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13. SUPPLEMENTARY NOTES

14. ABSTRACT

In response to radiation therapy (RT), many breast cancer patients experience early adverse skin reactions (EASRs) due to inflammation. Therefore, we test alternative medicine with anti-inflammatory properties, Calendula officinalis and Ching Wan Hung, in RT-induced EASRs. We have tested two animal models with two ionizing radiation (IR) sources. First, C57/BL6 mice were used and IR was performed in a clinical facility (Varian 2100C Linear Accelerator). Visible signs of radiation dermatitis, such as blood vessel dilation, erythema, scales, moist desquamation, were observed from day 8 to 20 with a peak on day 16. The mice treated with Calendula Officinalis shown a faster recovery compared to those treated with Ching Wan Hung. Due to new clinical regulation, we had to change IR source. Second, SKH-hr1 hairless mice were used and IR was performed in a research facility (a 100 KV X-ray machine). No significant skin lesions or signs of radiation dermatitis were observed in all groups of animals. Mild skin reactions, such as reddening and scales, were observed in medicine-treated group around days 8~14. In summary, appropriate radiation dosage and topical medicine control will need to be evaluated in future studies as proposed in our no-cost-extension period.

15. SUBJECT TERMS
Breast Cancer; Radiation Sensitivity; Alternative Medicine.

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Comprehensive and Alternative Medicine in Preventing Radiotherapy-Induced Adverse Skin Reactions

Progress Report

a) INTRODUCTION:

The proposed research has three obj ectives: (1) To test the molecula r mechanisms involved in ionizing radiation (IR)-induced molecular ch anges; (2) To com pare the protec tive effects of two CAM products, Calendula officinalis or Ching W an Hung, previously us ed in the treatm ent of burns; and (3) To identify molecular m echanisms involved in their protective eff ects, including three targeted pathways, apoptosis, proliferation, and inflammation, as well as genome-wide expression profiles to identify new targets.

b) BODY:

Tissue and blood sam ple collections: For both control and experim ental groups, half of the m ice were euthanized at day 5, and the rest ha lf were euthanized at day 16. Bloo d sam ples were collected, followed by centrifugation at 3000 rpm for 15 m in at 4 °C to separate serum from the blood cells. After centrifugation, the serum was a liquoted and frozen in -80 °C for later ELISA assays. Skin biops ies from the ir radiated area on th e hind limbs were taken and divided into three parts: 1) For R NA isolation, skin was rinsed qu ickly in co ld PBS, chopped into tiny pieces and imm ediately stabilized in RNAlater (Qiage n). Total RNA was isolated from approximate 25 m g skin tissue using Illustra RNAspin Mini RNA isolation Kit (GE Healthcare), following the manufacturer's instructions. 2) Part of skin biopsies were fixed in l0% neutral buf fered for malin (EMD) for histology analysis and 3) One third of skin biopsies were embedded in Tissue-Tek OCT (Sakura Finetek, C A) and stored in -800C for future immuno-histochemistry studies.

Mice were exposed to ionizing ra diation (IR) at the posterior dorsal region to 10 Gy/day for 4 consecutive days. Durin g each irrad iation, anesthetized m ice were p laced on a 1.5 cm thick Lucite plate an d irradiated w ith a 9 MeV Electron Beam irradiator. Hind li mbs of m ice were exposed to irradiation; the rest of the body was shielded by a 5 mm lead to protect vital or gans (**Figure 1**). Total of eight mice were used for each experiment (table). Two groups of c ontrol m ice (2 m ice/group) were treated without or with irradiation. Two groups of experim ental m ice (2 mice/ group) were treated by irradiation a nd topical application of either *Calendula officinalis* or *Ching Wan Hung* on both hind lim bs at two dif ferent time points, imm ediately prior to irradiation (left leg) or immediately post irradiation (right leg). The physical changes of the mo use skin at the irradiated region were photographed every two days usi ng SONY cybershot cam era coupled to a Der mLite II pro dermoscopy (3Gen, CA). In the firs t pilot test, C57/BL6 m ice were use d. The hairs on the m ice hind limbs were removed with Nair (Church & Dwight Co., NJ) two days befo re the irradiation. The IR was performed in clinical facilities (V arian 2100C Lin ear Accelerator) of Departm ent of Ra diation O ncology, Sylvester Cancer Center, at University of Miam i. In the secon d expe riment, SKH-hr1 hairless m ice were used. The IR wa s performed in the research facilities (a 100 KV X-ray m achine) of the D epartment of Radiation Oncology , Dr. Ahmed Mansoor's research lab, Papanicolaou Bldg, room 118, University of Miami

Figure 1: Overview of the Irradiation Setup

1st Trial: T otal eight C57/BL6 m ice were used for this experim ent. The physical appearance of skin at the irradiation affected area was phot ographed (Figure 2) to evaluate any apparent effects of Calendula officinalis or Ching Wan Hung on prevention irradiation-induced early adverse skin reactions (EASRs). Visible signs of radiation derm atitis, such as blood vessel dilation, ery thema, scales, m oist desquam ation, were observed from day 8 to day 20 with a peak on day 16. In ge neral, the sings of irradi ation-induced dermatitis were gradually dim inished after day 22, and the m ice tr eated with Calendula offici nalis shown a faster and better recovery com pared to those treated with Ch ing Wan Hung. In both treatm ent groups, no significant difference was observed between the left and right lim bs, indicating the timing to apply the drug, either prior to irradiation or post irradiation, has no apparent difference as shown in this experim ent. All experim ental mice treated with either topical medicine shown a better recovery compared to the controls.

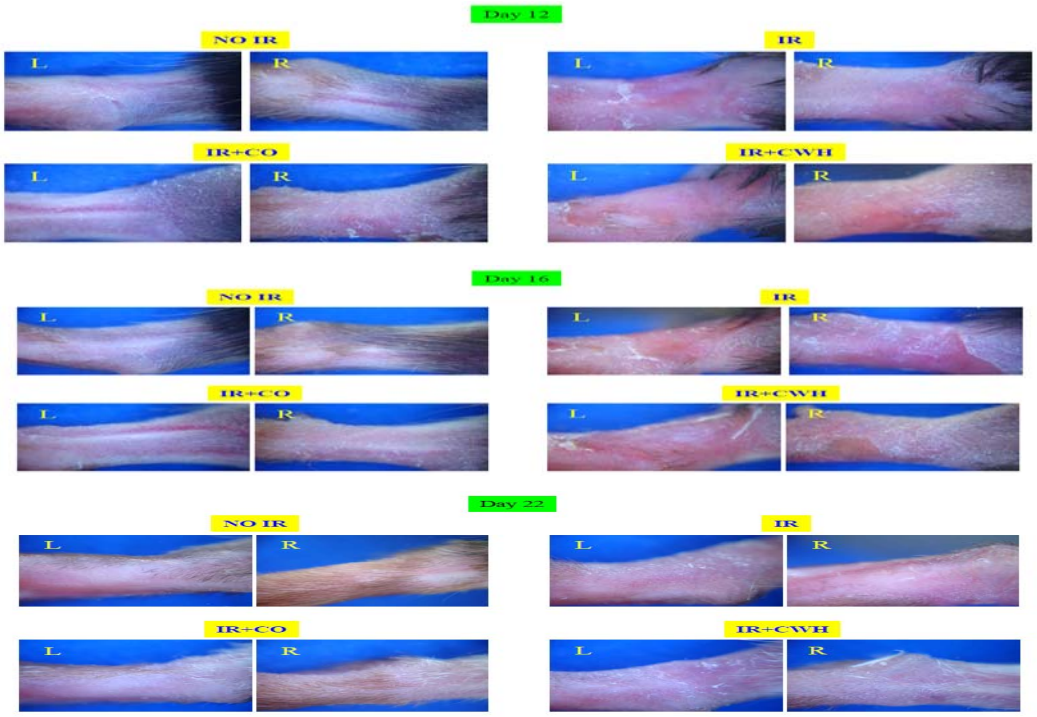

Figure 2: Mice skin images at the irradiated areas. (No IR: no irradiation; IR: irradiation without medical treatment; IR+CO: irradiation plus *Calendula officinalis* treatment; IR+CWH: irradiation plus *Ching Wan Hung* treatmen; L: left hind limb; R: right hind limb.)

2nd Trial: Total eight SKH-hr1 hairless mice were used in this experiment. To our surprise, no significant skin lesions or signs of radiation derm atitis were observed in all groups. Mild skin reactions, such as reddening and scales, were observed in m edicine-treated group around days 8~14. Much m ilder and shorter (days 12~ 14) reactions w ere observed in the control m ice, which receiv ed irradiation without any drug treatm ent. Since no obvious les ions or sign s of radiation-derm atitis were obs erved in this experim ent, an appropriate radiation dosage m ay need to be further determ ined. Be sides, fo r the topica l medicin e control, m ice treated witho ut irradiation but with the medicines may be included.

c) KEY RESEARCH ACCOMPLISHMENTS:

- Multiple 5 mm thick lead shield specific for mouse animal models were designed and build to prepare mouse for the proposed research.

- Two experiments were conducted with two animal models and two irradiators: sample collections and skin reactions were successfully established. However, another IR source (recently installed at the animal facility) will be used to deliver higher energy source.

d) REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

- NIH/NCI Grant Application ID: 1R01CA135288-01A 1 (12/01/2009 to 11/30/2014); Impact of Genomics on Disparities in Breast Cancer Radiosensitivity (priority score: 23 at 6%, within the fundable range)

e) CONCLUSI ONS:

- The 5 mm thick lead sh ield was effective in p reventing injury in internal organs from IR. This device can be used for all future animal research.
- Procedures for sample collections and skin reaction evaluations were successfully established. However, another IR source (recently installed at the animal facility) will be used to deliver IR with higher energy source in order to induce skin reactions critical for the proposed research.

f) REFER ENCES:

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g) APPENDICES:

Not Applicable.