Cytokines in combination to treat radiation-induced myelosuppresssion: evaluation of SCF + glycosylated EPO + pegylated G-CSF as an emergency treatment in highly irradiated monkeys

Multicytokine therapy may be useful to counteract radiation-induced myelosuppression. We assessed the stem cell factor + glycosylated erythropoietin + pegylated granulocyte colony-stimulating factor combination (SEG) as an emergency treatment. SEG in highly irradiated monkeys efficacy appeared to be restricted to granulopoiesis. Early administration of Erythropoietin did not prevent radiation-induced anemia.

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Counteracting the hematopoietic syndrome following accidental or intentional irradiation remains the first therapeutic challenge for physicians. Granulopoietic factors have been used for some years in victims irradiated at intermediate dose levels. This appears pertinent in the event of non-uniform exposure. Therefore, a recent consensus recommends the administration of a single granulopoietic cytokine (G-CSF/pegylatedG-CSF).[1] The experience of our group in animal models led us to propose an emergency antiapoptotic cytokine therapy which consists in the preservation and then stimulation of residual hematopoietic stem and progenitor cells (HSPC) following irradiation. Based on studies of our group and others,[2-6] we evaluated the stem cell factor + Flt-3 ligand + thrombopoietin + interleukin-3 (SFT3) combination. This was capable of abrogating thrombocytopenia when given as an early single administration in 7 Gy irradiated monkeys 2 hrs after total body irradiation (TBI). Recent data have reported the use of SEG combination made of cytokines available in the clinic, i.e. SCF + erythropoietin + PegG.[7] The aim of this study was to test SEG in our monkey model. We also evaluated the benefit of adding erythropoietin to the SFT3 reference combination.

Adult cynomolgus macaques (5.2±0.7 kg, n=5) were housed at the Centre de Recherches du Service de Santé des Armées in accordance with the European guidelines on animal care. Cohorts were restricted due to ethical constraints. Anesthetized monkeys were placed in a restraining chair and were exposed to a unilateral front TBI with a ^{60}Co gamma source at a total midline tissue dose (measured in air at the anterior iliac crest level) of 7 Gy (dose rate of 20 cGy/min.).

Recombinant human (rh) SCF, Flt3-ligand (FL), Tpo, interleukin-3 (IL-3, R&D systems, Abingdon, UK), glycosylated erythropoietin (Epo) and pegfilgrastim (PegG) (courtesy of Amgen, France) were used. After irradiation, the monkeys were injected with SEG (n=3) or SFT3 + Epo (n=2). SCF, FL, Tpo and IL-3 (50 µg/kg of each cytokine) were administered intravenously 2 hrs. after irradiation as survival factors. PegG (300 µg/kg subcutaneously) and glycosylated Epo (2.25 µg/kg subcutaneously in SEG, 4.5 µg/kg in SFT3 + Epo) were administered at 2 hrs. and 8 days, to provide a prolonged effect based on the biological half-life of pegylated/glycosylated cytokines. Supportive care is mandatory for this high dose radiation model. The animals were transfused with fresh, irradiated whole blood and an antibiotic regimen was provided as needed. Durations of neutropenia, thrombocytopenia, lymphopenia and anemia were defined respectively as the times when absolute neutrophil count (ANC), platelet (PLT) count, lymphocyte (Ly) count and hemoglobin (Hb) were lower than 0.5, 50, 1×10^9/L and 10 g/dL. The non-parametric Mann-Whitney rank test was used to test the significance between SEG and historical untreated controls. In comparison with 7 Gy irradiated historic untreated controls,[8] SEG treated animals displayed a non-significant reduction in the period of neutropenia (10.7±3.8 days *vs.* 15.1±1.6 days for ANC $<0.5\times10^9$/L and 13.3±4.1 days *vs.* 17.9±2 days for ANC $<1\times10^9$/L) and thrombocytopenia (only for PLT$<20\times10^9$/L/L with 9±8.6 days *vs.* 19.3±25.1 days in controls). There was no benefit from SEG treatment regarding the time when PLT $<50\times10^9$/L. Although

Table 1. Effect of treatments on blood hematologic parameters.

Parameters	*SEG (n=3)*	*SFT3 + Epo (n=2)*	**SFT3 (n=4)*	**PegG (n=2)*	**Controls (n=8)*
Duration of neutropenia (ANC $<0.5\times10^9$/L)	10.7±3.8 (8-15)	11 (10-12)	13±3.2 (9-16)	10.5 (9-12)	15.1±1.6 (13-17)
Duration of neutropenia (ANC $<1\times10^9$/L)	13.3±4.1 (10-18)	17 (17-17)	18.8±1.7 (17-21)	13 (11-15)	17.9±2 (14-20)
Duration of lymphopenia (lymphocytes $<1\times10^9$/L)	38±3.4 (34-40)	33.5 (30-37)	30.5±9.5 (20-40)	29.5 (27-32)	42.6±13.9 (27-71)
Duration of thrombocytopenia (platelets $<20\times10^9$/L)	9±8.6 (4-19)	1 (0-2)	0.8±1.5 (0-3)	8 (4-12)	19.3±25.1 (5-80)
Duration of thrombocytopenia (platelets $<50\times10^9$/L)	24.3±8.8 (10-44)	10 (5-15)	6.1±3.6 (4-11)	34.5 (9-60)	22.6±25.6 (7-80)
Duration of anemia (hemoglobin < 10 g/dL)	37±18.3 (16-50)	28.5 (19-38)	22.3±8.8 (10-31)	30.5 28-33)	35.3±26.7 (13-80)
Blood transfusion dependence (in blood units)	2.7±1.5 (1-4 units)	1 (1-1 units)	0.5±1 (0-2 units)	2.5 (1-4 units)	3.1±2 (1-7 units)

*SFT3: SCF + Flt3-ligand + Tpo + IL-3 (50 µg/kg each) given intravenously 2 hrs. after TBI. Peg-filgrastim (PegG; 300 µg/kg under sc route) and glycosylated Epo (2.25 µg/kg under sc route in SEG and 4.5 µg/kg in SFT3+Epo) were administered at 2 hrs. and 8 days. Duration in days is given as mean ± standard deviation, and range. *SFT3, PegG and controls are historic animals.*

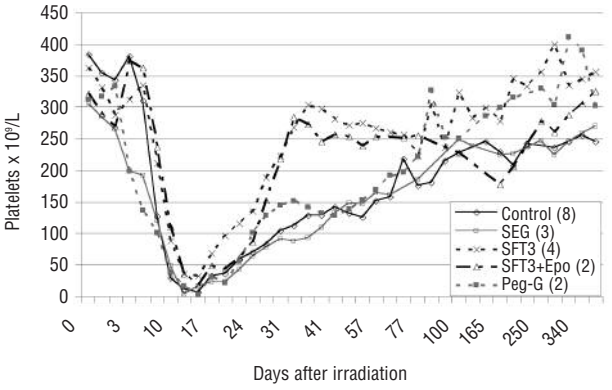

Figure 1. Effect of different cytokine treatments on platelet count in 7 Gy irradiated animals. SFT3 (50 µg/kg each) was given intravenously 2 hours after TBI. Peg-filgrastim (PegG; 300µg/kg under sc route) and glycosylated Epo (2.25 µg/kg under sc route in SEG and 4.5 µg/kg in SFT3 + Epo) were administered at 2 hours and 8 days. SFT3, PegG and untreated controls are historic animals. Data represent mean values.

animal cohorts were small, no difference in the former parameters was observed between historical PegG[8] and SEG groups. No reduction in blood transfusion needs was observed. At day 250 there was no difference in blood cell counts between groups. As previously reported for historical SFT3 treated monkeys,[9] SFT3+Epo treated animals had a reduced period of thrombocytopenia (1 day for PLT<20×10⁹/L and 10 days for PLT<50×10⁹/L) when compared with controls (19.3±25.1 days and 22.6±25.6 days respectively), PegG or SEG animals (Table 1 and Figure 1). SFT3 + Epo animals also showed a steeper slope of PLT recovery than other groups. However, they displayed a similar period of neutropenia to SEG and PegG groups (11, 10.7 and 10.5 days respectively for ANC <0.5×10⁹/L), a trend of reduction comparable to controls (15.1±1.6 days). Epo addition to SFT3 did not appear to reduce the duration of anemia (28.5 days *vs.* 22.3±8.8 days respectively) or blood cell transfusion needs (1 *vs.* 0.5 units).

SEG is made of granulopoietic G-CSF, Epo and SCF which has been reported to promote the survival of HSPCs and may act as a cofactor. Epo is a pleiotropic cytokine[10] which stimulates erythropoiesis (mainly CFU-e) and may protect various tissues from radiation toxicity. Interestingly, the recent clinical use of SEG in two accidentally irradiated victims (estimated dose 5 Gy; 1 pure hematologic syndrome and 1 associated with a cutaneous radiation syndrome; treatment initiated 26 and 34 days respectively after exposure) contributed to prompt recovery from BM aplasia. In a previous study, we tested the capacity of SEG to rescue lethally irradiated mice from death when administered 2 hrs. after irradiation.[11] We showed a beneficial effect in terms of survival but less than with SFT3. Here SEG activity appears restricted to granulopoiesis and our data suggest no improvement when compared with PegG. HSPC exhaustion observed in mice one year after irradiation was not confirmed.

Furthermore, the addition of Epo to SFT3 tested in 2 supplementary animals did not improve Tpo stimulation of erythropoieis. As far as a tissue protective effect is concerned, we did not observe any early endothelial progenitor cell mobilization or reduction of inflammatory parameters (*data not shown*). In conclusion, this study shows that SEG would be a poor alternative to SFT3±PegG[8, 9] as an emergency treatment. In our conditions, Epo did not improve erythropoietic recovery. The efficacy of SFT3±Epo on PLT recovery emphasizes the benefit of Tpo inclusion in cytokine combination. Thrombopoietic factors are indispensable in this context and Tpo mimetics should be evaluated in highly irradiated monkeys.[12]

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