

## Coniferyl Aldehyde Ameliorates Radiation Intestine Injury via Endothelial Cell Survival

Ye Ji Jeong<sup>a,c</sup>, Myung Gu Jung<sup>a</sup>, Yoon-Jin Lee<sup>a</sup>, Yun-Sil Lee<sup>b</sup>, Young-Gyu Ko<sup>c,\*</sup> and Hae-June Lee<sup>a,\*</sup>,

<sup>a</sup>Division of Radiation Effect, Korea Institute of Radiological and Medical Sciences, Seoul, Korea

<sup>b</sup>College of Pharmacy & Division of Life & Pharmaceutical Sciences, Ewha Woman's University, Seoul, Korea

<sup>c</sup>Department of Life Sciences, Korea University, Seoul, Korea

\*Corresponding author: ygko@korea.ac.kr, hjlee@kirams.re.kr,

### 1. Introduction

Radiation therapy is a widely used cancer therapy, but it causes side effect even when localized radaitothearyp is used. The small intestine is often exposed by radiation during radiation therapy for tumors not only in the gastrointestinal tract, but also liver, kidney, pancreas, biliary tract, and so on. Cancer treatments related gastrointestinal toxicity has also been recognized as a significant economic burden [1]. Especially, extensive apoptosis of microvascular endothelial cell of the lamina propria is the primary lesion initiating intestinal radiation damage after abdominal radiation therapy [2].

Coniferyl aldehyde (CA) is phenolic compounds isolated from cork stoppers, and one of the major pyrolysis products of lignin [3]. Shi H. was support for the empirical use of CA as a medicinal food for cardiovascular diseases [4]. CA is extracted many plant, such as Cinnamomum cassia, it also has platelet anti-aggregation activities [5]. CA inhibited prostaglandin synthetase four times than aspirin and electrically induced contractions of the guinea pig ileum in a dose-dependent way [6].

CA has positive effect in broad way but there is no consequence in radiation induced intestine damage. Here, we investigate effect of CA on small intestine after abdominal IR to mice in this study.

### 2. Methods and Results

#### 2.1 Experimental Design

All mouse procedures in this study were approved by the Institutional Animal Care and Use Committee of the Korea Institute of Radiological and Medical Sciences. Mice were fed a normal diet and autoclaving water. 7 weeks male C3H mice (Dooyeol Biotech co., ltd., Korea) were housed in a climate-controlled environment on a 12 h light/dark cycle.

Corniferyl Aldehyde (CA), was purchased from Sigma (US). CA (10 mg/kg) was given in *i.p.* injection the following schedule described in Fig 1. Animals randomly assigned into 4 group (n=6-12/group); non-IR control, CA alone, IR, and IR with CA treatment. Irradiation (13.5 Gy) was conducted with Xrad-320 for the abdominal local irradiation (IR). Animals sacrificed following the experimental schedule.

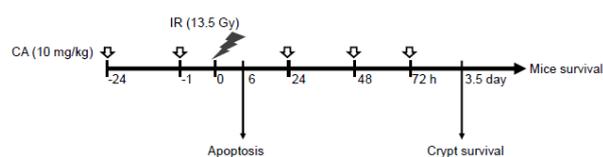


Figure 1. Experimental schedules. CA was treated by *i.p.* injection for 10 mg/kg. Radiation was treated 13.5 Gy abdominally.

#### 2.2 Mice survival

To examine effect of CA for suppressing side effect of radiation therapy, we performed survival test in mice with abdominal irradiation. CA treatment was given 5 times (pre-24, 1 and post-24, 28, 72 h against IR).

Survival was monitored every 12 hr after irradiation. The survival of mice with CA treatment increased from a mean of  $201 \pm 15$  h in control mice to  $231 \pm 13$  h.

#### 2.3 Crypt survival assay and histological evaluation

For the crypt survival assay, 6 mice were sacrificed at 3.5 days after irradiation. We collected jejunum from guts and fixed at 10% neutral formaldehyde from each groups.

Paraffin-embedded sections were dewaxed and rehydrated. Immunohistochemistry for ki-67 was performed with Vectastain Elite ABC kit (Vector Laboratories Inc.) following the manufacturer's protocol. For antigen retrieval, the sections were placed in citrate buffer (pH 6.0) and heated in a microwave oven for 10 minutes. For immunoperoxidase labeling, endogenous peroxidase was blocked by 0.3%  $H_2O_2$  in absolute methanol for 15 minutes at room temperature. The sections were then incubated overnight at 4°C with anti-ki-67 antibody (Acris Antibodies, Germany) and washed with PBS containing 0.05% Triton X-100. Incubation with corresponding secondary antibody for 30 min. Afterward the sections were counterstained by hematoxylin.

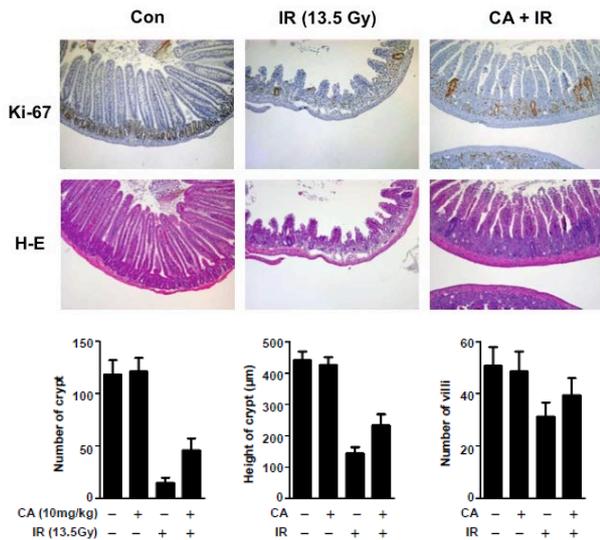


Figure 2. Morphological changes in small intestine with CA treatment following IR (Upper; ki-67, lower; H&E, X100)

At 3.5 day after IR, number of survived crypts, heights of crypt and villi were significantly decreased by IR compared to non-IR. CA alone did not effect any morphological changes in the intestine (data not shown). CA treatment to mice reduced radiation injury including crypt survival, heights of crypt and villi, depression of ki-67 expression (Fig. 2).

### 2.3 Endothelial cell survivals

To detect cell death in small intestine, mice were sacrificed at 6 h and after IR, then fixed in 10% neutral formalin, paraffin-embedded to sections. The sections were then incubated overnight at 4°C with anti-CD31 antibody (Santa Cruz Biotech, USA) and washed with PBS containing 0.05% Triton X- 100. Incubation with anti-rabbit 594 for 30 min and slides were applied with Dead-End Fluorometric TUNEL system (Promega) following the manufacturer's protocol. Then counterstained with VECTASHIELD containing DAPI. Sections were observed by confocal fluorescence laser microscope (CFLM) under X400 magnification.

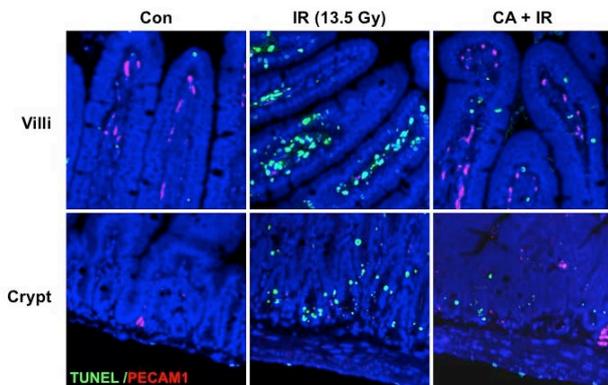


Figure 3. Apoptosis Cells are decreased and Epithelial Cells are increased in CA pre-treated before irradiation group than irradiation group. (TUNEL; Green, PECAM1; Red, X400)

IR induced severe apoptosis in crypt and villi including lamina propria. CA treatment did reduce not only epithelial cell death in villi and crypt, but also endothelial cell in lamina propria. It correlates with PECAM1, which is an endothelial cell marker, positive signals. This result suggests that CA is effective in avoiding apoptosis fate on the part of irradiation, especially for endothelial cells, consequently may contribute to prolonged survival of mice.

To confirm the effect of CA on endothelial cell survival *in vitro*, we conducted tube formation assay in HUVECs (human umbilical vein endothelial cells). Cells were exposed to 10 Gy radiation, trypsinized and counted cell numbers.  $4.5 \times 10^4$  cells were seeded on 48 well plates coated with cold Matrigel in complete EGM media.

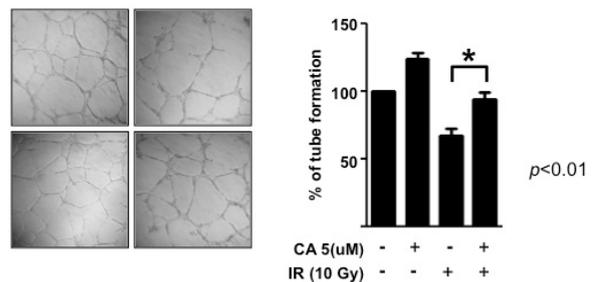


Figure 4. Effect of CA on the capillary-like tube formation by HUVEC. HUVEC was incubated on Matrigel for 18 h.

10 Gy radiation interrupted the tube formation of HUVEC. CA alone showed enhancement of tube formation compared with control, and also CA treatment preserved tube formation activity against IR.

### 3. Conclusions

In this study, CA increased the survival rate in C3H mice against 13.5 Gy abdominal IR. We found CA protects small intestine via preventing endothelial cell apoptosis and enhancing their angiogenic activity. CA also showed protective effect on crypt cell survival. Endothelial cell survival may affect crypt cell protection against IR [7, 8]. From this data, we concluded that CA is effective for protection against abdominal radiation injury. CA could ameliorate side-effect of radiation therapy.

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