G-CSF DURING LARGE FIELD RADIOTHERAPY REDUCES BONE MARROW RECOVERY CAPACITY


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Abstract
Objective: Side effects of chemo- and radiotherapy are granulo- and thrombocytopenia. However, the long-term effects of in vivo granulocyte-colony-stimulating factor (G-CSF) stimulation of the hematopoietic system during radiotherapy are not yet completely understood. In the present study, we sought to determine the bone marrow effect of G-CSF during radiotherapy.

Material and methods: In a prospective, randomized clinical trial 10 patients (6 m, 4 f, 30-64 yrs, mean 50.6 yrs) were assigned to large field radiotherapy (RT). 7 patients (pat.) with non-Hodgkin lymphoma, one patient with Hodgkin‘s disease and 2 patients with small-cell carcinoma of the lung were included. The patients were randomized to either radiotherapy alone (group A) or radiotherapy with simultaneous G-CSF (group B) treatment and assessed for acute and late toxicity. Blood samples were drawn and analyzed before and after G-CSF stimulation. The mobilization effectiveness of G-CSF on CD34+ progenitor cells was measured using flow cytometry and colony forming units (CFU) testing on admission and during the complete follow-up period (1, 3 and 18 months post RTx).

Results: Overall, 50 pat. were intended to be included to the protocol. However, the preliminary analysis revealed a significant decrease of thrombocytes and CD34+ progenitor cells in the G-CSF treatment group. According to the study protocol further treatment was stopped. Peripheral leukocyte counts ranged between 2800 - 4375 /µl in 9/10 pat. In group B mean thrombocyte levels dropped below 30,000 /µl and CD34+ progenitor cells to 50% (interruption criteria, p<0.02, Student’s t-test). Hemoglobin values did not vary. Differential blood smears showed differences in granulocyte counts and a higher proportion of neutrophils in group B. Lymphocyte counts of patients randomized to group A were significantly decreased when compared to group B. In group A, 3/5 pat. developed an overshooting reaction (4.7 x increase) after G-CSF-stimulation. In arm B circulating CD34+ progenitor cells dropped. In arm A, 3/5 pat. had an initial overshoot reaction when compared to none in group B. CFU (> 40 cells) and cluster (4 -39 cells) showed considerable variations.

Conclusion: Our results demonstrate that simultaneous treatment with G-CSF during radiotherapy reduces the mobilization of CD34+ progenitor cells and exhaust the bone marrow capacity while peripheral leukocyte counts remain at baseline levels.

Key words: chemotherapy, radiotherapy, granulocytopenia, thrombocytopenia, stem cell pool capacity, granulocyte-colony-stimulating factor, myelopoiesis, CD34

INTRODUCTION
Granulo- and thrombocytopenia are critical side effects of chemo- and radiotherapy [1, 30, 31, 33, 32]. In the complex hematopoietic system, circulating CD34+ precursor cells are specific indicators for bone marrow injury [30, 34]. Because of the specificity, CD34+ counts are used more frequently or in addition to peripheral blood cell counts. Little is known about the long-term effects of in vivo granulocyte-colony stimulating factor (G-CSF) treatment on the hematopoietic system during fractionated radiotherapy. Although the major effect of G-CSF is restricted to late granulopoietic precursor cells, increased proliferation and amplified differentiation of these cells during fractionated radiotherapy result in release of their reserve capacity. New precursor cells are destroyed during migration into the irradiation volume. A vicious circle of extension and migration further potentiates this expansion phenomenon. As a consequence, myelopoiesis is reduced and an "exhaust phenomenon" on early stem cells is triggered. We designed this prospective, randomized study in an effort to evaluate the effect of G-CSF treatment during fractionated large-field radiotherapy (RT).
AIMS OF THE STUDY

1. Does G-CSF given during fractionated large-volume irradiation cause an improved mobilization effect with rapid recovery of hematopoietic stem cells?
2. Is G-CSF responsible for therapeutic failures due to stimulation of precursor cells leading to an exhaustion of the reserve capacity of the bone marrow?
3. What are the long-term effects on the hematopoietic system?

MATERIAL AND METHODS

We quantified progenitor cells via assessment of the mobilization of CD34+ progenitor cells after G-CSF stimulation. Because of considerable interindividual variations in control patients and tumor patients, CD34+ cells were measured on admission and during follow-up (1, 3 and 18 months). Since many various parameters influence the mobilization of CD34+ progenitor cells they can only be used as an indirect parameter for bone marrow capacity.

STUDY DESIGN

Prospective randomized study for large-field radiotherapy with (group B) or without (group A) G-CSF stimulation during therapy. All procedures performed and treatment protocols used were approved by the University of Duesseldorf Institutional Review Board and Ethics Committee on Clinical Trials.

INCLUSION CRITERIA

• Age: 18-75 years
• solid or hematological tumors, except ALL / AML or multiple myeloma
• prognosis > 3 months
• large volume radiotherapy
• chemotherapy within 4 weeks before G-CSF stimulation
• stable hematopoietic recovery, defined as WBC >3000/µl, platelet count > 100 000/µl
• Signed consent to the protocol

Stopping rules were:

1. The decrease of CD 34+ progenitor cells of more than 50% of the initial base level in 1 arm (A or B)
2. Thrombopenia (< 30 000 /µl) in Arm A or B.

CRITERIA FOR LARGE-FIELD RADIOThERAPY

To analyze the impact of irradiation on CD34+ progenitor cells, small volume irradiation was excluded because of high hematopoietic recovery in non-irradiated bone marrow. A large volume was defined as a volume including at least eight thoracic vertebrae, the complete vertebral column, abdomen or pelvis. Calculation of the irradiated bone marrow volume was based on measurements according to age, gender and body weight.

MOBILIZATION OF CD34+ PROGENITOR CELLS

Hematopoietic precursor cells are located in the bone marrow and peripheral blood. The concentration of circulating CD 34+ cells is low and difficult to detect. Therefore, G-CSF (rhu G-CSF, Filgrastim; Neupogen®; Amgen/Roche Corp., Munich, Germany) was administered to all patients to mobilize CD34+ progenitor cells.

Patients were subcutaneously injected G-CSF 12.5 µg/kg body weight from day 1-4. CD34+ and progenitor cells were determined at day 4. In addition to that, a differential blood cell count (CBC count) was routinely performed.

ASSESSMENT OF CD34+ PROGENITOR CELLS

The mobilization impact of G-CSF on CD34+ progenitor cells in adults is an indirect parameter for stem cell pool capacity. Therefore, the mobilization of CD34+ cells was measured on admission, 1, 3 and 18 months after therapy.

Immune-specific cell surface antigens were analyzed using an immunofluorescent assay. Detection of CD34+ progenitor cells has been done using specific antibodies conjugated to either Fluorescin Isothiocyanate (FITC) or Phycoerythrin (PE).

COLONY ASSAY

After G-CSF stimulation, peripheral blood samples with progenitor cells were collected and transferred to a methylcellulose-Agar plate. 20 x 10³ cells were inoculated. Colony growth was controlled on day 7, 10 and 14. Final counts of the Colony Forming Units (CFU) were performed on day 14. Aggregates with 4 - 39 cells were defined as clusters and more than 40 cells as colonies.

RESULTS

Thirteen patients (pat.) were included in the study protocol within 11 months. Since two patients suffered tumor progression and succumbed to their disease (high-grade NHL) during therapy and another patient was lost to follow-up after 2 months, these 3 patients were excluded from the study. Six men and 4 women (range 30-64, mean 50.6 yrs.) with NHL (n = 7), M. Hodgkin (n = 1) and small-cell lung carcinoma (n = 2) were included in the first interim analysis. Further evaluations of these patients were carried out at 1, 3 and 18 months after radiotherapy. By that time, 5 patients in each arm were assessable. A survey of the patient characteristic is shown in Table 1.

Irradiated mean bone marrow volume was calculated at 22.5% in group A and 25% in group B. In group B the CD34+ progenitor cells dropped below 50% (p<0.02, Student’s t-Test). All patients received G-CSF to mobilize CD34 progenitor cells.

Results

Hemoglobin values remained unchanged. In group B mean values of thrombocytes dropped down to 20 000/µl. In 9/10 patients, peripheral leukocytes were found in a clinically acceptable range (>2500/µl). In
Table 1. Demographic and clinical data of all patients.

<table>
<thead>
<tr>
<th></th>
<th>Arm A</th>
<th>Arm B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>48 (30-64)</td>
<td>55 (30-64)</td>
</tr>
<tr>
<td>Gender</td>
<td>3 m / 2 f</td>
<td>2 m / 3 f</td>
</tr>
<tr>
<td>Initial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>NHL</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>M. Hodgkin</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>NHL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irradiated regions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mediast.</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>abd. bath</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>paraaort. lym</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Target volume dose (Gy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1x 39.6 (5x1.8)</td>
<td>1x 30 (5x1.5)</td>
<td>1x 30 (5x1.8)</td>
</tr>
<tr>
<td>1x 50.4 (5x1.8)</td>
<td>1x 39 (5x1.5)</td>
<td>1x 50.4 (5x1.8)</td>
</tr>
<tr>
<td>1x 39 (5x1.5)</td>
<td>1x 30 (5x1.8)</td>
<td>1x 30 (5x1.5)</td>
</tr>
<tr>
<td>Percentage of irradiated bone marrow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>of total mount of bone marrow volume</td>
<td>22.5%</td>
<td>25.3%</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mediast.</td>
<td>6x ACO 4x CHOP</td>
<td>6x CHOP 1x none</td>
</tr>
<tr>
<td>abd. bath</td>
<td>6x CHOP 1x none</td>
<td></td>
</tr>
<tr>
<td>paraaort. lym</td>
<td>6x ABVD 4x ABVD</td>
<td></td>
</tr>
</tbody>
</table>

*median values and ranges

Abbreviations: m = male; f = female; LC = lung cancer; NHL = Non Hodgkin Lymphoma; M. Hodgkin = Morbus Hodgkin; mediast. = mediastinum; abd. bath = abdominal bath; paraaort. lym = paraortic lymphnode; ACO = Adriblastin, Cyclophos-phamid, Oncovin; CHOP = Cyclophosphamid, Adriblastin, Oncovin, Prednison; ABVD = Adriblastin, Bleomycin, Vinblastin, Darcabacin; ACE = Actinomycin D, Cyclo-phosphamid, Etoposid.

Table 2. CD34+ progenitor cells in the peripheral blood.

<table>
<thead>
<tr>
<th>Group A</th>
<th>C0 abs</th>
<th>rel%</th>
<th>C1 abs</th>
<th>rel%</th>
<th>C2 abs</th>
<th>rel%</th>
<th>C3 abs</th>
<th>rel%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>13.13</td>
<td>100%</td>
<td>1.38</td>
<td>10.51%</td>
<td>10.74</td>
<td>81.80%</td>
<td>12.71</td>
<td>96.80%</td>
</tr>
<tr>
<td>Patient 2</td>
<td>1.38</td>
<td>100%</td>
<td>2.07</td>
<td>150%</td>
<td>6.39</td>
<td>477.5%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patient 3</td>
<td>10.74</td>
<td>100%</td>
<td>14.23</td>
<td>132.5%</td>
<td>6.43</td>
<td>59.8%</td>
<td>6.05</td>
<td>56.33%</td>
</tr>
<tr>
<td>Patient 4</td>
<td>35.27</td>
<td>100%</td>
<td>39.39</td>
<td>111.7%</td>
<td>20.28</td>
<td>57.5%</td>
<td>18.03</td>
<td>51.1%</td>
</tr>
<tr>
<td>Patient 5</td>
<td>4.26</td>
<td>100%</td>
<td>2.8</td>
<td>65.72%</td>
<td>3.07</td>
<td>72.06%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Group B

<table>
<thead>
<tr>
<th>Group B</th>
<th>C0 abs</th>
<th>rel%</th>
<th>C1 abs</th>
<th>rel%</th>
<th>C2 abs</th>
<th>rel%</th>
<th>C3 abs</th>
<th>rel%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>23.59</td>
<td>100%</td>
<td>1.57</td>
<td>6.6%</td>
<td>8.25</td>
<td>34.97%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patient 2</td>
<td>24.94</td>
<td>100%</td>
<td>9.92</td>
<td>39.77%</td>
<td>7.23</td>
<td>28.98%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patient 3</td>
<td>36.25</td>
<td>100%</td>
<td>12.55</td>
<td>34.62%</td>
<td>7.68</td>
<td>21.18%</td>
<td>12.70</td>
<td>35.03%</td>
</tr>
<tr>
<td>Patient 4</td>
<td>12.48</td>
<td>100%</td>
<td>4.3</td>
<td>34.45%</td>
<td>6.40</td>
<td>51.28%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patient 5</td>
<td>6.44</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>2.02</td>
<td>31.36%</td>
<td>1.3</td>
<td>20.18%</td>
</tr>
</tbody>
</table>

Absolute und relative counts of CD34+ cells in all patients (n = 5 in each group). The mobilization of CD34+ cells was measured on admission (C0), one (C1), three (C2) and 18 months (C3) after therapy. Group A: Values without G-CSF Group B: G-CSF treatment.
group A leukocytes ranged from 2800 to 4375/µl. Group B leukocytes showed larger differences. The proportional distribution of the differential blood count showed a significant difference for granulocytes. A higher proportion of neutrophils in group B was detected (>90% in group B, 53% in group A). The lymphocyte subpopulation in group A was found to be significantly lower (Figs. 1 and 2). Data of CD34+ progenitor cells in the peripheral blood are shown in table 2. In group A 3/5 pat. developed an overshooting reaction after G-CSF-application. In arm B CD34+ progenitor cells were declining in contrast to arm A, where 3/5 pat. demonstrated an initial overshoot reaction. However, in arm B we observed no increased reaction but values declined to a nadir of 10%. Reaching our stopping rules, we decided to discontinue the study.

**DISCUSSION**

Our results demonstrate that simultaneous G-CSF application during radiotherapy reduces the capacity of bone marrow recovery. After low-dose radiotherapy an increased endogenous G-CSF production and up-regulation of G-CSF receptors on bone marrow cells were described in literature [2]. Fushiki et al found an increased spleen weight and a rapid recovery from neutropenia when irradiated mice were injected with G-CSF [3]. Other investigators have demonstrated an expansion of early precursor cells after radiotherapy and stimulation with G-CSF [4-6]. All mice survived after the application of growth factors 20 h before and 2 h after a lethal dose of total body irradiation, whereas unprotected, non-treated animals died within 12 days after radiotherapy. The effect of radiotherapy on precursor cells was predominantly analyzed in animal models. Many authors assume that G-CSF has a protective effect against radiation induced myelosuppression [7-9]. Neta and co-workers described a significant radio-protective effect of G-CSF when treatment with Interleukin-1 and Interleukin-6 was initiated simultaneously with radiotherapy [10, 11].

In clinical studies G-CSF was used for the treatment of radiation-induced neutropenia. A rapid recovery of leukocytes was observed and a treatment recov-
ery interval was not necessary [12, 13]. Fushiki and Abe demonstrated in a randomized trial that G-CSF significantly reduces incidence and extent of radiation-induced neutropenia [14]. However, even though initial results encouraged further investigations, during the course of further evaluation of this novel therapeutic strategy, further progress to clinical application was hampered by various side effects. Documented risk factors when using G-CSF in connection with radio-chemotherapy. After the treatment of lung carcinoma with G-CSF and radio-chemotherapy long lasting thrombopenia was observed [15-17].

For the treatment of patients the timing, duration and dosage of G-CSF application are crucial factors for a successful treatment and a favorable outcome. G-CSF applied after radiotherapy has accelerated the recovery of the hematopoietic system [7, 8]. One single application of 1-2 µg G-CSF before or directly after radiotherapy did not protect against the hematopoietic syndrome [18, 19]. A dose-response relationship was seen after G-CSF application in the overall survival time of mice. Optimal results were achieved when G-CSF was administered at 3h and 24h after radiotherapy [5].

Application of 3µg G-CSF on day 1 to 4 followed by total body irradiation with 6.5 Gy three hours after the last administration of G-CSF results in a myelopoi- etic depression in mice. For the first time we saw an adverse effect of G-CSF in combination with radio-therapy [20]. Immediately after radiotherapy, precursor cell counts were found to be increased in spleen and bone marrow of animals that had been pretreated with G-CSF. Fourteen days after radiotherapy the precursor cells significantly decreased. At the same time the spleen could only compensate for reduced granulopoiesis in the early phase of irradiation. Erythroid cells predominantly repopulated the irradiated spleen. After radiotherapy the number of peripheral neutrocytes in the pretreated animals declined on day 14 and the monocyte count dropped on day 18 [20].

The mechanism of decreasing circulating CD34+ progenitor cells after radiotherapy is not yet fully under-stood. The administration of G-CSF in combina- tion with radiotherapy induces an amplified damage for precursor cells which is limited to the granulocyte subpopulation. Additionally, G-CSF is a potent stimu-lator of CFU-G formation from the pluripotent CFU-GM cells. Baird and coworkers postulated that radiation stimulates the normal bone marrow with an en-larged differentiation of granulocytes. It was hypothe-sized that increased differentiation and loss of self re-newal capacity followed radiation induced bone mar-row stimulation [21]. Other investigators found that the multi-potent, early precursor cells (CFU-GEMM) were extremely sensitive to radiotherapy and did not have any recovery capability [22]. Further studies demonstrated that during G-CSF application the GM- CFC (granulocytes - macrophages colony forming cells) had a decreased sensitivity to radiotherapy [23]. The D0 values for GM-CFC were 1.98 Gy for controls and 2.47 Gy in animals that were preconditioned with G-CSF [20]. In a clinical setting, no direct measure-ment of CD34+ cells is feasible. Peripheral circulating CD34+ cells were counted after previous stimulation with G-CSF. Under steady state conditions effectiveness depends on the G-CSF dose, the mode of appli-cation and the interval between G-CSF-administration and blood sample [24-26]. Subcutaneous injection of G-CSF is generally preferred for pharmacodynamic reasons [27]. The highest proportion of CD 34+ cells is expected 4-7 days after G-CSF injection [24, 25, 28]. Even in healthy individuals a variation of CD34+ cell production depending on age, dose and therapeutic timing is seen. Increased interindividual variation is documented in tumor patients [27]. In our study, vari-ables such as age, gender, and previous treatment modalities were comparable in both groups. For stimu-lation of CD34+ cells all patients received 12,5 µg G- CSF per kg body weight subcutaneously for 4 days [27, 29], 4/5 patients in each group underwent prior chemo-therapy. To minimize interindividual variations, CD34+ progenitor cell counts were assessed on admission and all further values were later compared to this baseline value. Long-term follow-up (18-25 months after the end of radiotherapy) was possible in 3/5 pts. in group A and in 2/5 pts. in group B. Results did not show any recovery of CD34+ cells in group B. A long-time reduction of the progenitor cell mobiliza-tion after radiotherapy was demonstrated. Some inves-tigators observed a recovery up to 40 days after radio-therapy. Their hematologic values remained at 50 - 60% of the baseline level and were comparable to our results [30].

A rapid decrease of the GM-CFC-population was observed in dogs who received a partial or total body irradiation without G-CSF. After 24 hours and during the week following total body irradiation, the values for the GM-CSF population decreased more com-pared to lymphocytes. Bone marrow recovery was evi-dent by both expansion of GM-CFC- and BFU-E population 14 days after total body irradiation. Several months after radiotherapy these values remained on a low subnormal level [30]. In further experiments, 3 dogs received 2 x 15 µg of rhu-G–CSF/kg/day on seven consecutive days following partial body irradiation. When compared to the control group, the G- CSF-group demonstrated an early expansion of the GM-CSF population. The regeneration of the GM- CSF and of BFU-E compartments was accelerated by early expansion of precursor cells, migration and set-tlements into the irradiated bone marrow. G-CSF re-sulted in an expansion of precursor cells [30].

Our results are not directly comparable to these studies due to the experimental conditions of the above mentioned studies. The irradiated bone marrow volumes were smaller and the applied radiation doses were higher. Also, G-CSF was given over a longer time period. However, some parallel effects were seen. In summary, we found a significant decrease of circulating peripheral progenitor cells in the G-CSF treatment group. This effect became clinically evident at the first CD34+ count determination which was performed 4 weeks after the end of the therapeutic course and per-sisted for more than one year. In contrast to the de-scribed animal models we did not find an adequate de-crease of circulating peripheral granulocytes. De-creased CD34+ levels persisted even 3 and 18 months after termination of radiotherapy. This was compara-
ble to experiments that have been performed using animal models.

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