Saving Lives One at A Time

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1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol mitigates the hematopoietic syndrome of lethal acute radiation syndrome in mice

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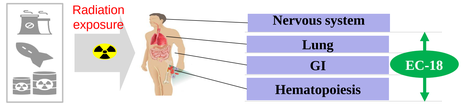
Abstract

The acute radiation syndrome (ARS) is a broad term used to describe a range of signs and symptoms that reflect severe damage to specific organ systems and that can lead to death within hours to several months after exposure. In this study, we investigated the efficacy of EC-18 for the development of a medical countermeasure for ARS by analyzing ionizing radiation (IR)-induced mortality and morbidity. First, we established a murine model of the ARS by exposing eleven week old male and female BALB/c mice to 6.0-6.5Gy doses of total body irradiation (TBI; γ-ray, ^{60}Co, 1553R/min), and assessed for 30 day survival, mean survival time and lethality dose (LD). The $LD_{70/30}$ with confidence interval (CI) was 6.11Gy (5.98-6.22Gy). To determine the efficacy of EC-18 in IR-induced mortality, we exposed BALB/c mice to a 6.11Gy dose ($LD_{70/30}$) of TBI and orally administered 10-250 mg/kg/day of EC-18, starting one day after irradiation. As a result, 6.11Gy of γ-radiation caused the death of 80% of the animals of positive control group within 23days, with an average life span (ALS) of 17.9days. The percentages of survival of the irradiated mice with EC-18 10, 50, and 250mg/kg were 20%, 40%, and 80% with ALS of 19.3, 22.3, and 28.2days, respectively. Moreover, the LD70/30 dose of γ-ray irradiation caused a substantial decrease in the body weight of the mice. The administration of EC-18 effectively prevented severe weight loss induced by irradiation. Next, we investigated the efficacy of EC-18 for hematopoietic ARS (H-ARS) by analyzing the kinetics of white blood cells (WBC), red blood cells (RBC), and platelets. A single whole body exposure of γ-radiation (6.11Gy) rapidly exhausted all kinds of WBC counts, and the administration of EC-18 significantly attenuated γ-radiation-induced depletion of WBCs in the irradiated mice. Especially, the administration of EC-18 substantially reduced γ-radiation-induced reduction of the absolute neutrophil counts (ANC). The mean first day of neutropenia (ANC<500cells/μL) of control and EC-18-treated cohorts was 1.8±1.09 and 2.2±1.09 days, respectively. Although EC-18 did not protect the irradiated mice from experiencing severe neutropenia, it effectively reduced the duration of severe neutropenia from 13.0 days to 7.2±1.79days. In addition, EC-18 significantly increased the mean nadir of ANC after γ-ray irradiation from 4.0±5.48 cells/μL to 20.0±10.00 cells/μL. In addition, the administration of EC-18 in the irradiated mice remarkably attenuated the rapid reduction of RBCs and hemoglobin. When exposed to a supra-lethal dose (8Gy) of γ-radiation, the two of five mice in the control cohort experienced severe skin discoloration and edema formation on the front right feet and hemorrhagic telangiectasia on the tales on day10. EC-18 remarkably improved γ-radiation-induced skin damage in the irradiated mice. Based on the observations in this study, we concluded that EC-18 has potential as a medical countermeasure for ARS.

Introduction

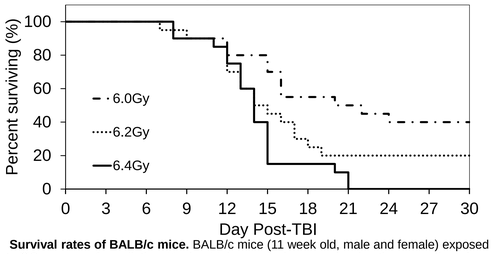
- The acute radiation syndrome (ARS) is a broad term used to describe a range of signs and symptoms that reflect severe damage to specific organ systems and that can lead to death within hours or up to several months after exposure. The ARS occurs after whole-body or significant partial-body irradiation of greater than 1 Gy, over a short time period (high dose rate). **López, Mario, and Margarita Martín. Reports of Practical Oncology & Radiotherapy 16.4 (2011): 138-146.**
- Since the risk of exposure to radiation continues to increase, there has also been an increasing interest in the search of ways of protection against the effects of acute irradiation by ionizing radiation in accidental condition. **Aminin, Dmitry L., et al. Natural product communications 6.5 (2011): 587-592.**



Overview of ARS and Symptoms

Results

1. Determination of Lethal Dose (LD)XX/30 in γ-radiation-induced acute radiation syndrome mice model

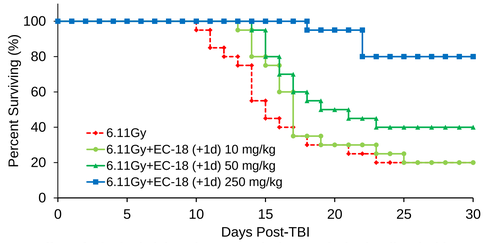


Survival rates of BALB/c mice. BALB/c mice (11 week old, male and female) exposed to ^{60}Co source of γ-radiation. Kaplan-Meier survival curves showing the proportion of mice surviving at each time points for each radiation dose of γ-ray.

LD XX/30	LD estimate (Gy)	Lower 95% CI (Gy)	Upper 95% CI (Gy)
LD30/30	5.31	4.98	5.56
LD50/30	5.79	5.59	5.96
LD70/30	6.11	5.98	6.22
LD95/30	6.39	6.30	6.48

Table 1. Estimated lethal dose in BALB/c mice after ^{60}Co γ-radiation.

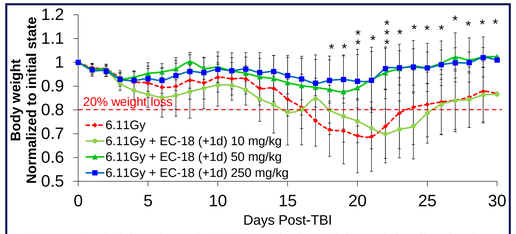
2. Dose effect relationship of EC-18 on the survival rate and body weight loss under γ-ray-induced acute radiation syndrome (ARS)



Dose effect of EC-18 administration on survival rates of mice irradiated with a dose of 6.11Gy of γ-radiation.; *P=0.0011, 6.11Gy + EC-18 50mg/kg versus 6.11Gy; *P<0.0001, 6.11Gy + EC-18 250mg/kg versus 6.11Gy (Log rank test)

Treatment	Mice Survived /total	Survival rate	Mean survival time (days)	Median Survival (days)	Log-rank test p*
6.11Gy	4/20	20%	17.9	15	
6.11Gy + EC-18 10mg/kg	4/20	20%	19.3	17	0.4425
6.11Gy + EC-18 50mg/kg	8/20	40%	22.3	20	0.0464
6.11Gy + EC-18 250mg/kg	16/20	80%	28.2	30	<0.0001

Table 2. Dose effect relationship of EC-18 on survivability and average life duration of irradiated mice

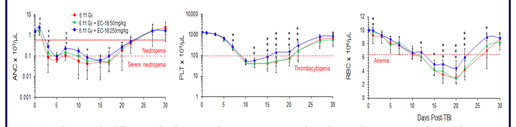


Effects of administration of EC-18 on body weights of irradiated mice. Normalized body weights of irradiated mice with a 6.11Gy dose of γ-radiation.

Treatment	10% Body Weight Loss		20% Body Weight Loss	
	n	%	N	%
6.11Gy	16	80%	8	40%
6.11Gy + EC-18 10mg/kg	17	85%	14	70%
6.11Gy + EC-18 50mg/kg	11	55%	7	35%
6.11Gy+ EC-18 250mg/kg	3	15%	3	15%

Table 3. PLAG significantly mitigates body-weight loss in mice exposed to the LD70/30 dose of γ-radiation.

3. PLAG mitigates the depletion of ANC, PLT, RBC, HGB in mice exposed to LD70/30 dose of γ-radiation.



EC-18 showed efficacy in improving neutropenia, thrombocytopenia and anemia in 24 h-delayed treatment model.

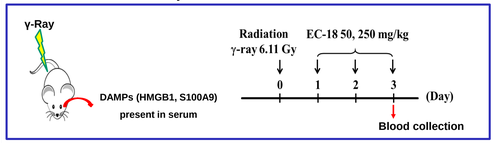
Treatment	Severe Neutropenia (ANC < 10^2 cells/μL)		Thrombocytopenia (PLT<10^5 cells/μL)		Anemia (HGB < 12 g/dL)	
	Mean First Day (±SE, range)	Mean Duration in Days (±SE, range)	Mean First Day (±SE, range)	Mean Duration in Days (±SE, range)	Mean First Day (±SE, range)	Mean Duration in Days (±SE, range)
6.11Gy	5.6±0.9 (3-17)	14.5±1.6 (4-23)	11.7±0.2 (7-12)	11.6±1.3 (5-18)	11.3±0.6 (3-17)	16.4±1.1 (10-18)
6.11Gy + EC-18 50mg/kg	9.0±1.1 (3-17) (*P* = 0.0271)	11.4±1.4 (4-19) (*P* = NS)	12.0±0.0 (12-12) (*P* = NS)	12.5±1.3 (5-18) (*P* = NS)	12.5±0.3 (12-17) (*P* = NS)	15.1±1.5 (5-18) (*P* = NS)
6.11Gy + EC-18 250mg/kg	12.1±0.8 (3-17) (*P* < 0.0001)	8.8±0.7 (5-14) (*P* = 0.0025)	12.0±0.0 (12-12) (*P* = NS)	6.0±0.5 (5-10) (*P* < 0.0001)	14.5±0.6 (12-17) (*P* = 0.0005)	9.1±1.0 (5-18) (*P* = 0.0004)

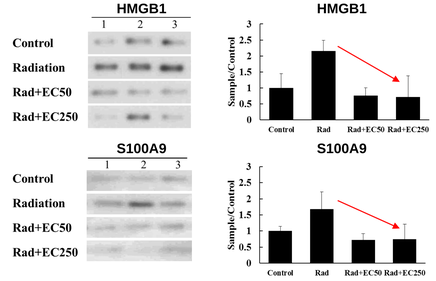
Table 4. Mean first day and mean duration of severe neutropenia (ANC <100 cells/μL), thrombocytopenia (PLT <100 x 10^3 cells/μL) and nemia (HGB <12 g/dL) in control and EC-18-treated mice exposed to lethal radiation dose

Treatment	Nadir of ANC (cells/μL)	Mean Number of Days to recovery ANC ≥ 500cells/μL (±SE, range)	Nadir of platelet (10^3 cells/μL)	Mean Number of Days to recovery platelet ≥ 10^6cells/μL (±SE, range)	Nadir of RBC (10^6 cells/μL)	Mean Number of Days to recovery RBC ≥ 6.3 x 10^6 cells/μL (±SE, range)
6.11Gy	29.0±4.3	25.2±1.9 (22-30)	35.4±3.7	23.2±1.3 (17-30)	3.8±0.4	28.0±1.3 (22-30)
6.11Gy + EC-18 50mg/kg	42.0±6.3 (*P* = NS)	25.4±1.1 (22-30) (*P* = NS)	38.0±3.5 (*P* = NS)	24.5±1.3 (17-30) (*P* = NS)	3.7±0.2 (*P* = NS)	29.1±0.9 (22-30) (*P* = NS)
6.11Gy + EC-18 250mg/kg	72.5±5.2 (*P* < 0.0001)	26.2±0.9 (22-30) (*P* = NS)	58.5±4.2 (*P* = 0.0002)	18.0±0.5 (17-22) (*P* = 0.0002)	4.8±0.2 (*P* = 0.0225)	25.3±1.0 (22-30) (*P* = NS)

Table 5. Mean nadir and mean number of days to recovery of ANC, platelets and RBCs in control and EC-18-treated mice exposed to lethal radiation dose

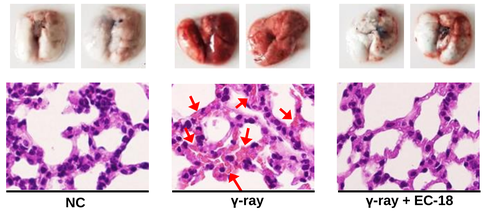
4. Effect of EC-18 on γ-radiation-induced DAMP release





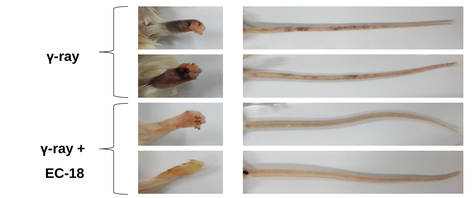
Effect of EC-18 administration on the release of HMGB1 and S100A9 in blood of mice irradiated with a dose of 6.11Gy of γ-radiation.

5. Effect of EC-18 on γ-radiation-induced lung injury.



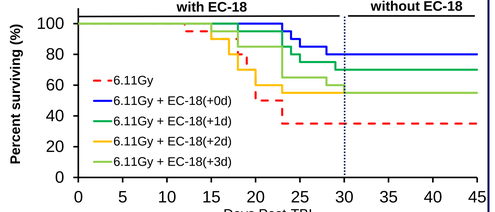
Effect of EC-18 administration on vascular leakage in lung of mice irradiated with a dose of 6.11Gy of γ-radiation.

6. Effect of EC-18 on γ-radiation-induced hemorrhagic telangiectasia and edema.



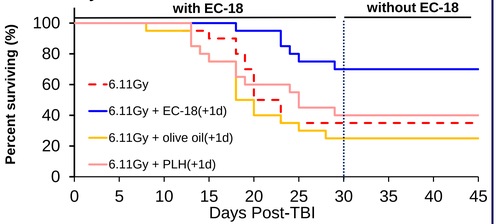
Effect of EC-18 administration on skin discoloration and edema formation of mice irradiated with a dose of 8Gy of γ-radiation.

7. Delayed administration effect of EC-18 on Survivability



Radiation only vs. EC-18 (250mpk) co-treatment, * *P* < 0.001; Radiation only vs EC-18 24h post-IR, *** *P* < 0.001; Radiation only vs EC-18 48h post-IR, ** *P* < 0.01; Radiation only vs EC-18 72h post-IR, ** *P* < 0.01**

8. Comparison of EC-18 with olive oil and PLH on Survivability



Olive oil (same calorie) and palmitic linoleic hydroxyl glycerol (PLH) showed little effect on survival in 24h delayed treatment. EC-18 has a distinctive mechanism of action for improving survivability in γ-radiation-induced ARS.

Conclusion

- Under γ-radiation-induced ARS condition, the administration of EC-18 significantly attenuated the radiation-associated mortality and loss of body weight in a dose-dependent manner.
- γ-radiation induced the rapid exhaustion of all kinds of blood cells, which is defined by γ-radiation-induced hematopoietic injury. The administration of EC-18 significantly attenuated γ-radiation-induced reduction of ANC, PLT and RBC counts.
- Based on the observations in this study, we concluded that EC-18 has therapeutic potential for improving survivability and reducing hematological damage in γ-radiation-induced ARS.