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Preclinical toxicity of DATR, a recombinant soluble human TRAIL mutant, in rats and cynomolgus monkeys

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ABSTRACT

The recombinant soluble human TRAIL mutant (DATR), derived from tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), is a promising agent for cancer therapy. The present study evaluated the toxicity of DATR in rats and monkeys.

Based on the results, the safety and toxic doses of DATR intravenously injected to rats for 50 days were 60 and 180 mg/kg, respectively, and when delivered intravenously guttae to monkeys for 50 days, these levels were 10 and 30 mg/kg, respectively.

The main toxic effects in rats were red blood cell count and haemoglobin decreases; blood urea nitrogen and creatinine increases. The main toxic effects in monkeys included red blood cell count and haemoglobin decreases; alanine aminotransferase and aspartate aminotransferase increases; high proliferation of karyocytes of the erythrocyte series; and regional hydropic degeneration of hepatic parenchymal cells.

The TUNEL assay showed both 90 mg/kg DATR- and TRAIL-induced apoptosis of the liver in monkeys, which confirmed the hepatotoxicity of DATR.

These findings indicated that the target toxic organs of DATR might be the haematological system. Furthermore, kidney in rats and liver in monkeys are also likely target toxic organs. The toxicity was reversible and did not differ from that associated with TRAIL administered at the same dosage.

1. Introduction

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) belongs to the tumour necrosis factor family (Wiley et al., 1995; Pitti et al., 1996). It has been identified as a powerful activator of programmed cell death, or apoptosis, in tumour cells, while sparing normal cells (Walczak et al., 1999; Ashkenazi et al., 1999; Ganten et al., 2005). Because of its wide range of antitumour activity in preclinical models, TRAIL has been studied for several years as a promising agent for cancer therapy.

DATR, recently investigated by Chengdu Diao Pharmaceutical Group Co., Ltd. (Chengdu, China), is a recombinant human 114–281 peptide of wild-type TRAIL without Pro at site 119 and Glu at site 120. Our previous study showed that liver, renal and haematological systems might be the acute toxic effectors of DATR (Zou et al., 2010; Walczak et al., 1999).

The purpose of the present study was to evaluate the preclinical subchronic toxicity of DATR and its reversibility in rats and cynomolgus monkeys. This work could lead to clinical applications of DATR.

2. Materials and methods

2.1. Chemicals

DATR and TRAIL were provided by Chengdu Diao Pharmaceutical Group Co., Ltd. (Chengdu, China) and stored at −20 °C.

2.2. Animals

Eighty Sprague–Dawley rats, aged 5–7 weeks, were provided by Sino-British Sippr/BK LAB Animal Co., Ltd., in equal amounts of each sex. All rats were housed at 5 animals per cage at a temperature of 23 ± 3 °C and a relative humidity of 55 ± 15%, with 10 h/day of light and air exchange at 20 times/h. Feed and water were freely available. A one week quarantine was needed before the experiments.

Thirty cynomolgus monkeys of equal sex were provided by Xishan Zhongke Laboratorial Animal Co., Ltd. (Suzhou, China). All monkeys were housed one animal per cage at a temperature of 23 ± 5 °C and a relative humidity of 65 ± 25%, with 10 h/day of light

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and air exchange at 10 times/h. Feed and water were freely available. A four week quarantine was needed before the experiments.

All animals used in our study were humanely cared for according to institutional animal care guidelines, and the relative protocols were licensed by the Local Institutional Committee of the Second Military Medical University.

2.3. Experimental design

Eighty healthy rats were randomly arranged into four groups. Each group contained 20 rats of an equal number of each sex. The thirty monkeys were similarly assigned into five groups.

There were three rationales for the chosen dosages. First, doses for the pharmacodynamic study in mice were 5, 15 and 45 mg/kg. Second, a clinical dose of TRAIL was used (Herbst et al., 2006; Herbst et al., 2010). Third, the "no observed adverse effect" level and the "lowest observed adverse effect" level of DATR in monkeys were 90.0 and 135.0 mg/kg, respectively. Therefore, we determined the dosages in rats and monkeys as described below. For further evaluation, a TRAIL group of 90 mg/kg was added to the monkey groups as a positive control.

The four groups of rats were intravenously injected (iv) DATR in the tail at concentrations of 0, 20, 60 and 180 mg/kg, respectively. All rats were treated for 7 consecutive days with 7 day intervals, for 49 days in total. At the end of the experiment (d 50), a 14 day recovery period was added to determine the reversibility persistence and delayed occurrence of toxic effects.

The five groups of monkeys were intravenously guttae (iv gtt) at 0, 10, 30 and 90 mg/kg DATR and 90 mg/kg TRAIL, respectively. To augment the possible toxicity, we selected 49 days administration period in rats and cynomolgus monkeys. It is also required by the State Food and Drug Administration of China guidelines ([H]GPT2-1, [S]GPT1-1).The administration lasted for 7 consecutive days, with 7 day intervals, for 49 days in total. A 14 day recovery period was added to the end of the experiment. On d 22, 50 and 64, and twice before administration as d 0, electrocardiogram (ECG) and body weight were recorded, and haematology and serum biochemistry were examined.

On d 50, half of the animals of each group were sacrificed under anaesthesia with 2% sodium pentobarbital (intraperitoneally injected into rats and iv into monkeys). All required organs and tissues were immediately isolated. The remaining animals were treated the same way at the end of the recovery period (d 64). The indexes, mentioned in the following, of each animal were examined when sacrificed. Furthermore, if death occurred at any time during the experiment, the animal was dissected to determine the cause of death.

All of the work mentioned above was performed according to the "Guidelines for Repeated Dose Toxicity Tests of Chemicals" provided by the State Food and Drug Administration of China under Good Laboratory Practice Regulations.

2.3.1. Clinical observations and test parameters

Throughout the entire experiment, the side effects caused by the administered agents were recorded daily.

2.3.2. ECG

ECGs of monkeys were collected with an ECG system (XD-7100, Shanghai Kohden Medical Electronic Instrument Corp., Shanghai, China) on d 22, 50 and 64, and twice before administration as d 0.

2.3.3. Haematology

Blood was sampled from the abdominal aorta of rats after anaesthesia before dissection, from the vein of monkeys on d 22, 50 and 64, and twice before administration. Blood samples were collected in tubes with ethylenediamine tetraacetic acid. A haematology autoanalyser (BC-3000, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) was used to analyse the haematological parameters. The reticulocyte (RET) levels were manually counted with a microscope after staining with methylene blue. Serum coagulation times were examined with a blood coagulation analyser (C2000-4, Beijing Precil Instrument Co., Ltd., Beijing, China).

2.3.4. Serum biochemistry

Blood samples were obtained in the same way described in the haematology section. After clotting, the blood was centrifuged and the serum was separated into new Eppendorf tubes. A 7080 automatic analyser (Hitachi High-Technologies Corp., Tokyo, Japan) was used to determine the serum biochemistry parameters. Chloride ($Cl-$), sodium ($Na+$) and potassium ($K+$) were determined with a Na/K/Cl analyser (EasyLyte PLUS, Medica Corp., Massachusetts, USA).

2.3.5. Immunology

The immunological parameters of the monkeys were also determined. A 7080 automatic analyser (Hitachi High-Technologies Corp., Tokyo, Japan) was used to determine the relevant parameters, including IgG, IgA and IgM of serum immune globulin, and C3 and C4 of serum complement. An ultraviolet spectrophotometer (UV-7504) was used to determine the content of immune complexes (CIC). Flow cytometer (Fc 500) was used to determine lymphocyte surface markers of peripheral blood, including CD3, CD4, CD8 and CD20.

2.3.6. Urinalysis

Urine samples of monkeys were analysed with an autoanalyser (Micro AUTION MA-4260, Arkray Global Business, Inc., Kyoto, Japan).

2.3.7. Bone marrow examination

Upon dissection, the breast bones of monkeys or thighbones of rats were dislodged and the bone marrow was extruded onto a carrier plate, where it was smeared in a uniform fashion. Absolute methanol was used to fix the bone marrow for 30 min. After air drying, bone marrow samples were dyed with Wright-Giemsa stain for 40 min, washed with tap water and air dried again.

2.3.8. Histopathology

Tissues and organs were isolated from each animal just after euthanasia. All of the tissues and organs were fixed with 10% neutral buffered formalin and routinely processed by embedding in paraffin, sectioning at 4 μm and staining with haematoxylin-eosin for histopathological examination.

2.4. TUNEL assay

Based on our previous subchronic toxicity studies, the liver of monkeys and kidney of rats were suspected to be the target organs of toxicity. Considering that TRAIL can induce apoptosis, we suspected that the toxicity was due to this effect. To confirm this theory, we designed a TUNEL experiment.

The pathological sections of liver from monkeys and kidneys from rats were examined with the TUNEL method with an in situ apoptosis detection kit according to the manufacturer's instructions; the kit was provided by Nanjing Keygen Biotech. Co., Ltd. (Nanjing, China).

The apoptosis rates were determined as the average of five random visual fields.