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Intravenous Single and Two Week Repeated Dose Toxicity Studies of Rice Cells-derived Recombinant Human Granulocyte-Macrophage Colony Stimulating Factor on Rats

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Recombinant human granulocyte-macrophage colony stimulating factor (hGM-CSF) regulates proliferation and differentiation of hematopoietic progenitor cells and modulates function of the mature hematopoietic cells. In the previous study, we reported that hGM-CSF could be produced in transgenic rice cell suspension culture, termed rhGM-CSF. In the present study we examined the single and repeated dose toxicity of rice cells-derived hGM-CSF in SD rats. During single dose toxicity study for 7 days, there were no any toxic effects at any dose of from 10 to 1000 μg/kg. The lethal dose (LD_{50}) was not found in this range. Moreover, repeated dose toxicity study of 14-days period and at the doses of 50 and 200 μg/kg (*i.v.*) of rhGM-CSF did not show any changes in food and water intake. There were also no significant changes in both body and organ weights between the control and the test groups. The hematological and blood biochemical parameters were statistically not different in all the groups. These results suggest that rhGM-CSF has no toxicity in SD rats.

Key words: Recombinant hGM-CSF, Rice cells, Single dose toxicity, Repeated dose toxicity, SD rats.

INTRODUCTION

Human granulocyte-macrophage colony-stimulating factor (hGM-CSF) was the first CSF to be purified, cloned and expressed using recombinant DNA technology (Armitage, 1998). This protein is known to have the activity of proliferation and differentiation of hematopoietic progenitor cells and modulates function of the mature hematopoietic cells (Wadhwa *et al.*, 1999; Morrissey *et al.*, 1987). hGM-CSF has increasing clinical applications in the treatment of neutropenia and aplastic anemia and has greatly reduced the infection risk associated with bone marrow transplantation by accelerating the response of neutrophils (Rasko *et al.*, 1994). Either alone or in combination with other therapeutic agents, hGM-CSF is essential for cancer treatment. This protein is produced as a biologically active form by using various hosts such as bacteria *Escherichia coli (E. coli)*, yeast *Saccharomyces cerevisiae* and mammalian cells Chinese hamster ovarian (CHO) cells, which are termed molgramostim, sargramostim and regramostim, respectively (Armitage, 1998). Recently, hGM-CSF was also successfully produced as a biologically active protein in transgenic tomato suspension cultures (Kwon *et al.*, 2003). This recombinant cytokine was synthesized by the transgenic cell culture and secreted into the growth medium at 45 mg/l after 10-days cultiva-

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Abbreviation: recombinant human granulocyte-macrophage colony stimulating factor (hGM-CSF), lethal dose (LD_{50}), *Escherichia coli* (*E. coli*), Chinese hamster ovary (CHO), rice cells-derived hGM-CSF (rhGM-CSF), phosphate buffered saline (PBS), ethylenediaminetetraacetic acid (EDTA), red blood cell (RBC), white blood cell (WBC), neutrophil (NEU), lymphocyte (LYM), monocyte (MONO), eosinophil (EOS), basophil (BASO), platelet (PLT), hematocrit (HCT), mean corpuscular volume (MCV), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CRE), glucose (GLU), total cholesterol (CHO), total protein (PRO), creatine phosphokinase (CPK), albumin (ALB), total bilirubin (BIL), yeast-derived hGM-CSF (yhGM-CSF), intravenous (*i.v.*), body weight (b.wt.), standard deviation (SD).

tion. On the other hand, the same research group has expressed hGM-CSF in a higher level in transgenic rice cell suspension culture by using a rice amylase expression system and obtained a biologically active form from the culture media (Shin *et al.*, 2003).

The products differ in the specific amino acid sequences and degree of glycosylation; *E. coli*-derived molgramostim is non-glycosylated, whereas those from yeast, CHO cells and the plant cells are glycosylated. The degree of glycosylation may have important clinical implications, since it might affect antigenicity, toxicity and pharmacokinetics (Armitage, 1998; Wadhwa *et al.*, 1999; Hovgaard *et al.*, 1993; Lieschke and Burgess, 1992; Ragnhammar *et al.*, 1994).

Glycosylation pattern of each of the hGM-CSF recombinant proteins is important for varying their molecular weights. Whereas the predicted molecular weight of the mature protein in the absence of glycosylation is 14.5 kDa, extensively glycosylated hGM-CSF has been reported with a molecular weight of approximately 28 kDa (Ganz *et al.*, 1996). It is known that glycosylation of hGM-CSF is heterogeneous and is not required for the activity, although it may act to modulate the activity and stability of the protein *in vivo* (Van den Steen *et al.*, 1998).

The rapid accumulation of knowledge about the mechanisms of plant gene regulation has enabled development of economically competitive plant cell systems for production of bioactive recombinant proteins of commercial value (Daniell *et al.*, 2001). Although a possible contamination in the product by various secondary metabolites including mycotoxins is an important consideration in the use of plant cell culture systems to produce such recombinant proteins, these systems are known to offer some advantages over animal cell culture systems (Doran, 2000). First, recombinant proteins produced by transformed plant cells are more likely to be safe for human consumption, because plant pathogens, such as fungi and viruses, are easily monitored and usually not pathogenic to humans. Second, sexual crossing can generate multiple transgenic plants. Third, the ease of downstream purification and the low cost of plant culture media, which contain inexpensive major components such sucrose and salts but no macromolecules, making plant cell culture an economically attractive alternative. Therefore, plant cell culture systems may be the most favorable means of producing small-to-medium quantities of high-priced, high-purity, specialty recombinant proteins, despite the fact that these systems are relatively new (Doran, 2000). Thus, although it is generally accepted that culture systems of plant cells take some advantages compared to those of animal cells, it is essential to evaluate whether the rice cells-derived hGM-CSF reveals a toxic effect due to a different pattern of glycosylation or an unknown factor.

In the present study, we examined the single and two-week repeated dose toxicity of rhGM-CSF produced by rice cell suspension culture.

MATERIALS AND METHODS

Drugs and chemicals. Rice cells-derived hGM-CSF (rhGM-CSF) was produced as described previously (Shin. *et al.*, 2003) and purified to near homogeneity of <95% purity from the cell culture media (Han *et al.*, unpublished data). The amount of the protein was measured by Lowry method (Lowry *et al.*, 1951) and dissolved in phosphate buffered saline (PBS). All chemicals were kept under an aseptic condition.

Experimental animals. For the single dose toxicity study, SD rats of either sex weighting 160 ± 10 g (female), 230 ± 10 (males) (6 weeks-old) were obtained from Japan SLC, Inc. (Shizuoka, Japan). For the repeated dose toxicity study, SD rats of either sex weighting 90 ± 5 g (female), 95 ± 5 g (male) (4 weeks-old) were purchased from Japan SLC, Inc. (Shizuoka, Japan). The animals were housed under standard conditions of a good laboratory practice (GLP)-based facility and kept in a well-ventilated and specific pathogen-free room where controlled with 12 hr light and 12 hr darkness and room temperature of 25 ± 2°C. All the animals were allowed sterilized tap water and commercial rodent chow (Japan SLC, shizuoka, Japan) ad libitum. The study has got the approval from the department's ethical committee for the use of the animals and the study design. Sterile solution of rhGM-CSF in saline were administrated intravenously (*i.v.*) via the tail vein at indicated doses and control group received 0.5 ml of the vehicle alone. The animals were observed continuously for the test period for any signs of behavioral changes, toxicity and mortality.

Single dose toxicity study. LD_{50} (*i.v.*) was determined in rats by the method of Lorke (Lorke *et al.*, 1983). Total 40 SD rats were used and divided into 20 male and 20 female rats. First, the rats were divided into 8 groups [aGroups I-VIII] of 5 animals each and housed under the same conditions as described above. 6 groups [aGroup II-IV, aGroup VI-VIII] as rhGM-CSF-treated groups were divided into two groups of male and female rats. Each group was again divided into three groups and administrated at doses of 10, 100 and 1000 μg/kg b.wt. (*i.v.*), respectively. The remaining two

groups (aGroup I; male, and aGroup V; female) received 0.5 ml of the vehicle alone as control groups, respectively. During 7 days, food and water intake, body weight, and general behavior were monitored daily. Animals were sacrificed at the 7th day.

Repeated dose toxicity study. 18 male and 18 female SD rats were divided into three groups according to the dose, respectively, and were intravenously (*i.v.*) administrated for 14 days daily. The treated groups were administrated at the doses of 50 and 200 μg/kg b.wt. and the control groups received 0.5 ml of the vehicle alone. Food and water intake, body weight, and general behavior were observed daily. Each animal was sacrificed at 14th day and the organs of each rat were extracted for examining the morphological changes and weights. Blood from each animal was collected using EDTA-anticoagulated tubes to obtain sera for hematological and biochemical assays. Sera were from the supernatants obtained by centrifuging the blood samples at 10,000 x g for 10 min at 4°C and stored at -70°C before measuring.

Hematological assay. EDTA-anticoagulated tubes were used to collect whole blood for investigation of these. The values of the hematological parameters, RBC (red blood cell), WBC (white blood cell), NEU (neutrophil), LYM (lymphocyte), MONO (monocyte), EOS (eosinophil), BASO (basophil), PLT (platelet), WBC differential count, HCT (hematocrit), and MCV (mean corpuscular volume) were determined according to the methods described in Schalm's Veterinary Hematology of Jain (1986) (Song *et al.*, 2006). HGB (hemoglobin), MCH (mean corpuscular hemoglobin), and MCHC (mean corpuscular hemoglobin concentration) were measured spectrophotometrically using the cyanmethemoglobin method of Cannon (1958).

Biochemical assay. EDTA-anticoagulated tubes were used to collect whole blood for investigation of the blood biochemical parameters. The levels of AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), BUN (blood urea nitrogen), CRE (creatinine), GLU (glucose), CHO (total cholesterol), PRO (total protein), CPK (creatine phosphokinase), ALB (albumin), BIL (total bilirubin) were determined using an Technicon RA-XT autoanalyzer (Technicon Corp., New York, USA) (Kim *et al.*, 2007; Han *et al.*, 2004).

Statistical analysis. Statistical analysis was performed using student's *t*-test. The significant difference between the groups are considered at $p < 0.05$ level. The values are expressed as mean ± standard deviation (*SD*).

RESULTS AND DISCUSSION

First, we have examined singe dose toxicity of rhGM-CSF. There was no mortality or any signs of behavioral changes or toxicity observed after intravenous injection of rhGM-CSF upto the dose level of 1000 μg/kg b.wt. (data not shown). Thus, the lethal dose (LD_{50}) was not found. Table 1 shows the effect on the change in body weights of rats treated with rhGM-CSF. There was no significant change in the body weights observed for 7 days after the administration of the high dose level of 1000 μg/kg b.wt. As shown in Table 2, rhGM-CSF did not affect food and water intake even at the dose of 1000 μg/kg b.wt.

Yeast-derived hGM-CSF (Leucogen®), termed yhGM-CSF, is known to show a protective effect against ulcerative mucositis in hamster buccal pouch (Cho *et al.*, 2006). The report shows that the topographical administration of ~2.5 μg/b.wt. (100 ± 20 g) as the minimal dose of yhGM-CSF gives a good healing effect against ulcerative mucositis. Thus, the dose of 1000 μg/kg b.wt. in the rats could be higher by ~40-fold than the effective one in hamster. The results suggest that considering the efficacy of yhGM-CSF being currently used for cancer patients, rhGM-CSF may be non-toxic in both male and female rats.

To perform repeated dose toxicity study for rhGM-CSF, the rats were administrated via the *i.v.* route for 14

Table 1. Body weight changes of rats treated with rhGM-CSF in single dose toxicity

Day	aGroup I	aGroup II	aGroup III	aGroup IV	aGroup V	aGroup VI	aGroup VII	aGroup VIII
	M				F			
1	233.1 ± 31.11	227.06 ± 23.02	240.8 ± 31.47	228.26 ± 21.71	161.74 ± 4.61	158.06 ± 9.64	158.32 ± 6.94	162.8 ± 5.28
7	270.5 ± 28.79	274.2 ± 17.95	268.46 ± 24.49	275.88 ± 25.29	183.88 ± 12.49	185.72 ± 14.6	181.38 ± 11.84	188.31 ± 10.05

Values are expressed as mean ± S.D. for 5 rats. Comparisons were made between aGroup I (control) and aGroup II (10 μg/kg b.wt.), III (100 μg/kg b.wt.), IV (1000 μg/kg b.wt.), and between aGroup V (control) and aGroup VI (10 μg/kg b.wt.), VII (100 μg/kg b.wt.), VIII (1000 μg/kg b.wt.). M is male and F is female.

Table 2. Food (g) and water (g) Intake changes of rats treated with rhGM-CSF in single dose toxicity

Parameters	aGroup I	aGroup II	aGroup III	aGroup IV	aGroup V	aGroup VI	aGroup VII	aGroup VIII
	M				F			
Food intake	21.16 ± 0.01	21.78 ± 1.59	21.55 ± 1.06	22.25 ± 0.49	16.7 ± 0.42	16.33 ± 1.09	16.92 ± 0.16	18 ± 1.13
Water intake	63.49 ± 3.52	62.42 ± 1.43	61.67 ± 5.95	58.08 ± 1.81	54.27 ± 4.62	53.61 ± 1.21	48.37 ± 0.75	46.28 ± 2.43

Values are expressed as mean ± S.D. for 5 rats. Comparisons were made between aGroup I (control) and aGroup II (10 μg/kg b.wt.), III (100 μg/kg b.wt.), IV (1000 μg/kg b.wt.), and between aGroup V (control) and aGroup VI (10 μg/kg b.wt.), VII (100 μg/kg b.wt.), VIII (1000 μg/kg b.wt.). M is male and F is female.

Table 3. Body weight changes of rats treated with various doses of rhGM-CSF in two-week repeated dose toxicity

Sex	Group	Days				
		0	7	14	21	28
M	Group I	96.7 ± 3.4	156.7 ± 7.9	217 ± 14.7	nil	nil
	Group II	96.2 ± 4.4	150.2 ± 6.6	194.7 ± 4.8	nil	nil
	Group III	95.2 ± 2.6	145.2 ± 9.6	192.7 ± 10.5	nil	nil
F	Group IV	88.0 ± 6.7	132.0 ± 5.1	161.7 ± 8.1	nil	nil
	Group V	88.8 ± 5.3	128.5 ± 5.8	155.7 ± 4.9	nil	nil
	Group V	87.8 ± 5.9	133.5 ± 5.0	161.5 ± 6.3	nil	nil

Results were expressed as mean ± SD for 6 rats. Control was treated with the same volume of PBS. Group I, were control, Group II, were treated with rhGM-CSF 50 μg/kg b.wt., and Group III, were treated with rhGM-CSF 200 μg/kg b.wt. M is male and F is female. Body weights were estimated at 0, 7th, 14th day after the treatment of rhGM-CSF.

Table 4. Organ weight changes of rats treated with various doses of rhGM-CSF in two-week repeated dose toxicity

Sex	Group	Organ					
		Liver	Spleen	Heart	Kidney	Lung	Testis
M	Group I	11.89 ± 1.22	0.6 ± 0.1	1.00 ± 0.14	1.12 ± 0.18	1.41 ± 0.23	1.34 ± 0.29
	Group II	11.40 ± 1.02	0.6 ± 0.10	0.97 ± 0.18	1.01 ± 0.1	1.38 ± 0.30	1.33 ± 0.20
	Group III	11.27 ± 1.06	0.60 ± 0.07	0.87 ± 0.13	1.04 ± 0.19	1.36 ± 0.25	1.29 ± 0.22
F	Group IV	8.09 ± 0.87	0.49 ± 0.04	0.76 ± 0.06	0.88 ± 0.08	1.21 ± 0.15	mil
	Group V	7.42 ± 0.57	0.45 ± 0.11	0.73 ± 0.14	0.75 ± 0.07	1.1 ± 0.12	nil
	Group VI	7.87 ± 0.44	0.55 ± 0.17	0.74 ± 0.08	0.88 ± 0.07	1.27 ± 0.15	nil

Results were expressed as mean ± SD for 6 rats. Each organ was extracted and weighed after blood sampling when the final injection finished. Group I and were controls, Group II and were treated with rhGM-CSF 50 μg/kg b.wt., and Group III and were treated with rhGM-CSF 200 μg/kg b.wt. M is male and F is female. Rats were sacrificed at 14th day.

Table 5. Food and water consumption changes of rats treated with rhGM-CSF in two-week repeated dose toxicity

	Group I	Group II	Group III	Group IV	Group V	Group VI
	M			F		
Food	357.9 ± 37.5	343.8 ± 37.9	347.9 ± 32.5	332.2 ± 39.7	333.5 ± 38.4	319.9 ± 70.2
Water	351.1 ± 41.6	315 ± 51.1	348.1 ± 35.1	338.6 ± 37.1	365.7 ± 27.4	331.1 ± 40.5

Results were expressed as mean ± SD for 6 rats. Each value represents weight (gram) of average consumptions of food and water for 3 days. Group I and were controls, Group II and were treated with rhGM-CSF 50 μg/kg b.wt., and Group III and were treated with rhGM-CSF 200 μg/kg b.wt. M is male and F is female. Rats were sacrificed at 14th day.

days daily. Effects of rhGM-CSF on body and organ weights in both male and female rats were determined, respectively. When the body weights were evaluated at the 7th and 14th days after the *i.v.* injection, the weights in the treated male rats, not in the female rats, was slightly decreased (Table 3). However, No statistically significant differences existed in the absolute and relative weights of all the isolated organs between the treated and control rats (Table 4). Furthermore, no lethality was recorded for any dose and any route for 14 days via the *i.v.* route. We examined a difference in consumption of food and water between the treated and control rats. As shown in Table 5, there was no significant difference in any intake of the food and water in

both male and female rats.

Next, the influences of rhGM-CSF on hematological and biochemical parameters were determined. First, hematological parameters and their comparisons among all groups are presented in Table 6 and 7. First, the values of hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were measured as hematological parameters (Table 6). The results show that there is any difference between the control and the treated rats in all the parameters in both male and female rats, suggesting that rhGM-CSF may be hematologically safe even if 200 μg/kg b.wt. was injected daily for 14 days. This dose could be higher by ~8-fold than the single effective dose in hamster. On the other hand, the counts of blood cells were examined after rhGM-CSF was injected daily for 14 days. As shown in Table 7, the numbers of all types of blood cells measured, platelets (PLT), red blood cells (RBC), white blood cells (WBC),

Table 6. Effect of rhGM-CSF on hematological parameters in two-week repeated dose toxicity

Sex	Group	HGB g/dl	HCT %	MCV fl	MCH pg	MCHC g/dl
M	Group I	13.8 ± 1.40	38.9 ± 5.04	55.2 ± 1.55	19.7 ± 1.02	35.6 ± 1.14
	Group II	12.9 ± 2.03	37.8 ± 7.17	56.0 ± 1.11	19.8 ± 0.68	35.4 ± 0.92
	Group III	13.1 ± 1.85	37.8 ± 5.10	54.9 ± 1.40	19.5 ± 0.56	35.6 ± 0.92
F	Group IV	13.8 ± 0.67	38.7 ± 2.53	55.7 ± 1.49	19.9 ± 0.75	35.7 ± 0.66
	Group V	13.4 ± 1.43	37.9 ± 3.86	55.7 ± 1.00	19.9 ± 0.26	35.3 ± 0.48
	Group VI	12.9 ± 2.74	38.3 ± 5.00	55.9 ± 1.38	20.1 ± 0.89	35.9 ± 1.08

Results were expressed as mean ± SD for 6 rats. Group I and were controls, Group II and were treated with rhGM-CSF 50 μg/kg b.wt., and Group III and were treated with rhGM-CSF 200 μg/kg b.wt. M is male and F is female. Hematological parameters were measured within 4 hr after blood sampling. Blood was collected in EDTA-anticoagulated tubes. Rats were sacrificed at 14th day.
*HGB (hemoglobin), HCT (hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration).

Table 7. Effect of rhGM-CSF on hematological parameters in two-week repeated dose toxicity

Sex	Group	PLT $10^3/mm^3$	RBC $10^6/mm^3$	WBC $10^3/mm^3$	NEU $10^3/mm^3$	LYM $10^3/mm^3$	MONO $10^3/mm^3$	EOS $10^3/mm^3$	BASO $10^3/mm^3$
M	Group I	1148.8 ± 110.09	7.07 ± 1.07	6.59 ± 0.88	0.75 ± 0.20	6.30 ± 1.96	0.40 ± 0.23	0.06 ± 0.01	0.08 ± 0.04
	Group II	1180.2 ± 84.73	6.14 ± 0.97	6.51 ± 0.66	0.55 ± 0.24	4.56 ± 1.39	0.27 ± 0.19	0.05 ± 0.03	0.07 ± 0.06
	Group III	1088.9 ± 115.42	6.74 ± 0.78	6.23 ± 0.87	0.89 ± 1.08	3.94 ± 1.96	0.29 ± 0.25	0.05 ± 0.03	0.06 ± 0.04
F	Group IV	1142.7 ± 104.00	6.97 ± 0.57	5.76 ± 1.75	0.53 ± 0.22	4.39 ± 1.76	0.18 ± 0.08	0.05 ± 0.03	0.11 ± 0.05
	Group V	1160.8 ± 164.70	6.81 ± 0.77	5.99 ± 0.87	0.57 ± 0.21	4.66 ± 1.02	0.16 ± 0.09	0.04 ± 0.02	0.06 ± 0.03
	Group VI	1058.2 ± 56.93	6.64 ± 1.42	5.87 ± 1.24	0.51 ± 0.23	3.56 ± 1.34	0.18 ± 0.13	0.05 ± 0.06	0.06 ± 0.03

Each result was expressed as mean ± SD for 6 rats. Group I and were controls, Group II and V were treated with rhGM-CSF 50 μg/kg b.wt., and Group III and were treated with rhGM-CSF 200 μg/kg b.wt. M is male and F is female. Hematological parameters were measured within 4 hr after blood sampling. Blood was collected in EDTA-anticoagulated tubes. Rats were sacrificed at 14th day.
*PLT (platelets), RBC (red blood cells), WBC (white blood cells), NEU (neutrophil), LYM (lymphocyte), MONO (monocyte), EOS (eosinophil), BASO (basophil).

Table 8. Effect of rhGM-CSF on biochemical parameters in two-week repeated dose toxicity

Sex	Group	AST IU/l	ALT IU/l	ALP IU/l	T-BIL mg/dl
M	Group I	71.95 ± 8.22	44.35 ± 4.32	482.70 ± 67.38	0.25 ± 0.05
	Group II	70.45 ± 7.21	43.50 ± 6.12	501.75 ± 99.01	0.29 ± 0.11
	Group III	79.87 ± 13.48	43.27 ± 5.75	544.67 ± 100.46	0.34 ± 0.12
F	Group IV	62.92 ± 6.74	37.68 ± 4.87	448.82 ± 65.66	0.25 ± 0.09
	Group V	59.30 ± 7.10	34.33 ± 4.90	312.65 ± 28.40	0.20 ± 0.05
	Group VI	58.60 ± 5.87	33.55 ± 3.25	374.6 ± 88.59	0.26 ± 0.05

Results were expressed as mean ± SD for 6 rats. Group I, were control, Group II, were treated with rhGM-CSF 50 μg/kg b.wt., and Group III, were treated with rhGM-CSF 200 μg/kg b.wt. M is male and F is female. Rats were sacrificed at 14th day. Biochemical parameters were measured using sera of rhGM-CSF treated rats. Sera were from the supernatants obtained by centrifuging the blood samples at 10,000 x g for 10 min at 4°C and stored at -70°C before measuring.
*AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), T-BIL (total bilirubin).

neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS) and basophils (BASO) were not significantly influenced ($p < 0.05$) by the *i.v.* injection of rhGM-CSF, further supporting that the daily treatment of rhGM-CSF of 200 μg/kg b.wt. for 14 days may be hematologically safe in both male and female rats.

Second, we examined the effect of rhGM-CSF on biochemical parameters. Table 8 shows the effects of rhGM-CSF on some biochemical parameters, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin (T-BIL). The treatment of rhGM-CSF to male rats did not significantly affect the level of AST, whereas that to female rats decreased slightly its level. In the case of ALT, whereas its level in male rats was not influenced by rhGM-CSF, that in female rats decreased slightly. A significant and dose-dependent increase ($p < 0.05$) in ALP and T-BIL levels was observed in the high dose-male Group III, compared to the control rats, but not in the female Group VI. On the other hand, blood urea nitrogen (BUN), creatinine (CRE) , glucose (GLU), total cholesterol (CHO), total protein (PRO), creatine phosphokinase (CPK), albumin (ALB) were also determined, but all of these parameters were not significantly different (data not shown).

In recent study, we found that the glycosylation pattern of rhGM-CSF was different from that of yhGM-CSF, based on analytical investigation for the purified recombinant protein obtained from rice cells; in particular, rhGM-CSF is more heavily glycosylated than yhGM-CSF or CHO cells-derived one (data not shown). The results suggest that rhGM-CSF may be more hydrophilic and retainable to the mucus of buccal pouches, and thus could be more effective in ulcerative mucositis of cancer patients induced by a chemo-therapeutic agent than yhGM-CSF or CHO cells-derived one. Furthermore, the heavier glycosylation of rhGM-CSF may render its blood half-life to be longer. Our recent results indicated that rhGM-CSF remains longer in the serum with a higher level compared to yhGM-CSF (data not shown). As described previously, plant cell culture systems could be the most favorable means of producing small-to-medium quantities of high-priced, high-purity, specialty recombinant proteins, despite the fact that these systems are relatively new, (Doran, 2000) which becomes now one of the emerging bio-fields, termed "molecular farming".

In summary, there were no significant changes in both body and organ weights between control and test groups. Also the hematological and blood biochemical parameters were statistically not different in all the groups. Together, these results suggest that rhGM-CSF has no toxicity in SD rats.

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