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Enhanced hematopoietic protection from radiation by the combination of genistein and captopril

R.M. Day [a,*], T.A. Davis [b], M. Barshishat-Kupper [a], E.A. McCart [a], A.J. Tipton [a], M.R. Landauer [c]

[a] Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA
[b] Department of Regenerative Medicine, Naval Medical Research Center, Silver Spring, MD, USA
[c] Armed Forces Radiobiology Research Institute, Bethesda, MD, USA

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ABSTRACT

The hematopoietic system is sensitive to radiation injury, and mortality can occur due to blood cell deficiency and stem cell loss. Genistein and the angiotensin converting enzyme (ACE) inhibitor captopril are two agents shown to protect the hematopoietic system from radiation injury. In this study we examined the combination of genistein with captopril for reduction of radiation-induced mortality from hematopoietic damage and the mechanisms of radiation protection. C57BL/6J mice were exposed to 8.25 Gy ^{60}Co total body irradiation (TBI) to evaluate the effects of genistein and captopril alone and in combination on survival, blood cell recovery, hematopoietic progenitor cell recovery, DNA damage, and erythropoietin production. 8.25 Gy TBI resulted in 0% survival after 30 days in untreated mice. A single subcutaneous injection of genistein administered 24 h before TBI resulted in 72% survival. Administration of captopril in the drinking water, from 1 h through 30 days postirradiation, increased survival to 55%. Genistein plus captopril increased survival to 95%. Enhanced survival was reflected in a reduction of radiation-induced anemia, improved recovery of nucleated bone marrow cells, splenocytes and circulating red blood cells. The drug combination enhanced early recovery of marrow progenitors: erythroid (CFU-E and BFU-E), and myeloid (CFU-GEMM, CFU-GM and CFU-M). Genistein alone and genistein plus captopril protected hematopoietic progenitor cells from radiation-induced micronuclei, while captopril had no effect. Captopril alone and genistein plus captopril, but not genistein alone, suppressed radiation-induced erythropoietin production. These data suggest that genistein and captopril protect the hematopoietic system from radiation injury via independent mechanisms.

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1. Introduction

With the rise of potential radiation exposure, either as the result of a nuclear accident or terrorist activity, there is increased need for effective, nontoxic radiation countermeasures. In addition, protection of normal tissue during radiotherapy remains an important clinical concern [1]. Radiation countermeasure agents have been categorized into three main areas: 1) radioprotectors or prophylactic agents that are administered prior to irradiation; 2) mitigators that are given during or shortly after radiation exposure but before the appearance of overt signs of radiation injury; and 3) radiation therapeutics or treatments that are given after the manifestation of clinical symptoms [2]. Currently, amifostine is the only radiation protector approved by the U.S. Food and Drug Administration [3,4]. However, amifostine has severe side effects including nausea, vomiting, and pronounced hypotension, making it unacceptable for wide use [5].

The combination of agents with different mechanisms of action can provide increased protection against toxic agents, as exemplified by the drug combination for the prevention and mitigation of soman nerve agent toxicity. In this model, pyridostigmine bromide (PB), a cholinesterase inhibitor, is used prophylactically with post-exposure administration of the anticholinergic atropine and the acetylcholinesterase reactivator oxime 2-pralidoxime chloride. While pretreatment with PB is not necessary, it greatly enhances the effectiveness of the post-exposure agents [6]. We hypothesized that this strategy may be applicable to an anticipated radiation exposure, such as clinical radiotherapy or entering a radiation contaminated area. Two candidate radiation countermeasures that do not have the severe side effects of amifostine are the isoflavone genistein and the angiotensin converting enzyme (ACE) inhibitor captopril [7,8].

The soy isoflavone genistein (4′,5,7-trihydroxyflavone) has a number of biological properties associated with radioprotection including

* Corresponding author at: Department of Pharmacology, Bldg C, Room 2023, 4301 Jones Bridge Rd, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799, USA. Tel.: +1 301 295 3236; fax: +1 301 295 3220.
E-mail address: regina.day@usuhs.edu (R.M. Day).

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antioxidant activity and free radical scavenging [9], estrogenic effects [10], anti-microbial activity [11], anti-inflammatory activity [12], and protein tyrosine kinase inhibitory properties [13]. In clinical trials, genistein was reported to reduce the adverse effects of chemotherapy and radiotherapy [14,15]. Our laboratory reported that a single subcutaneous injection of genistein administered 24 h prior to radiation exposure provides increased survival from acute radiation injury by protecting the bone marrow stem cell population [16–18]. Genistein transiently arrested long-term hematopoietic stem cells in the G_0/G_1 phases of the cell cycle and reduced radiation-induced genotoxicity, senescence, and stem cell pool exhaustion [18,19]. Genistein protection from total body irradiation (TBI) was associated with improved recovery of neutrophils and platelets [17]. Genistein also reduced radiation-induced chromosome breakages associated with the process of the appearance of micronuclei in primary lung fibroblasts [20,21]. Although genistein protects normal tissue from radiation, it was demonstrated to radiosensitize a variety of tumors [22], including those of the prostate [15,23].

Captopril, a sulfhydryl-containing analog of proline, was demonstrated to reduce pulmonary-related mortality and chronic renal failure in oncology patients receiving radiation for hematopoietic stem cell transplantation [24]. Captopril competitively inhibits ACE protease activity, resulting in decreased activation of the vasoconstrictor angiotensin II (Ang II) and decreased inactivation of the vasodilator bradykinin. A clinical investigation showed that captopril reduced pulmonary-related mortality and chronic renal failure in oncology patients receiving radiation for hematopoietic stem cell transplantation [24,25]. Our laboratory and others have demonstrated the effectiveness of captopril for mitigating radiation-induced injuries to the hematopoietic [8], renal [26], and pulmonary systems [27–29]. Captopril and the ACE inhibitor perindopril increased survival from radiation hematopoietic injury through accelerated recovery of erythrocytes, reticulocytes, leukocytes, and platelets [8].

In our current work, we explored the use of the radiation prophylactic agent genistein in combination with the radiation mitigation agent captopril. We observed enhanced survival and hematopoietic protection with this drug combination.

2. Materials and methods

2.1. Animals and irradiation

Female C57BL/6J mice were purchased from The Jackson Laboratory, Bar Harbor, ME, USA. Mice were housed in groups of four in rooms maintained at 21 ± 2 °C, 50% ± 10% humidity, and 12-h light/dark cycle in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Commercial rodent ration (Harlan Teklad Rodent Diet 8604) and acidified water (pH = 2.5–3.0, to control opportunistic infections [30]) were freely available. Mice were 12–14 weeks old at the time of irradiation as described [18]. Mice received total body irradiation (TBI) in a bilateral gamma radiation field at the ^{60}Co facility at the Armed Forces Radiobiology Research Institute (AFRRI). 8.25 Gy (0.6 Gy/min) was administered for most experiments to provide a radiation dosage at which genistein or captopril alone would not provide full protection. A lower dose, 7.75 Gy (0.6 Gy/min), was used for the micronucleus study in order that sufficient spared lineage negative bone marrow cells (Lin^- cells) would be available for analysis. At this radiation dose, genistein or captopril alone results in 100% survival. Control animals were sham irradiated. Experiments were conducted in compliance with the Animal Welfare Act, in accordance with the principles in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 2011, and approved by the AFRRI Institutional Animal Care and Use Committee.

2.2. Genistein and captopril preparation

Genistein (Sigma-Aldrich, St. Louis, MO, USA) was freshly prepared in polyethylene glycol 400 (PEG-400, Sigma-Aldrich) as previously described [7]. Genistein and PEG-400 vehicle were injected subcutaneously in 0.1 ml. Captopril (Sigma-Aldrich) was provided in the acidified drinking water at 0.55 g/l (110 mg/kg/day). Captopril has been determined to be stable in acidified water up to 30 days and does not affect the amount of water consumed by the mice [31].

2.3. Animal survival studies

We previously determined that genistein-induced radioprotection was optimal at a dose of 200 mg/kg when administered 24 h prior to TBI [7], and that captopril (110 mg/kg/day) was an effective radiation mitigator when administered in the drinking water postirradiation [8]. In our ^{60}Co facility the $LD_{50/30}$ with 95% confidence limits for female C57BL/6J mice was 7.52 Gy (7.44 Gy, 7.59 Gy) [16]. Thirty-day survival experiments utilized the pooled data from two experiments. For times of captopril administration, a negative (−) number indicates administration before irradiation while a positive (+) number indicates administration postirradiation. Day 0 equals the day of irradiation and treatments on day 0 began 1 h postirradiation. Mice were randomized for assignment to the following experimental groups: 1) sham irradiation, no treatment (N = 8); 2) sham irradiation + genistein + captopril (0 to +30) (N = 12); 3) radiation only (8.25 Gy) (N = 8); 4) radiation + vehicle (N = 36); 5) radiation + captopril (day +1 to +30) (N = 20); 6) radiation + captopril (day 0 to +30) (N = 20); 7) radiation + genistein (N = 36); 8) radiation + genistein + captopril (+1 to +30) (N = 19); 9) radiation + genistein + captopril (day 0 to +30) (N = 19).

2.4. Peripheral blood analyses

EDTA-treated blood (~0.5 ml) was obtained from pentobarbital-anesthetized mice via cardiac puncture. Peripheral blood was analyzed for number of white blood cells (WBC), absolute neutrophil count (ANC), lymphocytes (LYMPH), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), platelets (PLT), and reticulocytes (RETIC) using a Baker Advia 120 Hematology Analyzer (Baker, Tarrytown, NY, USA). Separate groups of animals were used for each time point [18].

2.5. Hematopoietic progenitor colony-forming cell assay

Bone marrow was obtained from femurs of the mice as previously described [17]. Briefly, femurs were flushed with Iscove's modified Dulbecco's medium, 2% heat-inactivated fetal calf serum (Hyclone, Logan, UT, USA), 5×10^{-5} M β-mercaptoethanol, penicillin (100 U/ml)/streptomycin (100 μg/ml) (GIBCO, Grand Island, NY, USA). Spleens were removed and digitally photographed; areas were calculated using NIH Image J planimetry software (v1.45; available at http://rsbweb.nih.gov/ij/). Spleens were gently minced and squashed to allow splenocyte detachment from connective tissue. Erythrocytes were lysed with ACK lysing buffer (GIBCO), and the suspension was washed twice in immuno-selection buffer (phosphate-buffered saline, 0.5% bovine serum albumin (BSA), 0.6% acid citrate dextrose, and 4% sodium bicarbonate). Cellular aggregates and connective tissue were removed with a 100 μm nylon mesh strainer (BD Biosciences, San Diego, CA, USA). Cellularity was determined by manual hemocytometry.

Bone marrow cells and splenocytes were assayed in 1 ml methylcellulose with 30% FCS, 1% BSA, 10^{-4} M 2-mercaptoethanol, and 2 mM L-glutamine (StemCell Technologies, Vancouver, Canada). Cells were plated at $1–5 \times 10^4$ cells/ml in Methocult M3434 (Stem Cell Technologies). Cultures for colony forming units-erythroid