Radioprotection of Hematopoietic Tissue by Fibroblast Growth Factors In Fractionated Radiation Experiments

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Acidic and basic fibroblast growth factors ($FGF_{1/2}$) myeloprotect mice in single dose total body irradiation (TBI) experiments with a dose modification factor (DMF) of ≈ 1.15. CFU-C assay suggests that one of the mechanisms is augmentation of the shoulder of the radiation dose response curve, and thus protection could be greater with fractionation. Four equal fractions of TBI were delivered to C3H/He mice at times 0 h, 8 h, 24 h, and 32 h. $FGF_{1/2}$ dose was 3 μg per iv injection given 24 and 4 hrs before the first radiation dose. FGF_2 treated mice had a significant survival advantage over saline-treated mice with a DMF of 1.22 ± 0.07 ($p < 0.01$). Adding a third dose of FGF_2, had no additional benefit on $LD_{50/30}$ (dose of radiation lethal to 50% of animals measured at day 30) (DMF = 1.23 ± 0.06, $p < 0.01$). FGF_1 was not as effective with fractionation (DMF = 1.04 ± 0.03). Increased survival in FGF2 treated mice was due to the a more rapid recovery of bone marrow hematopoietic cells and peripheral WBC, RBC and platelets. FGF_2 may prove a useful treatment response modifier in clinical fractionated irradiation.

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Most radioprotectors are radical scavengers and must be present at the time of irradiation. There is typically little or no radioprotection if these compounds are not present concurrent with, or within milliseconds after, irradiation and radioprotection is reduced when radiation is given in multiple fractions. In contrast, cytokine radioprotectors are typically most effective if given 24 h before radiation, have minimal added effect if given during radiation, and are often effective even if given several hours after radiation (1–4). Partial myeloprotection is still observed up to 2 days after FGF. Among cytokines studied to date, IL-1, G-CSF and FGF2 are known to both augment the shoulder of the dose response curve and radioprotect in single fraction TBI experiments (5–8). Several mechanisms of cytokine-induced radioprotection have been proposed. These mechanisms include induction of progenitor cells to proliferate, increase of the fraction of stem cells in the more resistant portions of the cell cycle (eg S-phase), and direct alteration of intrinsic sensitivity by transient induction of enzymes, such as superoxide dismutase (9). FGF_2 has many properties consistent with these hypothetical mechanisms, and has additional functions including prevention of radiation induced apoptosis, augmentation of potentially lethal damage repair (7), promotion of hematopoietic proliferation (particularly of megakaryocytes (10–14)), and induction of other hematopoietic cytokines (12, 14, 15). In this study we compared the radiomodifying effects of FGF_1 and FGF_2 in one-fraction and 4-fraction total body irradiation experiments respectively.

MATERIAL AND METHODS

Mice. Female C_3H/HeNCr mice were supplied through the Frederick Cancer Research Center (Frederick, MD), and maintained in the Research Animal Facility of NIH. Animals were housed in polycarbonate cages at 5 mice/cage. Animal holding rooms were climate controlled and circadian rhythm adjusted. All mice were 8–12 weeks of age at the time of TBI. The weight of the animals ranged from 19 to 23 g. Tested groups were matched for weight in each experiment.

Recombinant growth factors. Recombinant human FGF_1 and FGF_2 used in these experiments were provided by Pepro Tech Inc, Rocky Hill, NJ. Both FGFs (Lot No. 308 for FGF1 and Lot No. 3412 for FGF_2) had a concentration of 1 mg/ml in H_2O, pH = 7.4. Endotoxin level for

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FGF_1 and FGF_2 were less than 0.1 ng/μg. The specific biological activity of both FGFs were determined by the dose-dependent stimulation of thymidine uptake by BALB/C 3T3 cells. Immediately before injection, FGF_1 or FGF_2 was diluted with sterile 0.9% sodium chloride to the appropriate concentration. The desired dose of FGF_1 or FGF_2 was then intraveneously administrated in a final volume of 0.2–0.3 ml. Another group of mice received 0.2 ml of saline as control. FGF_1 or FGF_2 was given 24 and 4 h preceding TBI at 3 μg/mouse/dose. In one experiment FGF_2 was given a third time 20 h after the first dose of irradiation.

TBI. Groups of 10 mice were placed in a Plexiglas container and were irradiated at doses ranging from 750 to 1640 cGy with a ^{137}Cs gamma cell 40 (Nordion International, Inc., Kanata, Ontario, Canada) as the ionizing radiation source. The irradiator was calibrated with thermoluminocent dosimetry chips planted in phantom mice. The dose rate used was approximately 1 Gy/min. The number of surviving mice after TBI from each group was recorded daily for 30 days post-TBI. In parellel experiments, mice were sacrificed and their hematological blood cell indices (WBC, RBC, platelets and hematocrits) were evaluated. There were a minimum of 10 mice per group.

Statistics. Values are means $\pm$ 1 standard error of the mean. Survival curves and $LD_{50/30}$ calculations were made using non-linear least squares fitting to a logit curve of the form:

$$\text{Lethality} = e^{(d-LD_{50/30})/a)}[1 + e^{(d-LD_{50/30})/a)}]^{-1}$$

where 'd' is the total radiation dose given, $LD_{50/30}$ is the radiation dose lethal to 50% of animals, and 'a' is a fit parameter that adjusts the slope of the dose response curve. Dose modifying factors are the ratios of $LD_{50/30}$s measured in FGF-treated animals compared to controls.

RESULTS AND DISCUSSION

In the present study both FGF_1 and FGF_2 significantly protected C3H mice in single fraction experiments confirming our previous results. Dose modifying factors are 1.16 ± 0.01 for FGF_2 and 1.14 ± 0.02 for FGF_1. FGF_1 lost its protective benefit in fractionated experiments (4 fractions of radiation given at t = 0, 8, 24 and 32 h) after 6 μg FGF_1 (3 μg iv at t = −24 and −4 h) with a dose modifying factor of 1.04 ± 0.04. In contrast, the dose modifying factor after FGF_2 increased compared to single fraction radiation studies (1.22 ± 0.07, Figure). The $LD_{50/30}$ values for radiation given in four fractions over 32 h were: 1020 ± 30 cGy saline control; 1064 ± 29 cGy FGF_1; and 1244 ± 61 cGy FGF_2. The raw data for relevant dose levels are shown in Table 1. There were no surviving animals in any groups at doses above 1360 cGy. Above 1360 cGy some animals died in under 7 days suggesting gastrointestinal syndrome. Among irradiated animals treated with FGF_2 and surviving at day 20, peripheral blood counts showed greatest preservation of platelets, red blood cells and hematocrit, with minimal change in the nadir of white blood cells (Table 2). The cause of the differential response to acidic and basic FGF is uncertain. Several authors have shown that C3H mice are more responsive to FGF_2 compared to FGF_1 (2). We have shown that maximal radioprotection by FGF_1 requires higher doses than FGF_2 in C3H mice, and that maximal effects of FGF_1 are a bit less than maximal FGF_2 effects. It is unclear whether FGF_2 stimulates additional radiation damage modulating pathways than FGF_1 or whether FGF_1 requires significantly higher doses than FGF_2 to exert similar responses.

In an attempt to further augment the effects of FGF_2 mediated radioprotection we delivered 3 doses of 3 μg (given iv at t = −24, −4 and +20 h). The additional FGF_2 did not substantially improve radioprotection ($LD_{50/30}$ 1257 ± 51 cGy, Figure). We had previously used iv FGF_2 doses up to 24 μg, and likewise observed no increase of effect at doses over 6 μg. The results suggest a saturable receptor based response which results in intrinsic changes of radiosensitivity of hematopoietic progenitor cells. In unirradiated animals we found little evidence that FGF_2 increased hematopoietic proliferation or S-phase fraction (16). The augmented shoulder on the dose response curve is therefore probably due to inhibition of radiation induced apoptosis (7, 17), induction of intracellular sulfhydryls or superoxide dismutase (9, 18), or other

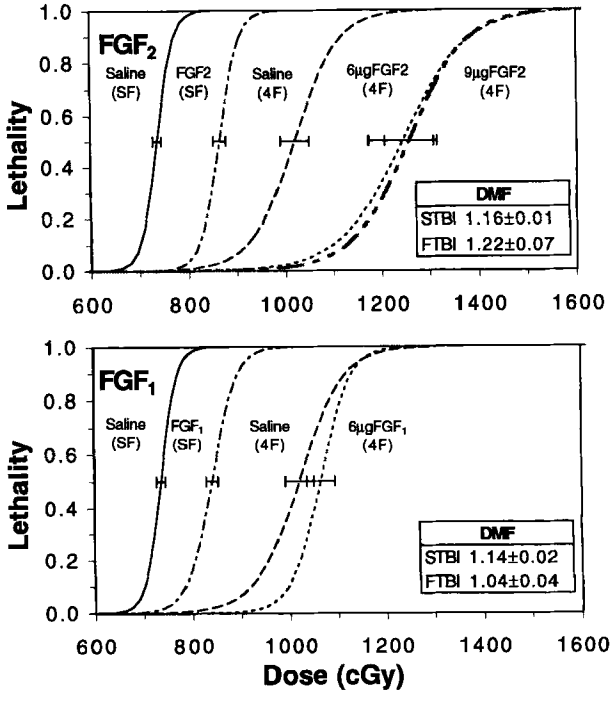

Figure. Survival curves were calculated as described in the text. Error bars are standard errors of the $LD_{50/30}$. STBI: Single fraction TBI, FTBI: Fractionated TBI.

Table 1

Effects of intraveneous administation of FGF_1 or FGF_2 on survival after single dose or fractionated TBI

Radiation (cGy)	Survival rate (%)			Median survival (days)		
	Saline	FGF_1	FGF_2	Saline	FGF_1	FGF_2
840-1STBI	1/20 (5)	11/20 (55)**	19/20 (95)**	14	>30	>30
840-FTBI	18/20 (90)	10/10 (100)	10/10 (100)	>30	>30	>30
960-1STBI	0/20 (0)	0/10 (0)	4/10 (40)*	10	12	17
960-FTBI	16/20 (80)	9/10 (90)	10/10 (100)	>30	>30	>30
1040-FTBI	4/10 (40)	7/10 (70)	10/10 (100)**	16	>30	>30
1160-FTBI	0/20 (0)	0/10 (0)	4/10 (40)*	14	16	22
1240-FTBI	0/10 (0)	0/10 (0)	3/10 (30)	14	14	18
1360-FTBI	0/20 (0)	0/10 (0)	0/20 (0)	12	12	14

Surviving fractions for C3H animals irradiated at mid-dose ranges. FGF_1 and FGF_2 were given as two iv doses of 3 μg/mouse as described in the text. Radiation was given in a single fraction (STBI) or in 4 equal fractions (FTBI). P value was calculated between the survival rate of FGF_1 or FGF_2-treated versus saline-treated mice. The χ^2 or Fisher exact tests was used, whichever was appropriate. *$p < 0.05$, **$p < 0.01$.

Table 2

Effects of intraveneous administation of FGF_2 on blood counts

Radiation (cGy)	Body Wt (gm)	WBC (10^3/mm)	RBC (10^6/mm)	Platelet (10^3/mm)	Hematocrit (%)
840-STBI/FGF_2	22.1 ± 2.4	2.3 ± 1.2	5.3 ± 2.7	148.5 ± 75	33.6 ± 16
840-FTBI/Saline	25.1 ± 3.6	2.5 ± 1.6	7.8 ± 0.1	891.7 ± 125	45.7 ± 1.9
840-FTBI/FGF2	23.7 ± 2.5	4.1 ± 3.2	6.7 ± 0.7*	970 ± 315	40.3 ± 3.1*
960-FTBI/Saline	23.5 ± 2.9	11.9 ± 7.3	2.3 ± 0.6	178 ± 24	13.9 ± 4.2
960-FTBI/FGF2	21.5 ± 2.5	4.5 ± 0.2*	7.2 ± 0.2**	531 ± 82**	42.8 ± 1.4**
1040-FTBI/Saline	23.4 ± 2.0	12.7 ± 2.1	2.9 ± 0.9	161 ± 78	17.5 ± 7.5
1040-FTBI/FGF2	20.1 ± 1.8	3.0 ± 0.3*	5.6 ± 1.3*	349 ± 103*	35.9 ± 6.2

Mice were sacrificed on day 20 after TBI and complete blood counts were performed. A single fraction (STBI) or 4 equal fractions (FTBI) of radiation were given at t = −24 and −4 h. Each FGF2 dose was 3 μg (total 6 μg/mouse); saline was used as control. *$p < 0.05$, **$p < 0.01$, T-test.

processes contributing to repair of radiation damage (19). The observation that FGF_2 yields improved protection with 4 fractions of radiation given over 32 h is relevant to clinical radiotherapy and distinguishes it among radioprotectors. Other cytokines that alter the shoulder of the dose response curve might also have improved effects with fractionation.

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