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Evidence of disseminated intravascular coagulation in a porcine model following radiation exposure

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ABSTRACT

Recent evidence has suggested that disseminated intravascular coagulation (DIC) plays an integral role in death at the LD_{50} dose of either gamma or solar particle event (SPE)-like proton radiation in ferrets. In these studies, Yucatan minipigs were evaluated to determine whether they were susceptible to the development of radiation induced DIC. Yucatan minipigs were exposed to a dose of 2.5 Gray (Gy) with X-rays and monitored over the course of 30 days. Evidence of DIC was evaluated by way of thromboelastometry parameters, platelet counts, fibrinogen concentration, and the d-dimer assay. Pigs exposed to X-rays developed signs of DIC within 2 days' post-irradiation. The development of DIC was exacerbated over the course of the studies, and one of the pigs died at day 14 and another had to be euthanized on day 16 post-irradiation. For both of these pigs, DIC was evident at the time of death. The following observations were indicated or were suggestive of DIC: whole blood clotting was impaired (as evidenced by thromboelastometry alterations), there were decreased platelet counts, elevated d-dimer concentrations in the blood, and/or hemorrhaging and the presence of fibrin in tissues observed during post-mortem examination. The extrapolation of data from these studies, in combination with other published data, have led to the hypothesis that there could be a correlation between the propensity to develop DIC, as indicated by hemorrhaging at death at relatively low doses of radiation, and the LD_{50} for a particular species. Our data suggest that the development of DIC may contribute to death at the LD_{50} dose in large mammals.

1. Introduction

Since the atomic bombings of Hiroshima and Nagasaki (Ishikawa and Swain, 1981), the consequences of radiation exposure have been a major concern for national security. Some relatively recent human radiation exposures of great significance have occurred as well; examples of these include the Chernobyl power plant meltdown of 1985 (Dallas, 2012), the Goiania accident in 1987 (IAEA, 1988), and the Fukushima–Daiichi meltdown of 2011 (IAEA, 2011). It is important for the national security of the United States that the effects of radiation exposure are known and methods are developed to mitigate any significant adverse biological effects of radiation exposure from a potential nuclear detonation or radiation accident (Weisdorf et al., 2006; Medalia, 2004). Several well-known non-terrestrial sources of radiation originate in the space environment, including solar particle event (SPE) radiation (Hu et al., 2009) and galactic cosmic radiation (Todd, 2003), which are of particular concern for astronaut health. An SPE involves the release of highly energetic charged particles with energies greater than 10 MeV/nucleon; protons that originate from the sun are the primary type of SPE radiation. For the planned exploration class missions, such as a trip to Mars, the length of time in space will be considerably longer than the time periods involved in previous space missions and this will result in considerably higher astronaut radiation doses. As part of space radiation research, there is currently much effort focused on ensuring astronaut safety on long-term space missions.

The Acute Radiation Syndrome (ARS) involves many different types of adverse health effects resulting from acute exposure to ionizing radiation (Dorr and Meineke, 2011; Hall and Giaccia, 2006). ARS is categorized into four areas: prodromal syndrome, gastrointestinal syndrome, cerebrovascular syndrome, and hematopoietic syndrome. The hematopoietic syndrome is observed after exposure to relatively low doses of radiation (1–5 Gy) and is thought to be the primary mechanism of radiation-induced death at the dose which kills half of the exposed population, which is known as the LD_{50}; death can be due to infection, presumably from the loss of white blood cells, and/or hemorrhaging, presumably due to the loss of platelets (Hall and Giaccia, 2006).

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In our previous studies, we have concluded that a coagulopathy, known as disseminated intravascular coagulation (DIC), occurred in irradiated ferrets at doses near the LD_{50} (Krigsfeld et al., 2014). In these studies, significant alterations in blood clotting parameters were observed in ferrets irradiated with SPE-like proton or gamma radiation, and the results were comparable for both types of radiation in terms of the onset and death from DIC Krigsfeld et al. (2014, 2012). Other studies revealed the activation of the coagulation cascade in response to radiation exposure, which is thought to have occurred through the increased presence of the biomarker fibrin (Krigsfeld et al., 2013). We have presented evidence (such as an increased soluble fibrin concentration in the blood, the presence of fibrin clots in tissues, and the inability to generate a stable clot) that doses near the LD_{50} can result in the development of DIC in ferrets (Krigsfeld et al., 2014). There is essentially no published evidence that radiation induced DIC occurs in other large mammals. Therefore, the current study was performed to determine whether there was evidence for the development of DIC in Yucatan minipigs exposed to ionizing radiation at a dose near the expected LD_{50} level, and a dose that astronauts could conceivably receive (e.g., during a worst case scenario such as that associated with the Carrington flare, by Stephens et al., 2005). The LD_{50} of Yucatan minipigs has not yet been reported. The average LD_{50} for other strains of pigs is 2.57 Gy (Morris and Jones, 1988), but LD_{50} values have been reported as low as 1.8 Gy in the Gottingen pig (Moroni et al., 2011). In this study, Yucatan minipigs were exposed to X-rays at a dose of 2.5 Gy.

2. Materials & methods

2.1. Animals and blood collection

Three Yucatan minipigs aged 8–14 weeks were purchased from Sinclair Bio Resources (Auxvasse, MO) and acclimated to the University of Pennsylvania Animal Facility. The Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania approved all animal procedures used in these studies. Approximately 1 week prior to radiation exposure, all animals were placed under anesthesia (isoflurane inhalant) and blood was collected from the superior vena cava and placed in either 3.8% sodium citrate or ethylenediaminetetraacetic acid (EDTA) – containing vacutainer tubes for analysis. Blood samples were also collected on days 2, 7, 10, 13, 16, and 30 post-irradiation.

2.2. Radiation procedures

Three pigs were irradiated with 6 MV X-rays generated by an iX linear accelerator from Varian (Palo Alto, California, USA) with an effective dose rate of 30 cGy per minute, at an extended source to surface distance of 370 cm, with collimator opening of 40×40 cm. 6 MV X-rays have a Bremsstrahlung spectrum with a peak energy of 6 MeV and an average energy below 3 MeV, delivering a close to exponential decay depth dose with initial buildup in the first 1.5 cm proximal depth, specified by percent depth dose at 10 cm depth as 67% for 100 cm source to surface to distance setup. In this configuration, 1450 machine monitor units (MU) were used to deliver 1.25 Gy to each side of the pigs using parallel opposed beams obtained by rotating the pig enclosures between irradiations. The output (cGy/MU) was determined using RadCalc® (Lifeline Software Inc. Austin, TA) dose calculation software. Calculation parameters were a maximal phantom depth of 10 cm (with variation from 5 to 10 cm), collimator opening of 40×40 cm, and a lateral phantom dimension of 55×50 cm, to approximate the irradiation geometry of the pigs. The calculation was verified with an ionization chamber measurement at a depth of 10 cm using 40×40 cm slabs of solid water. This measurement agreed within 3% of the expected calculation after accounting for the difference between the phantom dimensions used in the experiment and those used in the calculation. The measurement and calculations were performed at a phantom depth of 10 cm, so that the specified dose from the parallel opposed beams is defined at the center of a 20 cm thick phantom. A pig's head can have a diameter closer to 10 cm, instead of 20 cm; therefore, the head could get a dose 5% higher than the prescribed 2.5 Gy dose. The pigs' body radii vary between 5 and 10 cm. The dose was prescribed to an average radius (midline depth) of 8.5 cm to minimize the variation of dose received by different body parts. A head compensator was not used in this experiment for the sake of simplicity.

2.3. Thromboelastometry

Within one hour of blood collection, whole blood clotting was analyzed using a rotational thromboelastogram system (ROTEM, Munich, Germany), as previously described by Krigsfeld et al. (2014). Briefly, blood clotting was measured over the course of 90 min and data on the following parameters were recorded: coagulation time, clot formation time, α-angle, and maximum clot firmness.

2.4. Blood cell counts

For the whole blood collected in EDTA-containing tubes, a complete blood cell (CBC) count with differential analysis was performed within 24 hours of collection by an external laboratory using a Bayer Advia 120 Hematology Analyzer (Antech Diagnostics, Lake Success, NY, USA). Cell counts are referred to as the average absolute count $\pm$ standard deviation (SD).

2.5. D-dimer and fibrinogen ELISA

Plasma was isolated by centrifugation at 3000*G* for 15 min at 4°C; isolated plasma was stored at −80 °C. Thawed samples were used in the Porcine D-dimer ELISA assay (MyBioSource, San Diego, CA, USA) and the Porcine fibrinogen (Innovative Research Inc, Novi, MI, USA) ELISA assay following the vendors' protocols.

2.6. Statistical analysis

The thromboelastometry and CBC data were analyzed by the paired Student's t-test to determine whether there were statistically significant differences between the pre- and post-radiation time points using PRISM 5.0 (Graphpad, La Jolla, CA, USA) statistical and graphing software.

3. Results

3.1. Survival study and evidence of DIC

The radiation exposure resulted in death or euthanasia in 2 of the 3 irradiated pigs (Fig. 1). In this study, the pigs were monitored two–three times per day post-irradiation for any signs of distress. There was evidence of petechiae and ecchymosis in all three irradiated pigs at various time points. During this observation period, one pig was found dead at day 14 post-exposure, while a second animal exhibited signs of hemorrhaging, such as petechiae and purpura, beginning on day 14 post-exposure. After several days of worsening signs, which included severe ecchymosis, significant petechiae in the oral mucosa and skin, positive fecal occult blood test, inability to generate a stable blood clot, a very high D-dimer concentration in the blood (the highest concentration [145 ng/mL] was observed at day 1.5) and moribund condition, this pig needed