Critical care of sub-lethal irradiated transgenic mice using a complete soft food formula - DietGel76A™

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ABSTRACT
The objective of this research is to determine whether the administration of a complete soft food formula to sub-lethal irradiated animals from three different transgenic mice strains over a period of 21 consecutive days, will have a significant impact on the clinical signs, and the general survival rate of the animals. Our hypothesis was that using DietGel76A™, along with appropriate care of animals, would be significantly beneficial to experimental animals after a total body irradiation for allogeneic bone marrow transplantation.

For this experiment three different strains of mice were used: C57BL/6, (JAX stock 000664), NRG (JAX stock 007799) - immunodeficient mice model and CD45.1 (JAX stock 002014) - strain most commonly used for the transplant of bone marrow cells. In our experiment, no bone marrow transplants have been performed; therefore the reconstitution of hematopoietic system was done exclusively by self-replenishment.

Our hypothesis was that using DietGel76A™ along with antibiotic treatment, strict handling and manipulation procedures, the general mortality rate, as well as the onset of clinical signs between the treated animals and the control animals would be significantly lower.

This hypothesis was clearly confirmed for the C57BL/6 strain. The treatment with DietGel76A™ had a net positive impact on the recovery of more irradiation sensitive strains (CD45.1, and mostly NRG). However, in our opinion, mice belonging to more sensitive strains should be on DietGel76A™ for a longer period of time (7 days pre-irradiation to 42 days post irradiation).

MATERIALS AND METHODS
Animal Care Procedures
The protocol for this experiment was reviewed and assessed by the Animal Care Committee (ACC) of the IRCM. All the animals used in this experiment were cared for in compliance with the principles outlined in the current Guide to the Care and Use of Experimental Animals as published by the Canadian Council on Animal Care.

Objectives:
1. Objective 1: To determine the effect of DietGel76A™ on the survival rate of the animals.
2. Objective 2: To determine the potential differences between the used strains of mice in terms of sensitivity/sensitivity and response to gamma irradiation.
3. Objective 3: To investigate the effects of bone marrow (BW) on non-irradiated and sub-lethal irradiated mice, with the purpose to assess whether a protective weight loss is occurring, and to calculate the impact of this parameter over the animal wellbeing.

Experimental Design
Female mice, 4-6 weeks old from The Jackson Laboratories (Bar Harbor, Maine 04609 USA) were used. For this experiment three different strains of mice have been used:
- C57BL/6 (JAX stock 000664)
- NRG (JAX stock 007799)
- CD45.1 (JAX stock 002014)

Irradiation Procedures:
Upon receipt of the animals at the IRCM, the randomization, group assignment and pre-irradiation body weight measurements have been performed (Day 3) prior to irradiation. From Day 3 prior irradiation, the animals from SD groups (sub-lethal-Dietgel) received one can of Dietgel 76A in cage, for acclimatization. On the same day, all the animals started receiving an antibiotic treatment in water.

On irradiation day, all the animals (except controls) were sub-lethally irradiated using Gamma-radiation produced using an irradiator which source is constituted of Cedium-137. Doses of irradiation were based on the time of exposure calculated at the time of the experiment (conversion 1Gy/min=100 rad/min, source delivering is 120.84 rad/min). The amount of time spent inside the irradiator has been calculated depending on the dose and radiosotope decay charts. Irradiation doses were sub-lethal. Each animal received 40 Gy of Gamma-irradiation, equivalent to 3min 15sec of exposure (decay rate: 12.37 cGy/min).

Animal care and procedures, antibiotics and DietGel administration
Immediately after irradiation, all the animals were injected with 1 mL of sterile isotonic solution subcutaneously (Physiological Saline (0.9%), CDMV product # 1399, St-Hyacinthe (QC, Canada)). The injection was repeated after 24h. From Days 1 to Day 21 post irradiation, the following observations were made: daily animal check, mortality check, clinical signs check, endpoint scores. Special scoring sheets (Fig. 1) were designed to monitor the clinical signs and endpoints score. A decisional tree was designed to evaluate and take action according to the endpoints (Fig 2).

RESULTS
Mice from all groups pretreated with PBS and antibiotics displayed a survival rate of near to 100% over a 30 day period following irradiation. Following the irradiation of the three strains used in this experiment, we discovered that the most sensitive strain was the NRG (BW difference=1.95, R²=0.7565, i=+24.62), followed by the CD45 (BW difference =2.85, R²=0.7822, i=+20.52) and the C57 (BW difference =1.21, R²=0.7899, i=+19.80). Irradiated NRG mice supplemented with DietGel had a significant BW improvement in comparison with the non-treated cohort (NS=19.31 ±3.34) (p <0.05), see fig 2b). and fig 2h). Following irradiation, during the 21 days of the Dietgel 76 A treatment, there is a clear decreasing trend of BW for the treated group (IDG), versus non treated (INDG) group. The Dietgel was withdrawn after Day 22 of the study, which corresponds to a slight loss of the BW recovery trend in the treated group (fig 2).

DISCUSSION AND CONCLUSION
The analysis of the BW average gain between groups of irradiated animals treated and non-treated with Dietgel showed a higher variation (higher susceptibility) of NRG mice where the differences between two groups were more important. This is probably due to the increased cellular sensitivity to ionizing radiation of the NRG mice , according to the JAX description and phenotype database entry information for the strain.

Objective 1: The impact over the survival rate of the animals was clearly demonstrated. Although the animals were sub-lethally irradiated and no BMT was performed, the survival rate was exceptionally high.

Objective 2: C57BL/6 irradiated mice responded the most intensely to the treatment with DietGel76A™, although they seemed to be more resistant to the irradiation compared to the other two strains. In the case of NRG mice, which proved to be the most sensitive to the irradiation procedure, the difference was less significant. However, it seemed that the withdrawal of DietGel76A™ after 21 days had a negative impact over the general health of the irradiated NRG mice.

Objective 3: Following the irradiation of the three strains used in this experiment, we discovered that the most sensitive ones are the NRG mice, followed by the CD45.1 and the C57BL/6 mice.

Conclusion
The general objective of the experiment was to determine whether standard handling, sustained critical care and appropriate medical treatment along with a dietary supplement could improve the survival rate of irradiated animals form three different strains of mice.

Our Hypothesis was that using DietGel76A™ along with antibiotic treatment, strict handling and manipulation procedures, the general mortality rate, as well as the onset of clinical signs between the treated animals and the control animals will be significantly lower.

This hypothesis was confirmed for the CD45.1 strain. The treatment with DietGel76A™ had a net positive impact on the recovery of more irradiation sensitive strains (CD45.1 and mostly NRG). However, in our opinion, mice belonging to more sensitive strains should receive Dietgel76A™ for a longer period of time (42 days post irradiation).

References