Phase I and Early Phase II Studies on Human Urinary Colony Stimulating Factor

Kazuo Motoyoshi, MD, Fumimaro Takaku, MD, Kazuo Kusumoto, MD, Yasusada Miura, MD, Takeo Yamanaka, MD and Ken Kimura, MD

This report demonstrates the first trial for the clinical application of human urinary colony stimulating factor (CSFhu) which was highly purified and well characterized in our laboratory. In the Phase I study, 6 healthy volunteers were administered with 2.5 x 10^5 to 10^6 units of CSFhu intravenously. CSFhu did not show any severe side effects, although slight depression of maximum blood pressure was observed in the group injected with 10^6 units CSFhu and one volunteer who received 5 x 10^5 units CSFhu complained sweating and itching during the infusion. In the Phase II study, six cases suffering from leukocytopenia induced by anticancer drugs or irradiation were treated with 7 day intravenous CSFhu injections. Although recovery of leukocyte number was not observed in the group injected with 7 x 10^6 units CSFhu, complete or partial recovery of leukocyte and granulocyte number was observed in the group injected with 1.3 to 1.4 x 10^7 units CSFhu. Phase II study in a large scale is under way to evaluate further the effectiveness of CSFhu on leukocytopenic patients.

Key Words: Phase I and II studies, CSFhu, Leukocytopenia, Anticancer drugs

Human urinary colony stimulating factor (CSFhu) had been purified and its physico-chemical and biological characteristics were studied by several groups. In our laboratory, CSFhu was highly purified in a large scale and its biological functions were studied using in vitro agar culture system. Our previous experiments demonstrated that CSFhu could indirectly stimulate the granulocyte colony formation by human bone marrow cells through the stimulation of production of colony stimulating activity from the human bone marrow macrophages. In this report we describe the first trial of the intravenous injection of CSFhu to healthy volunteers and patients with leukocytopenia.

MATERIALS AND METHODS

Purification of CSFhu

The human urinary colony stimulating factor (CSFhu) was partially purified according to the procedures described previously. Briefly, pooled urine was concentrated by ultrafiltration using the Amicon Model DC-2 concentrator, and fractionated by the batchwise DEAE cellulose chromatography. By this method, colony inhibiting factor was completely excluded from the CSFhu fraction. CSFhu was further purified on Sephadex G-150 column and used in Phase I and early Phase II studies. The partially purified CSFhu used in this study has a specific activity of approximately 150,000 units/mg protein in a standard assay system. Acute, subacute and chronic toxicities of CSFhu on experimental animals were carefully examined in
the Green Cross Corporation and the existence of toxic substances in the CSF$_{HU}$ fraction was completely denied.

**Phase I study**

Six healthy volunteers listed in Table 1 who gave informed consent were intravenously injected for one hour with $2.5 \times 10^5$ to $1 \times 10^6$ units of CSF$_{HU}$, which was dissolved into 100 to 200 ml of saline. Blood pressure, pulse and respiration rates, and body temperature were measured at every ten minutes during the intravenous injection. General conditions were also observed carefully during and after the injection. Complete blood cell counting and differential counting of leukocytes were performed on the seventh day and at thirty minutes before and on the second and seventh days after the injection. Serum levels of total protein, GOT, GPT, Al-P, LDH, creatinine and BUN were measured on the seventh day before and on the second and seventh days after the injection. Urinalysis was also performed on the same days. Electrocardiogram was performed on the seventh day before and after the injections.

**Early Phase II study**

Six patients with leukopenia induced by anticancer therapy who gave their informed consent were treated with intravenous infusions of CSF$_{HU}$ as listed in Table 2. Cases 1 and 2 were treated with infusions of $10^6$ units CSF$_{HU}$/day for seven days. Case 3 was injected with $10^6$ units, $2 \times 10^6$ units and $3 \times 10^6$ units CSF$_{HU}$/day on the first, second, and third day respectively, and then with $2 \times 10^6$ units of CSF$_{HU}$/day during the fourth and seventh day. Cases 4 and 5 were treated with $10^6$ units CSF$_{HU}$/day on the first day then with $2 \times 10^6$ units CSF$_{HU}$/day for the following 6 days. Case 6 was injected with $2 \times 10^6$ units CSF$_{HU}$/day for 7 days.

Hematological parameters were measured on every two days until 15th day after the initial administration of CSF$_{HU}$.

**RESULTS**

1) **Phase I Study**

As shown in Table 1, six healthy volunteers injected with $2.5 \times 10^5$ to $10^6$ units CSF$_{HU}$ were observed with respect to blood pressure, pulse and respiration rates, body temperature and other physical findings during the drip infusions. Volunteers No. 1 and No. 2 showed no remarkable change. Volunteer No. 3 who received $5 \times 10^5$ units CSF$_{HU}$ complained itching on conjunctiva and sweating during the infusion, but these symptoms disappeared within several hours after the end of infusion. Volunteers No. 4, 5 and 6 who received $10^6$ units CSF$_{HU}$ showed slight decreases in maximum blood pressure during the infusions while volunteers No. 1, 2 and 3 who received $2.5 \times 10^5$

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Age</th>
<th>Sex</th>
<th>Dose of CSF$_{HU}$/administered</th>
<th>BP</th>
<th>Pulse rate</th>
<th>Respiration rate</th>
<th>BT</th>
<th>Other findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>43</td>
<td>M</td>
<td>250,000 units</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>(-)</td>
</tr>
<tr>
<td>No. 2</td>
<td>54</td>
<td>M</td>
<td>500,000 units</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>(-)</td>
</tr>
<tr>
<td>No. 3</td>
<td>46</td>
<td>M</td>
<td>500,000 units</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>Sweating</td>
</tr>
<tr>
<td>No. 4</td>
<td>40</td>
<td>M</td>
<td>1,000,000 units</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>Itching</td>
</tr>
<tr>
<td>No. 5</td>
<td>51</td>
<td>M</td>
<td>1,000,000 units</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>(-)</td>
</tr>
<tr>
<td>No. 6</td>
<td>57</td>
<td>M</td>
<td>1,000,000 units</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>(-)</td>
</tr>
</tbody>
</table>

→ : no change, Δ : slightly depressed.
or $5 \times 10^6$ units CSF_HU did not show any changes in blood pressure (Figure 1). Although blood pressure of No. 5 recovered to the initial level at the end of infusion, blood pressure of No. 4 and No. 6 was still low at the end of infusion, and complete recovery of blood pressure to the initial level was achieved within several hours after the end of infusions. Serum levels of total protein, GOT, GPT, Al-P, LDH, creatinine and BUN of these 6 volunteers did not change after CSF_HU infusion. Concentration of glucose and protein in urine also did not change. No remarkable change was found in hemoglobin and hematocrit level, in the numbers of platelets, red blood cells and white blood cells and in differential counting of leukocytes. Pattern of electrocardiogram did not change after the infusions.

2) Early Phase II study (summarized in Table 2)

Case 1 (male, 84 years old) and Case 2 (female, 75 years old): They had been suffered from esophageal carcinoma and treated with 5-Fluoro-uracil (5 mg/kg/day) and Krestin (3 g/day). Because leukocytopenia gradually developed, chemotherapy was ceased on the 75th day (case 1) and on the 40th day after the initial administration of these anticancer drugs respectively. At the end of chemotherapy, their leukocyte numbers were $3,800/mm^3$ (case 1) and $3,600/mm^3$ (case 2), and trial injections of CSF_HU (10^6 units/day for 7 days, total $7 \times 10^6$ units) were done. Increment in leukocyte numbers was not observed on 8th and 15th days after the initial administration of CSF_HU (no response).

Case 3 (male, 66 years old): He was suffered from esophageal carcinoma and received X-ray irradiation (total; 2720 rad). Irradiation was stopped because pancytopenia developed, and CSF_HU was administered for 7 days (total; $1.4 \times 10^7$ units). As shown in Figure 2, the increment in leukocyte number accompanied with the increment in the

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<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Treatment</th>
<th>CSF_HU Injection</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>84</td>
<td>Esophageal Ca</td>
<td>SFU, Krestin</td>
<td>$10^6 u/day$</td>
<td>w 3</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>75</td>
<td>Esophageal Ca</td>
<td>SFU, Krestin</td>
<td>$10^6 u/day$</td>
<td>w 3</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>66</td>
<td>Esophageal Ca</td>
<td>Irradiation</td>
<td>$10^6 u/day$</td>
<td>w 3</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>17</td>
<td>Ca of nasal cavity</td>
<td>HDR, EDX, BLM, VDS, PSL</td>
<td>$10^6 u/day$</td>
<td>w 3</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>80</td>
<td>Multiple Myeloma</td>
<td>VCR, Melphalan</td>
<td>$10^6 u/day$</td>
<td>w 3</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>17</td>
<td>Ca of nasal cavity</td>
<td>MTX, ACNU</td>
<td>$10^6 u/day$</td>
<td>w 3</td>
</tr>
</tbody>
</table>

* -, +, and ++ in this column demonstrate no recovery, partial and complete recovery.

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granulocyte number was observed, and his leukocyte number reached to normal leveal on 15th day after the initial injection of CSF$_{HU}$ (complete response). His platelet and reticulocyte numbers and hemoglobin level were still low even on the 21st day after the initial CSF$_{HU}$ injection.

Case 4 (male, 17 years old): He was given CSF$_{HU}$ (total; 1.3X10$^7$ units) for 7 days to treat the pancytopenia induced by chemotherapy (Adriamycin 70 mg, Cyclophosphamide 1,050 mg, Bleomycin 14 mg, Vindesine 3 mg and Prednisolone 100 mg) against generalized lymphnode metastasis from undifferentiated carcinoma in his nasal cavity. As shown in Figure 3, his leukocyte and granulocyte number began to increase during CSF$_{HU}$ infusion therapy and reached to normal level on the 15th day after the initial administration of CSF$_{HU}$. His hemoglobin level and the platelet number gradually increased, but his platelet numbers did not reach to the prior level even on the 15th day after the initial injection (complete response).

Case 5 (female, 80 years old): She was suffered from multiple myeloma (Ig A, $\lambda$) and treated twice with VMC therapy (Vincristine 1 mg, Melphalan 4 mg and Carbarylquinone 2 mg). Because her leukocyte and granulocyte number gradually decreased after 2nd VMC therapy, CSF$_{HU}$ was administered (total 1.3X10$^7$ units). As shown in Figure 4, leukocyte and granulocyte numbers increased to some degree but did not reach to the normal levels on the 15th day (partial response).

Case 6 (the same patient as case 4): This trial injection of CSF$_{HU}$ was done after the chemotherapy which was performed after the complete recovery from the leukocytopenia by the administration of CSF$_{HU}$. Leukocyte number started to increase on the third day and reached to 2,900 on the 15th day after the initial injection of CSF$_{HU}$ (partial response).

**DISCUSSION**

We described the results of Phase I and early Phase II studies for clinical application of colony stimulating factor purified from normal human urine$^{2-4}$. In the previous in vitro studies$^{5-4}$, we found the following points:

1) CSF$_{HU}$ is a glycoprotein of which molecular weight is 85,000 daltons.
Phase I and II studies on CSF_HU

CASE 5: Y.Q (80y.o., female) (multiple myeloma)

<table>
<thead>
<tr>
<th>VCH</th>
<th>Melphalan</th>
<th>CSF_HU</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MARCH " APRIL " MAY 1982

**Fig. 4. Clinical course of Case 5.**

2) CSF_HU can stimulate the granulocytic colony formation by human bone marrow cells.

3) CSF_HU can not stimulate that when monocytes were excluded from the bone marrow cells.

4) ¹²⁵I-CSF_HU can bind to the human monocytes.

These observations suggest that CSF_HU can indirectly stimulate granulocytic colony formation through the stimulation of monocytes to produce the colony stimulating activity which is active on granulocyte colony forming cells.

On the bases of these observations, we have tried to apply the CSF_HU on the patients suffering from severe leukocytopenia induced by administration of anticancer drugs or x-ray irradiation. In the Phase I study, we found that CSF_HU did not give any severe side effects to the healthy volunteers, although slight and transient depression of maximum blood pressure was observed in the group injected with 10⁶ units CSF_HU. To overcome the depression of blood pressure, the speed of drip infusion of CSF_HU was slowed down from 10⁶ units CSF_HU/60 min to 10⁶ units CSF_HU/120 min in the Phase II study.

In the Phase II study, six trial administrations of CSF_HU were done to 5 leukocytopenic patients. In the two out of four patients who were injected with 1.3 to 1.4 ×10⁷ units CSF_HU, complete recovery of leukocyte numbers was observed on the 15th day after the initial administration of CSF_HU, and partial recovery was observed in the other two patients in this group. Recovery of leukocyte numbers, on the other hand, was not observed in the two patients injected with 7×10⁶ units CSF_HU. From these observations, effective doses of CSF_HU may be more than 10⁷ units. Phase II study in a large scale will be required to evaluate the effectiveness of CSF_HU on the leukocytopenic patients.

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**REFERENCES**