Efficacy of tea polyphenols (TP 50) against radiation-induced hematopoietic and biochemical alterations in beagle dogs

Dong Xianzhe, Wang Dongxiao, Han Jichun, Luo Qingliang, Guo Daihong, Liu Ping, Yan Can, Hu Yuan

INTRODUCTION

Beagle dogs were exposed to a single acute dose of whole-body γ-radiation (3 Gy) and orally administered TP 50 (80 or 240 mg·kg⁻¹·d⁻¹) for 28 consecutive days. A hemogram was obtained from experimental dogs every other day for 42 d. At the end of the experiment, enzyme activities of the antioxidants superoxide-dismutase and glutathione peroxidase, serum levels of inflammatory cytokines (tumor necrosis factor-α, interleukin-1β, and interleukin-6), colony-forming units of bone marrow hematopoietic progenitor cells, and organ coefficients were measured.

RESULTS: Dogs exposed to γ-radiation alone exhibited typical hematopoietic syndrome. In contrast, irradiated dogs that received TP 50 exhibited an improved blood profile with reduced leucopenia, thrombocytopenia (platelet counts), and reticulocyte levels. TP 50 also significantly elevated levels of the endogenous antioxidant enzyme superoxide-dismutase, reduced the increased levels of serum cytokine in response to radiation-induced toxicity, and increased colony-forming units of bone marrow hematopoietic progenitor cells. In addition, TP 50 repaired radiation-induced organ damage.

CONCLUSION: The current findings suggest that oral administration of TP 50 to beagle dogs effectively alleviated hematopoietic bone marrow damage induced by γ-radiation.

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gastrointestinal, or central nervous systems after whole-body exposure limits its therapeutic role in the treatment of human malignancies. As a result, finding a potential drug that could exert a protective effect against radiation, such that it would exhibit only low toxicity, is an urgent requisite. Natural products with antioxidant effects that can induce bone marrow recovery from radiation damage are potential candidates. Tea polyphenols (TP) are extracted from green tea, which has been the leading beverage in China for thousands of years. TP contains (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin-gallate, and (-)-epigallocatechin-gallate (EGCG). Recently, antimutagenic, anticancer, antioxidative, and radioprotective effects of TP have been identified. TP, which contains approximately 50% EGCG in addition to other catechins, was found to significantly revert radiation-induced decreases in levels of various hematological components. TP also exerted a protective antioxidant effect and significantly reduced the elevation of serum levels of inflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), and IL-6 in mice exposed to whole-body irradiation.

In this study, we investigated the potential role of TP in protecting beagle dogs from a moderate dose of γ (gamma-ray) radiation.

**MATERIALS AND METHODS**

**Chemicals**

TP 50 was purchased from HeTian Biomed Technol Co. (Hangzhou, China). All standard compounds had > 98% purity. Reagents and kits for detecting enzyme activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Kits for detecting serum cytokine levels (TNF-α, IL-1β, and IL-6) were provided by R&D (Research & Diagnostics, Minneapolis, MN, USA).

**Experimental animals and ethics evaluation**

A total of 24 beagle dogs (12 females and 12 males) weighing 10-12 kg, aged 11-12 months, were purchased from Beijing Keyu Laboratory Animals Company (Beijing, China) under a production license for experimental animals [SCXK (Jing) 2007-0003]. All dogs were housed individually in stainless steel cages in the animal facility and maintained under controlled conditions including temperature (21-23 °C), a 12-h light/dark cycle, standard dog food, and tap water ad libitum. All dogs were acclimatized to laboratory conditions for 5 d during which preventive measures were administered. All experimental protocols were reviewed and approved by the Animal Experimentation Ethics Committee of General Hospital of Chinese PLA. All animal handling procedures were performed in compliance with the "Principles of Laboratory Animal Care" and China Laboratory Animal Use and Care Act. Dogs were anesthetized with sodium pentobarbital before undergoing surgery and were subsequently sacrificed.

**Measures**

Each dog was administered 160 000 units of gentamicin (intramuscular injection) and 0.4 g metronidazole (orally) per day for 3 d before the start of the experiment. During the experiment, dogs with severe vomiting or diarrhea were administered sodium lactate Ringer’s injection and an intravenous glucose drip (50%). Each dog was injected with 800 000 units of penicillin intramuscularly per day when the basal peripheral white blood cell count (WBC) was < 4 × 10⁹ cells/L to prevent infection. In addition, 1.0 g cefotaxime sodium was injected intravenously once daily when the WBC count was < 2 × 10⁹ cells/L. No antibiotics were administered when the WBC count was > 4 × 10⁹ cells/L. Each dog received 1.0 g Yunnan Baiyao and 0.25 g vitamin C orally, and 0.05 g aminomethylbenzoic acid via intravenous injection one time per day when the basal peripheral platelet count was < 1 × 10⁵ cells/L, for the treatment or prevention of hemorrhage.

**Radiation exposure and drug administration**

After 5-day acclimatization, dogs were randomly divided into four groups. Group-1: Untreated and un-irradiated controls. Group-2: Administered single acute dose of 3 Gy γ-radiation only. Groups-3 and -4: Oral administration of TP 50 at 80 or 240 mg·kg⁻¹·d⁻¹ (volume of 2 mL/kg body weight) to 3 Gy γ-irradiated dogs, respectively. The dosage was selected according to our pre-experiment result, which showed that these two dosages were close to the half-effective and maximum non-toxic dosages, respectively. Dogs in Groups 2-4 were anesthetized by intravenous injection of pentobarbital sodium (3%, 30 mg/kg), prostrated, and whole-body exposed to a single 3.0 Gy dose of radiation at a rate of 31.18 cGy/min using a 60Co source at the Department of Radiotherapy and Oncology, The Military Medical Academy of Science (Beijing, China). Various doses of TP 50 were orally administered for 28 consecutive days post-irradiation. Each dog was also weighed every 3 d to adjust for the amount of TP 50 to be administered. The experimental procedure is shown in Figure 1.

**Collection of blood and tissue**

During the experiment, the appearance, activity, food intake, mucosal secretions in the mouth/penis, feces, and body weight of each dog were recorded. In addition, blood samples were collected from each dog on alternate days of the experiment to obtain hemogram data (white cell, platelet, and reticulocyte counts). Serum was separated from blood samples to determine levels of SOD, GSH-PX, NS malondialdehyde (MDA), as well as serum levels of inflammatory cytokines TNF-α, IL-1β, and IL-6 using commercially available kits, as
Previously described. All dogs were sacrificed by bleeding the femoral arteries and dissected on day 28 of the experiment post anesthetization. Kidney, liver, testes, lung, spleen, brain, heart, bone marrow, and lymph nodes were collected and weighed. Organ coefficients were calculated and tissue sections observed for pathological changes. Bone marrow smears were prepared, and bone marrow cells including CFU-GM, CFU-MK, CFU-E, BFU-E, and CFU-Mix were cultured to examine the presence of hyperplasia using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method.  

**Statistical analysis**  
Data are presented as mean ± standard error of mean. Statistical analysis was conducted by using SPSS 16.0 software (IBM Corp., Al Monk, NY, USA). Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA), followed by Dunnett’s test. *P* < 0.05 was considered as statistically significant.

**RESULTS**  

**Blood cells**  
The WBC count in dogs exposed to 3.0 Gy γ-radiation was significantly reduced on day 1, reached a minimum level on day 21 (1.39 × 10¹⁰ cells/L), and slowly recovered to 6.20 × 10⁹ cells/L by day 42. The mean duration of leukopenia was (28.7 ± 1.2) d. Dogs treated with 240 or 80 mg·kg⁻¹·d⁻¹ TP 50 exhibited a significant decrease in WBC count on day 1 after irradiation that reached a minimum on days 17 and 19, respectively. The mean duration of leukopenia was between (18.7 ± 0.9) and (20.8 ± 1.4) d, while the mean recovery was 78.9% and 78.6% of the WBC count on day 0, respectively. The recovery rate of WBC in TP 50-administered groups occurred more rapidly than observed in the γ-irradiation only group (Figure 2A). The recovery level (%) was defined as the ratio of WBC on day 42 to that of pre-irradiation. The duration of leukopenia was defined as the number of days when WBC was < 4 × 10⁷ cells/L.  

The platelet (PLT) number in dogs exposed to 3.0 Gy γ-radiation was significantly downregulated on day 7, decreased to a minimum on day 13 (9.50 × 10¹⁰ cells/L), and slowly recovered to 120.50 × 10⁹ cells/L by day 42. The mean duration of thrombocytopenia was (11.7 ± 1.5) d. Dogs treated with 240 or 80 mg·kg⁻¹·d⁻¹ TP 50 exhibited significantly decreased PLT on day 1 post-irradiation that reached a minimum on days 15 and 17, respectively. The mean duration of thrombocytopenia was (5.7 ± 1.3) and (9.8 ± 1.1) d, respectively. Mean recovery levels, as compared with the basic level (PLT number on day 0), were 109.18% and 89.01%, respectively. The recovery of PLT was faster in both TP 50-treated groups compared with the irradiation-only group (Figure 2B). The duration of thrombocytopenia was defined as the number of days when PLT was < 15 × 10⁷ cells/L.  

The reticulocyte (RET) count in dogs exposed to 3.0 Gy γ-radiation was significantly reduced on day 1, further decreased to a minimum on day 11 (109.35 × 10⁹ cells/L), and gradually recovered to 1171.46 × 10⁹ cells/L by day 42. The mean duration of aspherinia was (24.9 ± 1.3) d. Dogs treated with 240 or 80 mg·kg⁻¹·d⁻¹ TP 50 exhibited a significant decrease in RET count on day 1 post-irradiation that reached a minimum level on days 13 and 11, respectively. The mean duration of aspherinia was (21.7 ± 2.3) d. Dogs treated with 240 or 80 mg·kg⁻¹·d⁻¹ TP 50 exhibited a significant decrease in RET count on day 1 post-irradiation that reached a minimum level on days 13 and 11, respectively. The mean duration of aspherinia was (21.7 ± 2.3) d.
duration of aspherinia was (11.7 ± 1.8) and (16.7 ± 0.4) d, respectively, while mean recovery levels were 237.22% and 216.93% compared with respective baseline levels of RET on day 0. The recovery rate of RET was more rapid in both TP 50-treated groups compared with the irradiation-only group (Figure 2C). The duration of aspherinia was defined as the number of days when RET was < 300 × 10^9 cells/L.

**Altered SOD, GSH-PX, and MDA levels**

Levels of the anti-oxidase enzyme SOD in peripheral blood were lower in the irradiation-only group on day 1 post-irradiation, and were further reduced on day 7 compared with the unirradiated control group (P < 0.05). SOD levels gradually recovered to (54 ± 9) U/ml on day 42. Dogs treated with 240 mg·kg⁻¹·d⁻¹ TP 50 exhibited significantly higher SOD levels on
day 7 after irradiation compared with the irradiation-only group (P < 0.05). TP 50-treated dogs showed a minimum SOD level on day 14 that recovered to the pre-irradiation level on day 27. Levels of GSH-PX enzyme in peripheral blood were also slowly reduced in the γ-irradiation group from day 1 post-irradiation. However, no significant increase was observed in GSH-PX or MDA levels in dogs treated with 240 or 80 mg·kg⁻¹·d⁻¹ TP 50 (Figure 3).

Changes in TNF-α, IL-1β, and IL-6 levels

Levels of TNF-α, IL-1β, and IL-6 were significantly increased on day 27 in dogs exposed to 3.0 Gy γ-radiation. Indeed, both 240 and 80 mg·kg⁻¹·d⁻¹ TP 50 treatments elicited a significant reduction in TNF-α, IL-1β, and IL-6 levels of irradiated dogs at day 27 compared with irradiation-only dogs (Figure 4).

Activity of hemopoietic progenitor cells in bone marrow

Colony numbers of different types of hemopoietic progenitor cells including CFU-GM, CFU-MK, CFU-E, BFU-E, and CFU-Mix were enumerated in the bone marrow. Dogs exposed to 3.0 Gy γ-radiation exhibited a significant decrease in colony numbers of hemopoietic progenitor cells on day 1 compared with day 0 (before irradiation). Dogs treated with 240 or 80 mg·kg⁻¹·d⁻¹ TP 50 exhibited a significantly higher number of CFU-GM, CFU-Mix, and CFU-MK cells on day 30 compared with irradiation-only dogs (Figure 4).

Organ coefficient and pathological evaluation

No significant differences were observed in the body weight or organ coefficients of testes, lung, liver, adrenal gland, kidney, or spleen among the four groups of dogs (data not shown). However, animals exposed to 3.0 Gy γ-radiation exhibited a decreased ratio of hemopoietic/adipose tissue in myeloid tissue, as well as reduced hemopoietic tissue (Figure 6A) and numbers of spermatogenic cells and mature sperm in testes (Figure 6B). In addition, lymph sinususes hydrops were observed in the lymph nodes (Figure 6C), fibroplasia and phlogocyte imbibition were observed in the lung (Figure 6D). No significant changes occurred in the heart, brain, or liver among the four groups (Figure 6E-G), the number of acinus lichenalis (Figure 6H) was reduced.

DISCUSSION

Ionizing radiation by both therapeutic and accidental exposures causes damage to the hemopoietic system and other tissues, thereby exerting a negative impact on health. Thus, compounds that can reduce the deleterious effects of radiation are crucial for the application of therapeutic radiation for cancers and treatment of accidental exposures; for example, amifostine, exerts a protective effect against radiation-induced damage but its usage in clinical practice is limited due to significant toxicity. In recent years, compounds from natural plants with radioprotective effects have garnered increasing attention. In particular, green tea extracts have been implicated in the control, mitigation, and prevention of a number of different diseases or conditions. In earlier studies, we evaluated the radioprotective efficacy of green tea polyphenols and its ingredients against radiation-induced damage in mice, and elucidated the underlying mechanisms.

In this study, our results revealed that TP 50 induced an improved hemogram, as indicated by a short duration of leucopenia and thrombocytopenia, and increased level of RET. In addition, we observed a significant increase in the activity of hemopoietic progenitor cells including CFU-GM, CFU-Mix, and CFU-MK cells in the bone marrow. Notably, radiation-induced damage to both blood cells and bone marrow was reversed by TP 50 treatment, which also induced the recovery of erythroid and myeloid cells. These changes indicated that TP 50 could mitigate the hemopoietic syndrome associated with exposure to radiation. As oxidative stress effectuates radiation-induced damage via excessive generation of reactive oxygen species, it was speculated that the antioxidant SOD might mitigate the observed effect. Notably, an increase in SOD activity after TP 50 administration was previously shown to facilitate the scavenging of free radicals resulting from the effects of radiation.

Cytokines such as TNF-α, IL-1β, and IL-6 play a significant role in the development of radiation-related toxicity. In addition, hepatic irradiation triggers fat accumulation in mouse livers involving acute-phase processes. An anti-TNF-α-therapy prevented early radiation-induced expression of FAT/CD36 in vivo. Notably, as IL-6 levels are associated with GSK-3β activity, inhibition of GSK-3β by reducing IL-6 conferred resistance to lipopolysaccharide-induced shock, but fostered ionizing radiation-induced death of mice and cells. Altogether, in this study, TP 50 significantly reduced serum levels of TNF-α, IL-1β, and IL-6.

Our findings suggest that oral administration of TP 50 in beagle dogs facilitated the recovery of hemopoietic progenitor cell damage induced by γ-radiation in bone marrow. Importantly, administration of TP 50 to the dogs did not elicit obvious adverse effects on various organs or tissues according to the results of our pathological tests. Furthermore, TP 50 alleviated and restored radiation-induced organ damage and no toxic effects were observed. As such, TP 50 may be a promising candidate for the prevention and treatment of radiation-induced toxicity and pathological damage.

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Figure 3 Levels of GSH-PX, SOD, and MDA in beagle dogs after 3.0 Gy irradiation and TP 50 treatment
A: GSH-PX levels in different groups; B: SOD levels in different groups; C: MDA levels in different groups. Data represent mean ± standard error of mean, n = 6. Untreated group: dogs were untreated and un-irradiated; model (3 Gy) group: dogs were exposed single acute dose of 3 Gy γ-radiation at a rate of 31.18 Gy/min using a 60Co source; TP 50 80 mg/kg group: dogs were exposed to 3 Gy γ-radiation and oral administered of TP 50 at 80 mg · kg⁻¹ · d⁻¹ (volume of 2 mL/kg body weight) for 28 consecutive days post-irradiation; TP 50 240 mg/kg group: dogs were exposed to 3 Gy γ-radiation and oral administered of TP 50 at 240 mg · kg⁻¹ · d⁻¹ (volume of 2 mL/kg body weight) for 28 consecutive days post-irradiation. TP 50 240 mg/kg group compared with irradiation-only control, \( P < 0.05; \) TP 50 80 mg/kg group compared with irradiation-only control, \( P < 0.05; \) TP 50 80 mg/kg group compared with irradiation-only control compared with untreated group, \( P < 0.05; \) irradiation-only control compared with untreated group, \( P < 0.01. \) GSH-PX: glutathione peroxidase; MDA: malondialdehyde; SOD: superoxide dismutase; TP 50: tea polyphenols.

Figure 4 Levels of IL-6, IL-1β, and TNF-α in beagle dogs after 3.0 Gy irradiation and TP 50 treatment
A: IL-1β levels in different groups; B: IL-6 levels in different groups; C: TNF-α in different groups. Data represent mean ± standard error of mean, n = 6. Untreated group: dogs were untreated and un-irradiated; model (3 Gy) group: dogs were exposed single acute dose of 3 Gy γ-radiation at a rate of 31.18 Gy/min using a 60Co source; TP 50 80 mg/kg group: dogs were exposed to 3 Gy γ-radiation and oral administered of TP 50 at 80 mg · kg⁻¹ · d⁻¹ (volume of 2 mL/kg body weight) for 28 consecutive days post-irradiation; TP 50 240 mg/kg group: dogs were exposed to 3 Gy γ-radiation and oral administered of TP 50 at 240 mg · kg⁻¹ · d⁻¹ (volume of 2 mL/kg body weight) for 28 consecutive days post-irradiation. TP 50 240 mg/kg group compared with irradiation-only control, \( P < 0.05; \) TP 50 80 mg/kg group compared with irradiation-only control, \( P < 0.05; \) irradiation-only control compared with untreated group, \( P < 0.01; \) irradiation-only control compared with untreated group, \( P < 0.01; \) TNF-α: tumor necrosis factor alpha; TP 50: tea polyphenols.
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Figure 5 Colony-forming unit of bone marrow hematopoietic progenitor cells in beagle dogs after 3.0 Gy radiation and TP 50 treatment

A: colony-forming unit of bone marrow cells in different groups before irradiation; B: colony-forming unit of bone marrow cells in different groups after irradiation; C: colony-forming unit of bone marrow cells in different groups after irradiation and TP 50 treatment. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was used to examine the presence of hyperplasia of bone marrow cells including CFU-GM, CFU-MK, CFU-E, BFU-E, and CFU-Mix. Data represent mean ± standard error of mean, n = 6. Irradiation-only control compared with untreated group, P < 0.01; irradiation-only control compared with untreated group, P < 0.05; TP 50 80 mg/kg group compared with irradiation-only control, P < 0.01; TP 50 240 mg/kg group compared with irradiation-only control, P < 0.01; TP 50 240 mg/kg group compared with irradiation-only control, P < 0.05; TP 50 80 mg/kg group compared with irradiation-only control, P < 0.05. TP 50: tea polyphenols; CFU-GM: Granulocyte macrophage colony-forming unit; CFU-E: colony-forming unit erythroid; CFU-Mix: mix-colony forming unit; CFU-MK: Megakaryocyte colony-forming unit; BFU-E: burst forming unit-erythroid.

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