Mitigation of gastrointestinal acute radiation syndrome by PLAG via inhibition of necroptosis in mice (PS7-42)

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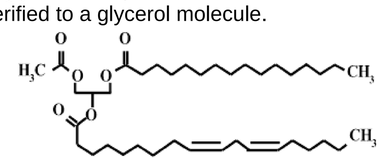
INTRODUCTION

- Gastrointestinal acute radiation syndrome (GI-ARS) is a life-threatening illness characterized by clonogenic crypt cell loss and villous atrophy, resulting in mucosal barrier destruction, malabsorption, microbial invasion, inflammation, and eventually leading to death.
- Several scientific evidences suggest that necroptosis is responsible for the sustained tissue damage and pro-inflammatory cytokine production caused by irradiation.
- PLAG (1-Palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol; known as EC-18) is a triacylglycerol composed of an acetic acid and two long chain fatty acids esterified to a glycerol molecule.

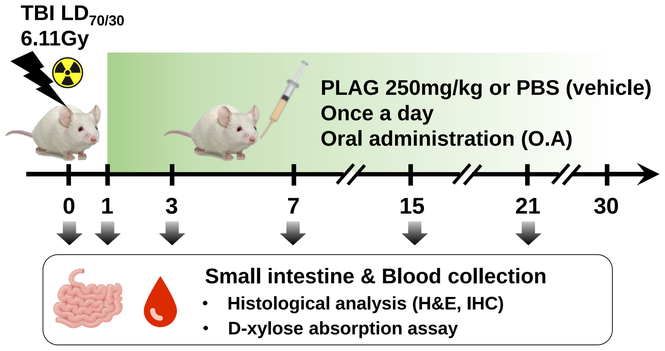


Chemical structure of PLAG (EC-18)
(1-Palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol; MW 639.99)

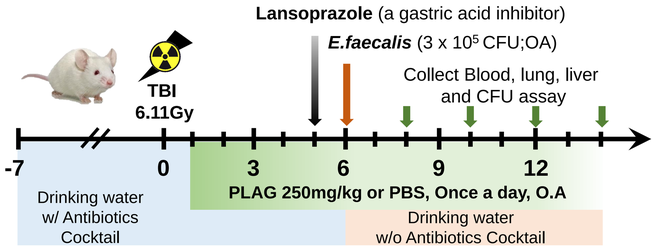
- We investigated the mitigating effects of PLAG on radiation-induced gastrointestinal injuries in the total body irradiation (TBI) mouse model.
- Our results show that PLAG facilitates the structural restoration of the intestinal tissues after irradiation, thus leading to the recovery of absorptive capacity and the protection from gut-microbial invasion.
- The current study also demonstrates the inhibitory effect of PLAG on radiation-induced necroptosis signaling activation in the intestinal epithelium.

METHOD

1. A murine model of TBI-induced gastrointestinal acute radiation syndrome (GI-ARS)

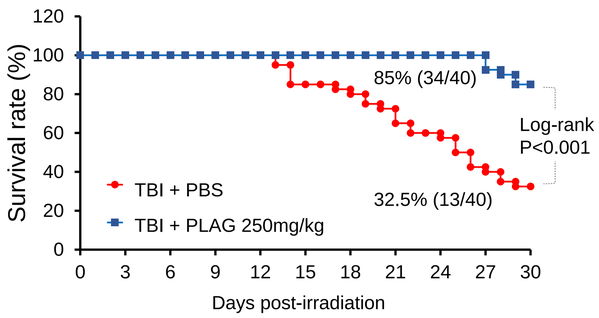


2. TBI-induced intestinal leakage mice model



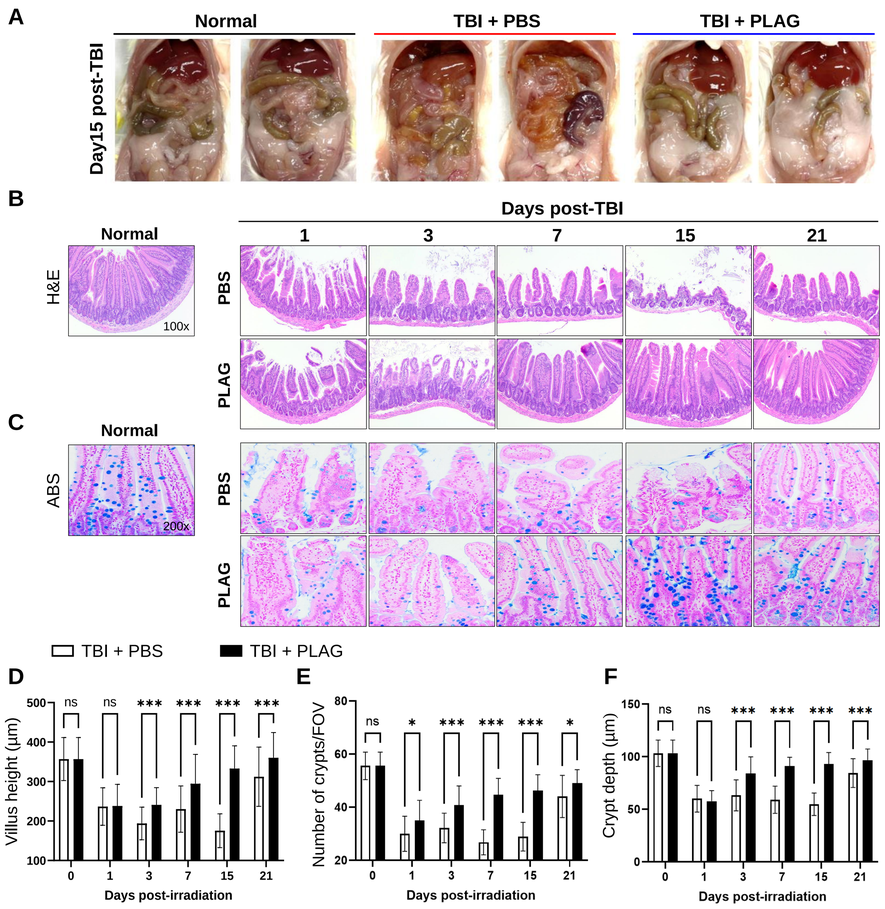
RESULTS & DISCUSSION

1. Effect of PLAG on survival rate under TBI-induced ARS



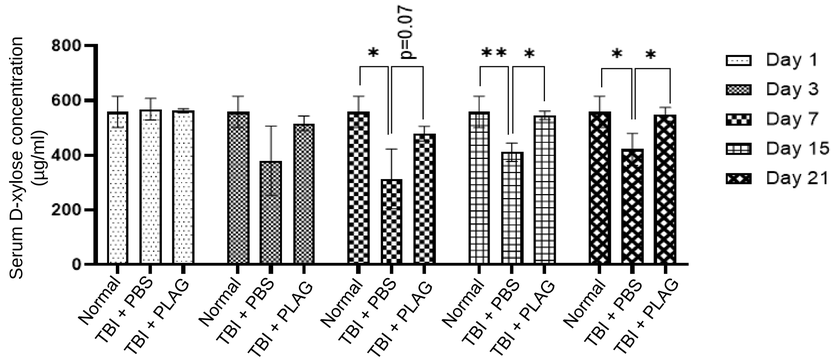
- PLAG significantly improved a 30-day survival rate of the TBI mice by 85% compared to 32.5% in the vehicle control group.

2. PLAG mitigates TBI-induced intestinal structural injury in mice.



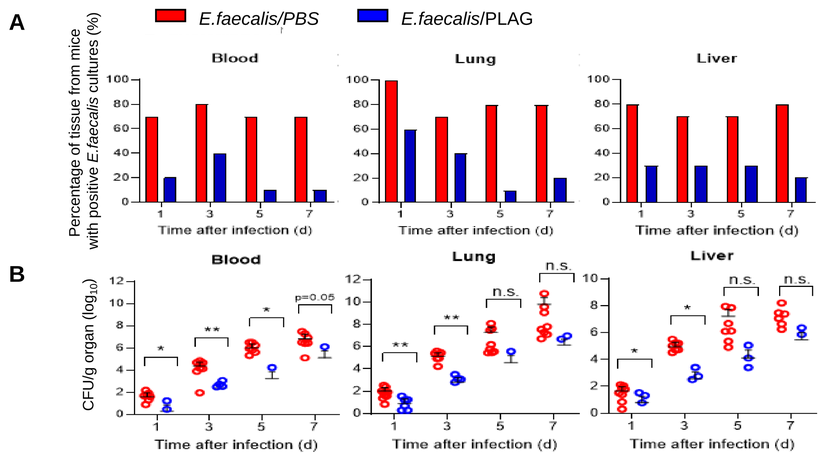
- Macroscopic observation of gut of the TBI mice on day 15 after irradiation indicates PLAG effectively alleviated radiation-induced gastrointestinal injury (A).
- PLAG facilitated the structural restoration of the intestinal tissues after irradiation by significantly increasing the villus heights, crypt number and crypt depth (B,D,E,F).
- PLAG promoted the repopulation of mucin-producing goblet cells in the intestinal epithelium of the TBI mice (C).

3. PLAG restores intestinal functionality in TBI-induced GI-ARS mice



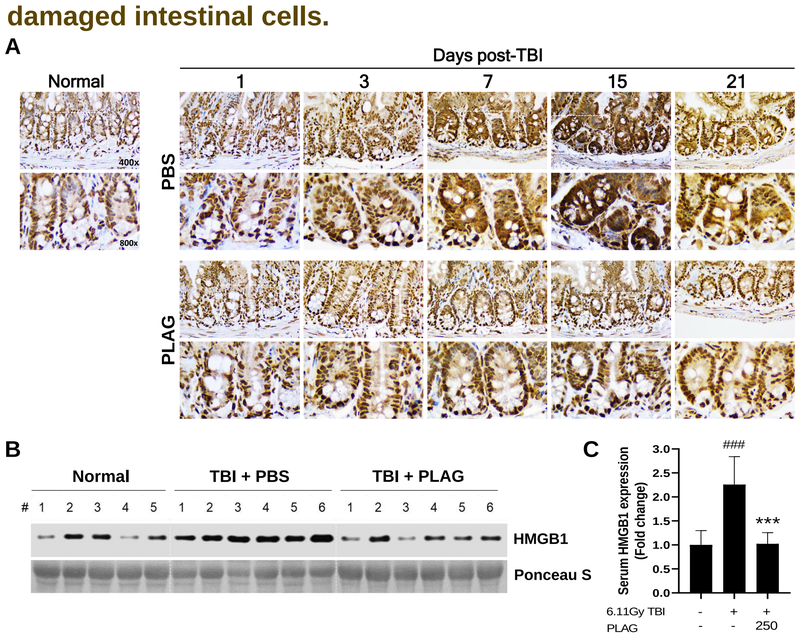
- D-xylose is a type of simple sugar that is easily absorbed by the small intestine. D-xylose absorption test checks the functionality of GI tract. PLAG significantly recovered the absorptive capacity of the xylose, which was severely impaired by the exposure to TBI.

4. Effect of PLAG on Intestinal leakage-induced bacterial translocation in GI-ARS model



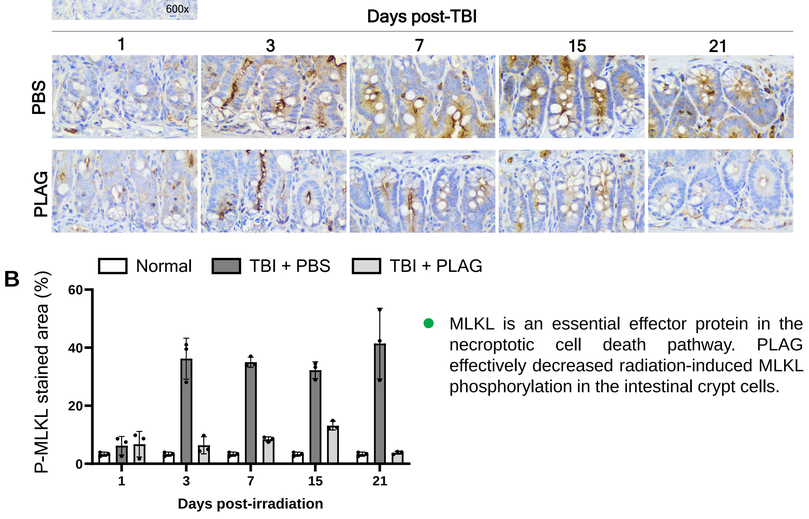
- Radiation-induced intestinal leakage increases risk of bacterial translocation from the intestinal lumen to the rest of the body. PLAG significantly reduced the occurrence rate with positive *E.faecalis* cultures (A) and the bacterial growth in the tissues (B) of the TBI-induced intestinal leakage mice.

5. Effect of PLAG on radiation-induced HMGB1 release from damaged intestinal cells.



- HMGB1 is a endogenous nuclear protein present in all cells. In dying cells, it can be passively released from the nucleus to the extracellular milieu and is sensed as a danger signaling molecule. PLAG reduced TBI-induced translocation of HMGB1 from nucleus to cytoplasm in the intestinal crypt cells (A) and the serum level of HMGB1 (B,C).

6. Effect of PLAG on radiation-induced necroptosis signaling activation in GI-ARS model



- MLKL is an essential effector protein in the necroptotic cell death pathway. PLAG effectively decreased radiation-induced MLKL phosphorylation in the intestinal crypt cells.

CONCLUSION

- PLAG facilitated the structural reconstitution of the intestinal tissues by increasing the villus heights, crypt number, crypt depths and mucin-producing goblet cells.
- PLAG significantly improved intestinal functionality and significantly reduced intestinal leakage-induced bacterial translocation.
- Overall, our findings support the radio-mitigative potential of PLAG against GI-ARS by regulating necroptosis signaling, which facilitates the restoration of the intestinal tissue and its function.
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REFERENCES

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2. Kim, Yong-Jae et al. *Radiation research* vol. 192,6 (2019): 602-611.

STATISTICAL ANALYSIS

- At least five mice in each group. All error bars indicate SD. Unpaired student T test or One-way ANOVA with turkey post hoc test . *P<0.05; **P<0.01; ***P<0.001

ENZYCHEM LIFESCIENCES

KRIBB Korea Research Institute of Bioscience & Biotechnology