Pergamon

European Journal of Cancer Vol. 31A, Suppl. 1, pp. S17–S21, 1995
Elsevier Science Ltd
Printed in Great Britain

0959-8049(95)00147-6

Amifostine Plus Granulocyte Colony-stimulating Factor Therapy Enhances Recovery from Supralethal Radiation Exposures: Preclinical Experience in Animal Models

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A murine model was used to explore whether the cytoprotective agent amifostine (WR-2721) can be used to protect a critical fraction of haemopoietic stem cells against radiation, and whether granulocyte colony-stimulating factor (G-CSF) can then be used to stimulate the protected cells to proliferate and reconstitute the haematopoietic system. Groups of C3H/HeN mice treated with 200 mg/kg amifostine i.p. 30 min before ^{60}Co irradiation and/or 125 μg/kg G-CSF subcutaneously from days 1–16 post irradiation were compared. The dose reduction factor (DRF) of the combination of amifostine and G-CSF from $LD_{50/30}$ values was greater than the sum of the DRFs for amifostine and G-CSF individually. Acceleration of recovery of bone marrow and splenic multipotent stem cells (CFU-s) and granulocyte-macrophage progenitor cells (GM-CFC), as well as of peripheral blood red and white cells and platelets, was greatest in mice treated with amifostine plus G-CSF. These studies suggest that amifostine and recombinant haematopoietic growth factors can be used in combination to reduce myelosuppression and lethality associated with radiation or radiomimetic drugs.

Key words: amifostine, cytoprotective agent, radiation, granulocyte colony-stimulating factors
Eur J Cancer, Vol. 31A, Suppl. 1, S17–S21, 1995

INTRODUCTION

AMIFOSTINE (WR-2721) has been shown by others to effectively protect against the haemopoietic toxicity associated with radio- and/or chemotherapy, and to allow the escalation of the survivable dose of these cytotoxic treatments [1–5]. In contrast, various biological response modifiers can be used to stimulate haemopoietic recovery once radiation injury has occurred [6]. In the last few years, a variety of haemopoietic cytokines have been identified and demonstrated to affect haematopoiesis at various specific pathways within the haemopoietic hierarchy. A number of these cytokines, both alone and in combination, have been shown not only to stimulate haemopoietic proliferation and differentiation *in vitro*, but also to stimulate haemopoietic regeneration when administered to myelosuppressed animals [7–12].

These findings led to exploration of the premise that safer, more effective radioprotection may be achieved by combining the radioprotective effects of amifostine with the ability of cytokines to stimulate regeneration of the protected stem and progenitor cells. Granulocyte colony-stimulating factor (G-CSF) is a haemopoietic cytokine that has been shown to enhance regeneration of neutrophils in animals with radiation-induced [7, 8] or drug-induced [13, 14] neutropenia. The studies reviewed below were conducted to investigate the ability of G-CSF to stimulate haemopoietic regeneration from stem and progenitor cells protected by amifostine.

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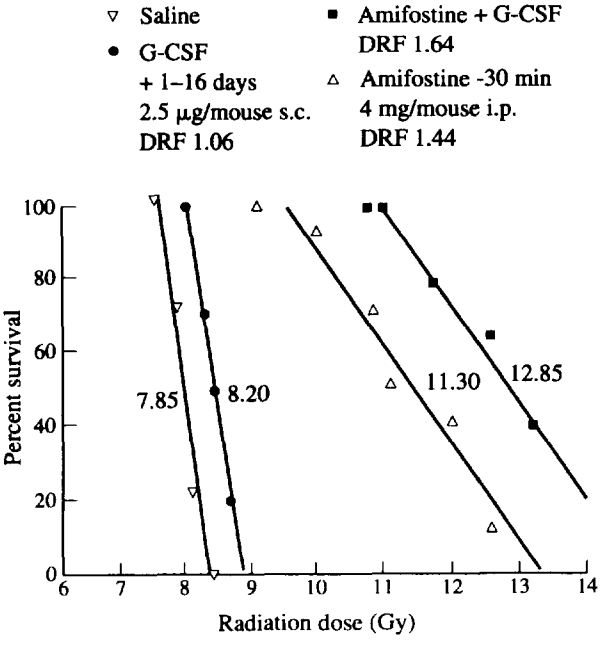

Figure 1. Effect of saline, G-CSF plus saline, saline plus amifostine, and G-CSF plus amifostine on survival of irradiated mice. C3H/HeN mice were administered amifostine (4 mg/mouse, i.p.) 30 min before ^{60}Co irradiation and G-CSF (2.5 μg/mouse/day, s.c.) on days 1–16 after irradiation. Each data point represents results obtained from 30 mice. Reprinted with permission from Patchen *et al. Int J Radiat Oncol Biol Phys* 1992, 22, 773–779 [15].

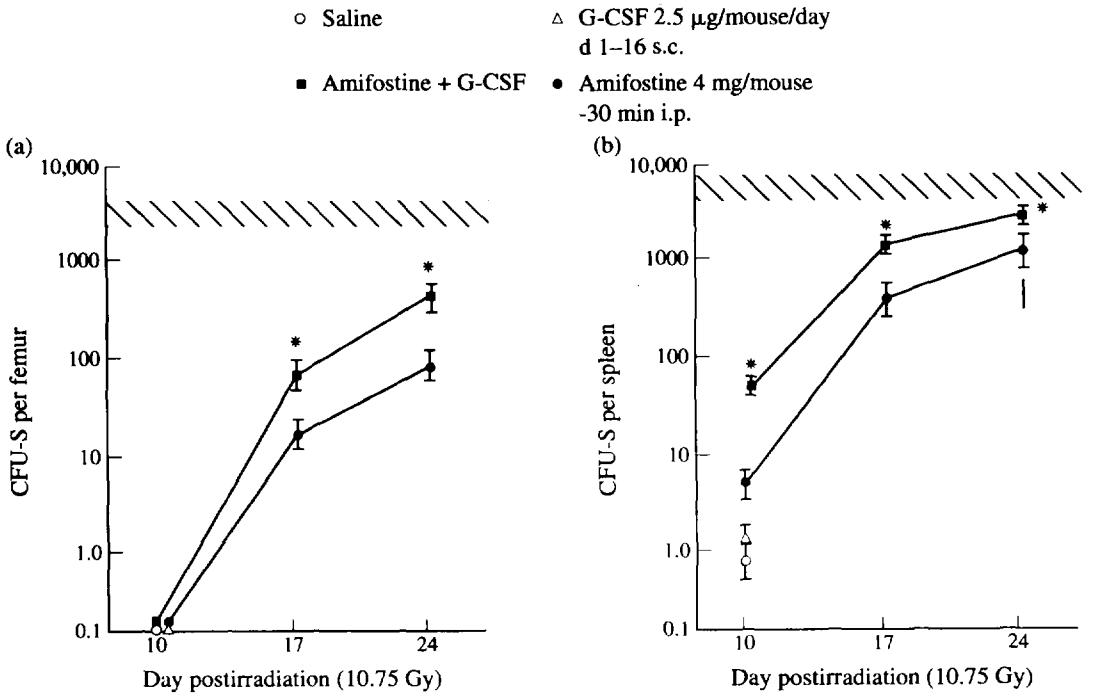

Figure 2. Effect of saline, G-CSF plus saline, saline plus amifostine, and G-CSF plus amifostine on CFU-s recovery in the bone marrow (a) and spleen (b) of mice exposed to 10.75 Gy. Asterisk indicates value that is significantly different from corresponding value for amifostine group ($P < 0.05$). Normal levels shown by hatched bar. Reprinted with permission from Patchen *et al. Int J Radiat Oncol Biol Phys* 1992, 22, 773–779 [15].

THE EFFECT OF PRE-IRRADIATION TREATMENT WITH AMIFOSTINE AND POSTIRRADIATION TREATMENT WITH GRANULOCYTE COLONY-STIMULATING FACTOR ON SURVIVAL IN MICE

Early studies were carried out to determine the effect of amifostine and G-CSF, individually and in combination, on survival in irradiated mice [15]. Group 1 received injections of saline (control); Group 2 received saline and G-CSF (amifostine control); Group 3 received amifostine and saline (G-CSF control); and Group 4 received amifostine and G-CSF. Amifostine was injected i.p. at a dose of 4 mg per mouse (~200 mg/kg) 30 min before irradiation. Concurrently, pre-irradiation amifostine control mice were similarly injected with saline. All mice then underwent bilateral whole-body ^{60}Co irradiation. On days 1–16 post irradiation, G-CSF was injected subcutaneously at a dose of 2.5 μg per mouse (~125 μg/kg) once daily. G-CSF control mice were similarly injected with saline on days 1–16.

Survival of irradiated mice given the four treatment regimens is presented in Figure 1. The $LD_{50/30}$ value (the radiation dose that killed 50% of the animals within 30 days after exposure) for the mice treated with saline alone was 7.85 ± 0.06 Gy. Administration of G-CSF alone significantly increased survival. The $LD_{50/30}$ for this group was 8.30 ± 0.08 Gy, giving a dose reduction factor (DRF) of 1.06. Treatment with amifostine was

*Table 1. Effect of G-CSF, amifostine alone and amifostine + G-CSF on splenic cellularity, CFU-s, and GM-CFC following a 10.75 Gy radiation exposure**

	Treatment			
	Saline	G-CSF	Amifostine	Amifostine + G-CSF
Cells per spleen ($\times 10^6$)				
Day 10	12.0 ± 0.2	13.0 ± 0.5	14.1 ± 0.6	15.2 ± 0.8
Day 17	– †	– †	69.9 ± 1.9	223.3 ± 23.2 ‡
Day 24	– †	– †	194.6 ± 16.3	153.4 ± 26.1
CFU-s per spleen				
Day 10	0.8 ± 0.4	1.3 ± 0.2	4.7 ± 1.2	54.8 ± 6.8 ‡
Day 17	– †	– †	488.0 ± 25.6	1382.0 ± 34.1 ‡
Day 24	– †	– †	2473.0 ± 490.0	4193.0 ± 526.7 ‡
GM-CFC per spleen				
Day 10	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Day 17	– †	– †	249.0 ± 35.5	13044.0 ± 1040.0 ‡
Day 24	– †	– †	13132.0 ± 1015.0	17776.0 ± 1208.0 ‡

* Mean ± standard error of values obtained from three experiments; † No mice survived to be assayed at this time point; ‡ $P < 0.05$, with respect to amifostine values (Student t-test). (In nonirradiated C3H/HeN mice, cellularity = 147 ± 10, CFU-s = 6907 ± 779, GM-CFC = 1650 ± 118).

even more effective at enhancing survival and altered the slope of the survival curve. For this group, the $LD_{50/30}$ was 11.30 ± 0.15 Gy, resulting in a DRF of 1.44.

THE EFFECT OF PRE-IRRADIATION TREATMENT WITH AMIFOSTINE AND POSTIRRADIATION TREATMENT WITH GRANULOCYTE COLONY-STIMULATING FACTOR ON HAEMOPOIETIC RECONSTITUTION IN MICE

Administration of amifostine and G-CSF yielded the greatest increase in survival, raising the $LD_{50/30}$ to 12.85 ± 0.24 Gy and resulting in a DRF of 1.64. The DRF of the combination of amifostine and G-CSF is greater than the sum of the DRFs for the two agents individually, indicating that the effects of amifostine and G-CSF on survival were more than additive when the agents were administered together.

The effects of amifostine, G-CSF, and amifostine plus G-CSF on the recovery of bone marrow and splenic multipotent haemopoietic stem cells (CFU-s) and granulocyte-macrophage progenitor cells (GM-CFC) and on the recovery of peripheral blood cellularity were assessed in mice following a 10.75 Gy dose

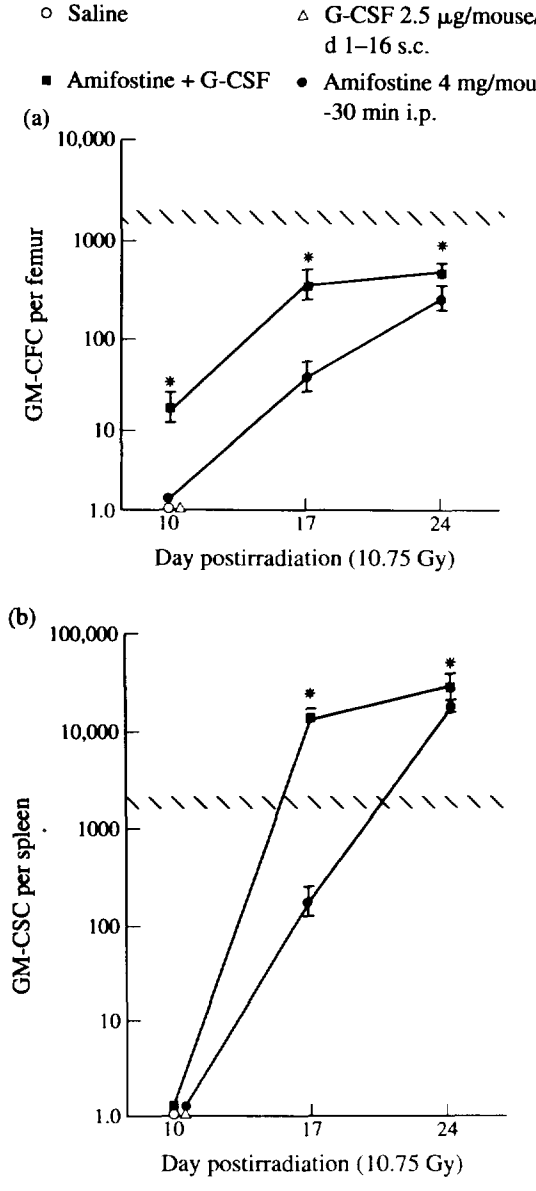

Figure 3. Effect of saline, G-CSF plus saline, saline plus amifostine, and G-CSF plus amifostine on GM-CFC recovery in the bone marrow (a) and spleen (b) of mice exposed to 10.75 Gy. Asterisk indicates value that is significantly different from corresponding value for amifostine group ($P < 0.05$). Normal levels shown by hatched bar. Reprinted with permission from Patchen *et al. Int J Radiat Oncol Biol Phys* 1992, 22, 773–779 [15].

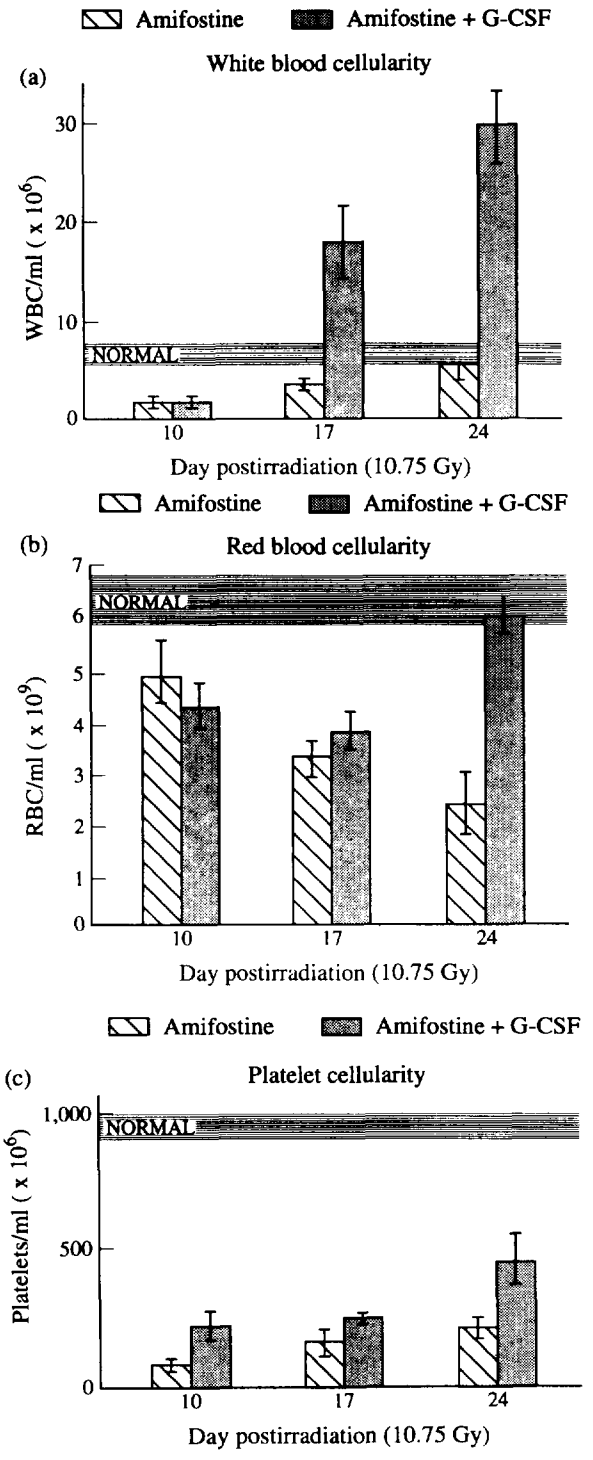

Figure 4. Effect of saline plus amifostine and G-CSF plus amifostine on recovery of peripheral WBCs (a), RBCs (b), and platelets (c) of mice exposed to 10.75 Gy. Data are presented as the mean ± standard error of values recorded during three replicate experiments. Reprinted with permission from Patchen *et al. Int J Radiat Oncol Biol Phys* 1992, 22, 773–779 [15].

of irradiation. Mice were treated with amifostine and/or G-CSF as described above. At 10, 17, and 24 days post irradiation, bone marrow and spleen were removed from irradiated animals and assayed for multipotent CFU-s and for committed GM-CFC progenitors according to previously described techniques [16, 17]. The cell suspensions used in the assays for CFU-s and GM-CFC employed tissues from three irradiated mice from each of the four treatment groups at each time point. Cells from non-irradiated mice were also assayed at each time point. Blood samples were taken by cardiac puncture, and white blood cell (WBC), red blood cell (RBC) and platelet counts were performed with a Coulter counter. This experiment was repeated three times.

Following the 10.75 Gy radiation exposure, all mice survived to be evaluated on day 10 post irradiation. However, only mice treated with amifostine and amifostine plus G-CSF were alive for evaluation on days 17 and 24. The effect of the four treatment regimens on bone marrow and splenic CFU-s recovery is shown in Figures 2a and b, respectively. Table 1 presents numerical values for spleen cellularity and CFU-s. On day 10, the numbers of bone marrow CFU-s were undetectable in all treatment groups. Thereafter, CFU-s recovery was observed in mice given amifostine. Recovery was greater still when G-CSF was combined with amifostine ($P < 0.05$, days 17 and 24). Recovery of CFU-s occurred more rapidly in the spleen, also a major haemopoietic organ in the mouse. As early as day 10, there was a major acceleration in the recovery of CFU-s in mice treated with amifostine and amifostine plus G-CSF. Again, recovery was significantly greater in mice treated with amifostine plus G-CSF than in mice treated with amifostine alone ($P < 0.05$, at each determination). By day 24, splenic CFU-s levels in combination-treated mice were close to the levels observed in the non-irradiated animals.

Figures 3a and b show the effect of the four treatment regimens on the recovery of committed GM-CFC progenitors in the bone marrow and spleen, respectively. Table 1 presents numerical values for spleen GM-CFC progenitors. An accelerated recovery of bone marrow GM-CFC progenitors was seen in the groups treated with amifostine and amifostine plus G-CSF. Again, a much greater response was achieved when amifostine was combined with G-CSF than when amifostine was administered alone; the differences between these two groups were significant at each determination ($P < 0.05$). It is interesting to note that the curve for mice treated with amifostine plus G-CSF appears to reach a plateau after day 17. This finding may be related to the fact that G-CSF treatment was discontinued on day 16 in this study. The most striking effects of the various treatments were observed on splenic GM-CFC recovery. As shown in Fig. 3b and Table 1, splenic GM-CFC of mice in both the amifostine and amifostine plus G-CSF groups exhibited a dramatic recovery. By day 17, GM-CFC numbers in mice treated with amifostine plus G-CSF exceeded numbers in normal animals. Again, the curve for mice treated with the amifostine plus G-CSF combination appeared to reach a plateau after day 17, perhaps reflecting the discontinuation of G-CSF therapy on day 16.

Following radiation exposure, the disappearance of mature peripheral blood elements is related to their lifespan. WBCs decline first, followed by a decline in platelets, and later by a decline in RBCs [18]. Based on lifespan kinetics, WBC and platelet numbers generally decline to a nadir within the first week post irradiation, while RBC numbers do not reach a nadir until several weeks post irradiation. The response of peripheral blood elements to treatment with amifostine or amifostine plus G-CSF is shown in Figure 4. Following treatment with amifostine alone, WBCs had returned to normal by day 24 post irradiation; however, when G-CSF was added to amifostine, WBC counts had far exceeded normal levels by day 17 post irradiation. Likewise, recovery of platelets was best for animals that had received the combination of amifostine plus G-CSF. By day 24, platelet counts in mice treated with amifostine plus G-CSF had recovered to half normal values compared with only a quarter of normal values in mice treated with amifostine alone. As was expected, RBC numbers decreased more slowly than WBC or PLT numbers post irradiation. While RBC numbers in amifostine-treated mice continued to decrease through day 24 post irradiation, the onset of RBC recovery in amifostine plus G-CSF-treated mice became apparent as early as day 17. By day 24, RBC numbers in these mice had returned to normal levels.

The results of these studies in irradiated mice indicate that amifostine and G-CSF act synergistically to increase survival and protect against the deleterious effects of radiation on haematopoiesis.

CONCLUSIONS

The studies reviewed here indicate that low doses of amifostine followed by G-CSF therapy result in synergistic enhancement of survival after radiation exposure. Treatment with amifostine leads to survival of a small fraction of haemopoietic stem and progenitor cells following irradiation. G-CSF therapy then appears to stimulate the surviving haemopoietic stem and progenitor cells to proliferate and to repopulate the animal. This combination treatment ultimately results in the accelerated recovery of the mature peripheral WBCs, RBCs, and platelets that are necessary for protection against the infection and haemorrhages that are typically the cause of death following radiation exposure. These studies suggest that the cytoprotective agent amifostine and recombinant haemopoietic growth factors can be used in combination to reduce myelosuppression and lethality associated with radiation and radiomimetic drugs.

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