Effects of High-Protein Diet and/or Resveratrol Supplementation on the Immune Response of Irradiated Rats

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ABSTRACT: We investigated the effects of a high-protein diet and resveratrol supplementation on immune cells changes induced by abdominal irradiation in rats. Female Wistar rats were divided into 5 groups: 1) control diet, 2) control diet with irradiation 3) 30% high-protein diet with irradiation, 4) normal diet with resveratrol supplementation and irradiation, and 5) 30% high-protein diet with resveratrol supplementation and irradiation. We measured blood protein and albumin concentrations, lipid profiles, white blood cell (WBC) counts, proinflammatory cytokine production, and splenocyte proliferation in rats that had been treated with a 17.5 Gy dose of radiation 30 days prior. A high-protein diet affected plasma total cholesterol and very low density lipoprotein-cholesterol levels, which were increased by the radiation treatment. In addition, the lymphocyte percentage and immunoglobulin M (IgM) concentration were increased, and the neutrophil percentage was decreased in rats fed a high-protein diet. Resveratrol supplementation decreased the triglyceride (TG) level, but increased the IgM concentration and splenocyte proliferation. Proinflammatory cytokine production was lower in rats fed a high-protein diet supplemented with resveratrol than in rats fed a control diet. The results of the present study indicate that high-protein diets, with or without resveratrol supplementation, might assist with recovery from radiation-induced inflammation by modulating immune cell percentages and cytokine production.

Keywords: radiation treatment, high-protein diet, resveratrol, immune function, inflammation

INTRODUCTION

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene), an abundant phenolic phytoalexin found in grape skin, berries, and red wine (1), is well known as a radical scavenger (2,3). In addition, it has been reported to have antimicrobial (1), anti-inflammatory, anti-tumor, anti-diabetic, and cardioprotective effects (4). Resveratrol modulates lipid metabolism by suppressing low-density lipoprotein (LDL) peroxidation, inhibits platelet aggregation, and inhibits vascular cell adhesion molecule expression (4-7).

Radiation treatment is one of the most common therapies used to eliminate tumors from cancer patients (8,9). However, the reactive oxygen species (ROS) generated after radiation treatment may cause various complications (9,10). Proinflammatory cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta (TGF-β) are involved in the acute phase of the response to radiation

(8). The subchronic phase generally induces macrophage and monocyte migration, angiogenesis, and fibrosis (9). Radiation also modifies immune function by enhancing the expression of major histocompatibility complex class 1 and antigen-presenting tumor cells. In addition, radiation increases cluster of differentiation (CD) 4^+ and $CD8^+$ T cell infiltration (11,12).

In clinical situations, patients receiving radiation treatment are advised of the importance of adequate protein consumption. The results of several studies have provided support for a positive relationship between dietary protein intake and antioxidant biomarkers. In a previous study (13), mice fed a 33% protein diet had higher liver antioxidant levels [i.e., vitamin C, vitamin E, and reduced glutathione (GSH)], lower liver glutathione disulfide ratios [i.e., (GSSG)/(GSH+GSSG)], and higher plasma vitamin E levels than mice fed a 7% protein diet. Another study reported that splenic apoptosis and DNA fragmentation were lower in mice fed a 20% protein, standard vitamin E level diet than in mice fed a 1% protein, low

vitamin E diet (14). A previous study from our lab investigated the inflammatory response to radiation treatment. Ten days after exposure to a single dose of radiation, the concentrations of proinflammatory cytokines (i.e., IL-1β, IL-6, and TNF-α) were elevated (15).

The purpose of the present study is to determine the long-term effects of radiation on the nutrition status and immune status of rats. In addition, our study was designed to explore the scientific and practical aspects of the consumption of a high-protein diet with or without resveratrol. We hypothesized that a high-protein diet supplemented with resveratrol could protect against the chronic effects of ionizing radiation. The results of the present study indicate that 1) the experimental diets tested induce hematologic changes in irradiated rats; 2) high-protein diets, with or without resveratrol, can alter the plasma nutritional profile; and 3) the experimental diets had radioprotective effects.

MATERIALS AND METHODS

Animals, diet, and abdominal radiation treatment

This protocol was approved by the Sookmyung Women's University Committee for the Ethics of Animal Experiments (SMU-IACUC-2009-0817-001). Six- to seven-week-old female Wistar rats (body weight of 150~160 g) were purchased from Central Lab. Animal, Inc. (Seoul, Korea). Upon arrival, the rats were quarantined and conditioned for one week prior to the start of the experiment. After the adaptation period, the rats were randomly divided into five groups (n=6 per group): 1) non-irradiated rats fed a control diet [i.e., negative controls (NC)], 2) irradiated rats fed a control diet (C), 3) irradiated rats fed a 30% high-protein diet (HP), 4) irradiated

rats fed a control diet and supplemented with resveratrol (RES), and 5) irradiated rats fed a 30% high- protein diet and supplemented with resveratrol (HP+ RES). The compositions of the experimental diets are listed in Table 1.

The ventral side of the pelvis of each rat in the irradiated groups was exposed to a 17.5 Gy radiation dose (16). The radiation treatments occurred in the Department of Radiation Oncology, Ajou University of Medicine (Suwon, Korea).

Every other day, a 2 mg/kg bodyweight (b.w.) dose of resveratrol powder (3,5,4'-trihydroxy-trans-stilbene; Sigma-Aldrich, St. Louis, MO, USA) was administered to the rats in the resveratrol-supplemented groups (i.e., RES and HP+RES).

Blood analyses

Rats were euthanized 30 days after receiving the radiation treatment. Ethyl ether was used as a volatile anesthetic agent, and then fresh whole blood was drawn directly from the heart into tubes containing 18 mg of EDTA for whole blood hematology and sodium heparin for plasma fraction. An automatic hematology analyzer (Sysmex XE-2100D, Sysmex Corporation, Kobe, Japan) was used to perform a complete blood cell count on each blood sample; red blood cell (RBC) counts, platelet counts, white blood cell (WBC) counts, and WBC differentials were recorded.

Plasma total protein, albumin, TG, total cholesterol, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and very low density lipoprotein-cholesterol (VLDL-C) concentrations were measured by colorimetric assays using an auto-analyzer (Hitachi 7600-210, Hitachi Medical Corporation, Tokyo, Japan) and the following Clinimate test kits: Total Protein and Albumin, Pureauto S CHO-N, TG-N, Cholestest N HDL, LDL-C, and VLDL-C (Daiichi Sankyo, Tokyo, Japan). Serum IgM concentrations were measured by immunoturbidimetric assay using an IgM test kit (Roche Diagnostics Deutschland GmbH, Mannheim, Germany) and a Roche COBAS Integra 800 analyzer (Roche Diagnostics, Basel, Switzerland).

Serum proinflammatory cytokine concentrations

Production of proinflammatory cytokines (i.e., IL-lβ, IL-6, and TNF-α) was measured with anti-rat test kits (R&D Systems Inc., Minneapolis, MN, USA) according to the instructions provided by the manufacturer. Briefly, affinity-purified polyclonal rat-specific IL-lβ, IL-6, and TNF-α antibodies were pre-coated onto 96-well microplates. Standards or serum samples were added to each well of the antibody-coated plates. After incubation and washing, 100 µL of biotin conjugate was added to each well. This was followed by a 2-h, room temperature

Table 1. Diet compositions (unit: %)

Ingredients	Control diet[1] for rats in the C and RES[3] groups	High-protein diet[2] for rats in the HP and HP+RES groups
Casein	14	30
Dextrose	15	10.5
Sucrose	10	10
Corn starch	46.57	35.24
Cellulose	5	5
Soybean oil	4	4
Mineral mix	3.5	3.5
Vitamin mix	1	1
L-Cystine	0.18	0.51
Choline bitartrate	0.25	0.25
tert-Butylhydroquinone	0.0008	0.0008

[1] Composition of the AIN-93M diet for the maintenance of adult rodents.
[2] Modified AIN-93M purified rodent diet containing 30% protein by weight.
[3] Administered 2 mg/kg b.w. resveratrol in distilled water orally every other day.

incubation. Then each well was aspirated and the plates were washed. The substrate solution was added to each well and the plates were incubated for 30 min at room temperature in the dark. After incubation, 100 μL of stop solution was added to each well. Within 2 h of the addition of the stop solution, the optical density (OD) of each well was measured at 450 nm.

Splenocyte proliferation

Thirty days after the radiation treatment, spleens were excised from anesthetized rats, homogenized, and centrifuged. The pellets were resuspended in RPMI 1640 culture media, filtered through a 200-μm cell strainer, and centrifuged. The strained cells were separated from the supernatant cells, lysing buffer was added, and the mixtures were centrifuged at 3,000 rpm for 10 min. The supernatants were discarded, and the splenocytes were washed again. Splenocyte suspensions of 5.0×10^6 cells/mL of RPMI 1640 culture media containing 10% fetal bovine serum and 100 U/mL penicillin were plated in 96-well plates. We then added concanavalin A (ConA, 5 μg/mL) or lipopolysaccharide (LPS, 15 μg/mL), and incubated the plates at $37^{\circ}C$ and 5% CO_2 for 44 h. Next, we added 10 μL of MTT solution [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 5 mg/mL] and incubated the plates for 4 h in the dark. We then aspirated the supernatant and added 150 μL of DMSO to each well to dissolve the formazan crystals. The OD value of each sample was detected at 540 nm with an ELISA reader. The splenocyte proliferation index was calculated as follows: splenocyte proliferation index=mean OD of sample wells/mean OD of control wells.

Statistical analysis

All data are expressed as mean±standard deviation. Differences between the NC and C groups were assessed by Student's t-tests at $P < 0.05$. Differences among the irradiated groups were assessed by Duncan's multiple range tests at $P < 0.05$. All data were analyzed using IBM SPSS statistics 20 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Body weight and food intake

The changes in body weight and food intake are presented in Tables 2 and 3. Radiation treatment significantly reduced body weight from day 10 to the end of the experiment (day 30). Previously, Gridley and his colleagues examined the long-term effects of radiation (17) and reported that the body mass and albumin concentrations of 4 Gy-irradiated rats were reduced nine months after radiation.

The food intake of group C was lower than the food intake of group NC from 5 to 15 days after radiation. Food intake gradually increased from day 10 in the HP and C groups and from day 15 in the RES and HP+RES groups. One of the common side effects of radiation is a reduction in body weight. Inflammation after radiation treatments may cause a loss of appetite; this anorectic effect is thought to be due to the secretion of proinflammatory cytokines, such as IL-6 and TNF-α (18-20).

Table 2. Body weight changes (unit: g)

	Day −5	Day 0 (RT)	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
NC	167.28±8.69[a]	186.79±9.60[b]	192.15±8.39[b]	201.88±10.14[bc##]	209.43±9.28[cd##]	216.97±8.41[cde##]	224.32±5.66[de##]	231.91±11.86[e#]
C	173.41±7.29[bc]	189.59±14.64[cd]	183.48±7.14[c]	145.39±13.67[a]	146.12±10.95[a]	165.38±8.71[b]	184.64±6.48[c]	203.04±3.96[d]
HP	169.19±8.89[abc]	183.22±13.31[bc]	175.63±9.93[abc]	137.64±26.75[a]	150.73±37.27[ab]	169.00±12.98[abc]	180.00±20.13[bc]	207.00±16.17[c]
RES	166.53±12.05	189.05±15.01	186.67±10.38	148.45±22.66	157.82±24.60	154.60±16.98	151.38±9.35	173.89±19.03
HP+RES	174.39±7.44[ab]	187.24±14.16[ab]	181.55±9.60[ab]	152.93±17.42[a]	153.99±17.72[a]	164.15±21.14[a]	174.30±24.57[ab]	202.32±22.31[b]

NC, normal control diet; C, control diet; HP, 30% high-protein diet; RES, control diet with 2 mg/kg b.w. of resveratrol every other day; HP+RES, high-protein diet with 2 mg/kg b.w. of resveratrol every other day.
Within eachgroup, superscript letters (a-e) indicate significant differences at $P < 0.05$ by Duncan's multiple range tests and hashmark indicates significant difference between NC and C groups by t-test ([#]$P < 0.05$ and [##]$P < 0.01$).

Table 3. Food intake changes (unit: %)

	Day −5	Day 0 (RT)	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
NC	100.00±8.13[ab]	86.82±11.94[a]	100.11±12.36[ab##]	109.14±9.36[ab##]	116.62±18.99[bc#]	116.31±21.25[bc]	128.09±20.13[bc]	144.34±15.49[c]
C	90.12±11.09[cd]	84.21±9.11[cd]	31.06±9.78[a]	60.87±9.48[b]	65.84±9.09[bc]	86.72±15.67[cd]	97.87±13.29[de]	118.05±17.11[e]
HP	96.42±8.64[d]	93.31±12.41[cd]	41.54±6.54[a]	61.96±7.23[b]	75.40±10.44[bc]	92.19±13.82[cd]	109.78±10.77[de]	125.72±12.41[e]
RES	82.07±12.10[bc]	108.11±8.37[d]	35.64±8.82[a]	50.07±9.08[a]	72.61±11.19[b]	88.31±18.08[bcd]	85.12±12.85[bc]	100.06±11.37[cd]
HP+RES	94.06±8.33[bc]	106.80±9.36[cd]	37.64±9.27[a]	54.06±11.37[a]	75.97±12.59[b]	92.45±10.49[bc]	98.93±14.52[c]	122.26±10.49[d]

NC, normal control diet; C, control diet; HP, 30% high-protein diet; RES, control diet with 2 mg/kg b.w. of resveratrol every other day; HP+RES, high-protein diet with 2 mg/kg b.w. of resveratrol every other day.
Within each group, superscript letters (a-e) indicate significant differences at $P < 0.05$ by Duncan's multiple range tests and hashmark indicates significant difference between NC and C groups by t-test ([#]$P < 0.05$ and [##]$P < 0.01$).

A sustained decrease in food consumption due to inflammation, illness, and loss of appetite results in increased glucose tolerance and insulin sensitivity, which leads to muscle loss, fat loss, and cachexia (20,21). Previous work by our group investigated weight gain and food intake during a period of 10 days after exposure to radiation (15). In that study, we reported that 22% and 33% protein diets had no effect on radiation-induced body weight loss, while a 7% protein diet was more effective at decreasing weight loss than a 33% protein diet. A high-protein diet was more protective against radiation-induced body weight loss than a low-protein diet (13,22).

Nutrition indices

The results of the blood total protein, albumin, and lipid profile analyses are shown in Table 4. The total protein and albumin levels were significantly lower in the C group than in the NC group ($P<0.01$ for both). Several previous studies have outlined the effects of radiation on the plasma nutrition indices measured in the current study. In our previous study, total protein, albumin, and HDL-C levels were decreased and TG and LDL-C levels were increased in rats euthanized 10 days after a single dose of radiation (15). Another study (23) also reported that total protein, albumin, total globulins, and HDL levels were decreased, whereas total lipid, cholesterol, and TG levels were increased after radiation. In Pote's study (24), hepatic TG and plasma lipoprotein levels were elevated in irradiated mice. The activation of lipoprotein lipases (LPL) was also increased by radiation treatment. None of the experimental diets tested in the present study restored the radiation-induced decrease in total protein and albumin levels. Serum total cholesterol, LDL-C, and VLDL-C levels in the C group were higher than those in the NC group. The TG levels of the rats in the HP, RES, and HP+RES groups were lower than the TG level of the C group. In addition, serum total cholesterol and VLDL-C levels of the HP group were significantly lower than those of the C group (all $P<0.001$). In contrast, the total cholesterol, LDL-C, and VLDL-C

levels of rats in the RES group were higher than the total cholesterol, LDL-C, and VLDL-C levels of rats in the C group (total cholesterol and VLDL-C, $P<0.001$; LDL-C, $P<0.01$). The results of current study indicate that radiation-induced decreases in protein and albumin levels and increases in LDL-C levels might be sustained for at least one month after a radiation dose. There was no difference in the serum levels of TG and HDL-C between the NC and C groups. Therefore, we conclude that TG and HDL-C levels recover to normal levels within 30 days after radiation treatment, but protein status restoration may take longer.

Shin et al. (13) observed that oxidation-induced damage was greater in mice fed a lower protein diet. In the study by Shin et al., mice were fed a 7%, 20%, or 33% protein diet for 3 weeks and then treated with whole body irradiation. Liver and plasma lipid oxidation damage were higher in the 7% protein diet group than in the 33% protein diet group. Radiation may activate LPL, thus elevating plasma concentrations of LDL and TG (13,24). Reduced LDL receptor expression also inhibits the flow of plasma LDL into the liver. In irradiated rats, hepatic cholesterol and TG levels may be caused by increased 3-hydroxy-3-methylglutaryl-coenzyme A activation and decreased cholesterol 7α-hydroxylase activation, which are the rate-limiting enzymes for cholesterol and bile acid synthesis (23,25,26). Therefore, our study suggests that a high-protein diet may improve lipid status and, particularly, and lipoprotein metabolism.

Blood cell analysis and pro-inflammatory cytokine production

Radiation-induced changes in the complete blood cell count with WBC differential are shown in Table 5. Platelet counts in the C group were significantly lower than platelet counts in the NC group [(531.50±97.48) $\times 10^3/\mu L$ vs. (795.50±89.39)$\times 10^3/\mu L$, respectively; $P<0.01$)]. In addition, WBC counts were significantly higher in the C group compared to the NC group [(6.68±1.21) $\times 10^3/\mu L$ vs. 3.69±0.56)$\times 10^3/\mu L$; $P<0.001$)]. The WBC counts of rats in the RES group were significantly higher

Table 4. Plasma total protein and albumin levels

	Total protein (g/dL)		Lipid profile (mg/dL)				
	Total protein	Albumin	Triglyceride	Cholesterol	HDL-C	LDL-C	VLDL-C
NC	7.90±0.91##	3.50±0.37##	169.50±32.09	68.33±5.05##	26.67±4.08	4.00±0.89##	37.67±6.44#
C	6.22±0.29	2.78±0.23	139.83±33.63c	84.67±8.41b	30.50±3.39	7.17±1.60a	47.00±6.63b
HP	6.45±0.69	3.00±0.62	58.50±8.83a	66.00±4.34a	30.33±1.03	5.50±0.55a	30.17±4.17a
RES	6.03±0.69	2.50±0.49	99.29±12.99b	104.14±12.58c	32.43±5.56	9.00±1.91b	62.71±9.59c
HP+RES	6.17±0.82	2.73±0.46	81.71±11.28b	93.43±7.32b	29.00±2.83	6.14±1.21a	58.28±5.47c

NC, normal control diet; C, control diet; HP, 30% high-protein diet; RES, control diet with 2 mg/kg b.w. of resveratrol every other day; HP+RES, high-protein diet with 2 mg/kg b.w. of resveratrol every other day.
Within radiation treated groups, superscript letters (a-c) indicate significant differences at $P<0.05$ by Duncan's multiple range tests, hashtag indicates significant difference between NC and C groups by t-test (#$P<0.05$ and ##$P<0.01$).

Table 5. Blood cell counts and white blood cell differentials

	Blood cell counts			WBC differential (%)				
	RBC (1×10^6/μL)	Platelet (1×10^3/μL)	WBC (1×10^3/μL)	Lymphocyte	Monocyte	Neutrophil	Eosinophil	Basophil
NC	8.07±1.14	795.50±89.39[##]	3.69±0.56[###]	72.03±6.00[##]	4.53±0.55	21.37±6.26[##]	1.28±0.32	0.78±0.12
C	7.01±1.08	531.50±97.48	6.68±1.21[ab]	57.20±5.71[ab]	4.07±0.67[b]	36.87±5.44[bc]	1.52±0.24	0.98±0.14
HP	6.66±0.42	650.83±125.17	4.85±0.85[a]	67.52±3.79[c]	2.80±0.22[a]	27.18±3.63[a]	1.63±0.22	0.87±0.12
RES	7.30±1.42	561.86±95.59	10.41±2.86[c]	52.00±6.82[a]	3.79±0.62[b]	41.76±6.76[c]	1.53±0.43	0.84±0.20
HP+RES	7.41±0.65	671.00±86.41	8.43±1.34[bc]	63.36±8.23[bc]	3.09±0.29[a]	31.03±8.47[ab]	1.64±0.32	0.89±0.19

NC, normal control diet; C, control diet; HP, 30% high-protein diet; RES, control diet with 2 mg/kg b.w. of resveratrol every other day; HP+RES, high-protein diet with 2 mg/kg b.w. of resveratrol every other day.
Within radiation treated groups, superscript letters (a-c) indicate significant differences at P<0.05 by Duncan's multiple range tests and hashmark indicates significant difference between NC and C groups by t-test ([##]P<0.01 and [###]P<0.001).

Table 6. Serum IgM levels and proinflammatory cytokine production

	IgM (mg/dL)	Cytokines production (pg/mL)		
		IL-1β	IL-6	TNF-α
NC	14.50±2.88[##]	92.43±4.22[#]	358.95±31.18[#]	40.17±3.46
C	9.00±1.26[a]	107.63±11.81[b]	300.00±45.96[b]	46.80±6.43[b]
HP	14.33±1.21[b]	108.50±8.18[b]	464.81±41.64[c]	62.95±6.39[c]
RES	15.86±2.48[b]	100.84±4.83[ab]	286.87±41.98[ab]	41.78±5.02[ab]
HP+RES	11.43±3.74[a]	96.91±6.30[a]	245.46±18.08[a]	38.79±7.56[a]

NC, normal control diet; C, control diet; HP, 30% high-protein diet; RES, control diet with 2 mg/kg b.w. of resveratrol every other day; HP+RES, high-protein diet with 2 mg/kg b.w. of resveratrol every other day.
Within radiation treated groups, superscript letters (a,b) indicate significant differences at P<0.05 by Duncan's multiple range tests and hashmark indicates significant difference between NC and C groups by t-test ([#]P<0.05 and [##]P<0.01).

than the WBC counts of the C group (P<0.001). The WBC count of the HP group was the lowest WBC count among the irradiated groups. With respect to the WBC differential, the lymphocyte and neutrophil percentages were both significantly affected by radiation (P<0.01). The lymphocyte percentage was lower and the neutrophil percentage was higher in the C group than in the NC group. The lymphocyte percentage was higher and the monocyte and neutrophil percentages (P<0.01) were lower in irradiated rats fed a 30% high-protein diet than in irradiated rats fed the control diet. The HP+RES diet also significantly decreased the monocyte percentage.

In our previous study, we found that WBC, RBC, monocyte, and neutrophil counts were increased and platelet, lymphocyte, and basophil counts were decreased 10 days after radiation (15). In the present study, we found that RBC, monocyte, and basophil counts were restored at 30 days post-radiation. However, the platelet, lymphocyte, and neutrophil counts had not recovered by 30 days after radiation treatment. Radiation induces ROS and inflammation. It attacks DNA and cellular homeostasis, resulting in signal transduction pathway modification and immunosuppression (8,9,27,28). Radiation also increases peripheral blood lymphocyte and splenocyte apoptosis and destroys the lymphoid and hematopoietic systems (29,30). In a study by Gridley et al. (17), rats were exposed to 0, 1, 2, and 4 Gy radiation doses and euthanized 9 months after irradiation. Blood cell populations, lymphocyte levels, granulocyte levels, monocyte levels, and RBC counts were lowest in rats exposed to the 2 Gy radiation dose. Another study reported that resveratrol supplementation of irradiated rats increased WBC counts, bone marrow cell counts, and lymphocyte percentages (31).

Serum IgM and cytokine concentrations are presented in Table 6. The serum IgM level was significantly lower in the C group compared to the NC group (P<0.01). We also observed significant IgM level differences among the irradiated groups. The IgM level of the HP and RES groups was greater than the IgM level of the C group (P<0.001).

Radiation treatment significantly increased IL-1β production and decreased IL-6 production (both P<0.05). When compared with the C group, all proinflammatory cytokines levels were lower in the HP+RES group (IL-1β, P<0.05; IL-6 and TNF-α, P<0.001), while IL-6 and TNF-α production were higher in the HP group (both P<0.001). Radiation generates chemokines (32) and proinflammatory cytokines such as IL-1β and IL-6 (28,33). Th1 cytokines such as IL-2, IL-12, interferon (IFN)-γ, and TNF-β, which are involved in cell-mediated immunity, promote post-radiation activation of cell adhesion molecules, whereas Th2 cytokines such as IL-3, IL-4, IL-5, IL-6, and IL-13, which are involved in humoral immunity, promote angiogenesis (34). In a previous study (10), researchers found that radiation exposure

activated proinflammatory and pro-fibrotic genes such as IL-6, TNF-α, and TGF-β. Acute and subchronic radiation also activated connective tissue growth factor and inhibited thrombocytes and leukocytes.

Another study (28) demonstrated that mice exposed to radiation had a decreased WBC count, fewer bone marrow cells, and a lower IFN-γ concentration. In addition, irradiated mice had increased blood IL-1β and TNF-α concentrations. Hematocytes are sensitive to radiation: even low doses of radiation can damage hematocytes. Bone marrow is comprised of yellow marrow and red marrow; the hematopoietic system of young adult animals is located in the latter. Red marrow is found in the humerus, the femur, the flat bones of the skull, the ribs, the vertebrae, and the pelvic bones (35). Exposure to a 2 Gy radiation dose alters cytokine and chemokine production patterns. It increases serum expression of IL-3, IL-4, leptin, monocyte chemoattractant protein (MCP)-1, MCP-5, and macrophage inflammatory protein (MIP)-1α, but decreases blood levels of IL-13, IL-17, IFN-γ, platelets, and WBC (36). In Shin's study, chronic low-dose radiation promoted the differentiation of naïve T cells into Th2 cells (36). In our study, pelvic bone marrow was directly exposed to radiation treatment (RT) and vertebrae and femur bone marrow was indirectly exposed to RT. Therefore, the RT induced inflammation and affected bone marrow, thus affecting the immune system. The high-protein diet supplemented with resveratrol had anti-inflammatory effects and decreased proinflammatory cytokine production.

Splenocyte proliferation

The splenocyte proliferation index is shown in Table 7. Proliferation was only affected by radiation in ConA-treated splenocytes ($P<0.01$). The experimental diets had significant effects on the proliferation indices of splenocytes from the irradiated groups. In the mitogen-free- and LPS-stimulated conditions, splenocyte proliferation was higher in the HP and RES groups than in the C group. The splenocyte proliferation index of the RES group was also increased in the ConA-stimulated condition ($P<0.05$). Spleen cells eliminate old red blood cells, eliminate pathogens, and produce antibodies (37). In a previous study, the proliferation index of splenocytes excised from mice 30 days after radiation was decreased in the mitogen-free- and ConA-stimulated conditions (38,39). In addition, decreased spleen cell numbers have been reported in malnourished rats (40). Resveratrol has been reported to restore splenocyte and CD3$^+$CD4$^+$ T cell counts following restraint stress (41). In the present study, radiation did not influence splenocyte proliferation, but did increase T cell activation in ConA-stimulated splenocytes.

CONCLUSION

We investigated the effects of a high-protein diet and resveratrol supplementation on rats receiving radiation treatment. This study confirmed that radiation treatment affects body weight and nutritional status, particularly protein and lipoprotein profiles. Radiation treatment also decreased protein and albumin levels and increased total cholesterol, LDL-C, and VLDL-C levels. In addition, food consumption and body weight gain were decreased in radiation-treated rats. A 30% high-protein diet may improve the impaired lipid status of irradiated rats. The high-protein diet was particularly effective at restoring concentrations of TG, total cholesterol, and VLDL-C. Resveratrol supplementation and treatment with a 30% high-protein diet supplemented with resveratrol decreased the TG concentrations of irradiated rats. Radiation treatment also impaired the immune status of the rats in this study-lymphocyte, neutrophil, and proinflammatory cytokine levels were particularly affected. Radiation treatment was associated with decreased lymphocyte levels, increased neutrophil levels, and increased IL-1β production. Treatment with a high-protein diet restored radiation-induced changes in WBC and cytokine profiles faster than the other diets tested.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

Table 7. Splenocyte proliferation index

	Without mitogen	With mitogen	
		ConA	LPS
NC	1.00±0.16	1.26±0.17$^{\#\#}$	1.65±0.79
C	2.05±0.78^a	2.89±0.42^a	1.49±0.26^a
HP	4.19±0.82^b	4.84±2.58^{ab}	4.77±1.80^b
RES	4.19±0.82^b	7.91±1.75^b	4.77±1.80^b
HP+RES	3.00±0.94^{ab}	2.49±0.86^a	2.29±0.93^{ab}

NC, normal control diet; C, control diet; HP, 30% high-protein diet; RES, control diet with 2 mg/kg b.w. of resveratrol every other day; HP+RES, high-protein diet with 2 mg/kg b.w. of resveratrol every other day.
Within radiation treated groups, superscript letters (a,b) indicate significant differences at $P<0.05$ by Duncan's multiple range tests, hashtag indicates significant difference between NC and C groups by t-test ($^{\#\#}P<0.01$).

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