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RhG-CSF Improves Radiation-induced Myelosuppression and Survival in the Canine Exposed to Fission Neutron Irradiation

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Neutron irradiation/rhG-CSF/Myelosuppression.

Fission-neutron radiation damage is hard to treat due to its critical injuries to hematopoietic and gastrointestinal systems, and so far few data are available on the therapeutic measures for neutron-radiation syndrome. This study was designed to test the effects of recombinant human granulocyte colony-stimulating factor (rhG-CSF) in dogs which had received 2.3 Gy mixed fission-neutron-γ irradiation with a high ratio of neutrons (~90%). Following irradiation, rhG-CSF treatment induced 100% survival versus 60% in controls. Only two of five rhG-CSF-treated dogs experienced leukopenia (white blood cells [WBC] count < 1.0×10^9/L) and neutropenia (neutrophil [ANC] count < 0.5×10^9/L), whereas all irradiated controls displayed a profound period of leukopenia and neutropenia. Furthermore, administration of rhG-CSF significantly delayed the onset of leukopenia and reduced the duration of leucopenia as compared with controls. In addition, individual dogs in the rhG-CSF-treated group exhibited evident differences in rhG-CSF responsiveness after neutron-irradiation. Finally, histopathological evaluation of the surviving dogs revealed that the incidence and severity of bone marrow, thymus and spleen damage decreased in rhG-CSF-treated dogs as compared with surviving controls. Thus, these results demonstrated that rhG-CSF administration enhanced recovery of myelopoiesis and survival after neutron-irradiation.

INTRODUCTION

Neutrons are non-charged particles, and classified as high linear energy transfer (LET) radiation. Extensive studies have shown the biological effect of fission-neutron irradiation and confirmed that the relative biological effectiveness (RBE) of fission-neutron irradiation for different animal models is greater than that observed with either X rays or γ rays.[1–4] Based on these studies, it was proposed that neutron irradiation caused more severe injuries to hematopoietic and gastrointestinal (GI) system as compared with the low-LET γ ray and X ray irradiation.[4,5] For instance, after neutron irradiation, the incidence and severity of the early signs of GI syndrome as well as early death increased, and the leucocyte count decreased very rapidly with much lower nadirs, which led to grave early infection and death.[5] Thus, it is especially difficult to treat the neutron-irradiation syndrome in view of these critical injuries, and so far few data are available on the therapeutic measures for neutron-radiation damage. Previous studies demonstrated that clinical support regimens[4] and bone marrow transplantation[3,6–8] effectively improved the survival and increased the $LD_{50/30}$ after mixed fission-neutron: γ irradiation. However, some protective drugs such as Amifostine and estradiol, which are effective in the treatment of γ-radiation injury, showed less or no effect on neutron-radiation damage.[9,10] The therapeutic efficacy of several hematopoietic growth factors (HGF) had also been examined in animal models exposed to mixed fission-neutron: γ irradiation.[11–13] But the percentage of neutrons in the mixed radiation was relative low and the specific effects of high-LET neutron irradiation in animals were not apparent in those studies.

Granulocyte colony stimulating factor (G-CSF) is an HGF that acts selectively on the neutrophil lineage and has been used extensively in clinical settings for accelerating hematopoietic recovery following chemotherapy or bone marrow

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transplantation, and decrease the period of neutropenia in the limited number of radiation accident victims studied.[14] Extensive studies have provided evidence that G-CSF is able to stimulate neutrophil recovery and to promote survival after lethal irradiation in murine,[15,16] canine,[17–19] and primate[20] models. Canine models have already been employed to study the effects of HGFs on haemopoiesis under normal conditions and in a state of perturbation due to exposures to ionizing radiation. It has been found that rhG-CSF stimulates hematopoiesis in normal dogs and can reverse the otherwise lethal myelosuppressive effect of radiation exposure when administered shortly after irradiation.[17] Similar results were reported by MacVittie *et al.*[18] Furthermore, in the canine, G-CSF administered early and continuously throughout the period of neutrophil recovery can rescue animals in the supralethal dose range of TBI.[19] In these cases, G-CSF has displayed evident efficacy in reducing neutropenia and enhancing survival of the canine model exposed to lethal doses of ^{60}Co gamma radiation.

In this study, we used the canine as a neutron-irradiated model given a dose with a high ratio of neutrons and evaluated the effects of rhG-CSF on the hematologic recovery and survival of irradiated dogs. The effects of the drug were further evaluated by studying the general conditions of the irradiated animals and the histopathology of surviving animals.

MATERIALS AND METHODS

Experimental animals

Healthy beagle dogs weighing 8.0–11.0 kg were all bought from the Experimental Animal Center of Academy. The certificate number for the quality of the experimental animals was SCXK (Jing) 2007-0003, and the certificate number for the raising conditions of the experimental animals was SYXK-(Jun) 2007-004. Canines were housed individually in stainless steel cages in rooms with a reverse-filtered air barrier, normal illumination rhythm, and stable temperature (18–22°C) and relative humidity (40–70%). They were fed commercially available primate chow and were provided with acidified drinking water. All canines were free of intestinal parasites and were seronegative for herpes B, simian T-lymphotrophic and simian immunodeficiency viruses. The housing, experiments and all other conditions were approved by an ethics committee in conformity with legal regulations in China.

Cytokine

The recombinant human G-CSF used in this study was purchased from Hangzhou Jiuyuan Gene Engineering Co. (Hangzhou, China), and was a sterile, clear and colorless solution at 250 μg/mL stock concentration.

Fission neutron irradiation

The Tsinghua University shielding experimental reactor was used as the source of mixed fission-neutron-γ radiation. This irradiation facility and the dosimetry have been described.[5] The maximum operational steady-state power of this reactor is 200 kW and the voltage of irradiation bomb was 50 kV. The neutron energy spectrum in the irradiation compartment has a average neutron energy of 1.4 MeV at the empty irradiation position. The reactor was operated at 50 kW, resulting in a dose rate of 20.97 cGy/min. Dosimetry was performed with IRM gas-flow type tissue-equivalent ionization chambers and graphite ionization chambers, which were used in a way known as the paired-chamber technique. Prostrate dogs placed in a rectangular perforated aluminum canister were irradiated unilaterally with their right sides facing the sources. They were exposed to mixed fission neutron and gamma radiation to a total midline tissue dose of 2.3 Gy. The neutron-γ ratio (9:1) was achieved by imposing a 10-cm-thick lead across the incident radiation beams. All irradiations in the present study were conducted with phantom midlines aligned at 110 cm from the tank wall. The size of the field of irradiation was approximately 100 × 100 × 45 cm. The midline, entrance, and exit doses, Dm, Den, and Dex, for neutron plus γ radiations, and the ratios Den/Dex and (Den-Dex)/Dm as an indication of nonhomogeneity in dose distribution, are listed in Table 1.

Study design

Fourteen canines were exposed to 2.3 Gy on day 0 and randomly assigned to three irradiation groups including two males in each group. In the rhG-CSF-treated group, Dogs (n = 5) were treated with 10 μg rhG-CSF/kg/d subcutaneously once a day starting within 1 hour after irradiation. Treatment lasted for 21 consecutive days. In the control groups, dogs were treated with the placebo solution and then received supportive care (n = 5) only or non-supportive care (n = 4).

Supportive care

Three days before irradiation, canines were placed in a laminar flow cabinet and the gastrointestinal tract was selectively decontaminated by administration of oral gentamicin and metronidazole (Hubei Huazhong Pharmaceutical Co., Hubei, China). An antibiotic regimen (penicillin 400,000

Table 1. Midline, entrance, and exit dose rates, and their ratios for different irradiation conditions

Parameters	Absorbed dose rate (mGy/min kW)
D_m	4.16
D_{en}	8.60
D_{ex}	2.47
D_{en}/D_{ex}	3.48
$(D_{en}-D_{ex})/D_m$	1.47

IU/dog [North China Pharmaceutical Co., Hebei, China], intramuscularly, every day [QD]) was initiated prophylactically at D3 after irradiation until the WBC was greater than 1×10^9/L for 3 consecutive days. Cefotaxime sodium (1.0 g/dog/d, [Yuekang Pharmaceutical Co., Beijing, China]) were administered intravenously when the WBC was less than 1×10^9/L and/or the rectal temperature of animals was higher than 40°C. Reptilase was used when the PLT declined to or below 100×10^9/L and/or macroscopic hemorrhage appeared on the skin. Fresh, irradiated (20 Gy ^{60}Co gamma irradiation) whole blood (approximately 60 mL/transfusion) from a random donor pool (canines of > 15 kg body weight) was administered when platelet (PLT) counts were below 50×10^9/L and the red blood cell (RBC) counts were below 3×10^{12}/L. Dehydration and electrolyte disturbances were treated with appropriate fluid and electrolyte administered intravenously.

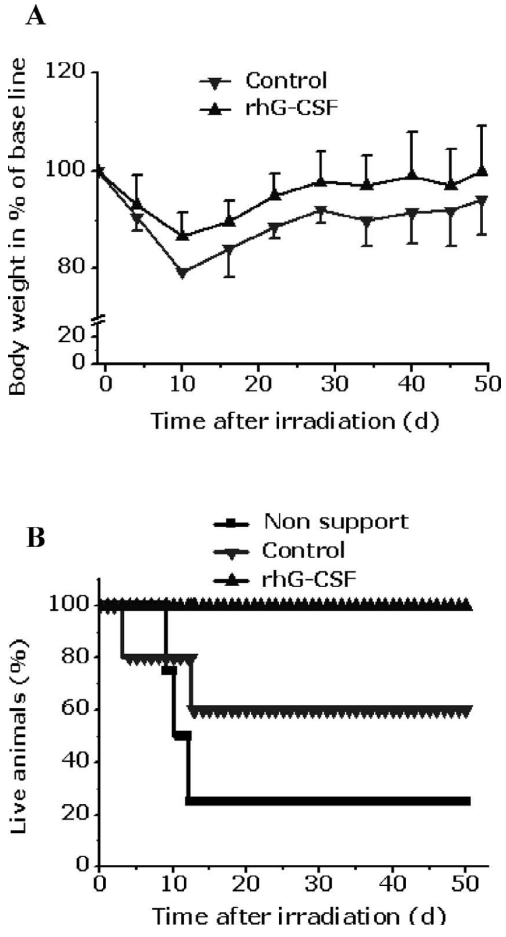

Fig. 1. Effect of rhG-CSF treatment on survival and body weight in 2.3-Gy fission neutron irradiated canines. Body weight (A) and percent survival (B) of fission neutron irradiated canines administered with non-supportive care (■, n = 4), supportive care alone (control group, ▼, n = 5) or supportive care plus rhG-CSF (rhG-CSF group, ▲, n = 5). Data represent mean values with standard deviations.

Hematological examination

A full set of blood cell counts was obtained for the blood samples collected during the study using a Sysmex XE-2100 Hematology Analyzer (Toa Medical Electronics Co., Kobe, Japan). Blood samples (0.5 ml) for determination of blood counts were taken prior to fission neutron irradiation and every other day for 50 days after irradiation in all animals. Assessment of hematologic evaluations has been previously described.[21)]

Histopathological observation

The dogs were anesthetized with sodium pentobarbital and killed humanely, and samples of the sternum and spleen were taken on day 50 after irradiation. Small and large intestine specimens were collected from dogs on the day of their death or day 50 after irradiation. Tissue specimens were fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with Hematoxylin-Eosin (H&E).

Statistical analysis

Results are expressed as mean ± standard deviation. The Kaplan-Meier Test was used to make pairwise comparisons of the onset of leukopenia and the durations of leukopenia and neutropenia and evaluate the statistical significance for survival rate. The unpaired Student's t-test was used to test

Table 2. Effect of different treatments on survival of dogs exposed to 2.3-Gy fission neutron irradiation

Group	Dog[#]	Survival (d)	Cause of death
Non support	4	50	Euthanized*
	5	12	Pneumorrhagia
	13	11	Pneumorrhagia
	19	9	Pneumorrhagia
Control	3	50	Euthanized*
	9	13	Pneumorrhagia
	15	50	Euthanized*
	16	50	Euthanized*
	17	4	Pneumorrhagia
RhG-CSF[#]	10	50	Euthanized*
	11	50	Euthanized*
	12	50	Euthanized*
	14	50	Euthanized*
	18	50	Euthanized*

* Euthanatized dogs were killed with sodium pentobarbital at the end of study. [#] Statistically different from Non-support controls ($P < 0.05$).

the significance between the 2 groups regarding other parameters. The tests were carried out using the software package SAS (SAS Institute, Cary, NC, USA).

RESULTS

The biological characteristics of 2.3 Gy neutron-irradiated animals

After 2.3 Gy neutron irradiation, all of the irradiated dogs showed early signs of gastrointestinal damage including reduced food intake, diarrhoea, and even bloody diarrhoea (Table S1 on web page). Consistent with previous study,[5] the incidence of GI syndrome significantly increased in neutron-irradiated animals than that of 4.5 Gy ^{60}Co γ-irradiated animals (Table S1 on web page). Due to severe GI syndrome, the animals displayed a short term of weight loss within 10 days after irradiation (Fig. 1A). It was also observed that, to some extent, rhG-CSF could attenuate weight loss and improve recovery of irradiated animals (Fig. 1A). In this study, one dog in the control group died at 4 days and the majority of deaths occurred from 9 to 13 days after exposure due to multiple haemorrhages, especially pneumorrhagia (Table 2). The mean survival time of died animal is earlier than that of 4.5 Gy ^{60}Co γ-irradiated animals (Table S2 on web page).

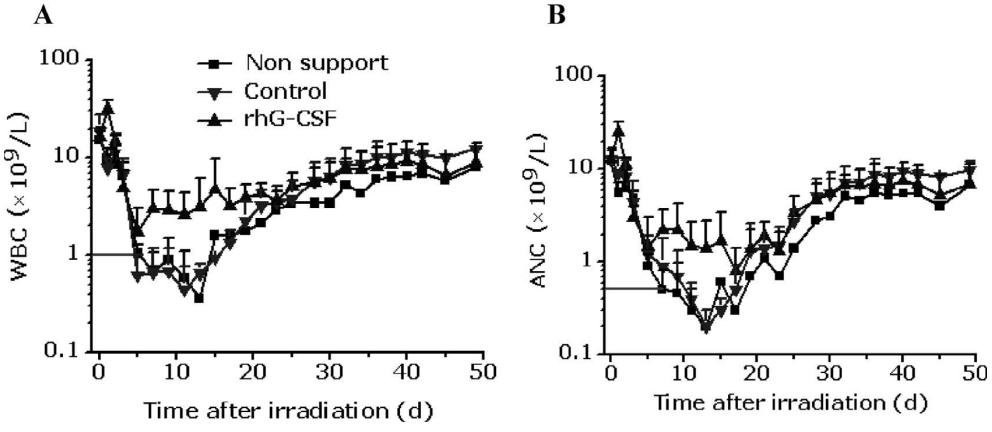

Fig. 2. Effect of rhG-CSF treatment on peripheral blood (A) white blood cells (WBC) and (B) neutrophils (ANC) count in the neutron-irradiated canines administered with supportive care plus rhG-CSF (rhG-CSF group, ▲, n = 5), supportive care alone (control group, ▼, n = 3–5), and non-supportive care (■, n = 1–4). Data represent mean values with standard deviations.

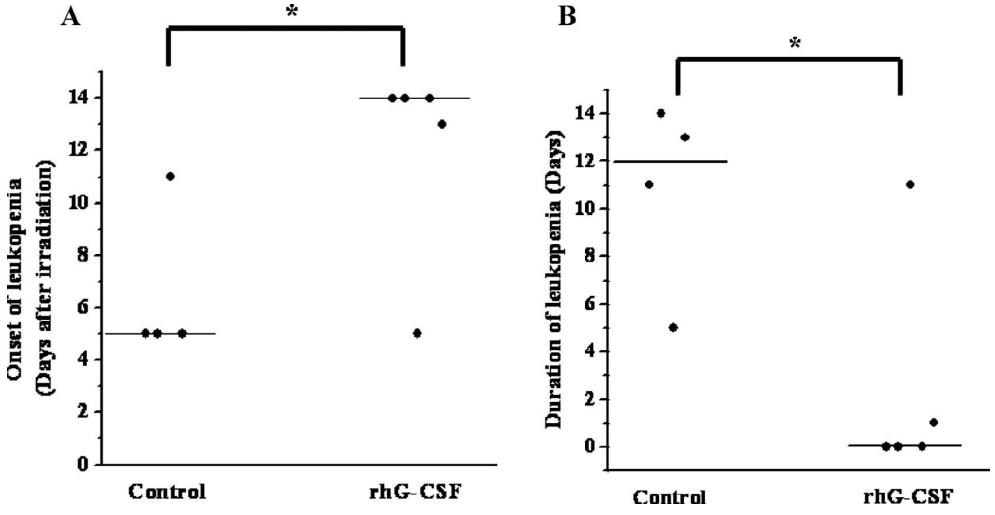

Fig. 3. Effect of rhG-CSF treatment on the onset of leukopenia (white blood cells [WBC] count < 1.0×10^9/L) (A) and duration of leukopenia (B) in the neutron-irradiated canines administered with supportive care plus rhG-CSF (rhG-CSF group, n = 5) and supportive care alone (control group, n = 4). Data represent mean values with standard deviations. * Statistically different from placebo-treated controls ($P < 0.05$).

RhG-CSF treatment enhances survival of neutron-irradiated canines

Previous study demonstrated that clinical support, including use of antibiotics, fresh irradiated platelet or whole blood transfusions, and fluids, significantly enhanced survival of canines exposed to lethal doses of mixed fission-neutron:gamma radiation.[4)] In this study, after receiving 2.3 Gy neutron-irradiation and clinical support, three of five dogs (60%) survived relative to one of four canines (25%) with non-supportive care (Table 1, Fig. 1B). Notably, rhG-CSF treatment increased the 50-day survival rate from 60 (3/5) to 100% (5/5) in the neutron-irradiated canines (Fig. 1B), suggesting that survival could be enhanced by rhG-CSF administration following neutron-irradiation.

RhG-CSF treatment improves neutrophil recovery in neutron-irradiated canines

Administration of rhG-CSF significantly reduced the severity and duration of leucopenia (Fig. 2A). All the surviving control dogs showed an evident phase of leukopenia or neutropenia (Fig. 2A, B). However, three of 5 treated dogs

Table 3. Effect of different treatments on clinical signs of dogs exposed to 2.3-Gy fission neutron irradiation. RhG-CSF treatment significantly reduced the duration of fever and days on antibiotics relative to control-treated animals

Parameters	Non Support (n = 4)	Control (n = 5)	rhG-CSF (n = 5)
Onset of fever (d)	7.3 ± 2.3	12.0 ± 5.5	16.7 ± 2.1
Duration of fever (d)	6.0	9.0 ± 2.6	3.2 ± 2.9*
The highest rectal temperature (°C)	40.9 ± 0.5	40.9 ± 0.3	40.2 ± 0.8
Antibiotics Required (d)[#]	–	15.7 ± 6.7	7.8 ± 2.8*

Canines were exposed to 2.3 Gy with neutron-irradiation and treated with rhG-CSF plus supportive care (rhG-CSF group) or placebo solution with supportive care (control group) or non-supportive care (non support group). Data represent mean values with standard deviations.

* Statistically different from placebo-treated controls ($P < .05$).

[#] Antibiotics used here only include cephalosporins. Other antibiotic regimen (penicillin) was initiated prophylactically on day three post irradiation and continued daily until the white blood cell count rose above that value for three consecutive days.

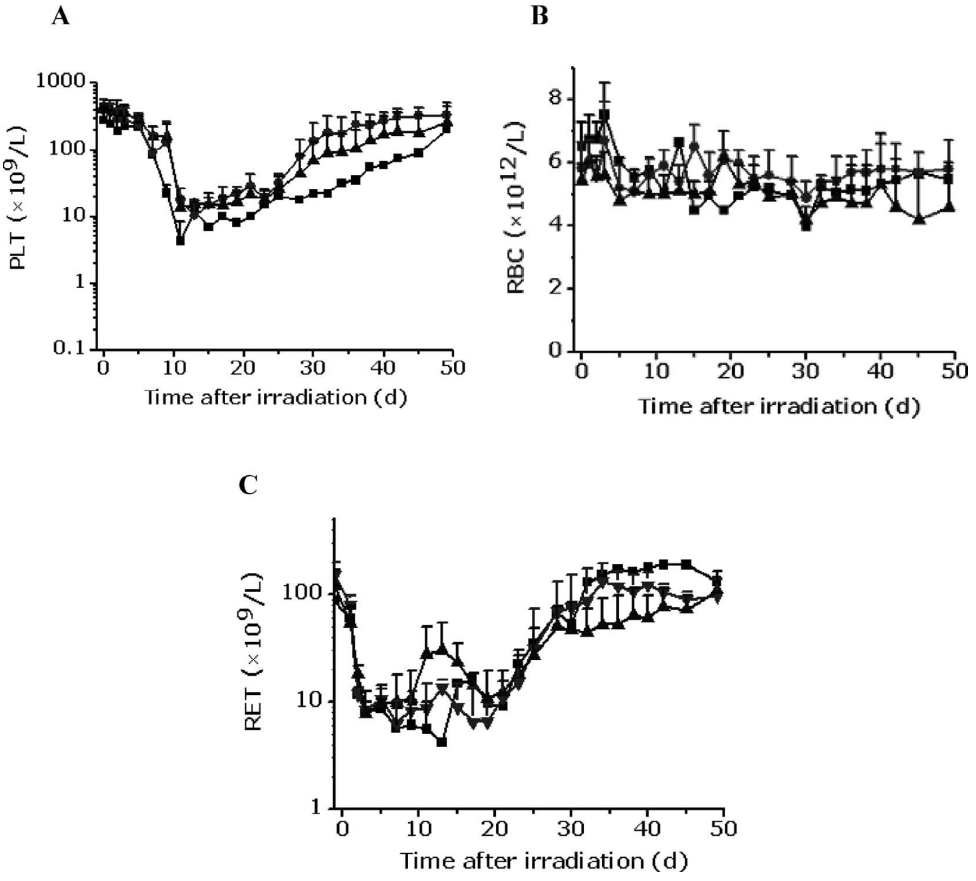

Fig. 4. Effect of rhG-CSF treatment on peripheral blood (A) platelets (PLT), (B) red blood cells (RBC), and (C) reticulocyte (RET) count in the neutron-irradiated canines administered with supportive care plus rhG-CSF (rhG-CSF group, ▲, n = 5), supportive care alone (control group, ▼, n = 3–5), and non-supportive care (■, n = 1–4). Data represent mean values with standard deviations.

exhibited no period of leukopenia or neutropenia (Fig. 2A, B). Furthermore, rhG-CSF treatment significantly delayed the onset of leukopenia (Fig. 3A) and reduced the duration of leucopenia (Fig. 3B) as compared with controls ($P < 0.05$). A shorter period of neutropenia with an average time to baseline neutrophil recovery was more pronounced in dogs treated with rhG-CSF versus control, but the difference did not reach statistical significance (data not shown). Con-

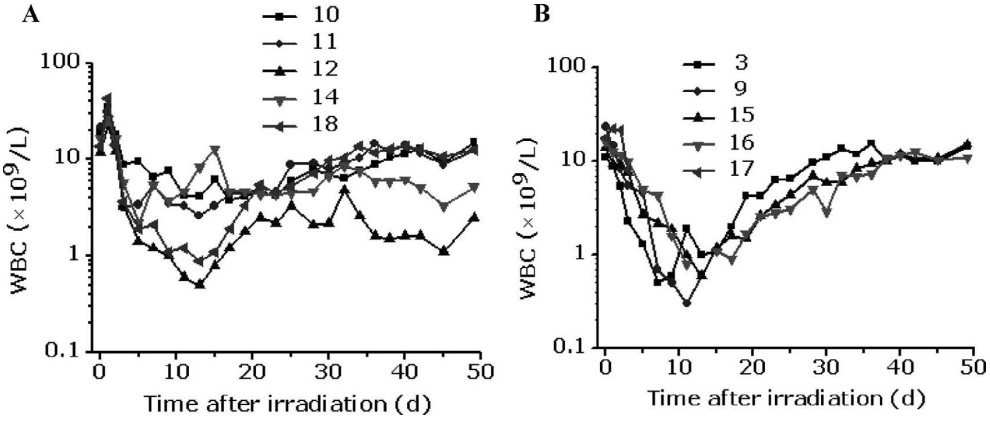

Fig. 5. Effect of rhG-CSF treatment on white blood cells (WBC) count in each individual animal after 2.3-Gy fission neutron irradiation. Each individual animal was administered with supportive care alone (A) or supportive care plus rhG-CSF (B) as described in Methods.

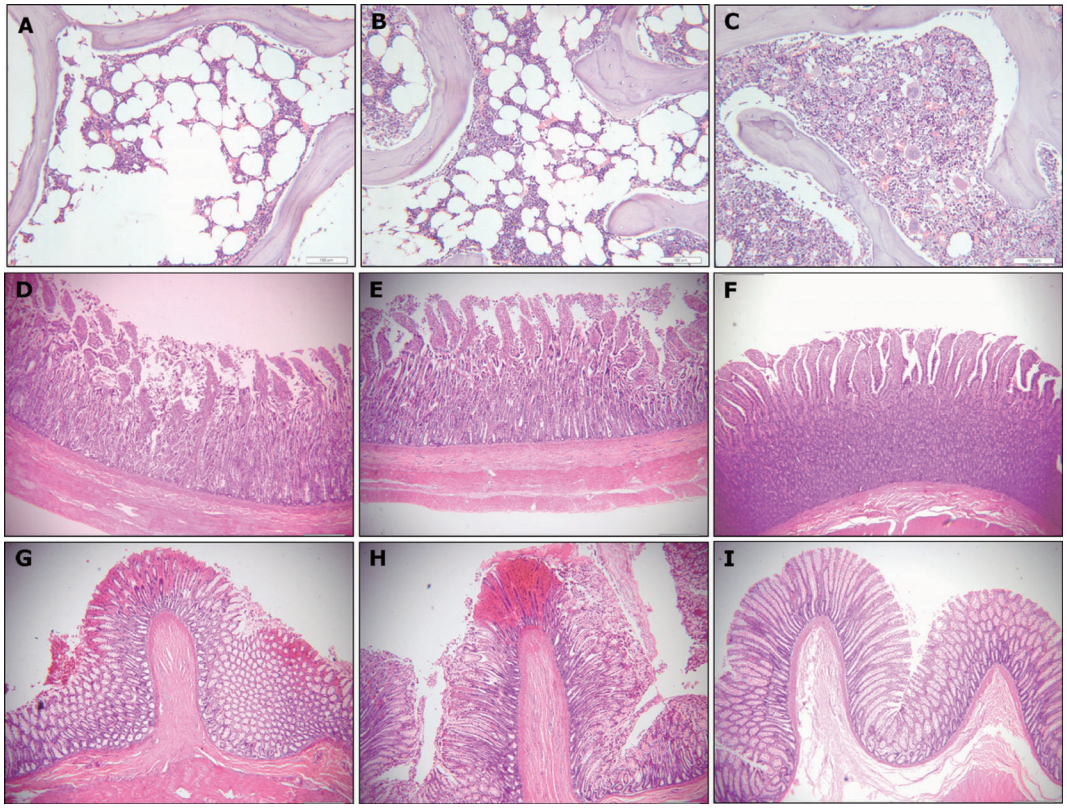

Fig. 6. (A–C) Bone marrow of the surviving neutron-irradiated dogs administered with non-supportive care (A), supportive care alone (B) or supportive care plus rhG-CSF (C). (D–I) Small intestinal mucosa (D–F) and colon (G–I) of the neutron-irradiated dogs with non-supportive care (dog No. 5, D, G), supportive care alone (dog No. 9, E, H) or supportive care plus rhG-CSF (dog No. 11, F, I). Images were examined under a Nikon 55i microscope (Nikon, Tokyo, Japan) and acquired through a SPOT RT camera and SPOT software (Diagnostic Instruments, Sterling Heights, MI). Images were taken at 200× (A–C) and 40× (D–I) magnification.

sequently, rhG-CSF treatment significantly reduced the fever duration and days on antibiotics relative to control-treated animals ($P < 0.05$) (Table 3).

After 2.3 Gy neutron irradiation, neither the duration of thrombocytopenia nor the recovery of platelet counts in the rhG-CSF-treated dogs differed from those in control-treated dogs (Fig. 4A). There was no dramatic fluctuation on red blood cells count in the different groups of animals (Fig. 4B). However, as early predictor of hematopoietic recovery,[22] measurement of peripheral blood reticulocytes indicated that rhG-CSF treatment effectively stimulated reticulocyte counts from 11 to 17 days after exposure and showed a biphasic response (Fig. 4C).

The individual differences in rhG-CSF responsiveness of the neutron-irradiated dogs

In the rhG-CSF-treated group, three of the five dogs (10#, 11# and 14#) exhibited no period of leukopenia or neutropenia and showed sustained increases in neutrophil count above 500/μL (Fig. 5A). However, the other two dogs (12# and 18#) showed significant decreased levels of neutrophil nadirs and experienced a profound period of neutropenia (Fig. 5A). These results indicate evident individual differences in rhG-CSF responsiveness of the neutron-irradiated dogs. In contrast, three survival control dogs exhibited a period of neutropenia with the ANC nadir at the same level, and showed similar endogenous hematopoietic recovery (Fig. 5B).

Histopathological observation

Further analysis with histopathological evaluation of bone marrow showed significant decrease of hematopoietic cells with marked lipid formation in surviving control dogs with or without supportive care (Fig. 6A–B). However, in the rhG-CSF-treated group, two dogs almost recovered to normal levels (Fig. 6C) while other dogs showed mild to serious damage of the structure and cellularity (Table 4). As shown in Table 4, the incidence and severity of thymus, spleen as well as bone marrow damage was reduced in surviving rhG-CSF-treated dogs as compared with surviving controls.

Table 4. Histopathology evaluation of dogs surviving to the end of the study (50 days after 2.3 Gy neutron-irradiation)

Group	Dog#	Spleen atrophy	Lymph gland atrophy	Bone Marrow Damage
Non support	4	–	+++	+++
Control	3	–	+++	+++
	15	+++	–	++
	16	–	–	+++
RhG-CSF	11	–	–	–
	18	–	–	–
	10	–	–	+
	12	–	–	++
	14	–	+++	+++

The incidence and severity of thymus, spleen and bone marrow damage was indicated as follows: –, none; +, minor; ++, moderate; +++, severe.

The histological changes in the small intestine were characterized by sticking of submucosa layers, sloughing crypts, and ulcers and ruptured villi in died control dogs with or without supportive care (Fig. 6D–E). Most of these dogs also showed mulifocality haemorrhage in the mucous membrane of colon (Fig. 6G–H). However, animals receiving rhG-CSF had the most favorable histologic findings with normal mucosal thickness, minimal inflammatory changes, and preserved tissue architecture (Fig. 6F, I). Neither in the small intestine nor in the large intestine histopathological observation showed differences among the surviving animals. They all showed normal architecture, irrespective of treatments.

DISCUSSION

Our results demonstrate the therapeutic efficacy of rhG-CSF in a canine preclinical model of severe myelosuppression induced by neutron-irradiation. The therapeutic administration of rhG-CSF reduced the severity and duration of neutropenia and decreased the period of neutropenia after neutron-radiation exposure. Administration of rhG-CSF also dramatically increased survival, from 60 per cent to 100 per cent, for the animals exposed to 2.3 Gy of neutron-irradiation. To our knowledge, this is the first instance where rhG-CSF alone favorably affected survival in neutron-irradiated dogs.

The exposure of canines under our irradiation conditions were adopted with n/γ ratios at the center of the empty animal holder of 10.6/1 (about 90% neutrons). Previous study had found that the incidence of the early signs of GI syndrome increased after the mixed neutron-γ irradiation with a high ratio of neutrons.[5] Consistent with these data, our results indicated that the severity of injury in dogs after neutron-irradiation with evident gastrointestinal syndrome in the early stage. All of the irradiated dogs showed obvious signs of gastrointestinal damage including reduced food intake, diarrhoea, and even bloody diarrhoea. Consistently, pathologic findings in the small and large intestine indicated marked lesion in the died control dogs. However, those surviving dogs all showed normal architecture, irrespective of treatments.

In this study, we observed that rhG-CSF administration significantly delayed the onset of leukopenia and reduced the duration of leucopenia. Consistent with this, the neutrophil-related parameters including the duration of neu-

tropenia, recovery time of ANC to ≥ 500/μL, and neutrophil nadir were all improved. However, with regard to these parameters, there was no statistical significant difference between the rhG-CSF-treated group and control group. It may be due to the rhG-CSF administration failed to prevent the development of neutropenia and elevate the nadir of neutrophil in 2 dogs of the five receiving rhG-CSF. These data suggest individual differences in cytokine responsiveness in the neutron-irradiated animals. In contrast, such individuality did not appear in 4.5 Gy γ-irradiated dogs (Fig. S1 on web page), in which the radiation-induced effects were comparable to 2.3 Gy neutron-irradiation (Table S2 on web page). The actual reason for the "non-responder" dogs occurred after neutron-irradiation remains unknown. It is likely that the unilateral exposure of the neutron irradiation conditions, in contrast to the TBI by ^{60}Co γ rays, are non-uniform to varying degrees, which makes it difficult to determine the absorbed dose to the critical organ (the bone marrow). Perhaps some animals (non-responder) incured relative high dose of radiation due to this, and preserved minimal target hematopoietic stem and progenitor cell population responding to the growth factors i.e. rhG-CSF.

RhG-CSF has been used extensively in clinical settings for accelerating hematopoietic recovery following chemotherapy or bone marrow transplantation, and decrease the period of neutropenia in the limited number of radiation accident victims studied.[14] Speculated from its extensive uses and our results, the mechanism of rhG-CSF-mediated protection of animals from exposure to neutron-irradiation may be related to the following factors. One of the possible mechanisms is related to the enhanced effect of rhG-CSF on neutrophil recovery. It had previously been shown that survival in the lethal irradiated canine model may depend on recovery of neutrophil counts because the most frequent cause of death is sepsis and pneumonia.[17] Thus, in this study there is a clear benefit from the promotion of neutrophil recovery by rhG-CSF treatment in terms of prevention of lethal infections. A second possibility is that rhG-CSF may exert protective effect on the GI syndrome after neutron-irradiation. RhG-CSF has previously been shown to be efficacious in mice with DSS colitis[23] and rats with necrotizing enterocolitis.[24] Furthermore, pretreatment of G-CSF ameliorated radiation-induced morphological destruction of intestinal mucosa in rats.[25] In this study, it is of interest to note that, to some extent, rhG-CSF could attenuate weight loss and improve recovery of irradiated animals, suggesting a possible alleviating action on the GI syndrome. One of the probable mechanisms in the protective effect of G-CSF is its inhibitory effect on the inflammatory response, which is triggered by irradiation via the participation of the bacterial flora.[25] Indeed, rhG-CSF was shown to downregulate proinflammatory cytokines such as IL-12, IL-6, and IFN-γ in the prevention of TNBS-induced colitis.[26] Third, it is probable that rhG-CSF administration regulates the blood coagulation system and prevents hemorrhage, which may contribute to the survival of irradiated animals. In the present study, it was showed that after neutron-irradiation most of the control dogs died of various hemorrhagic occurrences, suggesting that hemorrhage may be a key event that is fatal to the neutron-irradiated dogs. Our data indicated that rhG-CSF treatment had no effect on the platelet recovery in dogs exposed to neutron-irradiation, although some reports mentioned a positive effect of G-CSF on thrombocyte production.[18] In the same subjects, we also observed that the blood coagulation system of irradiated dogs was in hypercoagulabale state in the early stage after neutron-irradiation, and became hypocoagulable at crisis in the later stage.[27] Importantly, rhG-CSF administration could reverse the abnormal changes of the coagulation system and improve coagulation disorders of these irradiated dogs.[27] Thus, all these results would favor rhG-CSF protective effects on the blood coagulation system as the principal mechanism protection in these animals. Consistently, several studies elsewhere provided evidence that rhG-CSF modulated coagulation parameters and had been associated with the induction of a hypercoagulable state in patients as well as peripheral blood stem donors.[28–30] The mechanism of regulation of coagulation system by rhG-CSF appears to be related to an increase in von Willebrand factor,[29] in thrombin–antithrombin complex,[28] in platelet aggregation,[31] and in platelet activation with enhanced expression of tissue factor (TF) after G-CSF treatment.[32]

In summary, this study demonstrated that G-CSF can effectively improve the survival of neutron-irradiated dogs and promote the recovery of hematopoiesis as evidenced by elevated neutrophil nadirs and decreased durations of neutropenia. Our results may help to better understand the pathophysiology of neutron-irradiation syndrome and to define the efficacy of therapeutic interventions such as the use of HGF in accident victims who received acute neutron radiation.

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