Journal of Pharmacological and Toxicological Methods 71 (2015) 46–53

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Review

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

Ovidiu I. Jumanca [a,*], Jay Palmer [b]

[a] Institut de Recherches Cliniques de Montréal (IRCM), Montreal, QC, Canada
[b] Clear H2O, Portland, ME, USA

ARTICLE INFO

Article history:
Received 2 October 2014
Accepted 9 December 2014
Available online 17 December 2014

Keywords:
Mice
Irradiation
Critical care
Specific-pathogen free
Body-weight recovery
Soft food

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 mouse 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 is that using DietGel76A™, along with an antibiotic treatment, strict handling and manipulation procedures, the general mortality rate, as well as the onset of the clinical signs between the treated animals and the control animals, will be significantly lower. This hypothesis was confirmed for the C57BL/6 mice. However, the treatment with DietGel76A™ had only a very limited impact on the recovery of more irradiation sensitive strains (CD45.1 and mostly NRG). Further studies must be conducted on mice from these strains in order to assess whether mice belonging to more sensitive strains should be on DietGel76A™ for a longer period of time (at least 42 days post irradiation).

Contents

1. Introduction

Radiation produces pathological changes in living animals through multiple mechanisms, including oxidation (Miura, 2004). In rodents, and particularly mouse, irradiation is a very important step in achieving partial or complete destruction of the immune system in order to proceed with Bone Marrow Transplant (BMT). Irradiation has been used as an effective conditioning regimen for BMT, since it rapidly kills proliferating immunocompetent cells, particularly T cells, and hemopoietic progenitor cells in the recipients (Cui et al., 2002). Mice do not respond identically when exposed to gamma radiation; several biological factors can potentially affect the murine response to ionizing radiation. The dose of gamma radiation and the strain of mouse (Duran-Struuck & Dysko,

* Corresponding author.
E-mail address: ovidiu.jumanca@ircm.qc.ca (O.I. Jumanca).

http://dx.doi.org/10.1016/j.vascn.2014.12.003
1056-8719/

2009; Jackson, Vujaskovic, & Down, 2010) are 2 additional factors that can dramatically affect the degree of sickness linked to irradiation.

Several husbandry concepts should be considered when caring for irradiated mice before and after BMT (Duran-Struuck & Dysko, 2009). Transplanted animals undergo a 5 to 10 day irradiation sickness period from which they generally recover within 14 days (Drobyski, Keever, Hanson, McAuliffe, & Griffith, 1994; Holland & Mitchell, 1976; Sacher, 1957).

The objective of this research was to determine whether the administration of a complete soft food formula to sub-lethal irradiated animals from three different inbred and congenic mice strains over a period of 21 consecutive days will have a significant impact over clinical signs and the general survival rate of the animals. Our hypothesis is that using DietGel76A™, along with a classical antibiotic treatment, strict handling and manipulation procedures, the general mortality rate as well as the onset of clinical signs in the treated animals compared to the control animals should be significantly lower.

To test this hypothesis, the project was divided into three objectives:

Objective 1: To determine whether the use of DietGel76A™ would have a significant impact on clinical signs and the general survival rate of the animals.

Objective 2: To determine the potential differences between the strains of mice used in terms of sensibility/sensitivity and response to gamma-irradiation.

Objective 3: To investigate the differences in body weight (BW) between non-irradiated and sub-lethal irradiated cohorts, with the purpose to assess whether a progressive weight loss is occurring, and to calculate the impact of this parameter over the animal wellbeing.

2. Methods and materials

2.1. 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* (Canadian Council on Animal Care, 1993) as published by the Canadian Council on Animal Care.

2.2. Experimental design

Fifty four (54) 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, NRG and CD45.1.

C57BL/6, (JAX stock 000664) is the most widely used "genetic background" for genetically modified mice for use as models of human disease. NRG (JAX stock 007799) is an immunodeficient mouse strain, commonly used for cell or tissue transplant studies. CD45.1 (JAX stock 002014) is a mouse strain most commonly used for the transplant of bone marrow cells. The animals were assigned in three groups: sub-lethal irradiation and given treatment with DietGel 76A ("SD" group), sub-lethal irradiation, without DietGel 76A treatment (group "S") and control animals, non-irradiated but receiving the DietGel 76A (Control Group). For group distribution, see Table 1.

Upon reception of the animals at the IRCM, the animals were acclimated to the facility for three days. Randomization, group assignment and pre-irradiation body weight measurements have been performed three days prior to irradiation. From Day 3 prior irradiation, the animals from groups "SD" and control received one can of DietGel 76A in the cage, for acclimatization. On the same day, all animals started receiving an antibiotic treatment in water.

The experiment has been divided in three different time periods (Fig. 1):

1. Acclimatization period and pre-treatment with antibiotics (Day −3 to Day 0)
2. Irradiation and post-irradiation observations (Day 0 to Day 21)
   a. Withdrawal of antibiotics (Day 14) and DietGel (Day 21)
3. Post study observations (Day 21 to Day 42).

2.3. Irradiation procedures

On irradiation day, the mice in the irradiation groups were placed in a specially designed, well-ventilated acrylic container (pie cages) and subjected to whole-body irradiation. All animals (except controls) were sub-lethally irradiated using Gamma-radiation produced by IRCM irradiator type J.L. Shepherd Mark1-68-A-1 which has a 82 TBq Cesium-137 source. Doses of irradiation were based on the time of exposure calculated at the time of the experiment (conversion 1 Gy/min = 100 Rads/min, source delivery is 120.84 Rads/min).

Mice were irradiated using a Braintree scientific irradiation pie cages(™) (Braintree scientific product # MPC-2 with top filter, Braintree Scientific, Inc., PO Box 361, Braintree, MA 02185, USA). The use of mouse pie cages with dividers (MPC) allowed us to conduct sequential irradiations on groups of nine mice without needing to autoclave cages between each use, in a sterile environment. In total, 36 mice in four groups were irradiated.

Time of exposure—Animals were generally irradiated for a short period of time (<15 min). The amount of time spent inside the irradiator was calculated depending on the dose and radioisotope decay charts. Irradiation doses were sub-lethal. Each animal received 4 Gy of gamma-irradiation, equivalent to 3 min 15 s of exposure (decay rate: 123.72 cGy/min).

Obviously, the main purpose of whole body irradiation procedure is to create a strong immunodeficient condition in a very short time. Whole-body irradiation is one of the most common tools for myeloablation of the recipient's bone marrow (Cui et al., 2002). In our experiment, it was decided not to perform a bone marrow transplant of the irradiated animals due the low doses of irradiation (sub lethal level) and the high possibility of rapid reconstitution of the hematopoietic system following whole body irradiation.

2.4. Animal care and procedures, antibiotics and DietGel administration

Immediately after irradiation, all animals including control group were injected with 1 mL of sterile isotonic solution subcutaneously

Table 1
Experimental design and animal group distribution (*N*). The animals were assigned in three groups: sub-lethal irradiation and given treatment with DietGel 76A ("SD" group), sub-lethal irradiation, without DietGel 76A treatment (group "S") and control animals, non-irradiated but receiving the DietGel 76A (Control Group).

Group numbers	Strain	Group "SD" females (*N*)	Group "S" females (*N*)	Control females (*N*)	Total number of animals females (*N*)
1	Mice C57BL/6J stock 000664	6	6	6	18
2	Mice NRG stock 007799	6	6	6	18
3	Mice CD45.1 stock 002014	6	6	6	18
	Total	18	18	18	**54**

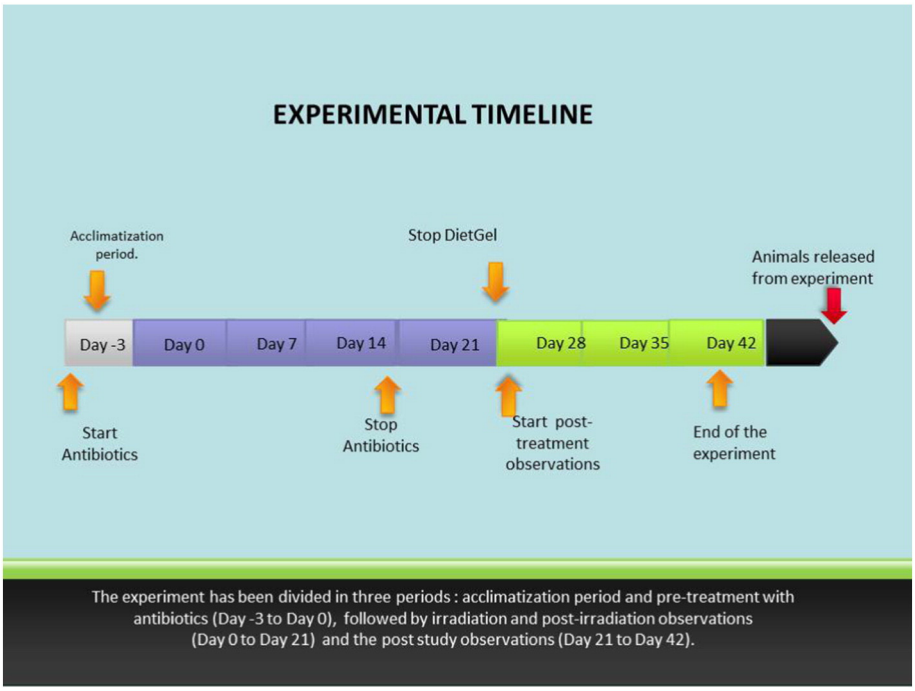

Fig. 1. Experimental timeline.

(Physiological Saline (0.9%), CDMV, product # 1399, St.-Hyacinthe (QC), Canada). The injection was repeated after 24 h. From Day 1 to Day 21 post irradiation, the following observations were made: animal body weight measurements, daily mortality check, daily clinical sign check, and endpoint scores. Special scoring sheets (Fig. 2) were designed to monitor the clinical signs and endpoint score. A decision tree was designed to evaluate and take action according to the endpoints (Fig. 3).

Endpoint Monitoring Form

Animal ID		Protocol	-	Cage /Rack/ room	/ /	Examined by / date	/

Appearerence	Score	Obs.	Body condition	Score	Obs.	Gastro-intestinal	Score	Obs.
Normal	0		Normal	0		Normal feces	0	
Fur dull, ungroomed	1		Slightly thin body condition	1		Small amount of normal feces	1	
Hunched back	2		Slightly overweight	1		Loose feces	2	
Prophyrin discharge	2		Thin body condition	2		Jaundice	2	
Closed Eyes	3		Obese	2		Diarrhea/liquid feces	3	
Moribund	**4**		**Severely emaciated**	**4**		**Bloody Feces**	**4**	

Skin	Score	Obs.	Behaviour	Score	Obs.	Respiratory	Score	Obs.
Normal	0		Normal	0		Normal Respiration	0	
Minor skin wound	1		Depressed	1		Slight increased respiratory rate	1	
Crusts , scabs	1		Isolated	1		Slight decreased respiratory rate	1	
Skin edema	2		Not moving	2		Dyspnoea /Gasping	2	
Multiple skin wounds	2		Hypersensitive to stimuli	2		Nasal discharge	2	
Ulcers	3		Laying on cage's bottom	3		Laborred/irregular	3	
Multiple ulcerations	**4**		**Self-mutilation**	**4**		**Abnormal sound (wheeze/rales)**	**4**	

Dehydration	Score	Obs.	Neurologic exam	Score	Obs.	Urinary	Score	Obs.
Normal hydration	0		Normal	0		Normal urine	0	
Skin turgor slow	1		Head tilt	1		Small amount of urine	1	
Skin turgor permanent	2		Lameness /Paresis/Gait	2		Frequent urination	2	
Shrunken eyes	2		Uncoordinated movements	2		Lack of urine	2	
Reduced activity	3		Paralysis	3		Red discharge of urine	3	
Animal lethargic	**4**		**Permanent convulsions**	**4**		**Blood dripping ext. sphincter**	**4**	
TOTAL COLUMN I			**TOTAL COLUMN II**			**TOTAL COLUMN III**		
Total score								

Procedure to be followed:

- Total score between 0-3 : No measures, the experiment can carry on as designed
- Total score between 3- 9: The animal has to be reevaluated in 72 h. Ask for a veterinary examination of the animal.
- Total score between 10-19: Advise the Principal Investigator. Ask for a veterinary exam, treatments might be suggested by the Vet.
- Total score between 20-24: AHT to advise the Veterinarian, ACC board has to be informed, verify protocol post-approval (PAM).
- Total score between 25-27 or at least one score of 4 : the experiment must be stopped immediately, advise the Veterinarian and ask for euthanasia

Fig. 2. Endpoint monitoring form for critical care of irradiated mice.

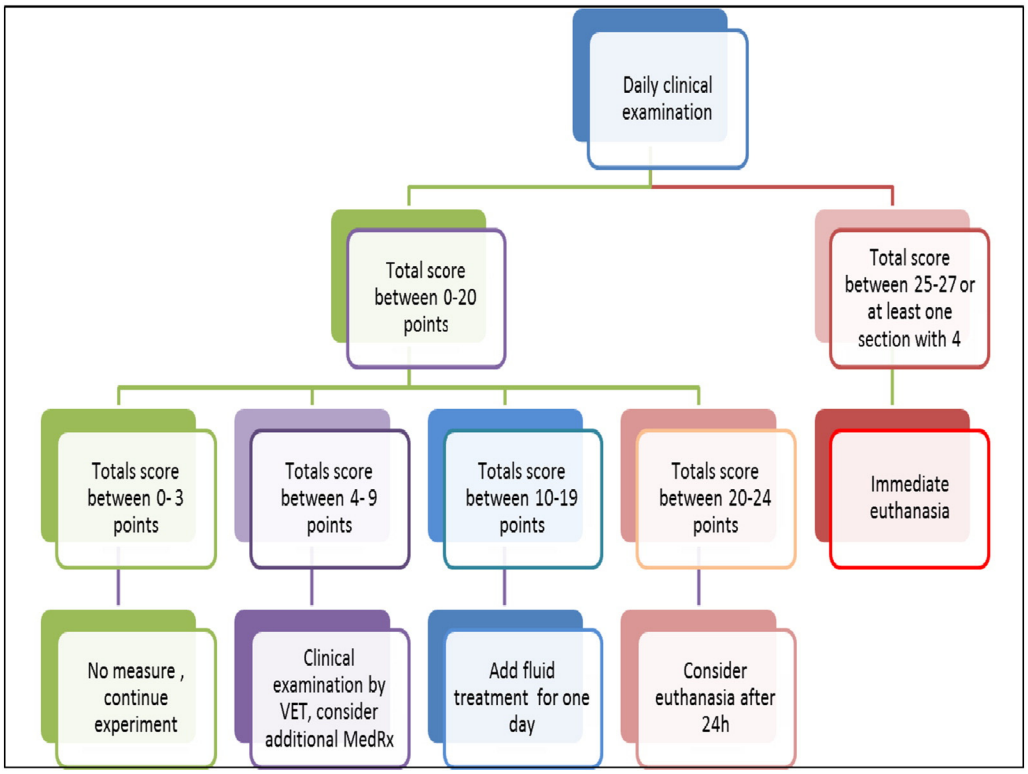

Fig. 3. Decisional tree for critical care of irradiated mice.

Antibiotic administration regimen: antibiotics were provided in the drinking water during the 3 days pre-irradiation and 14 days post irradiation. Red colored bottles were required because the antibiotics used are photosensitive. During first two weeks, a solution of sulfamethoxazole and trimethoprim (NOVO-TRIMEL DS (Sulfamethoxazole-Trimethoprim 200/40), oral suspension, fl. × 400 mL, prod NOVOPHARM, Toronto, ON (TMS) (120 mg/kg) was prepared by suspending 3.5 mL of TMS in 270 mL of drinking water. Each day, the water bottles were stirred to suspend the antibiotics. On the second week, an Enrofloxacin (Baytril™, Baytril Injectable Solution 50 mg/ml Vial/50 mL, CDMV, product # 11242, St.-Hyacinthe, Quebec) (25 mg/kg) was prepared by adding 1.0 mL of Enrofloxacin in 270 mL of drinking water. The solution was replaced with a fresh solution after 7 days.

From 3 days prior to irradiation until Day 21 post-irradiation, the mice were given DietGel® 76A (ClearH2O®, Portland, ME, USA).

DietGel 76A Barrier Packed is a nutritionally complete diet that combines hydration and nutrition in a single serving. A superior alternative to mash diets, it is formulated with purified ingredients, modeled on the 76A maintenance diet formulation. DietGel 76A is flavor-enhanced, resulting in increased consumption.

For the whole period of the experiment (42 days), all animals were evaluated daily and were weighted every other day. Data was recorded manually and statistically interpreted with the Microsoft Excel Analysis Tool Pack. The data are presented as mean and standard deviation (SD) of the mean. Statistical comparisons were applied between groups treated and non-treated mice, irradiated and non-irradiated mice as well as between different strains of transgenic mice. Comparisons were made using one factor analysis of variance (ANOVA).

2.5. Animal housing

Animals were housed on Allentown PNC (Model PNC—positive/negative control, 160 cages (P/NC) individually ventilated cage system with Edstrom™ automated watering system)—Allentown Inc, Allentown, NJ, USA ventilated racks, on negative pressure.

As mentioned in several previous studies (Cui et al., 2002; Drobyski et al., 1994; Hanson et al., 1987; Jackson et al., 2010), irradiated mice remain immune compromised for a period of time after irradiation, and therefore should be housed under strict barrier conditions. Animals in this experiment were handled only under HEPA-filtered, type II, class A Biosafety cabinets and in a 100% fresh filtered HEPA air ventilated room. The room housing the mice after irradiation was maintained under positive air pressure relative to the corridor, in order to minimize the risk of aerosol pathogens from entering the room. The dress code for all personnel (animal health technicians and animal caretakers handling these animals) was the following: sterile gown, gloves, hair bonnet, and surgical mask. The basic goal of these efforts was to prevent the transmission of any potential pathogen from humans or the environment to the transiently immune deficient mice.

3. Results

3.1. Mortality and clinical signs

Mice from all groups pretreated with PBS and antibiotics displayed a survival rate of near to 100% over a 30 day period following irradiation. One animal from the NRG group, irradiated with DietGel started to display clinical signs (dehydration, tremor) and to lose weight up to the end of the observation period. The animal was euthanatized two days before the end of the experiment.

3.2. Radiation sensitivity between NRG, CD45 and C57 mice

In the present study, we found a distinct difference in radiation sensitivity between NRG, CD45 and C57 mice when these mice were irradiated with 4 Gy in a single sub-lethal dose. These findings are consistent with what is reported in other experiments (Fox et al.,

2007; Hanson et al., 1987; http://jaxmice.jax.org/strain/002014.html; http://jaxmice.jax.org/strain/007799.html).

Following the irradiation of the three mouse strains used in this experiment, we discovered that the most sensitive strain was the NRG (BW difference = 1.95, $R^2 = 0.7565$, $\bar{x} = 24.62$), followed by the CD45 (BW difference = 2.85, $R^2 = 0.7822$, $\bar{x} = 20.52$) and the C57 (BW difference = 2.10, $R^2 = 0.7989$, $\bar{x} = 19.80$) (Fig. 4).

Body weight progress for each mouse strain is shown in Figs. 5, 6 and 7.

3.3. Positive effect of DietGel administration as a support treatment

The effect of DietGel administration along with the antibiotic treatment, strict handling and manipulation procedures was assessed as part of the study objectives.

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 most important (Fig. 6). This is probably due to the increased cellular sensitivity to ionizing radiation of the NRG mice (http://jaxmice.jax.org/strain/007799.html), according to the JAX description and phenotype database entry information for this strain.

Irradiated C57 mice supplemented with DietGel had a significant BW improvement in comparison with the non-treated cohort (SS = 19.31 ± 3.34) ($p < 0.05$), (Fig. 7). Following irradiation, during the 21 days of the DietGel 76 A treatment, there is a clear ascending trend of the BW for the treated group (SD), versus non treated (S) group. Variance within irradiated C57 mice group ($\sigma^2 = 0.51$) was greater than the variance within irradiated but not treated C57 mice ($\sigma^2 = 0.39$). 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. The non-treated group, however, had the most remarkable BW loss during the 10–12 days following irradiation, then, due to the reconstitution of the immune system; the mice continued their ascending trend of BW recovering.

NRG mice and to some extent CD45 mice responded less intensely to the addition of DietGel 76A than C57 mice. For CD45 mice, the BW variation between DietGel and non DietGel non-irradiated groups of treated animals was lower (MS = 0.69), while the variation was even less important (MS = 0.41) for the NRG mice.

Another interesting observation for the NRG group is the progression of BW at 21 days after irradiation and even after the retreat of DietGel, where the non-treated group seems to feel the effects of partial total body irradiation, while BW progression of the treated group continues (Fig. 6).

The analysis of the BW variation graphics show two distinct critical moments in the post-irradiation procedure:

- First moment: 4–6 days after irradiation where the mice in non-treated DietGel group showed a clear slowdown of their BW progression and even a certain loss in BW which was compensated in the following days.
- Second moment: following the withdrawal of DietGel and antibiotics (Day 21 post irradiation), which seems to have some effects of the BW variation, but growth BW improvement continues.

This is valid for C57 mice (Fig. 7), but similar patterns have been found in the other two lines as well.

Finally, the comparative data between DietGel treated and control animals (irradiated animals vs. non-irradiated animals) shows that, after 21 days, the effects of whole body irradiation had a significant impact on the animal BW (Fig. 8).

4. Discussion

For the purpose of the study and because the procedure was not followed by the bone marrow transplantation, we did not reach a lethal level of irradiation. It is worth mentioning that the term lethal irradiation vs. non-lethal irradiation are interpreted differently in literature (Cui et al., 2002), as is it is highly dependent on the strain, age and sex of the animals (Abrams, 1951; Holland & Mitchell, 1976; Sacher, 1957); therefore, the most appropriate was to use the irradiation dose (cG) as a unique parameter.

Radiation differs from most toxic agents. The difference is mostly in the very remarkable steepness of the slope of the cumulative curve or the narrowness of the distribution. Usually an increase in dosage of 30 per cent or less (increase from 600 to less than 800 R) will affect survival rate from 100 per cent to zero. Because of this, it makes little difference whether arithmetic or log dose is used in computations. For other toxic agents the distribution is nearly always normal with log dose and the transformation to log dose is required (http://www.informatics.jax.org/greenbook/chapters/chapter22.shtml; Sacher, 1956).

High radiation doses such as 1000 cGy have been documented to be relatively toxic when given to larger species such as dogs and humans (Deeg et al., 1988). However, many strains and stocks of mice have historically been more resistant to irradiation (e.g. C3H strains) while

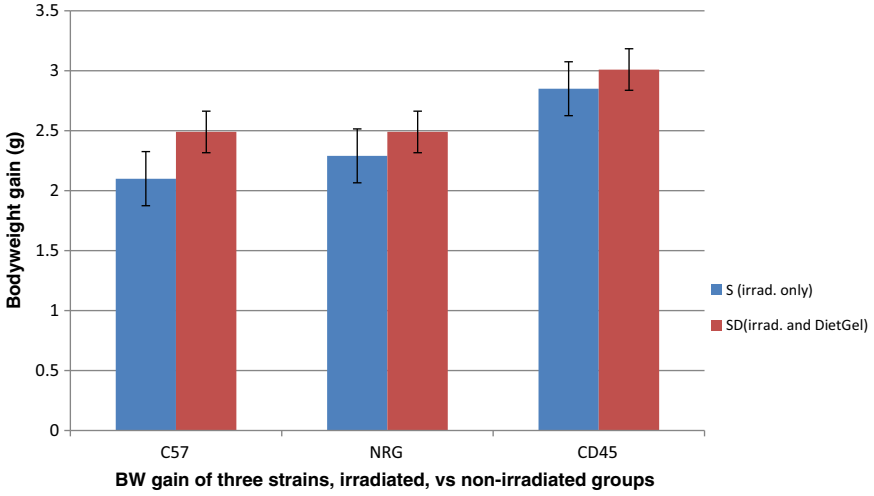

Fig. 4. Mean BW gain of the three strains, irradiated but no treated with DietGel vs. irradiated and treated with DietGel groups.

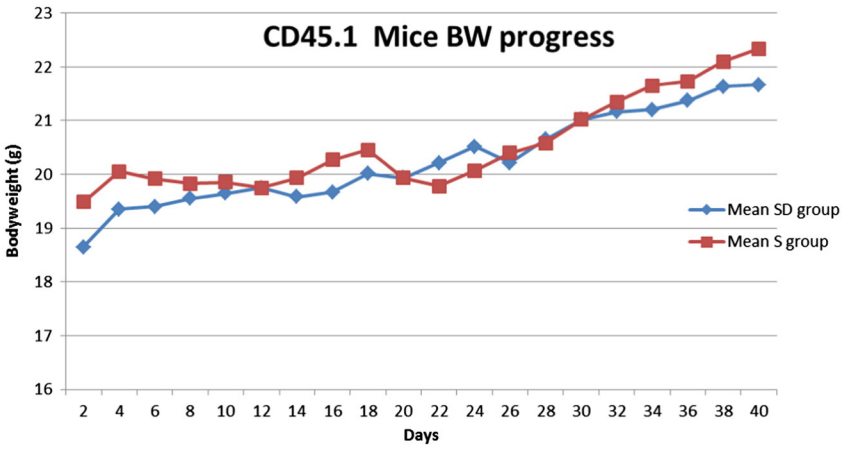

Fig. 5. CD45.1 mice BW progression.

others are significantly more sensitive (e.g. BALB/c) (Hanson et al., 1987; Weil, Stephens, Amos, Ruifrok, & Mason, 1996).

In whole body irradiated animals transplanted with a donor bone marrow, approximately 7 days after BMT, donor-derived cells such as monocytes, dendritic cells, and neutrophils can already be found in the spleen of recipient mice, and by day 21 after BMT, peripheral lympho-hematopoietic reconstitution of all cell lineages may be normal. However, many of these innate cellular effectors are yet not fully functional, and therefore BMT recipient animals are still at risk of opportunistic infection at this time (Auletta, Devecchio, Ferrara, & Heinzel, 2004; Cui et al., 2002; Jackson et al., 2010; Ojielo et al., 2003).

In our experiment, no bone marrow transplant was performed; therefore the reconstitution of hematopoietic system was done exclusively by self-regeneration. Also, the bone marrow transplant may increase the risk of secondary contamination through different bacterial and fungus organisms acquired during the processing and transplant procedures (Klein, Kadidlo, McCullough, McKenna, & Burns, 2006).

Our first objective was to determine whether the use of DietGel76A™ would have a significant impact on clinical signs and general animal survival rate.

The use of DietGel76A™ has significantly improved the recovery of C57BL/6 mice after sub-lethal irradiation. The same treatment had only limited benefits in the other two strains of mice used (CD45.1 and NRG) for the experiment, since no statistically significant difference was shown for body weight of Cd45.1 and NRG groups.

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.

The second objective was to investigate the differences in body weight (BW) between non irradiated and sub-lethal irradiated cohorts, with the purpose of assessing whether a progressive weight loss is occurring, and calculating the impact of this parameter over the animal wellbeing.

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.

One of the hypotheses to be explored is that the DietGel76A™ treatment was prematurely interrupted. A longer administration period of DietGel76A™ would have contributed to a complete recovery of the most sensitive mice to the irradiation procedures.

Our recommendation is to administer the DietGel76A™ to lethally and sub-lethally irradiated mice for a period of at least 42 days after the procedure.

Finally, the third objective was to determine the potential differences between the strains of mice used, in terms of sensitivity and response to gamma-irradiation.

In the present experiment, there were differences between the three strains used. These can be explained by the different genetic and physiological backgrounds of these strains. Genetic constitution is one of the major factors influencing radiation resistance in mice (http://www.informatics.jax.org/greenbook/chapters/chapter22.shtml). Differences between strains of mice are found in percentage of survival

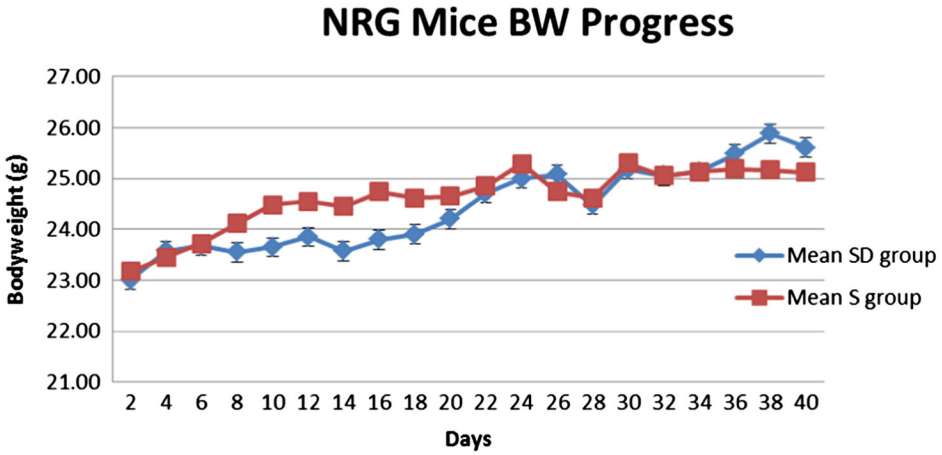

Fig. 6. NRG mice BW progression.

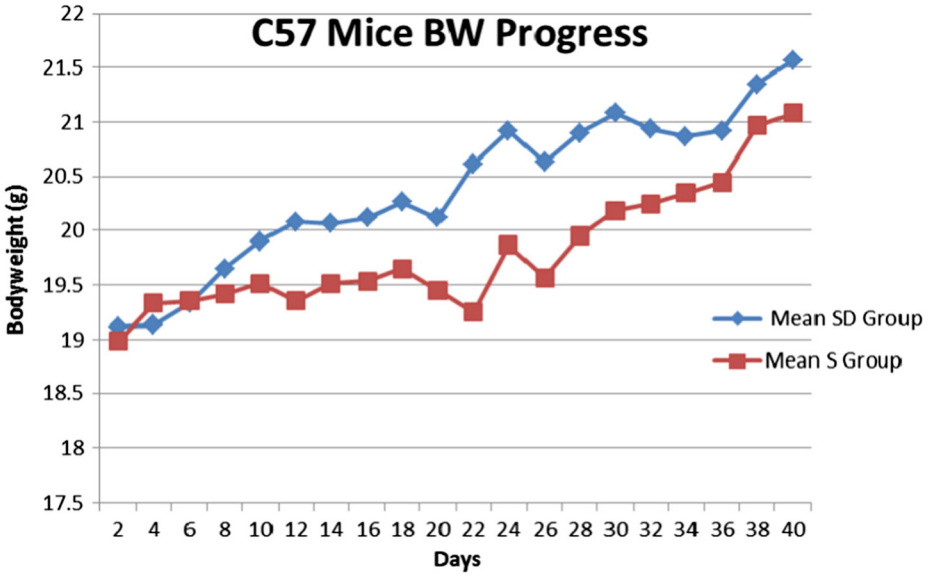

Fig. 7. C57BL6 mice BW chart-irradiated vs. non-irradiated group.

in the first days after irradiation, but also in the length of the recovery period and the recovery of initial BW.

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 C45.1 and the C57BL/6 mice.

5. 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 C57BL/6 strain. The C57 mice group recovered faster after irradiation, and the BW gain difference between DietGel treated animals vs. non treated animals was significantly higher.

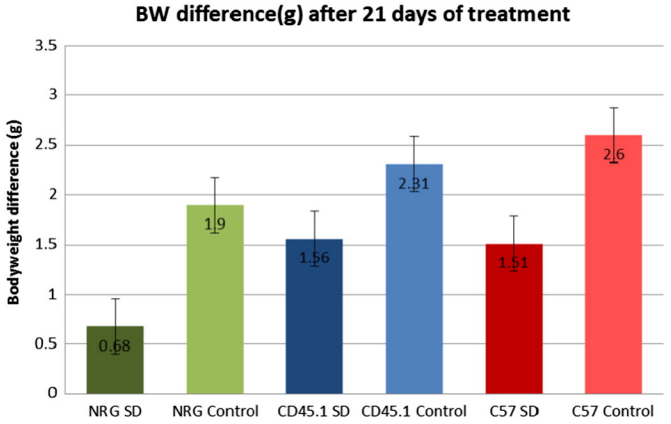

Fig. 8. Effects of irradiation: variation of BW average after 21 days—on 3 strains of mice treated with DietGel, irradiated (SD) vs. non irradiated (control) animals.

- However, the treatment with DietGel76A™ only a very limited impact on the recovery of more irradiation sensitive strains (CD45.1 and mostly NRG). Further studies must be conducted on mice from these strains in order to assess whether mice belonging to more sensitive strains should be on DietGel76A™ for a longer period of time (at least 42 days post irradiation).

Conflict of interest statement

This study was partially funded by Clear H2O Company (IRCM-ANI0036) (Portland, ME, USA), who provided financial support for the conduct of part of the research. The sponsor was not involved in the study design, collection, analysis and interpretation of data, and in the writing of the report. ClearH2O has been consulted in the decision to submit the article for publication. All correspondence for this article should be addressed to ovidiu.jumanca@ircm.qc.ca.

References

Abrams, H. L. (1951). Influence of age, body weight and sex on susceptibility of mice to the lethal effects of X-radiation. *Proceedings of the Society for Experimental Biology and Medicine*, *76*, 729–732.

Auletta, J. J., Devecchio, J. L., Ferrara, J. L., & Heinzel, F. P. (2004). Distinct phases in recovery of reconstituted innate cellular-mediated immunity after murine syngeneic bone marrow transplantation. *Biology of Blood and Marrow Transplantation*, *10*, 834–847.

Canadian Council on Animal Care (1993). *Guide to the care and use of experimental animals.*

Cui, Y. -Z., Hisha, H., Yang, G. -X., Fan, T. -X., Jin, T., Li, Q., et al. (2002). Optimal protocol for total body irradiation for allogeneic bone marrow transplantation in mice. *Bone Marrow Transplantation*, *30*, 843–849.

Deeg, H. J., Storb, R., Longton, G., Graham, T. C., Shulman, H. M., Appelbaum, F., et al. (1988). Single dose or fractionated total body irradiation and autologous marrow transplantation in dogs: Effects of exposure rate, fraction size, and fractionation interval on acute and delayed toxicity. *International Journal of Radiation Oncology, Biology, Physics*, *15*, 647–653.

Drobyski, W. R., Keever, C. A., Hanson, G. A., McAuliffe, T., & Griffith, O. W. (1994, October 1). Inhibition of nitric oxide production is associated with enhanced weight loss, decreased survival, and impaired alloengraftment in mice undergoing graft-versus-host disease after bone marrow transplantation. *Blood*, *84*(7), 2363–2373.

Duran-Struuck, R., & Dysko, R. C. (2009). Principles of Bone Marrow Transplantation (BMT): Providing Optimal Veterinary and Husbandry Care to Irradiated Mice in BMT Studies.

The mouse in biomedical research. Fox, J. G., et al. (Ed.). (2007). (2nd ed.). *ACLAM Series, Vol. 3.* (pp. 453). Academic Press.

Hanson, W. R., Fry, R. J., Sallese, A. R., Frischer, H., Ahmad, T., & Ainsworth, E. J. (1987). Comparison of intestine and bone marrow radiosensitivity of the BALB/c and the C57BL/6 mouse strains and their B6CF1 offspring. *Radiation Research*, *110*, 340–352, http://dx.doi.org/10.2307/3577002.

Hanson, et al. (1987). Comparison of intestine and bone marrow radio sensitivity of the BALB/c and the C57BL/6 mouse strains and their B6CF1 offspring. *Radiation Research*, *110*, 340–352.

Holland, J. M., & Mitchell, T. J. (1976, May). The relationship of strain, sex, and body weight to survival following sublethal whole-body X-irradiation. *Radiation Research*, *66*(2), 363–372.

Jackson, Isabel L., Vujaskovic, Zeljko, & Down, Julian D. (2010, January). Revisiting strain-related differences in radiation sensitivity of the mouse lung: Recognizing and avoiding the confounding effects of pleural effusions. *Radiation Research*, *173*(1), 10–20, http://dx.doi.org/10.1667/RR1911.1.

Klein, M. A., Kadidlo, D., McCullough, J., McKenna, D. H., & Burns, L. J. (2006). Microbial contamination of hematopoietic stem cell products: incidence and clinical sequelae. *Biology of Blood and Marrow Transplantation*, *12*, 1142–1149.

http://www.informatics.jax.org/greenbook/chapters/chapter22.shtml

http://jaxmice.jax.org/strain/002014.html

Miura, Y. (2004). Oxidative stress, radiation-adaptive responses, and aging. *Journal of Radiation Research*, *45*, 357–372.

http://jaxmice.jax.org/strain/007799.html

Ojielo, C. I., Cooke, K., Mancuso, P., Standiford, T. J., Olkiewicz, K. M., Clouthier, S., et al. (2003). Defective phagocytosis and clearance of *Pseudomonas aeruginosa* in the lung following bone marrow transplantation. *Journal of Immunology*, *171*, 4416–4424.

Sacher, G. A. (1956). On the statistical nature of mortality, with especial reference to chronic radiation mortality. *Radiology*, *67*, 250–257 (See also PubMed).

Sacher, G. A. (1957). Dependence of acute radiosensitivity on age in adult female mouse. *Science*, *125*, 1039–1040.

Weil, M. M., Stephens, L. C., Amos, C. I., Ruifrok, A. C., & Mason, K. A. (1996). Strain difference in jejunal crypt cell susceptibility to radiation-induced apoptosis. *International Journal of Radiation Biology*, *70*, 579–585, http://dx.doi.org/10.1080/095530096144789.