

WVU IACUC Model Guidance Sheet: Irradiation of Rodents and Bone Marrow Transplant

Purpose

Irradiation is commonly utilized in immunology and oncology research. Irradiation is used to kill rapidly dividing cells and can be used to kill bone marrow cells for immunosuppression or bone marrow transplant. Irradiation can be localized to a specific area of the body or applied to the entire body. When implementing radiation, it is important to consider the dose to be administered, potential adverse outcomes, supportive care, and humane endpoints.

Definitions

Sublethal radiation- Animal is transiently immunosuppressed

Lethal radiation- Complete myeloablation

Gamma irradiation- Exposure to ionizing radiation with gamma rays. Common isotopes include ^{137}CS or ^{60}Co

Relative biological effect- relative measure of damage done by a given radiation unit of energy deposited in biological tissue; cannot be accurately predicted and must be validated for different irradiator sources and experimental needs

X-ray irradiation- Penetrating form of high-energy electromagnetic radiation

Units-

Absorbed radiation: 1 gy (gray, SI unit) = 100 cGy = 100 rad

Dose rate: Gy/min, length of time subjects should be exposed to a beam for desired dose.

Biological effect measure: Sv (sievert, SI unit) or rem (roentgen equivalent man)

Fractionation of dose- The total irradiation dose is split up into different time intervals to minimize adverse outcomes

Bone Marrow Transplant (BMT)- Infuses blood forming cells from a donor animal into the body of a recipient which underwent whole body irradiation

Syngeneic graft- Graft transferred between genetically identical individuals

Allogeneic graft- Graft from another unrelated donor that is not genetically identical

Guidance

1. Irradiation Dosage Considerations

- a. Factors to consider when determining dose rate of an irradiator
 - i. Source of activity
 - ii. X-ray beam
 - iii. Position/distance
 - iv. Stationary or rotating subjects
 - v. Presence of attenuators or filters
- b. Accurate calibration is essential
- c. Factors which impact recommended dose
 - i. Radiation source
 - X-ray may require lower dosages
 - ii. Strain background
 - Relative strain sensitivity: $129S \leq SJL \leq C3H \leq C57BL/6 \leq BALB/c \leq SCID$
 - iii. Age
 - Neonate- relatively resistant
 - Weanling (20-30 days)- most sensitive
 - Young adult (3-4 months)- most resistant
 - Aged (6 months+)- declining resistance with age
 - iv. Health Status
 - Health issues can lead to increased sensitivity
 - v. Time of day
 - Diurnal variation to radiation sensitivity
 - vi. Number of doses
 - Fractionating a dose may reduce complications
- d. Pilot study recommended to determine appropriate dose given the animal model used, source of irradiation, and study goals

2. Bone Marrow Transplant procedure

- a. 7 days post BMT donor derived cells can be found in the spleen of the recipient mouse; 21 days post BMT all cell lines are reconstituted and may be normal, but not fully functional
- b. Genetically identical grafts have no risk of rejection (inbred animals or autologous bone marrow)
- c. Allogeneic grafts can lead to rejection and graft-versus-host disease
- d. Most commonly stem cells are administered via intravenous route (tail vein)
- e. Stem cells are isolated from a donor animals bone marrow or spleen
- f. Failure of engraftment is a possibility and should be considered if increased mortality is observed

3. **Monitoring and Supportive Care**

- a. Animals receiving whole body irradiation typically immunosuppressed and susceptible to opportunistic infection
- b. Whole Body Irradiation Side Effects
 - i. Weight loss
 - peaks 7 days post-irradiation
 - ii. Lethargy, inappetence, diarrhea
 - iii. Anemia
 - iv. Infection
 - v. Intestinal bleeding
 - vi. Graying of hair
 - vii. Secondary neoplasia
 - viii. Incisor damage
- c. Housing Considerations
 - i. Autoclaved cages/bedding
 - ii. Water: sterile +/- antibiotics; water acidification (pH 2.9-3.0)
 - iii. Irradiated food
 - iv. Diet gel (is irradiated and provides nutritional/hydration support)
- d. Monitoring- Daily
 - i. Evaluate Body Weight and Body Condition Score
 - ii. Scoring system recommended
 - iii. Evaluate for signs of rough coat, inactivity, hunched posture
 - iv. Localized irradiation can cause damage to organ of interest or localized skin reactions; monitor closely based on irradiation area

References

- <https://www.taconic.com/taconic-insights/oncology-immuno-oncology/rodent-irradiation-considerations.html>
- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5007026/>
- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2694700/>

Sample scoring system

Scoring System Whole Body Irradiation

Appearance:	Normal (smooth coat, clear eyes/nose)	0
	Hunched and/or fluffed	1
	Ocular discharge and/or edema	3
	Pale, white mucus membranes/skin**	6
	Blue mucus membranes/skin (cyanosis) [±]	12
Respiratory rate:	Normal breathing	0
	Increased breathing (double normal rate, rapid, shallow)	6
	Abdominal breathing (+/-gaspings or open mouth breathing) [±]	12
General behavior	Normal (based in baseline observations)	0
	Decreased mobility	2
	Ataxia, wobbly, weak ^{**}	6
	Inability to stand [±]	12
Provoked behavior	Normal (moves when cage is disturbed, runs from hand)	0
	Subdued; responds to stimulation (moves away briskly)	1
	Subdued even to stimulation (moves away slowly)	3
	Unresponsive to gentle prodding ^{**}	6
	Does not right when placed on side within 5 seconds [±]	12
Weight loss	< 20%	0
	20-25%	3
	26-30%	6
	31-35%	9
	≥35% [±]	12

Total Score

Score

< 6	Normal
6–11	Morbid: Monitor at least 3 times per day; notify appropriate personnel immediately
≥ 12	Moribund: Notify responsible personnel immediately for euthanasia if no single criterion is 12 [±] . Any single criteria of 12 [±] euthanize immediately; consider as 'found dead'.

** Regardless of score, notify appropriate person immediately.

* Regardless of score, immediately euthanize (death is imminent).