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

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This report demonstrates the first trial for the clinical application of human urinary colony stimulating factor (CSF_{HU}) which was highly purified and well characterized in our laboratory. In the Phase I study, 6 healthy volunteers were administered with 2.5×10^5 to 10^6 units of CSF_{HU} intravenously. CSF_{HU} did not show any severe side effects, although slight depression of maximum blood pressure was observed in the group injected with 10^6 units CSF_{HU} and one volunteer who received 5×10^5 units CSF_{HU} 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 CSF_{HU} injections. Although recovery of leukocyte number was not observed in the group injected with 7×10^6 units CSF_{HU}, complete or partial recovery of leukocyte and granulocyte number was observed in the group injected with 1.3 to 1.4×10^7 units CSF_{HU}. Phase II study in a large scale is under way to evaluate further the effectiveness of CSF_{HU} on leukocytopenic patients.

Key Words: Phase I and II studies, CSF_{HU}, Leukocytopenia, Anticancer drugs

Human urinary colony stimulating factor (CSF_{HU}) had been purified and its physico-chemical and biological characteristics were studied by several groups¹⁻⁶. In our laboratory, CSF_{HU} was highly purified in a large scale and its biological functions were studied using in vitro agar culture system^{2,4}. Our previous experiments demonstrated that CSF_{HU} 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 macrophages⁴. In this report we describe the first trial of the intravenous injection of CSF_{HU} to healthy volunteers and patients with leukocytopenia.

MATERIALS AND METHODS

Purification of CSF_{HU}

The human urinary colony stimulating factor (CSF_{HU}) was partially purified according to the procedures described previously². 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 CSF_{HU} fraction. CSF_{HU} was further purified on Sephadex G-150 column and used in Phase I and early Phase II studies. The partially purified CSF_{HU} used in this studies has a specific activity of approximately 150,000 units/mg protein in a standard assay system². Acute, subacute and chronic toxicities of CSF_{HU} on experimental animals were carefully examined in

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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 2.5×10^5 to 1×10^6 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, 2×10^6 units and 3×10^6 units CSF_{HU}/day on the first, second, and third day respectively, and then with 2×10^6 units of CSF_{HU}/day during the fourth and seventh day. Cases 4 and 5 were treated with 10^6 units CSF_{HU} on the first day then with 2×10^6 units CSF_{HU}/day for the following 6 days. Case 6 was injected with 2×10^6 units CSF_{HU} 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 2.5×10^5 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 5×10^5 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 2.5×10^5

Table 1. Changes of blood pressure, pulse and respiration rate, and body temperature during CSF_{HU} infusions.

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

→ : no change, ↘ : slightly depressed.

or 5×10^5 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

Fig. 1. Changes of blood pressure during CSF_{HU} intravenous infusion.

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 (3g/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/mm³ (case 1) and 3,600/mm³ (case 2), and trial injections of CSF_{HU} (10^6 units/day for 7 days, total 7×10^6 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; 1.4×10^7 units). As shown in Figure 2, the increment in leukocyte number accompanied with the increment in the

Table 2. Summary of early Phase II Study of CSF_{HU}.

Case	Sex	Age	Diagnosis	Treatment	CSF _{HU} Injection			WBC			
					Dose	Period	Total dose	day1	day3	day15	Recovery [*]
1	M	84	Esophageal Ca	5FU, Krestin	10^6 u/day	7days	7×10^6 u	3800	3300	3600	-
2	F	75	Esophageal Ca	5FU, Krestin	10^6 u/day	7days	7×10^6 u	3600	3900	3500	-
3	M	66	Esophageal Ca	Irradiation	10^6 u/day	1day					
					2×10^6 u/day	5days					
					3×10^6 u/day	1day	1.4×10^7 u	1700	2800	4600	++
4	M	17	Ca of nasal cavity	HONR, EDX, BLM	10^6 u/day	1day					
				VDS, PSL	2×10^6 u/day	6days	1.3×10^7 u	800	1600	5300	++
5	F	80	Multiple Myeloma	VCR, Melphalan	10^6 u/day	1day					
				CQ	2×10^6 u/day	6days	1.3×10^7 u	1600	2500	2600	+
6	M	17	Ca of nasal cavity	MTX, ACNU							
				MMC	2×10^6 u/day	7days	1.4×10^7 u	500	2100	2900	+

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

granulocyte number was observed, and his leukocyte number reached to normal level 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; 1.3×10^7 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, λ) and treated twice with VMC therapy

Fig. 2. Clinical course of Case 3.

CASE 4: S.H. (17yo, male)
(nasal tumor, undiff. Ca)

Fig. 3. Clinical course of Case 4.

(Vincristine 1 mg, Melphalan 4 mg and Carbazylquinone 2mg). Because her leukocyte and granulocyte number gradually decreased after 2nd VMC therapy, CSF_{HU} was administered (total 1.3×10^7 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^{2,4)}, we found the following points:

1) CSF_{HU} is a glycoprotein of which molecular weight is 85,000 daltons.

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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