Serum Concentrations of Granulocyte Colony-Stimulating Factor (G-CSF) in Antithyroid Drug-Induced Agranulocytosis

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Abstract. Granulocyte colony-stimulating factor (G-CSF) levels in serum were determined by a highly-sensitive chemiluminescent enzyme immunoassay (limit of detection, 0.5 pg/ml) in 54 patients with Graves’ disease including 6 patients complicated with methimazole-induced agranulocytosis. Serum G-CSF levels in patients with Graves’ disease were not different from normal subjects and did not correlate with serum FT4 level or circulating neutrophil counts. Before the onset of agranulocytosis, there was no difference in serum G-CSF level between the patients complicated with agranulocytosis and the uncomplicated patients. When circulating neutrophil counts decreased to less than 0.5 × 10⁹/L, serum G-CSF level elevated with the mean of 106.8 ± 82.2 (SD) pg/ml, but the level did not correlate with the duration of agranulocytosis. Interestingly, maximum serum G-CSF level during the treatment with recombinant human G-CSF (100 μg/day) was related to bone marrow finding at the onset of agranulocytosis and correlated with the duration of agranulocytosis (r = 0.824, p<0.05). In conclusion, measuring serum G-CSF levels with a highly-sensitive chemiluminescent enzyme immunoassay revealed that 1) thyrotoxicosis does not affect serum G-CSF level, 2) serum G-CSF level during antithyroid drug treatment does not play an important role in development of agranulocytosis, 3) the maximum serum G-CSF level in the course of agranulocytosis is related to the responsiveness of bone marrow to G-CSF and the recovery time from agranulocytosis.

Key words: Granulocyte colony-stimulating factor (G-CSF), Chemiluminescent enzyme immunoassay, Methimazole induced-agranulocytosis


AGRANULOCYTOSIS is the most serious and potentially fatal side effect of antithyroid drug therapy for hyperthyroidism due to Graves’ disease. It was previously suggested that toxic factors involving some essential metabolic pathway might be the underlying cause, while several studies have indicated that immune mechanisms probably mediate the occurrence of granulocytopenia [1, 2]. However, the pathogenic mechanism for the granulocyte depression induced by antithyroid drug has not been established.

Granulocyte colony-stimulating factor (G-CSF) is one of the hematopoietic cytokines, which plays an important role in granulocyte proliferation and activation [3]. Recombinant human G-CSF (rhG-CSF) was employed in treatment of antithyroid drug-induced moderate granulocytopenia and severe forms of agranulocytosis [4].

We developed a highly sensitive assay to determine G-CSF levels by chemiluminescent enzyme immunoassay, which is sensitive enough to accurately measure
serum G-CSF levels in normal subjects [5]. Although, serum G-CSF level in patients with Graves’ disease has been reported using this highly-sensitive assay [6], time course changes in serum G-CSF level in patients with antithyroid drug-induced agranulocytosis have yet to be well documented.

The present study was performed by serially measuring serum G-CSF levels by the highly-sensitive assay in patients with agranulocytosis to determine 1) whether G-CSF plays some role in developing agranulocytosis induced by antithyroid drug, and 2) whether serum G-CSF levels during rhG-CSF treatment correlate with the effectiveness of the therapy.

Materials and Methods

Subjects

Fifty-four patients with Graves’ disease (12 males and 42 females with mean age of 36.7 ± 12.6 (SD) years) including 6 patients with agranulocytosis induced by methimazole (MMI) for hyperthyroidism were studied. The clinical findings of the patients with agranulocytosis are summarized in Table 1. The institutional ethics board of the Kuma Hospital approved our research project, and we obtained informed consent from all patients.

Protocol for treatment of agranulocytosis

An automatic blood cell analyzer (Technicon H1; Bayer, Tarrytown, system, NY) was used to determine circulating white blood cell (WBC) and neutrophil counts. Agranulocytosis was defined as circulating neutrophil count of less than 0.5 × 10^9/L. When agranulocytosis developed, MMI was immediately discontinued, and rhG-CSF (100 μg) was given subcutaneously once a day until circulating neutrophil counts increased above 1.0 × 10^9/L. Recovery time was defined as the number of days required for the circulating neutrophils to attain a count of greater than 1.0 × 10^9/L. None of the subjects was given any other treatment for agranulocytosis in the course of the disease. Specimen of bone marrow was obtained at the time of agranulocytosis onset to determine the decrease in granulocyte series.

Serum G-CSF concentration was serially measured throughout the course of agranulocytosis: before the administration of MMI, during MMI treatment, at the time of agranulocytosis onset, and during rhG-CSF treatment until recovery of myelopoiesis. During the treatment with rhG-CSF, the serum was obtained immediately before the injection of rhG-CSF. The serum for G-CSF measurement was frozen at −40°C until utilized.

Determination of serum G-CSF levels

The serum G-CSF level was determined with a chemiluminescent enzyme immunoassay [5]. The sensitivity of this assay was 0.5 pg/ml and the measurable range in clinical application was from 1.0 to 10000 pg/ml. The intra-assay and inter-assay coefficients of variation were less than 5%. In the normal subjects, the G-CSF levels were distributed from 2.0 to 31.9 pg/ml (n = 116) with a mean of 13.8 ± 7.7 (SD) pg/ml, with no significant daily variations [5]. The serum samples for G-CSF measurement were stable for 6 months when stored at −30°C.

Statistical analysis

Data are expressed as mean ± SD. Statistical com-

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Weeks of MMI therapy</th>
<th>MMI dose (mg/day)</th>
<th>WBC at onset × 10^9/L</th>
<th>Neutrophil count at onset × 10^9/L</th>
<th>Serum G-CSF level at onset (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>f</td>
<td>19</td>
<td>4</td>
<td>20</td>
<td>2.1</td>
<td>0.44</td>
<td>88</td>
</tr>
<tr>
<td>2</td>
<td>f</td>
<td>54</td>
<td>4</td>
<td>30</td>
<td>1.8</td>
<td>0.36</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>f</td>
<td>53</td>
<td>8</td>
<td>20</td>
<td>2.3</td>
<td>0.29</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>f</td>
<td>55</td>
<td>12</td>
<td>15</td>
<td>2.8</td>
<td>0.22</td>
<td>220</td>
</tr>
<tr>
<td>5</td>
<td>f</td>
<td>31</td>
<td>8</td>
<td>30</td>
<td>1.6</td>
<td>0.09</td>
<td>197</td>
</tr>
<tr>
<td>6</td>
<td>f</td>
<td>26</td>
<td>6</td>
<td>30</td>
<td>3.9</td>
<td>0.07</td>
<td>51</td>
</tr>
</tbody>
</table>

f; female.
Comparisons of serum G-CSF levels between the groups were performed by Wilcoxon’s unpaired test. Comparison of changes in serum G-CSF level before and after MMI treatment in the patients complicated with agranulocytosis was made by Wilcoxon’s paired test. The relation between serum G-CSF level and serum FT$_4$ concentration, circulating neutrophil count or the recovery time required for circulating neutrophil to attain a count of greater than $1.0 \times 10^9$ /L was evaluated by linear correlation analysis. Statistical significance was considered where the possibility of chance occurrence was $p<0.05$.

**Results**

**Serum G-CSF levels in patients with Graves’ disease**

Mean of serum G-CSF levels in patients with Graves’ disease was $12.6 \pm 8.0$ (SD) pg/ml, which is not significantly different from normal subjects. Serum G-CSF level in patients with Graves’ disease did not correlate with serum FT$_4$ level or circulating granulocyte counts. In two of the untreated hyperthyroid patients, circulating neutrophil counts were less than $1.0 \times 10^9$ /L (0.96 $\times 10^9$ /L and 0.88 $\times 10^9$ /L), and their serum G-CSF levels were 6.9 pg/ml and 19 pg/ml. Their circulating neutrophil counts were increased to greater than $1.0 \times 10^9$ /L when serum FT$_4$ levels became normal with MMI treatment.

**Serum G-CSF levels during the clinical course of agranulocytosis**

**Representative Cases**

**Patient 1.** A 19-year-old woman was admitted to the Kuma Hospital because of methimazole (MMI)-induced agranulocytosis. Fig. 1 shows the clinical course of the subject. She was diagnosed with Graves’ disease and had been treated with MMI (20 mg daily) for one month before admission. On the day of admission, routine examination of blood cell count revealed that white blood cell (WBC) count was decreased to $2.0 \times 10^9$/L and neutrophil count was $0.44 \times 10^9$/L although she was asymptomatic. The bone marrow examination showed a prominent decrease in mature granulocytes (Table 2). After the second injection of rhG-CSF (100 µg a day), swift recovery of circulating neutrophil count was observed. Serum G-CSF level was 88 pg/ml on the day of agranulocytosis onset, but increased remarkably to 1100 pg/ml on the third hospital day.

**Patient 2.** A 54-year-old woman was admitted to the Kuma Hospital because of MMI-induced agranulocytosis. She had had undergone subtotal thyroidectomy for hyperthyroidism due to Graves’ disease 11 years earlier. One month before admission, she was found to have relapsed hyperthyroidism; at that time serum FT$_4$ level was 84.2 pmol/l and 24-hour radioactive iodine uptake rate was 53.3% and she was started on MMI (30 mg/day). On the day of admission routine examination of blood cell count revealed that the neutrophil count was $0.36 \times 10^9$/L although she was asymptomatic. The

![Fig. 1. Changes in serum G-CSF level (○—○) and circulating neutrophil count (■—■) in the course of rhG-CSF treatment in patients 1 and 2 with MMI-induced agranulocytosis. Arrows indicate 100 µg of rhG-CSF given subcutaneously once a day.](image-url)
neutrophil count remained decreased in spite of daily injection of rhG-CSF (100 μg), but after the 12th injection of rhG-CSF, circulating neutrophil count returned to 4.8 × 10^9/L (Fig. 1). Serum G-CSF level was 66 pg/ml on the day of admission, increased to the maximum of 5700 pg/ml when circulating neutrophil count became zero, and was steeply decreased to 19 pg/ml when circulating neutrophil count returned to normal. Bone marrow examination at the time of admission revealed severe maturation arrest in myeloid cells (Table 2).

Before the onset of agranulocytosis, serum G-CSF levels of the patients with agranulocytosis were 14.0 ± 4.9 (mean ± SD) pg/ml at untreated hyperthyroid state and 18.3 ± 6.8 (SD) pg/ml during MMI treatment. The difference in serum G-CSF level between before and after MMI treatment of the patients with agranulocytosis was not significant. Also during MMI treatment, there was no significant difference in serum G-CSF level between the patients with agranulocytosis and the patients uncomplicated with agranulocytosis (12.6 ± 8.2 (mean ± SD) pg/ml).

At the onset of agranulocytosis, serum G-CSF level ranged from 19 to 220 pg/ml, with the mean of 106.8 ± 82.2 (SD) pg/ml (Table 1). There was no correlation between serum G-CSF levels and the neutrophil count at the onset of agranulocytosis (r = −0.330, p = 0.523). The recovery time required for circulating neutrophil to attain a count of 1.0 × 10^9/L did not correlate with serum G-CSF levels or the neutrophil count at the onset of agranulocytosis.

After the start of treatment with rhG-CSF, serum G-CSF level remarkably increased in all subjects and there was a single peak in serum G-CSF level during the period of agranulocytosis: maximum serum G-CSF level was not sustained, however, and a rapid decrease of serum G-CSF level was observed immediately after the sharp increase of serum G-CSF level. In patients 1 and 6, the sharp decrease of serum G-CSF level was observed after the cessation of rhG-CSF treatment, while in the remaining patients, the decrease of serum G-CSF level occurred during rhG-CSF treatment. In particular in patient 2, the sharp decrease of serum G-CSF was observed although circulating neutrophil count remained zero. After sharp decrease of serum G-CSF level, the pronounced recovery of circulating neutrophil count occurred.

**Findings of bone marrow examination and serum G-CSF level**

In patient 3 in whom serum G-CSF level was 19 pg/ml at the onset of agranulocytosis, myeloid cells in granulocyte series remained normal (Table 2).

In the patients with prominent decrease in more immature granulocyte series in bone marrow (patients 2 and 4, Table 2), maximum serum G-CSF levels during rhG-CSF treatment were quite high and the duration of agranulocytosis was long. As shown in Fig. 2, maximum serum G-CSF level in the course of agranulocytosis correlated significantly with the recovery time (r = 0.824, p<0.05).

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**Table 2.** Results of bone marrow examination, maximum serum G-CSF levels and the recovery time

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCC × 10^9/L</td>
<td>4.8</td>
<td>13.1</td>
<td>22.3</td>
<td>6.6</td>
<td>7.9</td>
<td>6.7</td>
</tr>
<tr>
<td>M/E Ratio</td>
<td>0.5</td>
<td>0.1</td>
<td>2.4</td>
<td>0.1</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Differential cell count × 10^9/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloblast</td>
<td>0.19</td>
<td>5.18</td>
<td>4.41</td>
<td>2.89</td>
<td>2.08</td>
<td>1.6</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>1.65</td>
<td>(–)</td>
<td>12.32</td>
<td>0.13</td>
<td>2.24</td>
<td>4.8</td>
</tr>
<tr>
<td>Myelocyte</td>
<td>1.55</td>
<td>(–)</td>
<td>17.64</td>
<td>(–)</td>
<td>2.56</td>
<td>2.9</td>
</tr>
<tr>
<td>Metamyelocyte</td>
<td>2.33</td>
<td>(–)</td>
<td>19.32</td>
<td>(–)</td>
<td>0.64</td>
<td>(–)</td>
</tr>
<tr>
<td>Band form</td>
<td>(–)</td>
<td>(–)</td>
<td>38.72</td>
<td>(–)</td>
<td>(–)</td>
<td>(–)</td>
</tr>
<tr>
<td>Segmented</td>
<td>(–)</td>
<td>(–)</td>
<td>21.11</td>
<td>(–)</td>
<td>(–)</td>
<td>(–)</td>
</tr>
<tr>
<td>Maximum G-CSF level (pg/ml)</td>
<td>1100</td>
<td>5700</td>
<td>1800</td>
<td>2000</td>
<td>1800</td>
<td>1000</td>
</tr>
<tr>
<td>Recovery time (day)</td>
<td>3</td>
<td>13</td>
<td>5</td>
<td>11</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

* NCC indicates nucleated cell counts.
* M/E ratio indicates myeloid-erythroid ratio.
* Recovery time was defined as the number of days required for circulating neutrophil count to be >10 × 10^9/L.
Discussion

This study showed that serum G-CSF levels in patients with Graves’ disease were not different from normal subjects. Itaka et al. reported that serum G-CSF level in untreated patients with Graves’ disease was significantly higher than that in healthy subject, but did not correlate with serum thyroid hormone levels [6]. Our study also showed that serum G-CSF levels in patients with Graves’ disease did not correlate with serum FT₄ levels. These findings indicate that serum G-CSF level is not affected by serum FT₄ level.

This study showed that in two of the untreated patients in whom serum G-CSF levels were normal, circulating neutrophil counts were less than normal at untreated hyperthyroid state and increased to normal at euthyroid state with methimazole treatment. Itaka et al. also reported that in 17 untreated patients with Graves’ disease, the circulating neutrophil counts were relatively small (<2.0 × 10⁹/L), increased to normal after methimazole treatment, and were not related to changes in serum G-CSF level [6]. These findings suggest that in some untreated patients with Graves’ disease, thyrotoxicosis may become a cause of granulocytopenia unmediated by serum G-CSF level.

This study showed that in the patients with Graves’ disease complicated with agranulocytosis, no significant changes in serum G-CSF level during antithyroid drug treatment before the development of agranulocytosis. This study also showed that, during antithyroid drug treatment, serum G-CSF level in the patients complicated with agranulocytosis was not significantly different from that in the patients without agranulocytosis. We therefore concluded that an inability to secrete G-CSF is not the cause of agranulocytosis induced by antithyroid drug.

The relation between serum G-CSF level and circulating neutrophil count is complex. For example, it has been reported that in the patients with granulocytopenia due to aplastic anemia, serum G-CSF levels are quite high and correlate inversely with circulating neutrophil counts [7], while, in patients with inflammatory disease, both serum G-CSF levels and circulating neutrophil count are elevated as we have reported in patients with subacute thyroiditis [8]. In this study, although the mean of serum G-CSF level was elevated at the onset of agranulocytosis, there was no correlation between serum G-CSF level and circulating neutrophil count: for instance, in patient 3 with normal bone marrow finding, serum G-CSF level at the onset of agranulocytosis was not elevated. These results indicate that the increase of serum G-CSF level during the period of agranulocytosis might be related to the ability of the bone marrow to resume the generation of granulocyte in response to G-CSF rather than to circulating neutrophil counts. Interestingly, maximum serum G-CSF level in the course of agranulocytosis correlated with the recovery time for circulating neutrophil to attain a count of 1.0 × 10⁹/L. It was previously reported that in drug-induced granulocytopenia, the recovery time is strongly dependent on the findings of bone marrow examination, that is, the number and maturation of granulocyte precursor [9]. We thus concluded that maximum serum G-CSF level in the course of agranulocytosis provides the ability of the bone marrow to resume the generation of granulocyte in response to G-CSF; namely, the higher the maximum serum G-CSF level, the longer the recovery time of granulocytes.

It was reported that the half-life of serum G-CSF is 2.05 ± 0.33 (SE) hours in normal subjects when rhG-CSF is given subcutaneously [10]. It is also thought that the clearance of G-CSF from serum is strongly dependent on the binding of G-CSF to the receptor on neutrophil surface [11]. The half-life of serum G-CSF in patients with agranulocytosis is not known. Although we obtained the serum sample for G-CSF measurement approximately 24 hour after the last rhG-CSF administration during rhG-CSF treatment, serum G-CSF
level during rhG-CSF treatment for agranulocytosis might represent both endogenous G-CSF and exogenously administered rhG-CSF. Our observation of the sharp decrease in serum G-CSF level with pronounced recovery of circulating neutrophil count might be due to the binding of G-CSF to the receptor on neutrophil and myeloid precursor cell surface. The sharp decrease in serum G-CSF level of patient 2 when circulating neutrophil count remained zero might indicate maturation and proliferation of myeloid cell series in the bone marrow. Thus the sharp decrease of serum G-CSF level in patients with severe maturation arrest in granulocyte series in bone marrow may predict that the recovery of circulating neutrophil count will occur in the next few days.

It was reported that treatment with rhG-CSF in antithyroid drug-induced granulocytopenia might have two beneficial effects: shortening of the duration of complete agranulocytosis and acceleration of the increase of granulocytes in the recovery phase [12]. Since this was not a randomized study, we could not conclude that rhG-CSF treatment was responsible for the rapid recovery of granulocytes and the favorable outcome in our patients. However, in three of our patients (nos. 1, 5 and 6) with the prominent decrease in mature precursors in myeloid cell series, circulating neutrophil count recovered after 3, 6 and 3 days, respectively. These were much more rapid than expected based on the literature [8]. In patient 2 with no promyelocytes and more mature myeloid precursors in the bone marrow, the recovery of circulating neutrophil count was observed at 13 days. In this patient, whether rhG-CSF treatment shortened the recovery time is unclear. There are case reports that patients with drug induced-agranulocytosis, in whom bone marrow was as hypoplastic as in patient 2, were successfully treated with a relatively high dose of rhG-CSF (300 μg/day or 600 μg/day) [13, 14]. It is also reported that the dose of rhG-CSF with 75 μg/day is not sufficient in patients with severe agranulocytosis [4, 15]. In patient 2, there is a possibility that the dose of rhG-CSF was insufficient to activate the ability of the bone marrow to resume the generation of granulocyte. Serum G-CSF level of this patient reached to 5700 pg/ml which exceeded a peak of serum G-CSF level when rhG-CSF 100 μg is administered subcutaneously [9]. Maximum serum G-CSF level of patient 2 was twice as high as that in the patient who recovered spontaneously from MMI-induced agranulocytosis, which we reported previously [16]. If we had chosen a higher dose of rhG-CSF injection for the treatment of patient 2, the peak of serum G-CSF level would have reached to 5700 pg/ml more rapidly, and this patient’s duration of complete agranulocytosis might have been shortened.

Antithyroid drug treatment is the major therapy for Graves’ hyperthyroidism in Japan [17]. Thought rare, adverse side effects of antithyroid drugs including agranulocytosis and anti-myeloperoxidase anti-neutrophil cytoplasmic antibodies (MPO-ANCA) in serum induced by propylthiouracil occurs in some patients with Graves’ disease [18]. Development of new type of antithyroid drug without such potentially fatal side effects might be necessary.

In conclusion, measuring serum G-CSF levels with a highly-sensitive chemiluminescent enzyme assay revealed that serum G-CSF level during antithyroid drug treatment is unrelated to the development of agranulocytosis, but that maximum serum G-CSF level in the course of agranulocytosis is related to the responsiveness of bone marrow to G-CSF and the recovery time from agranulocytosis.

Reference


