STANDARD OPERATING PROCEDURE #615

RODENT IRRADIATION

1. PURPOSE

This Standard Operating Procedure (SOP) describes the procedures for the care of mice undergoing total body irradiation.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff, veterinary care staff.

3. MATERIALS

3.1. Antibiotics

3.2. Sterile isotonic solution for injection, e.g., 0.9% sodium chloride, Lactated Ringer's Solution

3.3. Carprofen

3.4. Antibiotic ointment, e.g., sulfadiazine ointment, triple antibiotic ointment

4. CONSIDERATIONS

4.1. Irradiation kills rapidly dividing cells, including bone marrow and epithelial cells of the gut and other organs. The most common application of rodent irradiation is to destroy the bone marrow and other hematopoietic progenitors (myeloablation), either for immunosuppression or before replacing the immune system with donor grafts.

4.2. The dose of radiation should take into consideration the strain and age of animals as well as the purpose of the experiment.

4.2.1. Different rodent strains will have varying sensitivity to radiation and some immunosuppressed animals can be particularly radiosensitive. For example, Balb/C mice are more sensitive to irradiation whereas C57B/6 mice are more resistant.

4.2.2. Older animals are typically more resistant to radiation.

4.2.3. Transient immunosuppression can be achieved with sublethal doses of irradiation whereas complete myeloablation requires lethal doses.

4.2.3.1. Partial myeloablation (sublethal dose) can be achieved with irradiation doses of <600 cGy.

4.2.3.2. Complete myeloablation (lethal dose) can usually be achieved with irradiation doses of 700 to 1300 cGy.

4.3. Radiation doses will vary according to the irradiator type and radiation source. If no literature references are available to determine the adequate dose of irradiation to use, conduct a pilot study.

4.4. Fractionated doses should be considered, if appropriate, to reduce morbidity and mortality. Fractionating a dose consists of delivering 2 half doses separated by an interval of 3 to 4 hours.

4.5. Irradiators should be routinely calibrated according to the manufacturer's recommendations.

5. PROCEDURES

5.1. Consider initiating antibiotic treatment approximately 3 days before irradiation. Provide one of the following antibiotics in the drinking water as the sole source of drinking water for two weeks following irradiation and label cages receiving treatment. Consider adding sucralose to the drinking water to make the antibiotic solution more palatable.

5.1.1. Sulfamethoxazole and trimethoprim (TMS):

5.1.1.3. Each mL of TMS oral suspension contains 40mg sulfamethoxazole and 8mg trimethoprim.

5.1.1.4. Add 6mL of TMS oral suspension per 250mL of drinking water. Re-suspend daily by shaking the water bottle.

5.1.1.5. Alternatively, an oral medication suspension medium such as MediDrop® Sucralose can be used instead of water. When using this product to prepare the antibiotic dilution, daily shaking of the water bottle is not necessary.

5.1.1.6. Discard solution and prepare fresh after 7 days.

5.1.2. Enrofloxacin:

5.1.2.1. Add 2.5mL of enrofloxacin (50mg/mL) per 250ml of drinking water and protect from light.

5.1.2.2. Alternatively, an oral medication suspension medium such as MediDrop® Sucralose can be used instead of water.

5.1.2.3. Discard solution and prepare fresh after 7 days.

5.2. Mice exposed to total body irradiation should be housed under sterile conditions (i.e., sterile feed, bedding, water, cages) until, if ever, they regain a functional immune system.

5.3. Mice can be irradiated in their home cage or appropriate container. Anesthesia is not required.

5.4. The animal is placed in the irradiator and irradiated at the dose specified in the Animal Use Protocol (AUP) as approved by the Facility Animal Care Committee (FACC).

5.5. Cages of irradiated mice are identified with the following information:

5.5.1. Dose of irradiation

5.5.2. Date of irradiation

5.6. Irradiated mice should be monitored on the day following irradiation and then at least three times per week for two weeks thereafter. Observations should be documented in the Humane Endpoints Monitoring Log.

5.7. Possible clinical signs of irradiation sickness following total body irradiation:

5.7.1. Weight loss: due to inappetence and diarrhea

5.7.2. Lethargy

5.7.3. Hunched posture

5.7.4. Rough coat

5.7.5. Skin burns

5.7.6. Anemia: nose and paws appear pale

5.7.7. Infection

5.7.8. Intestinal bleeding: feces may appear dark

5.7.9. Transplant failure: Graft Versus Host Disease

5.7.10. Graying of the hair coat, particularly in black haired mice

5.7.11. Development of secondary neoplasia

5.7.12. Damage to incisors

5.8. Provide 1ml of sterile isotonic fluids (preferable warmed to body temperature), subcutaneously, immediately before or after irradiation and repeat after 24 hours.

5.9. Wet food may be provided at the bottom of the cage, daily, for 7 days.

5.10. In case of skin burns, in consultation with veterinary care staff:

5.10.1. Provide carprofen 20mg/kg SC, once a day, for 2 to 5 days to alleviate discomfort.

5.10.2. Apply antibiotic ointment (e.g., sulfadiazine ointment) daily on the wound, until healed.

5.11. Humane intervention points:

5.11.1. When immune reconstitution has been provided by bone marrow transplant, mice usually recover within 2-3 weeks. Animals that have not received a bone marrow transplant will not recover.

5.11.2. If the general condition of the animal does not improve by 21 days post-irradiation, irradiated mice should be euthanized.

5.11.3. Euthanize animals presenting the following clinical signs:

5.11.3.1. Weight loss exceeding 20% of pre-irradiation weight.

5.11.3.2. Body condition score of less than 2.

5.11.3.3. No or weak response to external stimuli.

5.11.3.4. Hunched posture, lethargy, and lack of grooming.

5.11.3.5. Pale eyes and/or extremities.

5.11.3.6. Cold to touch

5.11.3.7. Severe skin ulcerations that do not respond to treatment.

6. REFERENCES

6.1. Duran-Struuck R, Dysko RC. Principles of bone marrow transplantation (BMT): providing optimal veterinary and husbandry care to irradiated mice in BMT studies. J Am Assoc Lab Anim Sci. 2009 Jan;48(1):11-22. PMID: 19245745; PMCID: PMC2694700.

6.2. Iwakawa M, Noda S, Ohta T, Ohira C, Lee R, Goto M, Wakabayashi M, Matsui Y, Harada Y, Imai T. Different radiation susceptibility among five strains of mice detected by a skin reaction. J Radiat Res. 2003 Mar;44(1):7-13. doi: 10.1269/jrr.44.7. PMID: 12841593.

6.3. Cui, YZ., Hisha, H., Yang, GX. et al. Optimal protocol for total body irradiation for allogeneic bone marrow transplantation in mice. Bone Marrow Transplant 30, 843–849 (2002). https://doi.org/10.1038/sj.bmt.1703766

6.4. Spalding, J. F., & Trujillo, T. T. (1962). Radiosensitivity of Mice as a Function of Age. Radiation Research, 16(2), 125–129. https://doi.org/10.2307/3571191

6.5. Duran-Struuck R, Hartigan A, Clouthier SG, Dyson MC, Lowler K, Gatza E, Tawara I, Toubai T, Weisiger E, Hugunin K, Reddy P, Wilkinson JE. Differential susceptibility of C57BL/6NCr and B6.Cg-Ptprca mice to commensal bacteria after whole body irradiation in translational bone marrow transplant studies. J Transl Med. 2008 Feb 28;6:10. doi: 10.1186/1479-5876-6-10. PMID: 18307812; PMCID: PMC2292684.

6.6. Gibson BW, Boles NC, Souroullas GP, Herron AJ, Fraley JK, Schwiebert RS, Sharp JJ, Goodell MA. Comparison of Cesium-137 and X-ray Irradiators by Using Bone Marrow Transplant Reconstitution in C57BL/6J Mice. Comp Med. 2015 Jun;65(3):165-72. PMID: 26141441; PMCID: PMC4485625.

6.7. P. Dubé, Considerations for rodent irradiation, https://www.taconic.com/taconic-insights/oncology-immuno-oncology/rodent-irradiation-considerations.html

SOP REVISION HISTORY

DATE	REVISIONS
2022.07.12	This Standard Operating Procedure (SOP) describes the procedures for the care of ~~irradiated~~ mice **undergoing total body irradiation**.
2022.07.12	~~3.1. Disinfectant~~
2022.07.12	3.5. Antibiotic ointment~~,~~ ~~(~~e.g., sulfadiazine ointment, ~~BNP~~ triple antibiotic ointment~~)~~
2022.07.12	3.2. Antibiotics ~~(sulfamethoxazole and trimethoprim or enrofloxacin)~~
2022.07.12	**4. CONSIDERATIONS** **4.1. Irradiation kills rapidly dividing cells, including bone marrow and epithelial cells of the gut and other organs. The most common application of rodent irradiation is to destroy the bone marrow and other hematopoietic progenitors (myeloablation), either for immunosuppression or before replacing the immune system with donor grafts.** **4.2. The dose of radiation should take into consideration the strain and age of animals as well as the purpose of the experiment.** **4.2.1. Different rodent strains will have varying sensitivity to radiation and some immunosuppressed animals can be particularly radiosensitive. For example, Balb/C mice are more sensitive to irradiation whereas C57B/6 mice are more resistant.** **4.2.2. Older animals are typically more resistant to radiation.** **4.2.3. Transient immunosuppression can be achieved with sublethal doses of irradiation whereas complete myeloablation requires lethal doses.** **4.3. Radiation doses will vary according to the irradiator type and radiation source. If no literature references are available to determine the adequate dose of irradiation to use, conduct a pilot study.** **4.2.3.1. Partial myeloablation can be achieved with irradiation doses of <600 cGy.** **4.2.3.2. Complete myeloablation can usually be achieved with irradiation doses of 700 to 1300 cGy.** **4.4. Fractionated doses should be considered, if appropriate, to reduce morbidity and mortality. Fractionating a dose consists of delivering 2 half doses separated by an interval of 3 to 4 hours.**

2022.07.12	5.1. Consider initiating antibiotic treatment approximately 3 days before irradiation. Provide one of the following antibiotics in the drinking water as the sole source of drinking water for two weeks following irradiation and label cages receiving treatment. **Consider adding sucralose to the drinking water to make the antibiotic solution more palatable.**
2022.07.12	**5.1.1.3. Alternatively, an oral medication suspension medium such as MediDrop® Sucralose can be used instead of water. When using this product to prepare the antibiotic dilution, daily shaking of the water bottle is not necessary.**
2022.07.12	**5.1.2.2. Alternatively, an oral medication suspension medium such as MediDrop® Sucralose can be used instead of water.**
2022.07.12	5.3. Mice can be irradiated in their home cage **or appropriate container**. Anesthesia is not required.
2022.07.12	5.7. Possible clinical signs **of irradiation sickness** following total body irradiation:
2022.07.12	5.6. Irradiated mice should be monitored on the day following irradiation and then at least three times per week for two weeks thereafter. Observations should be documented ~~on a~~ **in the Humane Endpoints** Monitoring Log.
2022.07.12	5.10. In case of skin burns, **in consultation with veterinary care staff**:
2022.07.12	5.10.2. Apply antibiotic ointment (e.g. sulfadiazine ointment~~, BNP~~) daily on the wound, until healed.
2022.07.12	5.11.2. If the general condition of the animal does not improve ~~after~~ **by** 21 days **post-irradiation**, irradiated mice should be euthanized.
2022.07.12	**5.11.3.6. Cold to touch** **5.11.3.7. Severe skin ulcerations that do not respond to treatment.**
2022.07.12	**6. REFERENCES** **6.1. Duran-Struuck R, Dysko RC. Principles of bone marrow transplantation (BMT): providing optimal veterinary and husbandry care to irradiated mice in BMT studies. J Am Assoc Lab Anim Sci. 2009 Jan;48(1):11-22. PMID: 19245745; PMCID: PMC2694700.** **6.2. Iwakawa M, Noda S, Ohta T, Ohira C, Lee R, Goto M, Wakabayashi M, Matsui Y, Harada Y, Imai T. Different radiation susceptibility among five strains of mice detected by a skin reaction. J Radiat Res. 2003 Mar;44(1):7-13. doi: 10.1269/jrr.44.7. PMID: 12841593.** **6.3. Cui, YZ., Hisha, H., Yang, GX. et al. Optimal protocol for total body irradiation for allogeneic bone marrow transplantation in mice. Bone Marrow Transplant 30, 843–849 (2002). https://doi.org/10.1038/sj.bmt.1703766** **6.4. Spalding, J. F., & Trujillo, T. T. (1962). Radiosensitivity of Mice as a Function of Age. Radiation Research, 16(2), 125–129. https://doi.org/10.2307/3571191** **6.5. Duran-Struuck R, Hartigan A, Clouthier SG, Dyson MC, Lowler K, Gatza E, Tawara I, Toubai T, Weisiger E, Hugunin K, Reddy P, Wilkinson JE. Differential susceptibility of C57BL/6NCr and B6.Cg-Ptprca mice to commensal bacteria after whole body irradiation in translational bone marrow transplant studies. J Transl Med. 2008 Feb 28;6:10. doi: 10.1186/1479-5876-6-10. PMID: 18307812; PMCID: PMC2292684.** **6.6. Gibson BW, Boles NC, Souroullas GP, Herron AJ, Fraley JK, Schwiebert RS, Sharp JJ, Goodell MA. Comparison of Cesium-137 and X-ray Irradiators by Using Bone Marrow Transplant Reconstitution in C57BL/6J Mice. Comp Med. 2015 Jun;65(3):165-72. PMID: 26141441; PMCID: PMC4485625.**
2022.09.14	**6.7. P. Dubé, Considerations for rodent irradiation, https://www.taconic.com/taconic-insights/oncology-immuno-oncology/rodent-irradiation-considerations.html**
2023.02.14	Rodent ~~Post-~~Irradiation ~~Care~~
2023.02.14	**4.5 Irradiators should be routinely calibrated according to the manufacturer's recommendations.**
2023.02.23	5.9. ~~Provide~~ Wet food **may be provided** at the bottom of the cage, daily, for 7 days.