (5 weeks), each dog was given a complete physical examination; all health parameters were normal. The animals were housed in a room maintained at a temperature of 23±3°C and a relative humidity of 50±10% with artificial lighting from 07:00 to 19:00 and with 13-18 air changes per hour. Only healthy animals were assigned to the study. The dogs were housed singly in a stainless wire cage. Food (Japan Oriental Yeast Company, Tokyo, Japan) was restricted to 300 g per day (the remaining food was weighed to measure net food intake/animal) with water ad libitum. This experiment was conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International), and animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council).

Test chemical
CKD-602, 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. CKD-602 was dissolved in distilled water with D-mannitol 50 mg, tartaric acid 0.06 mg in 1 ml and adjusted to pH 3.5 and was prepared immediately before the treatment. Those of lower groups were prepared by stepwise dilution of that of the highest dose group.

Experimental groups
Healthy males and females were randomly assigned to three experimental groups of CKD-602 receiving

![Fig. 1. Chemical structure of CKD-602.](image-url)