Mitigating effect of EC-18 on gamma radiation-induced systemic inflammatory response syndrome

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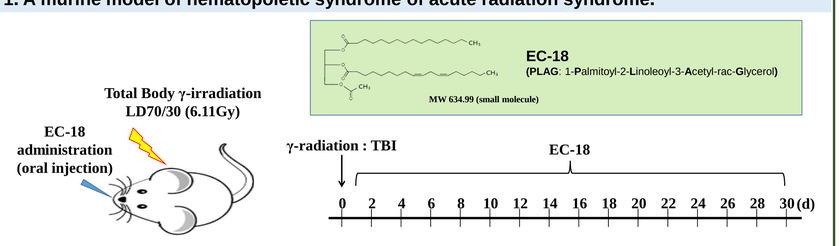
Abstract

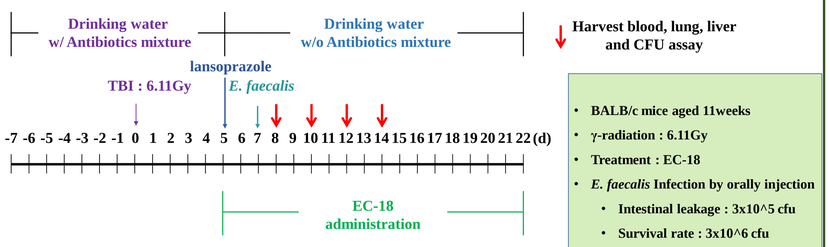
Acute radiation syndrome (ARS) is a collection of adverse health effects caused by exposure to high doses of ionizing radiation in a short time. Previously, we verified the therapeutic potential of EC-18 as a radiation countermeasure by mitigating radiation-associated mortality and immunodeficiency in BALB/c mice after exposure to total-body irradiation (TBI) with gamma radiation. In this study, we show that EC-18 prevents gamma radiation-induced systemic inflammatory response by reducing vulnerability to pathogenic infections. The murine model of ARS was established as described previously. Briefly, eleven-week-old male and female BALB/c mice were exposed to the lethal dose (LD) 70/30 of TBI. Then, the mice were orally administered with either PBS (vehicle control) or EC-18 at the doses of 50, 100, 250, and 500 mg/kg beginning 24 hr post-exposure and continuing daily to the end of the experiment. A single exposure to LD70/30 of TBI induced an immediate increase in the blood levels of CXCL1 (2.9 fold), CXCL2 (1.6 fold), and IL-6 (20.9 fold) at 6 hr post-TBI, but the cytokine levels returned to the baseline afterward. When the irradiated mice started to die around 15 days post-TBI, they exhibited a second surge in the blood levels of CXCL1 (28 fold), CXCL2 (42.6 fold), IL-6 (91 fold), and C-reactive protein (5.4 fold). However, EC18-treated groups showed a significant decrease in the blood levels of them ($p < 0.001$). In order to investigate the cause of death in our ARS mice model, we hypothesized that the proinflammatory cytokine surge of irradiated mice might be caused by IR-induced disruption of intestinal epithelial barrier function, thereby, external or internal pathogens might invade other sterile organs. To test our hypothesis, irradiated mice were orally infected with *Enterococcus feacalis* (*E. faecalis*) and bacterial growth was observed in multiple organs including blood, liver, and lung. The results demonstrated that *E. faecalis* grew progressively in organs of the infected mice, whereas the infected mice administered with EC-18 250 mg/kg exhibited considerably fewer occurrences and lower bacterial growth rate ($p < 0.05$). Based on these observations, we believe that EC-18 has high potential as a radiation countermeasure for gamma-radiation ARS.

Experimental Design

1. A murine model of hematopoietic syndrome of acute radiation syndrome.



2. Intestinal leakage (bacterial growth) and survival rate of mice with γ-Radiation and bacterial translocation model

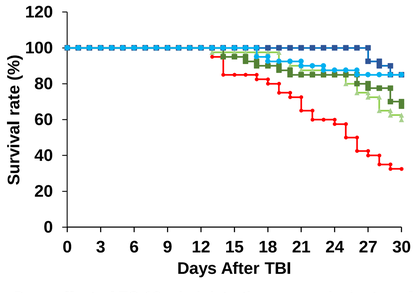


- BALB/c mice aged 11weeks
- γ-radiation : 6.11Gy
- Treatment : EC-18
- *E. faecalis* Infection by orally injection
  - Intestinal leakage : 3x10^5 cfu
  - Survival rate : 3x10^6 cfu

Drinking water w/ antibiotics mixture : 4mg/ml penicillin, streptomycin and bacitracin

Results

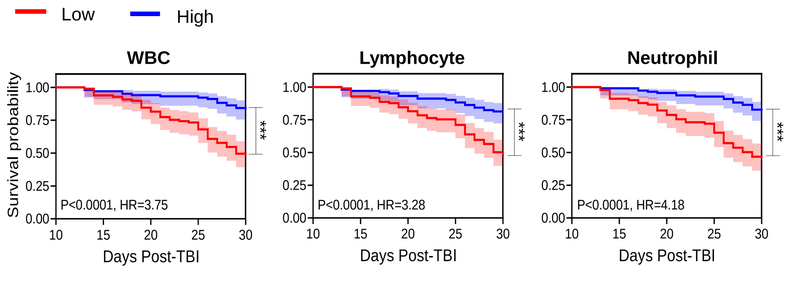
1. Dose effect relationship of EC-18 on the survival rate under γ-ray-induced acute radiation syndrome (ARS)

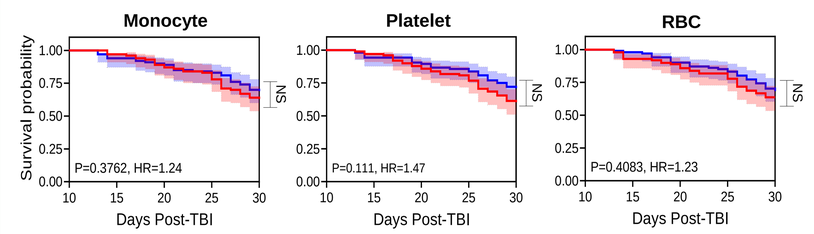


	Mice Survived /total	Survival rate	Log-rank Test p*
6.11Gy	13/40	32.5 %	
6.11Gy + EC-18 50	24/40	60 %	** (0.0041)
6.11Gy + EC-18 100	27/40	67.5 %	*** (0.0008)
6.11Gy + EC-18 250	34/40	85 %	*** (< 0.001)
6.11Gy + EC-18 500	34/40	85 %	*** (< 0.001)

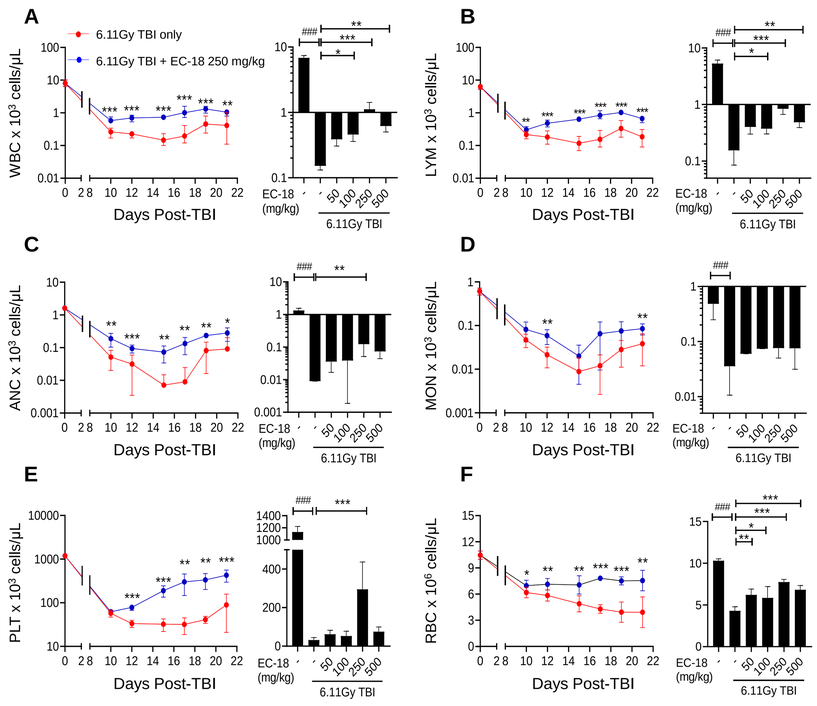
Dose effect of EC-18 administration on survival rates of mice irradiated with a dose of 6.11Gy of γ-radiation.
* indicates 6.11Gy + EC-18 versus 6.11Gy, *,*P* <0.05, **,*P* <0.01, ***,*P* <0.001. (Log rank test)

2. Relationship of hematologic damage on the survival rate under γ-ray-induced ARS model



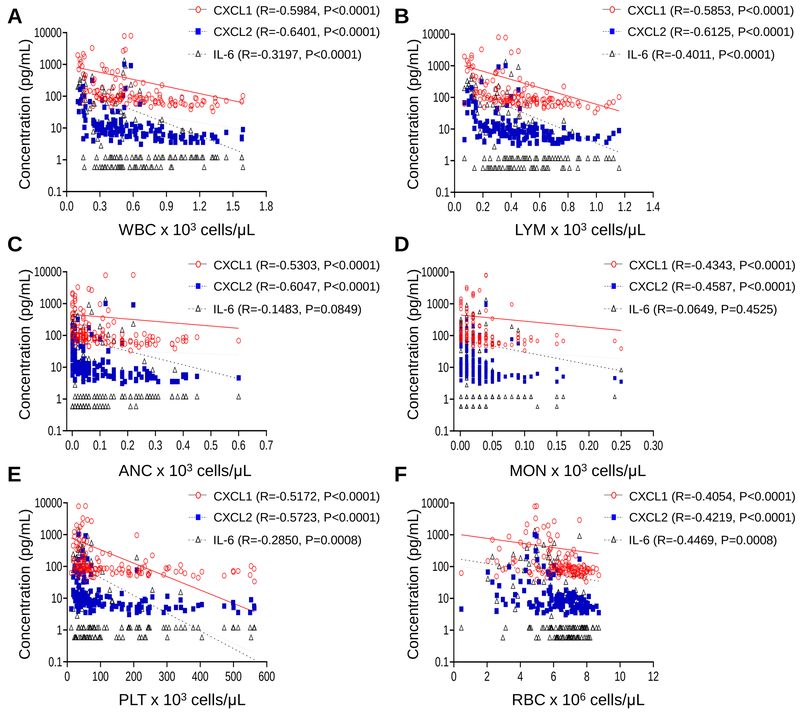


Blood cells and survival. Survival curves were based on each blood cell measured (n=200). Cox regression model showing overall survival with CIs for each blood cell based on time from TBI to last follow-up date (death or still survived), with significance indicated by P value and hazard ratio (HR). The number of cells were measured using CBC analyzer, and the high versus low values were determined using a cutoff above the median for each blood cell. There was worse survival if WBCs, lymphocytes and neutrophils were low (red, below cutoffs of 0.38 x 10^3 cells/μL for WBC, 0.23 x 10^3 cells/μL for lymphocytes, 0.07 x 10^3 cells/μL for neutrophils) versus high (blue, above cutoffs). However, there were little relationship between survival and other blood cells (cutoff value of 0.04 x 10^3 cells/μL for monocytes, 45 x 10^3 cells/μL for platelets, and 6.52 x 10^6 cells/μL for RBC). Each line indicates the predicted survival probability over follow-up time, with the error band indicating the corresponding two-sided 95% CI.

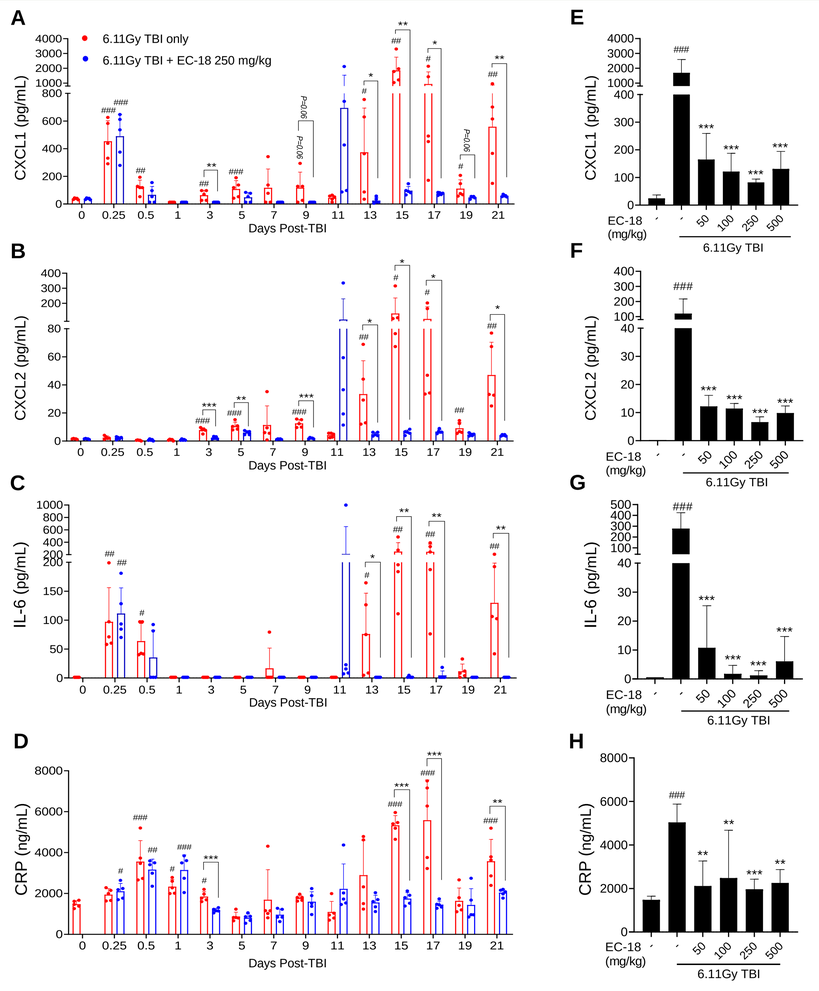


EC-18 mitigates the hematopoietic syndrome in irradiated mice. Mice (n=6-7 males per group) were exposed to the LD70/30 (6.11Gy) of gamma radiation and administered 50, 100, 250 and 500mg/kg/day of EC-18 by oral gavage on days 1-21 beginning ~24 hr after TBI. # indicates negative control vs. 6.11Gy TBI only, and * indicates 6.11 Gy TBI only vs. 6.11 Gy + EC-18-treated groups. #/*P < 0.05, ##/**P < 0.01, ###/***P < 0.001. The complete blood counts (CBC) data are representative of five independent experiments with six to seven mice per group.

3. Association between hematological nadirs and inflammatory cytokine/chemokines

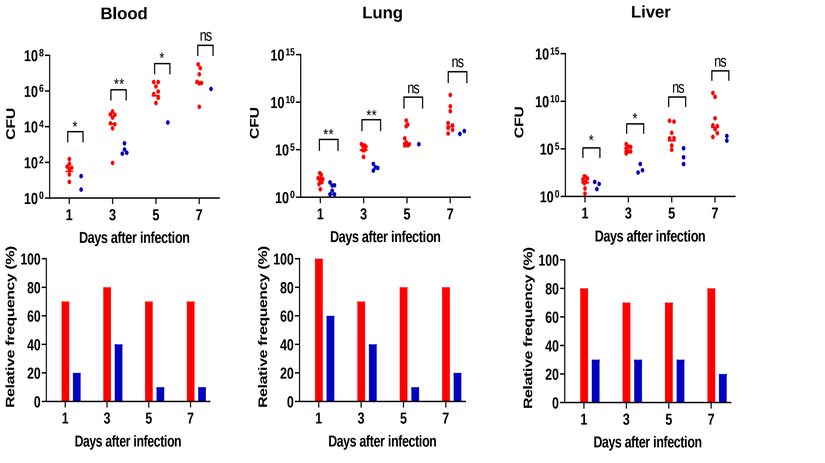


Spearman's rank correlation for hematology and cytokines/chemokines in irradiated mice. The blood samples were harvested from mice (n=136 males per day) on days 15-21 after a single LD70/30 (6.11Gy) of TBI. Correlation plot between (A) WBCs, (B) lymphocytes, (C) ANCs, (D) monocytes, (E) platelets, and (F) RBCs and pro-inflammatory cytokine/chemokines CXCL1, CXCL2 and IL-6 (spearman's rank correlations).



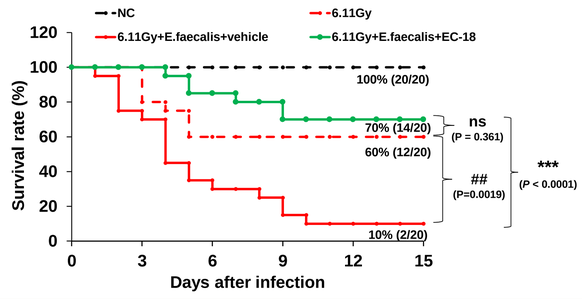
PLAG attenuates blood levels of inflammatory cytokines/chemokines and C-reactive protein (CRP) in irradiated mice. Effect of EC-18 administration on the kinetics of (A) the chemokine (C-X-C motif) ligand 1 (CXCL1), (B) CXCL2, (C) interleukin-6 (IL-6) and (D) CRP in blood after TBI. Dose effect of PLAG administration on the blood level of (E) CXCL1, (F) CXCL2, (G) IL-6, and (H) CRP on day 15 after TBI. # indicates negative control vs. 6.11Gy TBI only, and * indicates 6.11 Gy TBI only vs. 6.11 Gy + PLAG-treated groups. #/*P < 0.05, ##/**P < 0.01, ###/***P < 0.001.

4. Effect of EC-18 on bacterial translocation and survival rate under γ-ray-induced ARS mice with *E. faecalis* infection model



Bacterial growth in tissues and occurrence rate with positive *E. faecalis* cultures of lethally-irradiated mice orally infected with E.faecalis.

Effect of EC-18 administration on survival rates of mice exposed to LD70/30 (6.11Gy) of TBI with γ-ray and orally infected with *E. faecalis*.
# indicates 6.11Gy versus 6.11Gy + E.faecalis + vehicle , and * indicates 6.11Gy + *E.faecalis* + EC-18 versus 6.11Gy + *E.faecalis* + vehicle; #/*, *P* <0.05, ##/**,*P* <0.01, ###/***,*P* <0.005. (Log rank test)



Conclusion

- Under γ-radiation-induced ARS condition, we found that low hematological nadirs (high cytokine levels) are significantly associated with high mortality (low hematological nadirs).
- The administration of EC-18 significantly attenuated the hematopoietic syndrome and systemic inflammatory responses in a dose-dependent manner.
- The administration of EC-18 significantly reduced γ-radiation-induced enterobacterium translocation and γ-radiation and enterobacterium-induced lethality.
- Based on the observations in this study, we concluded that EC-18 has therapeutic potential for improving survivability and reducing hematological and intestinal damage in γ-radiation-induced ARS.