

Column E Explanation

1. Registration Number: Armed Forces Radiobiology Research Institute, Certificate #51-F-0003
2. Number of animals used in this study: 74
3. Species (common name) of animals used in the study: Pig
4. Explain the procedure producing pain and/or distress.

Irradiation is not a painful procedure in itself. Animals are sedated prior to being moved to the irradiation chamber to reduce stress, and are recovered in their home cages or in procedures rooms equipped with thermal support (for instance, (b) (4)). Although irradiation in itself does not cause significant stress, sequelae of radiation exposure may lead to suppression of the bone marrow and changes in function and structure of the gastrointestinal (GI) system, potentially leading to infections, fever, lethargy, anorexia, bleeding, vomiting, constipation and diarrhea which can cause pain and distress in the animal, if supportive therapy is not provided.

Antibiotics, analgesics, anti-emetics, anti-diarrheal, gastrointestinal protectants, food supplements, probiotics, fiber supplements and oral electrolyte supplements were used in these studies for minimal symptomatic supportive care. Around 21-28 days post-irradiation, the animal's immune system, bone marrow and gastrointestinal system typically starts recovering toward normal status. If the animals did not recover or reached a point where pain and distress could not be medically managed, they were humanely euthanized. Animals were closely monitored post irradiation multiple times a day until they completely recovered from acute radiation syndrome (ARS).

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Depending upon the irradiation dose, irradiated animals succumb to death due to compromised immune responses, opportunistic infections and damage to the GI. The percentage of surviving animals is the indicator of the efficacy of a countermeasure.

We use a non-steroidal anti-inflammatory drug to relieve pain and inflammation associated with radiation injury. This drug is given three days post radiation exposure and continued until 30 days post-irradiation when the animal is well out of the critical period (period during which clinical symptoms are manifested). We also use inhalational and/or injectable anesthesia at the time of moderately invasive procedures such as blood collection and euthanasia. Use of supportive care in the form of antibiotics, analgesics, gastrointestinal protectants, food supplements, probiotics, fiber supplements and oral electrolyte supplements is expected to mitigate the pain and stress associated to the sequelae of irradiation.

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1. Registration Number: Armed Forces Radiobiology Research Institute, Certificate #51-F-0003
2. Number of animals used in this study: 129
3. Species (common name) of animals used in the study: Nonhuman primates; Rhesus macaques (*Macaca mulatta*)
4. Explain the procedure producing pain and/or distress.

Pain is not expected from the irradiation procedure itself, but the sequelae of radiation exposure at the levels used in this study can cause pain and distress. Animals were sedated with anesthetic agent ((b) (4)) throughout the irradiation procedure to reduce stress. Seven to ten days post-irradiation, various changes occur in the body (e.g. compromised immune system leading to immunosuppression, bone marrow suppression, gastrointestinal upset, etc.). These changes can potentially lead to bacterial infections, fever, anorexia, bleeding, nausea, vomiting, constipation, and diarrhea which can cause pain and distress in the animal. If pain or distress was observed, the principal investigator and attending veterinarian were notified to evaluate and determine the appropriate treatment.

Antibiotics, analgesics, antipyretic agents, nutritional supplements, and oral electrolyte supplements were used to provide relief from any discomfort or pain. Blood transfusion, intravenous fluids, anti-diarrheal, anti-ulcer, and anti-emetics were provided to some animals. These medical interventions provided relief from any discomfort or pain the animals may have experienced. Around 30 days post-irradiation, the animal's immune system, bone marrow, and gastrointestinal system typically start recovering. Animals were closely monitored post-irradiation and multiple times a day until they completely recovered from acute radiation syndrome (ARS).

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Nonhuman primates are necessary for the pre-clinical development of radiation countermeasures intended for use in humans because drug metabolism and physiology in nonhuman primates are similar to humans. There is a greater than 95% DNA sequence similarity between nonhuman primates and humans. This animal model is considered as the gold standard by the US Food and Drug Administration (FDA) for drug development. Testing a drug with potential for human application in nonhuman primates ensures safety and specificity prior to the drug entering into the clinic for human trials. Rhesus macaques are the model of choice for investigations of radiation injury and countermeasures because of the large database available from the existing literature that allows for robust comparison. The FDA has accepted rhesus macaques as the appropriate animal model for pilot and pivotal efficacy testing of radiation countermeasures under the Animal Efficacy Rule, where drug efficacy cannot be performed in humans.

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