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4-WEEK SUBCUTANEOUS TOXICITY STUDY OF RECOMBINANT GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (LBD-005) IN RATS

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ABSTRACT: *Recombinant granulocyte-macrophage colony stimulating factor was subcutaneously administered to both sexes of Sprague-Dawley rats at the doses of 0, 0.5, 1 and 2 mg/kg of body weight five days per week for 4 weeks to evaluate the subchronic toxicity. There were decreased segmented neutrophils in the blood in the females dosed at 1 and 2 mg/kg. In the serum, changes were decreased alkaline phostatase(ALP) in the female groups dosed at 1 and 2 mg/kg, and increased glutamic oxaloacetic transaminiase (GOT) in the male groups dosed at 0.5 and 2.0 mg/kg. Mild to moderate inflammation was observed at the injected sites of the animals of both sexes administered with 2.0 mg/kg. No change was found in body weight, clinical signs, food consumption, water consumption, gross necropsy findings and organ weights. Based on the results, it is concluded that the maximum tolerated dose is no less than 2 mg/kg/day and no effect level is less than 1 mg/kg/day in rats.*

Key Words: *Granulocyte-macrophage colony stimulating factor, subchronic toxocity, rats.*

INTRODUCTION

Granulocyte-macrophage colony stimulating factor(GM-CSF) is one of several hematopoietic growth factors which have now been purified, cloned and produced in the biotechnology industry. It is now clear that the factor is able not only to stimulate the proliferation of granulocyte and macrophage colonies(Burgess & Metcalf, 1980; Sieff *et al.*, 1985; Macalf *et al.*, 1986) but also to regulate some of the functional activities of stem cells(Metcalf, 1986). The possible relationships of GM-CSF in combating myeloid leukemias and other leukocyte deficiency diseases(Gasson, 1984; Barlogie *et al.*, 1990) have stimulated cellular and molecular biologists to carry out a great deal of research. Demands in the clinical

field have also accelerated the researchers to produce many kinds of recombinant GM-CSF.

Human clinical trials, using different recombinant forms of GM-CSF, are still continuing, with varing degrees of success(Goldstone and Khwaja, 1990; Lieschke *et al.*, 1989). Previous researches and the possible hope of overcoming myelogenic diseases such as leukopenia, aplastic anemia and leukemia, or at least accelerating myelopoiesis following cytotoxic chemotherapy and/or radiation therapy(Drings and Fischer, 1990), have led to the development of another recombinant GM-CSF (LBD-005) by Lucky R & D Center, Biotechnology(Daejeon, Korea).

This paper presents the data from 4 weeks, repeated dose study of LBD-005 in rats.

MATERIALS AND METHODS

Test Materials

Recombinant GM-CSF(LBD-005) with a protein content of 0.2 mg/ml(w/v), pH 7.4 and 328 mOsm was produced and supplied from Lucky R & D Center, Biotechnology (84, Jang-Dong, Yousung-Koo, Daejeon, Korea).

The vehicle, phosphate buffered saline(pH 7.4) was supplied from Lucky R & D Center, Biotechnology.

Animals and Maintenance

Specific-pathogen-free(SPF) Sprague-Dawley rats of both sexes were obtained at 4 weeks of age from the Laboratory of Animal Breeding, Korea Research Institute of Chemical Technology. They were acclimatized for about 1 week prior to administration of the test materials under the barrier-sustained animal room maintained at a temperature of 23±3°C, a ralative humidity of 50±10% and illumination cycle of 12 hours light and 12 hours dark(07:00-19:00). The rats were housed in stainless-steel wire cages(220×410×200 mm). Standard rat and mouse pellets(Jaiil Feed Co., Ltd., Yuseong, Korea) sterilized by gamma-irradiation at dose of 2 Mrad and tap water sterilized by an ultra-violet sterilizer were fed *ad libitum*.

Experimental Procedure

Groups of 10 male and 10 female rats received subcutaneous doses of 0(vehicle control), 0.5, 1 and 2 mg LBD-005/kg of body weight five days per week for 4 weeks. The animals were inspected daily for clinical signs. Body weights, food consumption and water consumption were determined once a week during the study. Urine samples were collected and analyzed for specific gravity, pH, protein, ketone body, occult blood, bilirubin, urobilinogen, nitrite using colorimetric strip (Multistix, Ames) and urine volume for 24 hours at the last week of the study. All animals were anesthetized with ether, collected blood for hematology and blood biochemistry through the posterior vena cava, and necropsied at the termination of the study.

In hematology, leucocyte count, erythrocyte count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpscular hemoglobin concentration and platelet count were determined by a hematological autoanalyzer (S-880 Coulter Counter, Coulter Electronics).

Differential leucocyte count was determined on the blood smears. Prothrombin time of plasma was determined by fibro-coagulation analyzer (COBAS).

Blood biochemistry was conducted to quantify serum levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, glucose, total protein, blood urea nitrogen, creatinine, total cholesterol and total bilirubin by biochemical autoanalyzer(JCA VCX-1000, Jeol Co.), sodium and potassium by flame photometer(IL943, Instrumentation Laboratory), and chloride by chloridemeter(C-200AP, Jookoo Co.).

Organ weights were determined for kidney, liver, spleen, heart, lung, thyroid, adrenal gland, brain, testis and ovary. These tissues for organ weights, sternum, skin and grossly abnormal tissues were taken and fixed in 10% neutral buffered formalin. The tissues were processed for standard paraffin embedding prior to sectioning at 5 μm and stained with hematoxylin and eosin. They were light microscopically examined.

RESULTS

Clinical Findings

Hair loss on the head was found in 2 males of control group and dark red materials around eyes in 1 female of 1 mg/kg group.

Body Weights, Food Consumption and Water Consumption

No change was found which was considered to be treatment-related(Fig. 1, 2 and 3).

Urinalysis

In the urine sediments, there were increased numbers of leukocytes in the

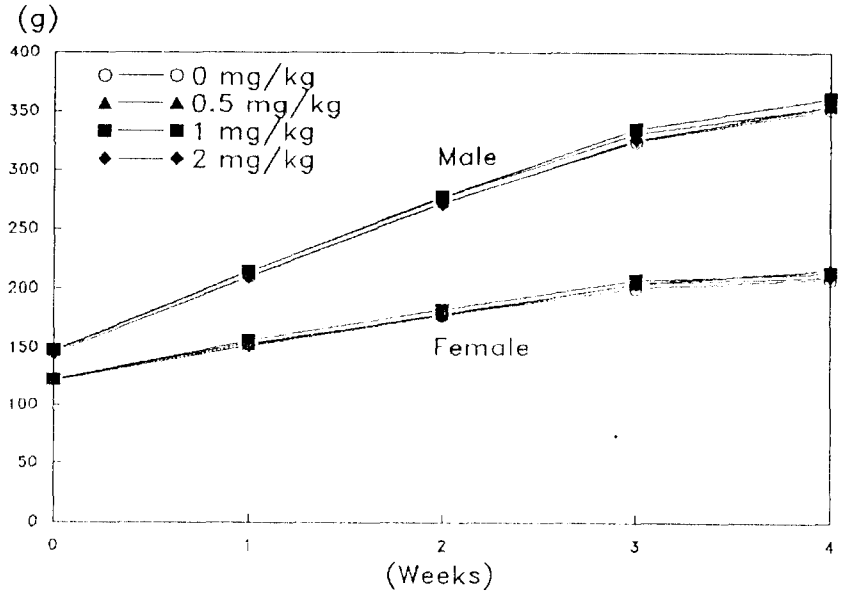

Figure 1. Body weight changes in rats during the period of 4 weeks of subcutaneous administration of LBD-005.

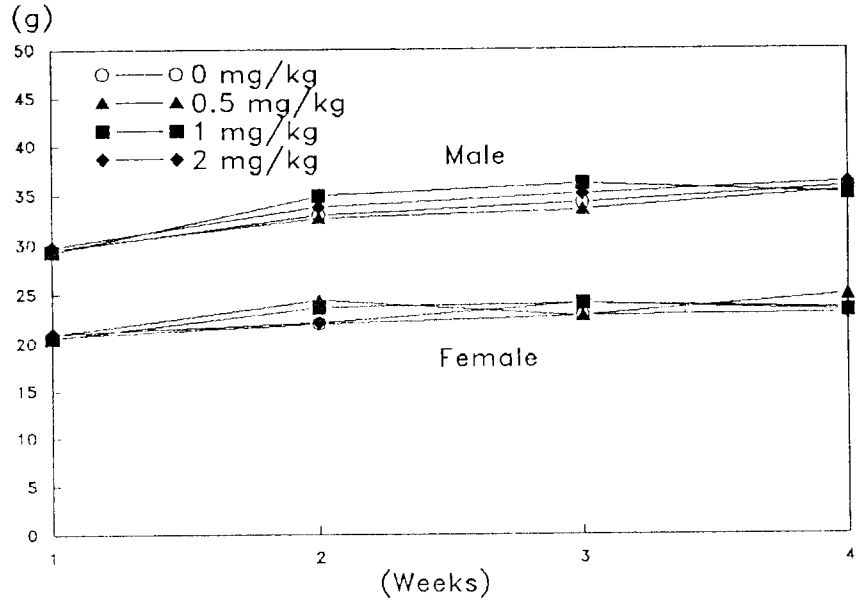

Figure 2. Food consumption in rats during the period of 4 weeks of subcutaneous administration of LBD-005.

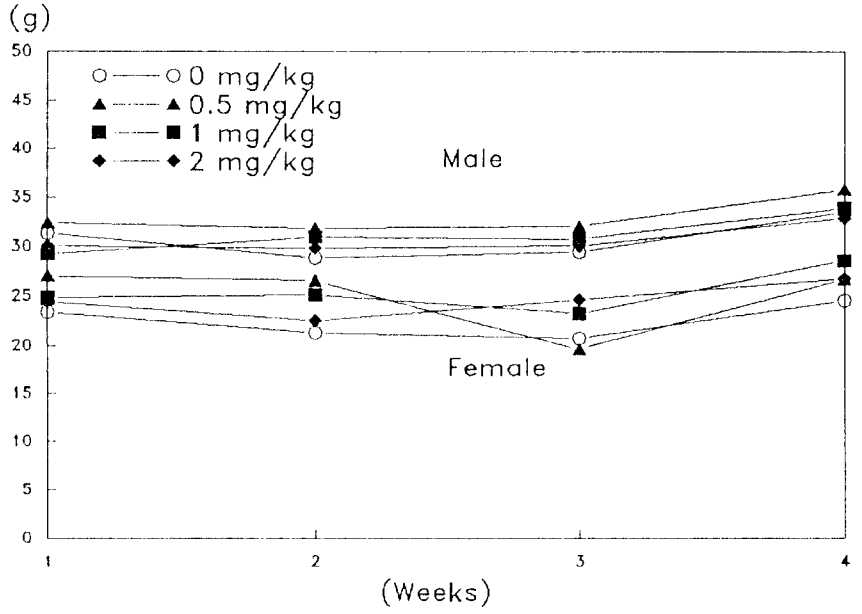

Figure 3. Water consumption in rats during the period of 4 weeks of subcutaneous administration of LBD-005.

treated female groups without statistical differences. Increased urine volumes were found in the treated groups of both sexes without statistical differences (Table 1).

Hematology

Table 1. Urinalysis in rats after 4 weeks of subcutaneous administration of LBD-005 for 5 days per week

Sex		Male				Female			
Dose (mg/kg)		0	0.5	1	2	0	0.5	1	2
No. of rats		10	10	10	10	10	10	10	10
Urine volume (ml/day)		16.2 ±2.8	23.5 ±10.6	19.9 ±7.0	20.9 ±11.5	11.5 ±4.6[a]	16.6 ±6.1	16.5 ±5.7	14.0 ±6.0
1) Specific gravity	0	1	2	2	1	0	2	3	0
		3	2	2	3	1	1	2	3
	2	4	0	2	3	1	2	2	2
	3	1	3	2	3	1	2	3	2
	4	1	2	2	0	2	1	0	0
	5	0	1	0	0	5	2	3	3
2) pH	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	2	0	1	0
	3	0	0	0	0	4	1	2	2
	4	0	1	1	0	0	2	2	3
	5	1	3	3	2	2	2	2	3
	6	5	1	3	3	2	2	1	2
	7	4	5	3	5	0	2	2	0
	8	0	0	0	0	0	1	0	0
3) Protein	0	0	0	0	0	1	4	3	3
	1	2	0	0	1	4	4	3	1
	2	8	10	10	9	4	0	3	4
	3	0	0	0	0	1	2	1	2
	4	0	0	0	0	0	0	0	0
4) Glucose	0	0	10	10	10	10	10	10	10
	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
5) Ketone bodies	0	6	5	6	5	7	9	9	9
	1	4	5	4	5	3	1	1	1
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
6) Bilirubin	0	0	10	10	10	7	7	8	9
	1	0	0	0	0	3	3	2	1
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
7) Occult blood	0	7	6	9	6	10	10	10	9
	1	3	4	1	4	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0

[a] No. of animals examined is 9.

Continued

Sex		Male				Female			
Dose (mg/kg)		0	0.5	1	2	0	0.5	1	2
No. of rats		10	10	10	10	10	10	10	10
8) Nitrite	0	10	10	10	10	10	10	10	10
	1	0	0	0	0	0	0	0	0
9) Urobilinogen	0	10	9	10	10	9	6	7	9
	1	0	1	0	0	1	3	2	1
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0[b]	0[b]	0
10) Sediment									
CASTS	−	10	9	10	10	10	10	10	10
	±	0	1	0	0	0	0	0	0
RBC	−	6	4	7	8	4	5	10	5
	±	4	6	3	2	6	5	0	5
WBC	−	7	8	9	7	10	6	5	6
	±	3	2	1	3	0	4	5	4
EPI	−	9	10	9	9	10	9	9	10
	±	1	0	1	1	0	1	1	0

1) 0: <1.005
1: 1.010
2: 1.015
3: 1.020
4: 1.025
5: >1.030

2) 0: 5.0 6: 8.0
1: 5.5 7: 8.5
2: 6.0 8: 9.0
3: 6.5
4: 7.0
5: 7.5

3) 0: −
1: +/−
2: 30 mg/dl
3: 100 mg/dl
4: >300 mg/dl

4) 0: −
0: 0.1 g/dl
2: 0.25 g/dl
3: 0.5 g/dl
4: 1.0 g/dl

5) 0: −
1: +/−
2 +
3: ++
4: +++

6) 0: −
1: +
2: ++
3: +++

7) 0: −
1: +/−
2: +
3: ++
4: +++

8) 0: −
1: +

9) 0: 0.5 EU/dl
1: 1.0 EU/dl
2: 2.0 EU/dl
3: 4.0 EU/dl
4: >8.0 EU/dl

10) One microscopic field(×400)

	Casts	RBC	WBC	Epithelium
−	0	0	0	0
±	≤1	≤4	≤5	severals

[b] One sample is not detected.

Decreased segmented neutrophils were observed in the females dosed at 1 and 2 mg/kg. Mean corpuscular volume and mean corpsucular hemoglobin were decreased in the males dosed at 0.5 mg/kg(Table 2).

Blood Biochemistry

Glutamic-oxaloacetic transaminase was increased in the males dosed at 0.5 and 2 mg/kg. Alkaline phosphatase was increased in the males dosed at 1 mg/kg but decreased in the females dosed at 1 and 2 mg/kg. Blood urea nitrogen was decreased in the females dosed at 0.5 mg/kg(Table 3).

Necropsy Findings

Table 2. Hematology in rats after 4 weeks of subcutaneous administration of LBD-005 for 5 days per week

Sex	Dose (mg/kg)	No. of rats	WBC ($\times 10^3$ μl)	RBC ($\times 10^3$ μl)	Hemoglobin g/dl	Hematocrit %	MCV fl	MCH pg	MCHC g/dl	Platelet ($\times 10^3$ μl)
Male	0	10	8.38	7.18	14.79	44.48	61.91	20.60	33.27	1113
			±1.65	±0.39	±0.80	±2.34	±0.81	±0.53	±0.55	±59
	0.5	10	9.85	7.40	14.76	44.69	60.32*	19.92*	33.03	1230
			±3.47	±0.46	±1.27	±3.50	±1.40	±0.65	±0.46	±23
	1	10	9.65	7.29	14.77	44.63	61.21	20.26	33.11	1191
			±3.17	±0.38	±0.84	±2.49	±1.68	±0.54	±0.23	±42
	2	10	11.26	7.37	15.26	45.99	62.35	20.70	33.20	1143
			±4.36	±0.39	±0.73	±2.35	±0.66	±0.44	±0.60	±73
Female	0	8	11.41	7.80	15.70	47.06	60.33	20.14	33.40	1166
			±4.74	±0.26	±0.53	±1.93	±1.40	±0.78	±1.22	±68
	0.5	10	11.14	7.56	15.24	45.05	59.54	20.13	33.82	1199
			±3.94	±0.41	±1.00	±2.40	±1.41	±0.54	±0.87	±26
	1	10	9.10	7.48	15.17	44.90	59.99	20.28	33.80	1185
			±1.96	±0.42	±0.83	±2.79	±1.22	±0.48	±0.58	±84
	2	9	12.19	7.74	15.97	46.84	60.46	20.62	34.12	1159
			±3.81	±0.25	±0.39	±2.11	±1.56	±0.39	±92	±92

Sex	Dose (mg/kg)	No. of rats	Differential Leukocyte Count ($\times 10^3$ μl)						Prothrombin time (second)
			Segmented.	Bands.	Eosinophils	Basophils	Lymphocyte	Monocytes	
Male	0	10	1.007	0.000	0.088	0.000	7.137	0.150	19.8
			±0.321	±0.000	±0.081	±0.000	±1.443	±0.102	±1.0
	0.5	10	1.175	0.000	0.072	0.000	8.437	0.163	20.2
			±0.640	±0.000	±0.097	±0.000	±2.934	±0.156	±0.9
	1	10	1.268	0.000	0.023	0.000	8.145	0.209	19.1
			±0.545	±0.000	±0.037	±0.000	±2.745	±0.155	±0.4
	2	10	1.173	0.000	0.061	0.000	9.856	0.168	19.7
			±0.700	±0.000	±0.118	±0.000	±3.861	±0.183	±1.0
Female	0	8	0.680	0.000	0.111	0.000	10.441	0.181	20.8
			±0.220	±0.000	±0.081	±0.000	±4.453	±0.203	±7.4
	0.5	10	0.591	0.000	0.058	0.000	10.360	0.126	19.0
			±0.190	±0.000	±0.080	±0.000	±4.009	±0.112	±0.8
	1	10	0.445*	0.000	0.050	0.000	8.439	0.164	18.7
			±0.198	±0.000	±0.066	±0.000	±1.991	±0.134	±1.1
	2	9	0.398*	0.000	0.087	0.000	11.454	0.247	19.9
			±0.137	±0.000	±0.118	±0.000	±3.779	±0.105	±2.0

Indicates a siginificant difference from control group by Dunnett's t-test (:P<0.05)

Table 3. Serum biochemistry in rats after 4 weeks of subcutaneous administration of LBD-005 for 5 days per week

Sex	Dose (mg/kg)	No. of rats	GOT IU/l	GPT IU/l	ALP IU/l	BUN mg/dl	CRE mg/dl	GLU mg/dl
Male	0	10	79.81	36.00	439.70	14.33	0.15	139.60
			±5.59	±5.83	±80.22	±1.46	±0.05	±23.13
	0.5	10	92.46**	36.00	456.86	14.39	0.15	125.68
			±9.62	±5.32	±50.58	±1.46	±0.04	±14.55
	1	10	87.63	37.51	529.05*	14.23	0.15	145.01
			±6.88	±4.29	±120.23	±1.32	±0.02	±23.19
	2	10	93.67**	34.88	485.45	14.41	0.16	145.36
			±9.87	±3.42	±51.13	±1.99	±0.05	±35.28
Female	0	10	104.86	25.66	302.81	14.96	0.17	102.43
			±23.51	±5.17	±81.26	±1.84	±0.06	±25.95
	0.5	10	96.95	27.18	253.54	12.91*	0.20	120.44
			±9.72	±4.77	±59.09	±1.85	±0.06	±27.21
	1	10	106.67	24.60	230.98*	14.26	0.18	114.81
			±15.63	±4.10	±24.00	±1.41	±0.05	±22.36
	2	10	95.45	26.22	236.50*	14.06	0.19	104.90
			±14.19	±5.34	±47.85	±2.03	±0.05	±13.44

Sex	Dose (mg/kg)	No. of rats	TCHO mg/dl	TP g/d	TBIL mg/dl	CL mmol/l	NA mmol/l	K mmol/l
Male	0	10	89.57	6.15	0.66	103.3	140.1	4.80
			±13.02	±0.19	±0.11	±1.8	±2.7	±1.00
	0.5	10	80.57	6.19	0.67	104.7	140.1	4.17
			±10.38	±0.30	±0.07	±1.4	±3.0	±0.23
	1	10	89.55	6.19	0.67	103.6	139.1	4.41
			±16.26	±0.28	±0.08	±2.5	±3.1	±1.09
	2	10	81.10	6.25	0.70	103.4	140.4	4.70
			±11.87	±0.21	±0.06	±2.3	±4.7	±0.96
Female	0	10	73.54	6.06	0.78	104.8	141.9	4.84
			±14.05	±0.23	±0.14	±1.7	±4.5	±0.99
	0.5	10	77.64	5.90	0.73	105.0	142.1	4.51
			±15.17	±0.21	±0.09	±2.5	±4.7	±0.27
	1	10	79.79	6.10	0.70	140.0	140.1	4.87
			±14.73	±0.40	±0.12	±1.9	±5.3	±1.05
	2	10	81.67	6.17	0.79	105.6	141.9	4.43
			±10.98	±0.27	±0.14	±2.9	±5.8	±0.31

Indicates a significant difference from control groups by Dunnett's t-test (: $P<0.05$, **: $P<0.01$).

Diffuse red spots were found in 1 male of 1 mg/kg group. Congestion on the site of administration were found in 1 male of control group and 1 female of 2 mg/kg group.

Organ Weights

Relative organ weights of kidney were increased in females dosed at 0.5 mg/kg (Table 4 and 5).

Table 4. Absolute organ weights of rats after 4 weeks of subcutaneous administration of LBD-005 for 5 days per week

(unit: g)

Sex	Male				Female			
Dose (mg/kg)	0	0.5	1	2	0	0.5	0	2
No. of of rats	10	10	10	10	10	10	10	10
Body weight	331.0 ±5.7	329.6 ±8.1	338.3 ±25.9	329.6 ±27.1	196.8 ±8.5	204.8 ±13.5	203.3 ±16.1	199.8 ±10.5
Brain	1.945 ±0.043	1.962 ±0.084	1.970 ±0.071	1.947 ±0.068	1.839 ±0.052	1.812 ±0.095	1.811 ±0.057	1.806 ±0.072
Kidney-L	1.331 ±0.096	1.334 ±0.116	1.362 ±0.160	1.299 ±0.140	0.821 ±0.055	0.782 ±0.059	0.816 ±0.071	0.828 ±0.072
Kidney-R	1.352 ±0.084	1.354 ±0.089	1.376 ±0.148	1.326 ±0.151	0.850 ±0.063	0.794 ±0.039	0.844 ±0.084	0.846 ±0.064
Kidneys-total	2.682 ±0.177	2.688 ±0.202	2.738 ±0.306	2.625 ±0.286	1.671 ±0.112	1.577 ±0.095	1.660 ±0.151	1.675 ±0.134
Heart	1.151 ±0.100	1.165 ±0.074	1.226 ±0.128	1.155 ±0.111	0.761 ±0.078	0.779 ±0.066	0.748 ±0.077	0.758 ±0.049
Lungs	1.305 ±0.113	1.343 ±0.139	1.344 ±0.247	1.346 ±0.162	0.972 ±0.058	0.987 ±0.100	1.078 ±0.113	1.005 ±0.086
Spleen	0.638 ±0.048	0.676 ±0.112	0.646 ±0.102	0.667 ±0.120	0.403 ±0.050	0.420 ±0.053	0.430 ±0.061	0.446 ±0.054
Liver	11.011 ±0.741	10.870 ±0.660	11.248 ±1.526	10.693 ±1.620	5.933 ±0.488	5.923 ±0.481	6.142 ±0.798	6.057 ±0.498
Thyroid-L	0.00967 ±0.00185	0.00948 ±0.00121	0.00879 ±0.00161	0.00786 ±0.00262	0.00704 ±0.00102	0.00685 ±0.00148	0.00583 ±0.00139	0.00564 ±0.00123
Thyroid-R	0.01065 ±0.00144	0.00940 ±0.00221	0.01039 ±0.00205	0.00935 ±0.00226	0.00792 ±0.00213	0.00703 ±0.00209	0.00707 ±0.00161	0.00688 ±0.00163
Thyroids-total	0.02033 ±0.00233	0.01888 ±0.00272	0.01918 ±0.00338	0.01721 ±0.00492	0.01496 ±0.00240	0.01388 ±0.00320	0.01290 ±0.00249	0.01252 ±0.00219
Adrenal-L	0.03230 ±0.00379	0.03154 ±0.00384	0.03324 ±0.00677	0.03085 ±0.00798	0.03813 ±0.00396	0.03518 ±0.00614	0.03720 ±0.00472	0.03804 ±0.00514
Adrenal-R	0.03046 ±0.00279	0.02859 ±0.00344	0.03087 ±0.00459	0.02871 ±0.00458	0.03459 ±0.00355	0.03305 ±0.00410	0.03396 ±0.00646	0.03440 ±0.00518
Adrenals-total	0.06275 ±0.00610	0.06013 ±0.00640	0.06410 ±0.01064	0.05956 ±0.01224	0.07272 ±0.00732	0.06823 ±0.00963	0.07116 ±0.01064	0.07245 ±0.00986
Testis-L (Ovary-L)	1.602 ±0.138	1.631 ±0.149	1.672 ±0.085	1.539 ±0.093	0.04390 ±0.00407	0.04554 ±0.01004	0.04798 ±0.01045	0.04453 ±0.0141
Testis-R (Ovary-R)	1.588 ±0.127	1.621 ±0.164	1.665 ±0.095	1.535 ±0.120	0.04410 ±0.00761	0.04597 ±0.01141	0.04334 ±0.01044	0.04322 ±0.01561
Testes(Ovary) -total	3.190 ±0.262	3.252 ±0.310	3.336 ±0.172	3.074 ±0.210	0.08800 ±0.00876	0.09150 ±0.02007	0.09132 ±0.01997	0.08775 ±0.02857

Table 5. Relative organ weights of rats after 4 weeks of subcutaneous administration of LBD-005 for 5 days per week

(unit: g)

Sex	Male				Female			
Dose (mg/kg)	0	0.5	1	2	0	0.5	1	2
No. of of rats	10	10	10	10	10	10	10	10
Brain	0.589	0.596	0.586	0.594	0.938	0.887	0.896	0.906
	±0.031	±0.034	±0.056	±0.052	±0.059	±0.066	±0.071	±0.061
Kidney-L	0.402	0.405	0.402	0.394	0.418	0.384	0.402	0.415
	±0.025	±0.036	±0.026	±0.027	±0.021	±0.040	±0.021	±0.032
Kidney-R	0.409	0.411	0.406	0.402	0.432	0.389	0.415	0.424
	±0.025	±0.026	±0.024	±0.034	±0.021	±0.032**	±0.024	±0.030
Kidney	0.811	0.817	0.808	0.797	0.850	0.773	0.817	0.839
	±0.049	±0.062	±0.049	±0.060	±0.038	±0.070**	±0.040	±0.061
Heart	0.347	0.354	0.362	0.350	0.388	0.380	0.368	0.380
	±0.021	±0.021	±0.025	±0.020	±0.046	±0.016	±0.030	±0.026
Lungs	0.394	0.407	0.396	0.408	0.495	0.483	0.507	0.504
	±0.029	±0.026	±0.054	±0.023	±0.029	±0.045	±0.054	±0.041
Spleen	0.193	0.205	0.191	0.201	0.206	0.205	0.211	0.223
	±0.013	±0.029	±0.022	±0.024	±0.031	±0.021	±0.025	±0.025
Liver	3.327	3.298	3.315	3.230	3.017	2.894	3.015	3.035
	±0.175	±0.105	±0.250	±0.284	±0.190	±0.185	±0.236	±0.239
Thyroid-L	0.0293	0.00289	0.00260	0.00236	0.00360	0.00338	0.00287	0.00284
	±0.00059	±0.00045	±0.00043	±0.00070	±0.00059	±0.00087	±0.00066	±0.00068
Thyroid-R	0.00322	±0.00288	±0.00306	±0.00283	±0.00402	±0.00347	±0.00350	±0.00345
	±0.00044	±0.00078	±0.00049	±0.00062	±0.00108	±0.00115	±0.00079	±0.00082
Thyroids	0.00615	±0.00576	±0.00566	±0.00518	±0.00762	±0.00684	±0.00637	±0.00628
-total	±0.00156	±0.00141	±0.00108	±0.00113	±0.00102	±0.00187	±0.00127	±0.00075
Adrenal-L	0.00978	±0.00960	±0.00977	±0.00934	±0.01941	±0.01726	±0.01835	±0.01901
	±0.00126	±0.00133	±0.00151	±0.00224	±0.00204	±0.00325	±0.00228	±0.00203
Adrenal-R	0.00921	0.00868	0.00911	0.00872	0.01763	0.01620	0.01668	0.01717
	±0.00077	±0.00100	±0.00104	±0.00127	±0.00203	±0.00223	±0.00278	±0.00197
Adrenals	0.01899	0.01829	0.01888	0.01807	0.03704	0.03346	0.03504	0.03618
-total	±0.00167	±0.00251	±0.00249	±0.00357	±0.00410	±0.00519	±0.00477	±0.00362
Testis-L	0.484	0.469	0.469	0.496	0.02240	0.02240	0.02350	0.02226
(Ovary-L)	±0.034	±0.051	±0.035	±0.043	±0.00259	±0.00529	±0.00441	±0.00685
Testis-R	0.480	0.493	0.494	0.468	0.02250	0.02265	0.02138	0.02153
(Ovary-R)	±0.031	±0.056	±0.043	±0.050	±0.00414	±0.00607	±0.00521	±0.00743
Testes(Ovary)	0.963	0.989	0.990	0.938	0.04490	0.04505	0.04488	0.04379
-total	±0.063	±0.106	±0.077	±0.092	±0.00538	±0.01066	±0.00913	±0.01381

*Indicates a significant difference from control group by Dunnett's t-test(**: $P<0.01$).

Table 6. Histopathological findings in rats after 4 weeks of subcutaneous administration of LBD-005 for 5 days per week

Sex	Findings	Dose(mg/kg):	0	0.5	1	2
		No. of rats/sex:	10	10	10	10
Male						
	Kindney:					
	Interstitial nephritis		1(10%)	0(0%)	0(0%)	0(0%)
	Thyroid gland:					
	Ectopic thymus		0(0%)	0(0%)	0(0%)	1(10%)
	Skin:					
	Subcutaneous edema		0(0%)	1(10%)	0(0%)	0(0%)
	Subcutaneous inflammation		0(0%)	1(10%)	1(10%)	3(30%)
Female						
	Kidney:					
	Hydronephrosis		1(10%)	0(0%)	0(0)	0(0%)
	Nephrocaloinosis		0(0%)	0(0%)	1(10%)	2(20%)
	Thyroid gland:					
	Ectopic thymus		0(0%)	1(10%)	0(0%)	1(10%)
	Skin:					
	Subcutaneous edema		1(10%)	0(0%)	1(10%)	1(10%)
	Subcutanous inflammation		0(0%)	1(10%)	0(0%)	5(50%)

Histopathological Findings

Mild to moderate inflammation was observed at the injected sites of the animals of both sexes dosed at 0.5 and 2 mg/kg, and of males dosed at 1 mg/kg. Nephrocalcinosis was observed in 1 female dosed at 1 mg/kg and 2 females dosed at 2 mg/kg. Other changes, ectopic thymus of the thyroid, interstitial nephritis, hydronephrosis and edema at the injected sites were seen in a few cases without dose-dependency(Table 6).

DISCUSSION

In this study, no change produced by LBD-005 was observed after the administration of the test mateiral in clinical findings, body weights, food consumption, water consumption, gross necropsy findings and organ weights.

Although the decreased serum ALP in the female groups dosed at 1 and 2 mg/kg and the increased serum GOT in the male groups dosed at 0.5 and 2 mg/kg have been related with the dose, it is difficult to conclude that the changes are treatment-related toxicities without the related histopathological changes.

Although GM-CSF is a stimulating factor, in this study, the female rats showed a dose response in decreasing segmented neutrophil count but not in total WBC. Morstyn *et al.* (1988) also reported changes in white cells, he noticed a transient fall in the WBC level after both intravenous and subcutaneous administration which was followed by a susequent increase.

One of the problems occuring in clinical trials, using the subcutaneous route of administration, has been the association of local rashes(Morstyn and Burgess

1988). In this study the injection site inflammatory reactions were seen only at the high dose, which is 200 times the predicted clinical dose (Thompson *et al.*, 1989) using the subcutaneous route.

It is, therefore, concluded that in rats the maximum tolerated dose is no less than 2.0 mg/kg/dry and no effect level is less than 1.0 mg/kg/day. This is 100 times the clinical dose and with this safety margin, LBD-005 deserves further research.

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