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Preclinical pharmacology and toxicology study of Ad-hTERT-E1a-Apoptin, a novel dual cancer-specific oncolytic adenovirus

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ABSTRACT

Clinical studies have demonstrated that conditionally replicating adenovirus is safe. We constructed an oncolytic adenovirus, Ad-hTERT-E1a-Apoptin, using a cancer-specific promoter (human telomerase reverse transcriptase promoter, hTERTp) and a cancer cell-selective apoptosis-inducing gene (Apoptin). Ad-hTERT-E1a-Apoptin was proven effective both in vitro and in vivo in our previous study. In this study, the preclinical safety profiles of Ad-hTERT-E1a-Apoptin in animal models were investigated. At doses of 5.0×10^8, 2.5×10^9, and 1.25×10^{10} viral particles (VP)/kg, Ad-hTERT-E1a-Apoptin had no adverse effects on mouse behavior, muscle cooperation, sedative effect, digestive system, and nervous systems, or on beagle cardiovascular and respiratory systems at 5.0×10^8, 2.5×10^9, and 1.25×10^{10} VP/kg doses. In acute toxicity tests in mice, the maximum tolerated dose $> 5 \times 10^{10}$ VP/kg. There was no inflammation or ulceration at the injection sites within two weeks. In repeat-dose toxicological studies, the no observable adverse effect levels of Ad-hTERT-E1a-Apoptin in rats (1.25×10^{10} VP/kg) and beagles (2.5×10^9 VP/kg) were 62.5- and 12.5-fold of the proposed clinical dose, respectively. The anti-virus antibody was produced in animal sera. Bone marrow examination revealed no histopathological changes. Guinea pigs sensitized by three repeated intraperitoneal injections of 1.35×10^{10} VP/mL Ad-hTERT-E1a-Apoptin each and challenged by one intravenous injection of 1.67×10^8 VP/kg Ad-hTERT-E1a-Apoptin did not exhibit any sign of systemic anaphylaxis. Our data from different animal models suggest that Ad-hTERT-E1a-Apoptin is a safe anti-tumor therapeutic agent.

Introduction

In the field of anti-tumor therapy, more interest and grants are being devoted to gene therapy. Cancer virotherapy has now become the primary field in which gene therapy is being applied (Yu and Fang, 2007a). Since the early 1980s, adenoviral vectors have been widely used in gene transfer experiments (Kamen and Henry, 2004). Conditionally replicative adenoviruses (CRAd) differ from adenoviral vectors in their ability to replicate (Everts and Poel, 2005). CRAd can be genetically manipulated and exhibit multiple distinct anti-cancer mechanisms, and have attracted a great deal of interest as cancer therapeutics vectors (Choi et al., 2012). ONYX-015 is one adenovirus that has been studied in clinical trials in the US (Bischoff et al., 1996; Kim, 2001; Nemunaitis et al., 2001). In 2003, the ONYX-015 phase III clinical trial in combination with chemotherapy was suspended due to funding problems. Dose-limiting toxicity (DLT) was not identified at doses of up to 2.0×10^{12} viral particles (VP) by intratumoral injections (Ganly et al., 2000) and by hepatic artery administration (Reid et al., 2001), or at doses of up to 2.0×10^{13} VP by intravenous (iv) administration (Nemunaitis et al., 2000). Phase III clinical trials of the Oncorine, an E1B55-kDa gene-deleted adenovirus, have been completed, and it was released into the market in 2005 in China (Yu and Fang, 2007a; Su et al., 2008). It has been proven safe through a clinical trial involving

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five doses of 5.0×10^7 to 1.5×10^{12} VP/day over five consecutive days. DLT and serious adverse events were not observed in the course of treatment. The most frequent complications were fever, flu-like symptoms, and pain at the injection site. By 2009, over 350 protocols had been approved for clinical gene therapy trials using attenuated adenoviral vectors (Chailertvanitkul and Pouton, 2010). These clinical research results demonstrate the safety of this novel platform for cancer therapy, as well as the difficulties that must be overcome.

Apoptin (also known as VP3) is a type of viral protein from chicken anemia virus that can induce apoptosis in various tumor cells, including tumor cells from hepatoma carcinoma (Han et al., 2011), oral cancer (Schoop et al., 2008), gastric carcinoma (Liu et al., 2012), prostate carcinoma (Zhang et al., 2013), and ovarian cancer (Wang and Zhang, 2011). Due to its small size, the Apoptin gene can be inserted into adenoviruses, making it attractive for cancer gene therapy. Preliminary studies have demonstrated the effect of Apoptin insertion in various vectors on restricting manifold tumors, which makes it attractive for cancer gene therapy (Xiao et al., 2010). Apoptin is specifically active in malignant and transformed cells, but not in normal cells. In vitro, Apoptin fails to induce programmed cell death in normal lymphoid, dermal, epidermal, endothelial, and smooth-muscle cells (Danen-Van Oorschot et al., 1997; Zhan et al., 2012). Normal lymphocytes tolerate the expression of Apoptin during both activation and proliferation (Pietersen et al., 2005).This is one reason for selecting animal species.

In a previous study, we constructed a dual cancer-specific antitumor CRAd, designating it Ad-hTERT-E1a-Apoptin (Xiao et al., 2010). Briefly, transgene cassettes containing the E1a-driving human telomerase reverse transcriptase (hTERT) core promoter (the 5′ flanking region of the hTERT gene between positions −283 and −78) and the Apoptin-driving cytomegalovirus promoter were subcloned into the adenoviral genome via a shuttle vector. Then, the infectious adenovirus designated Ad-hTERT-E1a-Apoptin was packaged in HEK-293 cells (Xiao et al., 2010). This CRAd possesses the ability to induce both tumor-specific growth inhibition and tumor-specific replication. Further investigation showed that Ad-hTERT-E1a-Apoptin anti-tumor activity was significant in vivo and in vitro. The intended clinical dose of Ad-hTERT-E1a-Apoptin is 2.0×10^8 VP/kg (Yu and Fang, 2007b).

Due to its replication defectiveness, recombinant adenovirus can be modified to lack pathogenicity and to increase its safety (Palmer et al., 2002; Varnavski et al., 2005). A number of phase I and II clinical trials and preclinical experiments have demonstrated that the safety of such recombinant adenoviruses was further improved and that they produced anti-tumor effects (Bauerschmitz et al., 2002; Makower et al., 2003; Bauerschmitz et al., 2004). The nonclinical toxicity testing is normally performed in healthy animals with a rigorously controlled health status, one rodent and one non-rodent species are required for toxicity evaluation, and this is based on the known combined strength of two mammalian species in predicting human toxicities (Dixit and Boelsterli, 2007). The information obtained from dogs better predicts adverse effects in humans, relative to data from rodents and even monkeys (Greaves et al., 2004). The toxicities occur in both rats and dogs showed about a 70% concordance with humans (Litchfield, 1962). In this study, the potential adverse effects, or the safety, of Ad-hTERT-E1a-Apoptin were assessed in mice, rats, guinea pigs, and dogs in accordance with the Chinese Good Laboratory Practice standards and the Chinese guidance for human gene therapy and its product quality control.

Materials and methods

Animals. This study involved BALB/c mice (3–4 weeks old, male, 17–25 ± 0.3 g; female 16–24 ± 0.25 g), Wistar rats (5 weeks old; males, 171.5 ± 12.1 g; females, 144.5 ± 8.2 g), male Hartley guinea pigs (2–3 months old, 350–450 g), and beagles (16 weeks old, 5.7 ± 0.3 kg, males and females, four were 25 weeks old, 8–11 kg, males and females). The animals were housed in a light-, temperature-, and humidity-controlled room (22 ± 20 °C, 50 ± 5% relative humidity) and were given solid diet (Changchun billion Adams Laboratory Animal Technology Co., Ltd.) and tap water ad libitum. All animals were obtained from the Experiment Animal Center of the Chinese Military Medical Academy and fasted before the experiments. The Institutional Animal Care and Use Committee of the Academy of Military Medical Sciences approved this experiment. Human albumin was purchased from Beisheng Pharmaceutical Company.

Pharmacological safety study in mice and beagles. BALB/c mice (140 male, 140 female) received a single iv infusion of 5.0×10^8, 2.5×10^9, or 1.25×10^{10} VP/kg Ad-hTERT-E1a-Apoptin and 5 mg/kg 0.9% sodium chloride (control group) through the tail vein. The four 25-week-old beagles were respectively injected with 5.0×10^8, 2.5×10^9, or 1.25×10^{10} VP/kg Ad-hTERT-E1a-Apoptin (experimental groups), or 5 mg/kg 0.9% sodium chloride (control group) by single iv injection (Su et al., 2008; Lee et al., 2012). For the muscle cooperation and hot plate test in mice, the animals were evaluated 30 and 60 min after the administration.

Neurological safety pharmacology studies. There were 4 groups (1 control, 3 treatment) of mice with each group having 5 mice/sex/group. We observed the influence of Ad-hTERT-E1a-Apoptin on locomotor activity, writhing response, fighting, convulsion, tremor, exophthalmos, ptosis, piloerection, tail elevation, traction, motor incoordination, muscle tone, catalepsy, righting reflex, pain response, pinna reflex, skin color, respiration, lacrimation, salivation, diarrhea, vocalization, and death in 24 h.

Muscle cooperation in mice. There were 4 groups (1 control, 3 treatment) of mice with each group having 5 mice/sex/group. Mice were put on the Rota-rod to train them. The Rota-rod rotation stick was positioned upside down and rotated 16 times per minute. The time a mouse took to fall was measured, and if the animal fell within 2 min, motion hindrance was deemed to have occurred. We recorded the number of times motion hindrance occurred.

Hot plate test in mice. There were 4 groups (1 control, 3 treatment) of mice with each group having 5 mice/sex/group. For the hot plate test (Park et al., 2012), mice were individually placed on a 55 °C hot plate apparatus and the reaction time, starting from the placement of the mice on the hot plate to the time they began licking their front paw, was measured. The basal latency for the hot plate test was approximately 10 s.

Writhing reaction in mice. There were 4 groups (1 control, 3 treatment) of mice with each group having 5 mice/sex/group. For the writhing test, 0.1 mL/10 g 0.6% acetic acid was injected intraperitoneally (ip), and then mice were returned to the cage. The number of writhing reactions was recorded 15 min after the test. A writhe was defined as a contraction of the abdominal muscles accompanied by extension of the forelimbs and elongation of the body. Ad-hTERT-E1a-Apoptin was administered 20 min before the writhing test began.

Sleeping time in mice. There were 4 groups (1 control, 3 treatment) of mice with each group having 5 mice/sex/group. Pentobarbital (30 mg/kg) was injected into the abdominal cavity after Ad-hTERT-E1a-Apoptin administration via the tail vein. The index losing more than 5 s of righting reflex was used to estimate the start time and duration of sleeping (Lee et al., 2012).

Electroshock seizure test in mice. There were 4 groups (1 control, 3 treatment) of mice with each group having 5 mice/sex/group. Electroshock was applied via ear electrodes (forceps style) using an electric stimulator (Kitano et al., 1996). The electroshock consisted of a single train of