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Granulocyte colony-stimulating factor administered as a single intraperitoneal injection modifies the lethal dose$_{95/30}$ in irradiated $B_6D_2F_1$ mice

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Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic growth factor that stimulates the proliferation of progenitor myeloid cells. We have previously demonstrated that recombinant human G-CSF (rhG-CSF) significantly improves survival of lethally irradiated $B_6D_2F_1$ mice when administered as a single intraperitoneal dose of 1 mg/kg 2 hours after a lethal dose (LD)$_{95/30}$ irradiation. In our model, rhG-CSF is also able to modify the LD$_{95/30}$ in irradiated animals and 1.1 has been found to be the dose modification factor (the ratio of LD$_{95/30}$ for mice treated with rhG-CSF to that for control animals).

Granulocyte colony stimulating factor (G-CSF) is a hematopoietic growth factor that stimulates the *in vitro* proliferation of progenitor cells committed to the myeloid lineage.[1] In animal models, G-CSF is able to stimulate granulocyte recovery and to promote survival after lethal irradiation when administered as daily injections,[2,3] indicating a possible influence on more primitive progenitors. In these cases, G-CSF modifies both the lethal dose$_{95/30}$ and $_{50/30}$ (LD$_{95/30}$ and $_{50/30}$) providing evidence that G-CSF protects animals from the lethal effects of irradiation.[4-6] We have previously demonstrated that recombinant human G-CSF (rhG-CSF) administered as a single intraperitoneal dose of 1 mg/kg 2 hours after a LD$_{95/30}$ irradiation significantly improves survival of lethally irradiated $B_6D_2F_1$ mice (78% vs 7%, p<0.001).[7] Herein, we want to report the effect of rhG-CSF on survival after different doses of total body irradiation (TBI) and the LD$_{95/30}$ variation in our model.

Eight week $B_6D_2F_1$ female mice were maintained in a sterile unit with filtered air on hardwood chip contact bedding (Panlab, SL) from irradiation to day +30 and provided with commercial sterile rodent chow and sterile water supplemented with neomycin sulfate (Gibco Lab, 40 mg/L) and cotrimoxazol (Soltrim®, Almirall Lab, 1.6 g/L). A ^{60}Co source (*Alcyon II, Compagnie General de Radiologie, General Electric*) was used to deliver total-body ^{60}Co gamma irradiation (1.25 MeV). Mice were initially irradiated up to a total dose of 1000 cGys at a dose rate of 50 cGys/min, previously established as the LD$_{95/30}$.[8] Irradiation was progressively increased to a total dose of 1100 cGy at the same dose rate in order to find the LD$_{95/30}$ for rhG-CSF-treated animals and subsequently decreased to 925 cGy. rhG-CSF (provided by Amgen, Thousand Oaks, CA, USA) was administered as a single dose of 1 mg/kg (20 µg) and diluted in saline to a final volume of 250 µL, 2 hours after the irradiation. Control mice were injected with 250 mL of physiological saline. A minimum of 30 animals from both groups was used to analyze overall survival for each one of the total doses analyzed. Surviving animals were recorded daily for 30 days. Differences in survival of irradiated rhG-CSF-treated and controls were determined using the Mantel-Peto-Cox test.

Results are shown in Figures 1 and 2. Survival post-TBI significantly increases in the control group when reducing the total dose (40% at 925 cGy vs 7% at 1000 cGy, p<0.001) (Figure 1). Nevertheless, differences in survival between both groups of animals are still significant at the 925 cGys point (40% vs 95%, p<0.005).

In the rhG-CSF group, there is a progressive decrease in survival after TBI when total dose pro-

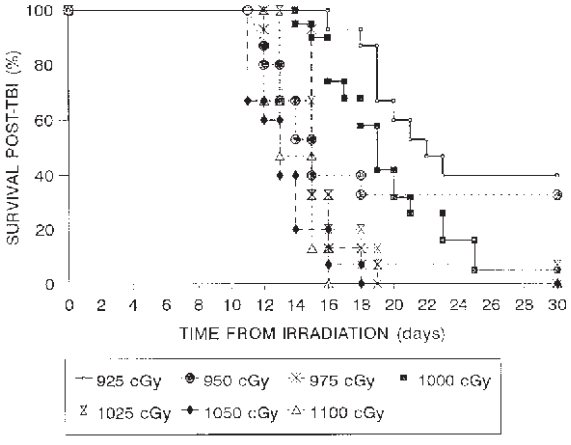

Figure 1. Survival of control irradiated mice receiving a total dose of 925 cGy, 950 cGy, 975 cGy, 1000 cGy, 1025 cGy, 1050 cGy and 1100 cGy on day 0. Control mice received 250 µL of physiological saline 2 hours after the irradiation procedure.

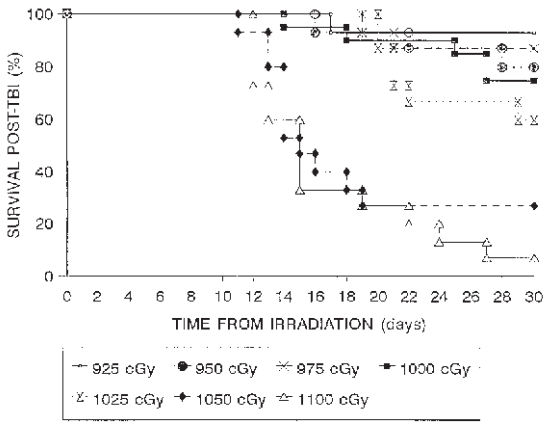

Figure 2. Effect of a single intraperitoneal injection of rhG-CSF (1 mg/kg, 2 hours after irradiation) on survival of irradiated mice with a total dose of 925 cGy, 950 cGy, 975 cGy, 1000 cGy, 1025 cGy, 1050 cGy and 1100 cGy.

gressively increases up to 1100 cGys (Figure 2); there are significant differences between survivals of rhG-CSF-treated and control animals at total doses of 1025 (60% vs 7%, p<0.001) and 1050 cGy (27% vs 0%, p<0.025). However, no significant differences can be observed at 1100 cGy (5% vs 0%, NS), as has been previously reported in a murine model with daily injections of G-CSF.[5] A dose of 1100 cGy can thus be considered the $LD_{95/30}$ in our model. Consequently, 1.1 has been found to be the dose modification factor (the ratio of $LD_{95/30}$ for mice treated with rhG-CSF to that for control animals).

In our model, rhG-CSF administered as a single intraperitoneal dose is also able to modify the $LD_{95/30}$ in irradiated animals, as demonstrated by others when rhG-CSF is administered in daily doses.[4-6] Nevertheless, rhG-CSF was not effective in enhancing survival when total dose was higher than 1050 cGys, suggesting that the radioprotective effect of G-CSF requires a certain number of residual surviving stem cells.

Key words

Granulocyte colony-stimulating factor, total body irradiation, hematopoietic injury

Funding

This work was partially supported by grant number 93/0381 from the FIS.

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