Antibiotic reduces irradiation-induced intestinal injury

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1. Introduction

The gastrointestinal (GI) system is highly radiosensitive and intestinal injury is one of the major side effects of radiation therapy for cancers in the abdominal and pelvic areas [1-4]. Rifaximin is an antibiotic drug that is mostly used to treat traveler's diarrhea caused by bacterial enteropathogens. The purpose of this study was to evaluate the feasibility of attenuating radiation-induced intestinal injuries with rifaximin.

2. Methods and Results

2.1 Animals

Female C57BL/6 mice (5-weeks old) were purchased from the Orient Bio Co. (Seongnam, Korea) and allowed to acclimate to the new environment for 1 week prior to experimentation. The animal room temperature and relative humidity were maintained at 22 ± 3℃ and 50 ± 20%, respectively. The animals were given water from reverse osmosis and food (Purina) *ad libitum*. All animal experiments were conducted following a protocol approved by the Institutional Animal Care and Use Committee.

2.2 Sequence of irradiation and rifaximin treatment

To determine the optimal sequence of rifaximin and radiation treatment, 4 experimental groups were set: *(i)* whole abdominal irradiation (WAI) alone, *(ii)* oral rifaximin (30 mg/kg/day) for 2 days alone, *iii)* rifaximin for 2 days before WAI, (*iv)* rifaximin for 2 days after WAI. The whole abdominal area was irradiated with 15 Gy in a single dose using a ^{60}Co irradiator (Thermatron 780, Atomic Energy of Canada) at a dose rate of 1.3 Gy/min. For irradiation, mice were placed in ventilated Plexiglas containers under light anesthesia with tiletamine/zolazepam (Virbac Zoletil^{TM} 50; Virbac Lab, Carros, France). Rifaximin (molecular weight: 785.88) was obtained from Sigma-Aldrich (St. Louis, MO, USA) and was administrated orally to mice at 30 mg/kg/day on 2 consecutive days before or after WAI.

2.3 Survival of mice and body weight

Animal survival and body weight were monitored daily for 55 days and 23 days after irradiation, respectively. All animals that became moribund were sacrificed immediately.

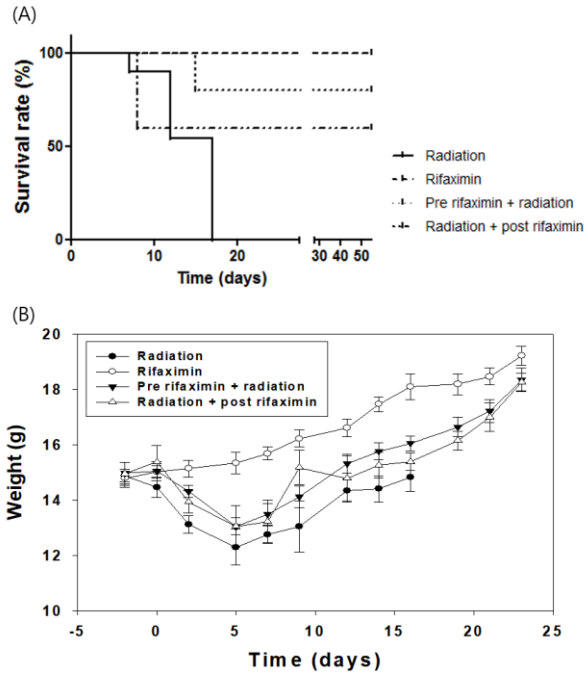

Fig. 1. Survival rate (A) and body weight (B) of mice (10 per group, means ± SEs) given 15 Gy of WAI alone, rifaximin for 2 days before WAI, or rifaximin for 2 days after WAI.

Female C57BL/6 mice (5-weeks old, purchased from the Orient Bio Co.) treated with 15 Gy of WAI alone began to die from day 7, and all mice in this group died by day 17 (Fig. 1). Forty percent of the mice given rifaximin after WAI died on the 8th day after WAI, and 20% of the mice given rifaximin before WAI died on the 15th day after WAI. Importantly, no additional deaths occurred in either rifaximin group until day 55, the end of the study period. The treatment with rifaximin alone had no effect on the survival or body weight of mice. Rifaximin treatment before or after WAI also attenuated the radiation-induced loss of body weight, although the differences between the two groups were not statistically significant (Fig. 1B). Accordingly, we investigated the effect of rifaximin administered for 2 days before WAI on the intestinal injuries in the subsequent experiments.

2.4 Jejunal crypt assay and morphological changes

Mice were randomly divided into four groups: *(i)* control group, *(ii)* whole abdominal irradiation (WAI), *(iii)* rifaximin for 2 days, and (*iv)* rifaximin for 2 days before WAI. Mice were sacrificed 1-7 days after WAI, and the jejunum were removed and fixed in neutral-buffered formalin after treatment as previously described. After paraffin embedding, tissue sections were stained with hematoxylin and eosin (H&E). The number of surviving crypts per transverse histological section was counted. A crypt that contained at least 10 surviving cells was defined as a "surviving crypt". Crypts in 5 to 8 circumferences per mouse were counted and averaged.

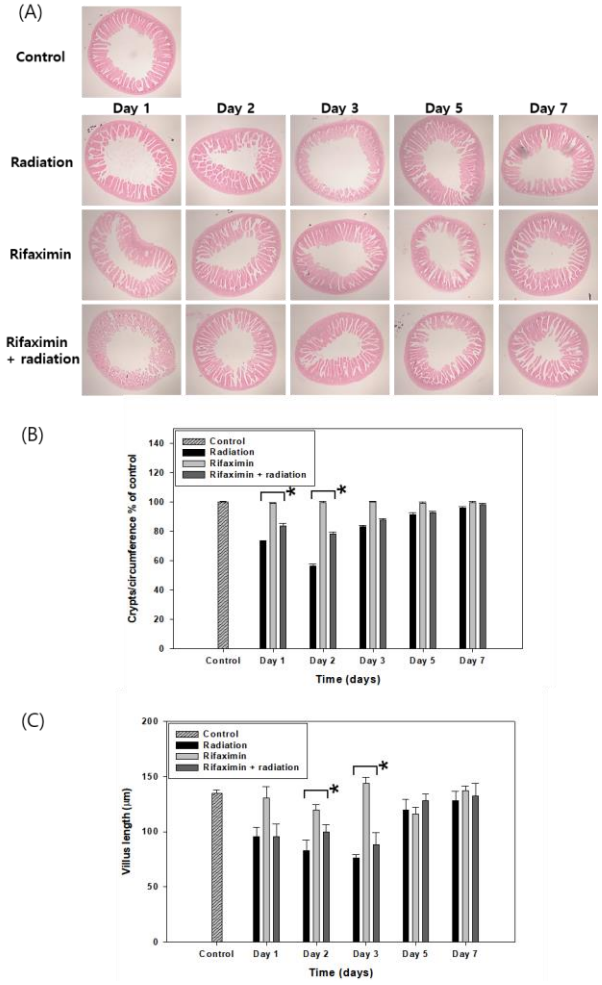

Fig. 2. Representative hematoxylin and eosin-stained transverse sections (40×) of the jejuna of mice given no radiation (control), WAI, rifaximin for 2 days and rifaximin for 2 days before WAI (A), quantitation of jejunal crypt cells of the full jejunum circumference (B), and quantitation of villi length in the jejunal mucosa (C). Data are means ± SEs. (*****$p < 0.05$)

Fig. 2A shows the effect of rifaximin on WAI-induced histological changes in the small intestinal mucosa. The tissues from the control group (no radiation) or that from the rifaximin treatment alone group showed a large number of crypts at the bases of the microvilli. The major changes induced by WAI were death of crypt cells and characteristic shortening of the villi. The average number of surviving crypts in control crypts was 104.1 ± 1.6/circumferences and that at 2 days after WAI was only 56.4 ± 1.5/circumference in the WAI group, but was 78.3 ± 1.8/circumference in the animals given rifaximin before WAI ($p < 0.05$). In the group treated with rifaximin before WAI, the length of villi on day 3 was 88.5 ± 10.1 μm. The length of villi of the WAI alone group was statistically smaller than that of rifaximin plus WAI group on day 2 and 3 ($p < 0.05$).

3. Conclusions

Oral administration of rifaximin to C57BL/6 female mice for 2 days before WAI with 15 Gy reduced the radiation-induced damage in the small intestine and improved the survival of mice. Rifaximin may be useful for the attenuation of the radiation-induced gastrointestinal damages.

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