FLT-3 Ligand Provides Hematopoietic Protection From Total Body Irradiation in Rabbits

By A. Gratwohl, L. John, H. Baldomero, J. Roth, A. Tichelli, C. Nissen, S.D. Lyman, and A. Wodnar-Filipowicz

Several hematopoietic cytokines have been investigated for their potential to provide protection from the lethal consequences of bone marrow aplasia after total body irradiation (TBI). Some can increase the dose of irradiation tolerated by the animals; none allow endogenous recovery after doses such as administered in clinical blood or marrow transplantation. We tested the radioprotective potential of FLT-3 ligand, an early acting hematopoietic cytokine, alone and in combination with a late acting cytokine, granulocyte-colony stimulating factor (G-CSF). Adult outbred New Zealand White rabbits were submitted to TBI of 1,200 or 1,400 cGy by a Co60 source. Recombinant human (rh) FLT-3 ligand at a dose of 500 µg/kg and/or rhG-CSF at a dose of 10 µg/kg were administered for 14 days subcutaneously, beginning either 2 days before or the day after TBI. All control animals given no growth factors died of aplasia at day 10 (range, 5 to 16). All 8 animals given G-CSF had severe aplasia and 7 died at day 8 (range, 5 to 10); 1 animal survived, with G-CSF being administered before TBI. In contrast, 11 of 12 animals given FLT-3 ligand, with or without G-CSF, survived. Radioprotection was best in the group given FLT-3 ligand together with G-CSF before TBI. In these animals median platelet counts were never <10 x 10⁹/L and median white blood cell counts never <0.5 x 10⁹/L. These data show that hematopoietic recovery can occur after 1,400 cGy TBI in rabbits, if protected by FLT-3 ligand, and suggest a radioprotective clinical potential of this cytokine.

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ANIMALS AND METHODS

Animals. Adult outbred New Zealand White rabbits (Biological Research Laboratories Ltd, Fullinsdorf, Switzerland) of both sexes were used. Animals were housed in single cages with standard pellet diet and water ad libitum in the supervised Animal Care Facility Center of the Department of Research at the Kantonsspital Basel.

Experimental design. Animals were treated with TBI in groups of two to four animals each, given either no growth factors (control animals) or growth factors for a period of 14 days, beginning 2 days before TBI (pre) or the day after TBI (post), as outlined in Table 1. The protocol was approved by the Ethical Committee for Animal Care of the Canton of Basel-Stadt.

TBI. TBI was administered from a Co60 source at a dose rate of 20 cGy/min in general anesthesia in two doses on 2 consecutive days, as previously described in our transplant protocol.26-27 Total doses of 1,200 and 1,400 cGy were examined. TBI was applied as a lateral beam from the top to animals lying on the floor. Animals were anesthetized by intramuscular (im) injection of Hypnorm (Janssen-Cilag Pharmaceuticals, Baar, Switzerland) and were administered 0.2 mg of dexamethasone to protect from immediate central nervous toxicity. Supportive care after TBI was comprised of Bactrim (Roche, Ltd, Basel, Switzerland) administered orally daily and an injection of normal saline subcutaneously (sc) if required. No red blood cell or platelet transfusions were given.

Growth factors. Recombinant human granulocyte colony-stimulating factor (rhG-CSF) (Neupogen; kindly provided by Roche Pharma, Reinach, Switzerland) at a dose of 10 µg/kg and FLT-3 ligand (kindly provided by Immunex Corp, Seattle, WA) at a dose of 500 µg/kg were used. These doses of human growth factors had been previously tested and shown to stimulate hematopoiesis in vitro and to synergize in vivo in the mobilization of hematopoietic precursor cells in rabbits. Growth factors were administered either 2 days before or the day after TBI. All control animals given no growth factors died of aplasia at day 10 (range, 5 to 16). All 8 animals given G-CSF had severe aplasia and 7 died at day 8 (range, 5 to 10); 1 animal survived, with G-CSF being administered before TBI. In contrast, 11 of 12 animals given FLT-3 ligand, with or without G-CSF, survived. Radioprotection was best in the group given FLT-3 ligand together with G-CSF before TBI. In these animals median platelet counts were never <10 x 10⁹/L and median white blood cell counts never <0.5 x 10⁹/L. These data show that hematopoietic recovery can occur after 1,400 cGy TBI in rabbits, if protected by FLT-3 ligand, and suggest a radioprotective clinical potential of this cytokine.

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Submitted February 9, 1998; accepted May 13, 1998.

Supported in part by the Swiss National Research Foundation Grant Nos. 3200-042431-94/1.

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0006-4971/98/9203-0036$3.00/0


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factors were injected daily sc in the shaved back of the animal. FLT-3 ligand and G-CSF were administered either singly or combined, as outlined in Table 1.

Laboratory examinations. Animals were inspected daily and their weight was recorded. Whole blood cell counts, including hemoglobin, reticulocyte, platelet counts, and white blood cell differential counts, were determined three times weekly.28 A postmortem examination was performed in all the deceased animals.

Statistical analysis. Mean, median, and range of numerical variables were calculated by the Excel spreadsheet program. Survival of the groups was compared by Fisher’s exact t-test, the blood values at nadir by unpaired t-test.

RESULTS

Survival. The outcome of animals following TBI is summarized in Table 1 and illustrated in Fig 1. All 8 control animals, subjected to 1,200 or 1,400 cGy but given no growth factors, died of aplasia between days 5 and 16 after TBI (median, day 10). No higher dose was considered because of excessive early toxicity of the central nervous system observed in previous studies.27 Seven of 8 animals given 1,400 cGy TBI and treated with G-CSF alone died between days 5 and 10 (median, day 8). In this treatment group, only one animal administered G-CSF after TBI recovered from severe pancytopenia and survived.

In contrast, 11 of 12 animals treated with FLT-3 ligand survived. The survivors included all 10 animals given FLT-3 ligand, with or without additional G-CSF, before TBI. Of the two animals subjected to 1,200 cGy and administered FLT-3 ligand and G-CSF after TBI, one animal failed to recover and died at day 8 in aplasia. The difference in survival between the control group and the FLT-3 ligand–treated group is highly significant ($P < .01$).

Extent of aplasia. As a result of TBI, all control animals entered severe pancytopenia with platelet counts $<10 \times 10^9/L$ and white blood cell counts $<0.2 \times 10^9/L$ (Table 1). The extent of pancytopenia was no different in animals treated with G-CSF alone. Even the 1 surviving animal had a nadir of $8 \times 10^9/L$ for platelets and $0.1 \times 10^9/L$ for white blood cell counts. In contrast, none of the 4 animals given the combination of FLT-3 ligand and G-CSF before TBI had white blood cell counts $<0.2 \times 10^9/L$ at any time point and only 1 animal had a white blood cell count $<0.5 \times 10^9/L$ for 1 day. A platelet count was $<10 \times 10^9/L$ in only 1 animal on 1 day. Remarkably, 2 of 4 animals never had platelet counts $<20 \times 10^9/L$ nor white blood cell counts $<1.0 \times 10^9/L$. The values of white blood cell counts and platelets at the time of nadir are significantly different between the control group and the FLT-3 ligand + G-CSF pretreated animals ($P < .05$).

The kinetics of entering and recovering from aplasia in terms of median leukocyte, platelet, reticulocyte counts, and hemoglobin values are illustrated in Fig 2. Control animals and those administered G-CSF alone entered rapid aplasia and failed to recover. Among animals given FLT-3 ligand alone and those given the combination of FLT-3 ligand and G-CSF, there was a difference concerning white blood cell kinetics. In animals administered FLT-3 ligand alone, median white blood cell counts were already $<1 \times 10^9/L$ at day 2 and recovered to $>1 \times 10^9/L$ at day 11 only. In contrast, in the combined treatment group, white blood cell counts went to $<1 \times 10^9/L$ at day 4 and recovered to $>1 \times 10^9/L$ at day 8.

DISCUSSION

The data from this study show that FLT-3 ligand, alone or in combination with G-CSF, can consistently rescue animals from otherwise lethal aplasia when administered before TBI. G-CSF alone is insufficient. All animals pretreated with FLT-3 ligand survived high-dose irradiation. The best results in terms of survival and the speed of recovery from aplasia were achieved when the two cytokines were combined and given before, not after, irradiation. With the combination, the nadir of white blood cell and platelet count was reduced in both intensity and duration. These remarkable radioprotective effects of FLT-3 ligand are by far superior to the effects of other growth factors and cytokines tested previously for their potential to protect animals against the toxicity of TBI. Of the hematopoietic cytokines, G-CSF was shown to have some radioprotective effect in mice when administered before TBI. Sixty percent of mice given high-dose G-CSF (40 µg/kg) before 700 cGy TBI survived in contrast to control animals. No protection was observed at 800 cGy.29 Similarly, modest effects were observed with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukins (IL)-3 and -6, in sublethally, but not in lethally, irradiated animals.8,30 Most data have been accumulated using IL-1 alone or in combination with G-CSF, GM-CSF, stem cell factor or tumor necrosis factor-α (TNF-α) or interferon-γ.22,31,32 Better results were obtained with the combination of IL-1 and stem cell factor administered before TBI.32 Even there, effects on protection were relatively marginal with an increase in LD$_{50/30}$ by factors of 1.2 to 1.3 from 800

### Table 1. Treatment and Outcome of Rabbits Subjected to TBI

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Growth Factor</th>
<th>Time of Injection</th>
<th>TBI (cGy)</th>
<th>N</th>
<th>Platelet (×10⁹/L)*</th>
<th>WBC (×10⁹/L)</th>
<th>Survival Outcome</th>
<th>Day of Death*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td>1,200</td>
<td>4</td>
<td>8 (7-11)†</td>
<td>0.13 (0.06-0.26)#</td>
<td>0/4</td>
<td>12 (9-16)</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td></td>
<td>1,400</td>
<td>4</td>
<td>2 (1-9)†</td>
<td>0.19 (0.11-0.23)#</td>
<td>0/4</td>
<td>8 (5-12)</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Post</td>
<td></td>
<td>1,400</td>
<td>4</td>
<td>5 (2-22)</td>
<td>0.07 (0.05-0.11)</td>
<td>0/4</td>
<td>9 (5-10)</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Pre</td>
<td></td>
<td>1,400</td>
<td>4</td>
<td>7 (3-46)</td>
<td>0.09 (0.07-0.14)</td>
<td>1/4</td>
<td>8 (7-8)</td>
</tr>
<tr>
<td>FL</td>
<td>Pre</td>
<td></td>
<td>1,400</td>
<td>4</td>
<td>7 (6-7)</td>
<td>0.10 (0.05-0.12)</td>
<td>4/4</td>
<td>NA</td>
</tr>
<tr>
<td>FL + G-CSF</td>
<td>Post</td>
<td></td>
<td>1,200</td>
<td>2</td>
<td>4.5 (4-5)</td>
<td>0.28 (0.14-0.41)</td>
<td>1/2</td>
<td>8</td>
</tr>
<tr>
<td>FL + G-CSF</td>
<td>Pre</td>
<td></td>
<td>1,200</td>
<td>2</td>
<td>37 (20-54)†</td>
<td>0.92 (0.3-1.54)#</td>
<td>2/2</td>
<td>NA</td>
</tr>
<tr>
<td>FL + G-CSF</td>
<td>Pre</td>
<td></td>
<td>1,400</td>
<td>4</td>
<td>11 (5-15)†</td>
<td>0.55 (0.36-2.39)#</td>
<td>4/4</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: Platelet, platelets; WBC, white blood cells; FL, Flt 3 ligand; NA, not applicable.

*Median (range).

Difference in platelet counts (†) and WBC (‡) between control group animals and FL + G-CSF pretreated animals according to unpaired t-test, $P = .05$ and $P = .02$, respectively.
to 950 cGy. In only one of the models tested did total doses of tolerated TBI exceed 1,000 cGy when mice were given stem cell factor before and after 1,150 cGy TBI.13

The mechanism of radioprotection by growth factors is unknown and the results appear, in part, surprising. In clinical studies of leukemia treatment, cytokines are used to prime target cells for higher susceptibility to chemotherapy.33 Therefore, an even more rapid decline in peripheral blood values could have been expected in cytokine-pretreated animals. To the contrary, in irradiated animals receiving FLT-3 ligand and G-CSF the decrease of peripheral blood values was not only less pronounced but also delayed. In addition, decline in whole blood leukocyte counts was significantly slower in animals given FLT-3 ligand and G-CSF than in animals given FLT-3 ligand alone. Dose of G-CSF and FLT-3 ligand and the timing of administration were selected on the basis of previous experiments in the same animal model.21 We found that FLT-3 ligand and G-CSF synergistically mobilize hematopoietic precursor cells in rabbits and have their most pronounced effect at 10 to 12 days’ administration, ie, at the time when aplasia after radiation is most severe. Effects of both cytokines on the kinetics of entry into and recovery from hematopoietic aplasia cannot be explained solely by expansion and proliferation of stem and progenitor cells.

Several decades ago, the property of bacterial lipopolysacharides to protect animals from lethal irradiation was described and for a long time provided the basis for considerations in radioprotection.34 It is now known that lipopolysaccharides elicit an inflammatory reaction by releasing a variety of cytokines, including IL-1, IL-6, CSFs, TNF, IFNs, and transforming growth factor, all shown to have some radioprotective activity of their own. Based on these effects, Neta and Oppen-heim8 postulated that an inflammatory response would assist in recovery from radiation by the removal of damaged tissue and by promoting restoration of normal function. Later, it was postulated that growth factors induce or enhance repair mechanisms and, thus, may protect stem cells from an irradiation insult.9,31 Radiation is a powerful stimulator of apoptosis.35,36
Aplasia after TBI might be the consequence of radiation-induced apoptosis in hematopoietic precursor cells. It has been shown that FLT-3 ligand promotes the maintenance of hematopoietic progenitors in vitro and has antiapoptotic effects. Protection of radiation-induced apoptosis by combined FLT-3 ligand, stem cell factor, and IL-3 has recently been shown for human and primate hematopoietic precursor cells in vitro. We postulate that similar mechanisms might take place in vivo in animals subjected to TBI.

Our data illustrate the profound radioprotective effects of FLT-3 ligand and extend information from previous studies concerning the potential of cytokine therapy in the treatment and prevention of radiation injury. The results of this study open the way for further analyses of radioprotective effects of FLT-3 ligand in growth factor combinations which should also include stem cell factor, thrombopoietin, and erythropoietin. The clinical goal is to design optimized radioprotective regimens to allow high-dose radiation for cancer patients or as intensive immunosuppression for solid-organ transplant recipients without the need for stem cell rescue.

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