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5-androstenediol improves survival in clinically unsupported rhesus monkeys with radiation-induced myelosuppression

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Abstract

We previously reported that five daily intramuscular doses of 5-androstenediol (AED), a naturally occurring adrenal steroid hormone, stimulated multilineage recovery of bone marrow in rhesus monkeys with radiation-induced myelosuppression after 4.0 Gy total body irradiation (TBI). Here we report the effect of AED on the survival of eighty rhesus macaques that received a 6.0 Gy dose of TBI in four sequential pilot studies. The drug was administered intramuscularly, based on body weight, 2–4 h after irradiation and continued once daily for a total of five injections. No clinical support in the form of antibiotics or transfusions was given to the animals at any time during the study. Five of the 40 (12.5%) treated animals died, compared to 13 of 40 (32.5%) of the animals in the control group ($p=0.032$). The combination of accumulated days of thrombocytopenia (<20,000 platelets/μL) up to day 14 (before the first death) together with treatment, accurately predicts mortality ($p<0.001$). The compound significantly reduced the duration of thrombocytopenia and neutropenia ($p<0.01$). The accumulation of days of neutropenia (ANC < 500 cells/μL) up to day 14 plays no major role in predicting death. AED shows significant activity in irradiated primates with acute hematopoietic radiation syndrome.
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Keywords: Acute radiation syndrome; AED; Androstenediol; Radiation protection; Rhesus macaques; Hematopoiesis

1. Introduction

There is an urgent need for a medical countermeasure to treat the acute radiation syndrome (ARS) that results from exposure to moderate and high levels of ionizing radiation. Many scenarios have been developed for terrorist attacks with nuclear devices [1]. According to these models, mass casualties are likely to occur with a wide variety of radiation exposures [2]. Total body irradiation (TBI) between 2 and 8 Gy delivered at relatively high dose rates (>0.2 Gy/min) [3] will induce the hematopoietic component of ARS, a condition that involves bone marrow suppression and compromises the lymphatic system [4]. There are no approved drugs to treat ARS or are there patients available to test the effectiveness of a potential treatment. In 2002 the United States Food and Drug Administration (FDA) adopted the so-called Animal Efficacy Rule, which allows the FDA to approve certain drugs demonstrating efficacy in relevant animal species and adequate safety in healthy human volunteers [5]. The FDA has indicated that the measure of efficacy

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for a countermeasure to ARS must be demonstrated as a survival advantage in irradiated monkeys.

Androst-5-ene-3β, 17β-diol, (AED) is a naturally occurring adrenal steroid hormone. In a sub-lethal murine radiation model, therapeutic doses stimulated myelopoiesis, increased numbers of circulating neutrophils, platelets and natural killer cells, and enhanced resistance to opportunistic infections [6,7]. Numerous studies demonstrated that the hormone increased survival of mice subjected to TBI-induced acute hematopoietic syndrome [6–10].

We have previously reported that following 4.0 Gy ^{60}Co TBI in monkeys, AED promotes multilineage hematopoietic recovery of neutrophils, platelets, and red blood cells with a significant reduction of the duration of days of neutropenia, thrombocytopenia and anemia when compared to vehicle controls [11]. ARS and the ensuing subsyndromes are a function of the absorbed radiation dose, among other factors [3]. The myelosuppression from radiation is dose dependent. In our previous studies, a 4.0 Gy TBI dose suppressed the bone marrow to a sufficient extent to permit the kinetics of bone marrow recovery to be followed through observation of blood counts. When the radiation-absorbed dose is increased to 6.0 Gy TBI, the extent of myelosuppression is dramatically increased, and because of the severity and prolongation of neutropenia and thrombocytopenia, there is a higher incidence of mortality secondary to bleeding and infection. Here we report the results of a planned sequence of four pilot studies that further evaluated AED in a model involving irradiated rhesus macaques. We report the collective evidence regarding the potential of this drug to restore hematopoiesis and therefore enhance the chances of survival, relative to irradiated controls, following myelosuppression in rhesus monkeys given 6.0 Gy TBI.

2. Materials and methods

2.1. Studies

These lethality studies (Table 2) are compliant with animal care guidelines and were conducted under good laboratory practice (GLP) procedures [12,13]. The first study, 2543, standardized and calibrated the telemetry and irradiation systems. The other three studies were similar in design, except in sample size and dose of AED.

2.2. Rhesus monkeys

Male and female rhesus monkeys, *Macaca mulatta*, 3.0 to 5.0 kg, were housed in individual stainless steel cages in environmentally controlled rooms set to maintain 18–29 °C with a relative humidity of 30–70% and a 12 hour on-and-off light cycle. Animals were fed one or two times daily with primate diet (Harlan Teklad), fresh fruits, vegetables and drinking water. All animals were seronegative for herpes B virus, simian T-cell leukemia viruses, simian immunodeficiency virus, and intestinal parasites. The respective Institutional Animal Care and Use Committee (IACUC) approved each experimental plan. Two contract research organizations conducted these studies under good laboratory practice procedures.

2.3. Irradiation

Preliminary estimates from the literature and calibration studies indicated that 6.0 Gy could result in 50% mortality of untreated primates (LD_{50}) [14]. Prior to irradiating the monkeys, and following established standards for clinical dosimetry of megavoltage therapy [15], all studies used water phantoms for calibration of the beam dose rate so as to ensure similar isodose curves at the animal's mid-plane depth. Monkeys were first acclimated to a custom, 1 cm thick, Lexan (GE Plastics, Pittsfield, MA) restraining device and irradiated without the use of anesthesia or sedation. On the day of irradiation, animals were placed in individual cages and transported via a licensed carrier in an environmentally controlled vehicle to the radiation facility. Animals were irradiated with either ^{60}Co photons or 6 MV X-rays through anterior/posterior and posterior/anterior parallel-opposed ports at a dose rate of approximately 60 cGy/min to a total mid-plane dose of 6.0 Gy. To ensure uniformity of radiation dose amongst groups, abdominal and chest circumference measurements were used to calculate the thickness of the animal to the mid-plane in order to determine exposure time to the radiation source.

2.4. Animal monitoring and supportive care

For each study, experimental animals were randomly assigned to parallel groups and stratified by gender and body weight. All studies were vehicle controlled. Between 2 and 3 weeks prior to irradiation, each animal underwent surgical implantation of a telemeter (model TA10TAD70, Data Sciences International, Arden Hills, MN) permitting remote, non-invasive monitoring of core body temperature. Core body temperature data were used to rapidly identify animals experiencing shock-related irreversible hypothermia, consequently requiring humane sacrifice. Neither transfusions nor antibiotics were given to the animals. Buprenorphine analgesia was provided, if needed, for pain control. Each animal was observed twice daily (a.m. and p.m.) to check survival and for evidence of pain and/or distress. All animals were examined postmortem.

2.5. Test article

AED, androst-5-ene-3β, 17β-diol, was prepared as a 100 mg/mL aqueous suspension using 7.4 mM sodium phosphate buffer, pH 6.0, containing 0.5% polysorbate 80, 0.02% benzalkonium chloride as a preservative and either 4.8% mannitol (studies 2543, 0973, 109) or 0.5% carboxy methyl

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