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Single Oral Dose Toxicity Studies of PGB-2, a Novel Polyglucosamine Polymer Produced from *Citrobacter* sp. BL-4 in Mice

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This study was conducted to obtain information of the oral dose acute toxicity of PGB-2, a novel polyglucosamine polymer produced from *Citrobacter* sp. BL-4 (a new strain) in male and female mice. Mortality, body weight changes, clinical signs were monitored during 14 days after single oral dose of test article at dose levels of 2000, 1000, 500, 250 and 125 ml/kg. Gross lesions, organ weight and histopathology of principal organs were examined after necropsy. As the results, we could not find any mortalities, clinical signs, changes in the body weight and gross findings except for white foci in the liver. In addition, no PGB-2-treatment related abnormal changes on the organ weight and histopathology of principle organs were detected except for atypical signs of liver. White liver foci were confirmed as focal infiltration of inflammatory cells. The results suggest that the PGB-2 is relatively safe in mice but the possibility of hepatotoxicity could not be excluded. The LD_{50} and approximate LD in mice after single oral dose of PGB-2 were considered over 2000 mg/kg, respectively. In future, the potential hepatotoxicity of PGB-2 should be evaluated through the repeat dose toxicity test prior to develop as a new agent.

Key words: Polyglucosamine, PGB-2, Polymer, *Citrobacter*, Single oral dose toxicity, Mice, Histopathology.

INTRODUCTION

The deacetylated form of chitin, chitosan (polyglucosamine), has unique properties which make it useful for a variety of industrial applications such as a viscosity control agent, adhesive, paper- strengthening agent, and flocculating aid, etc. The traditional industrial source of chitin is shellfish waste from shrimp, crab, and lobster processing. However, problems with seasonal and limited supply, confined production locations, product variability, and high processing costs associated with the chemical conversion of chitin to chitosan appear to have limited the potential industrial acceptance of this polymer (White *et al.*, 1979).

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Recent advances in fermentation technology suggest that large-scale culturing of an organism that contains chitosan might be an attractive route to the production of this polymer and microbial biopolymers have received considerable attention as flocculating agents; e.g., those from *Bacillus* (Yoon *et al.*, 1998; Deng *et al.*, 2003), *Phodovulum* (Watanebe *et al.*, 1999), *Rhodococcus* (Kurane *et al.*, 1986), *Klebsiella* (Dermlim *et al.*, 1999), *Paenibacillus* (Oh *et al.*, 2001), *Alcaligenes* (Toeda and Kurane, 1991), *Enterobacter* (Yokoi *et al.*, 2001) and *Citrobacter* (Fujita *et al.*, 2001; Jang *et al.*, 2001). The polysaccharide-type bioflocculants are more effective and stable than protein and glycoprotein-type bioflocculants. Also the cationic flocculants are known to be more powerful for treatment of negatively charged organic waste in sewage or the recovery of microbial cells. Most microbial polysaccharide are produced as a less effective anionic or natural type; nevertheless, the microbial cationic bioflocculant, except for the natural

biopolymer chitosan, has rarely been developed.

A novel polyglucosamine polymer, PGB-2, was produced extracellularly from a new strain *Citrobacter* sp. BL-4 using pH-stat fed batch cultivation. It was composed of 97.3% glucosamine and 2.7% rhamnose; its average molecular weight, solubility in 2% acetic acid and viscosity were 20 kDa, 5 g/l and 2.9 cps, respectively. FT-IR and ^{1}H NMR spectra of PGB-2 revealed a close identity with chitosan from crab shells (Kim *et al.*, 2006). However, there are no reports on the toxicological aspects of microbial biopolymers.

The purpose of the present study, therefore, is to obtain the primary safety information about PGB-2, a novel polyglucosamine polymer and further clarify it's safety for clinical use.

MATERIALS AND METHODS

Experimental animals. Each of thirty female and male ICR mice (6-week old upon receipt, SLC, Japan) was used after acclimatization for 8 days. Five animals were allocated per polycarbonate cage in a temperature (20~25°C) and humidity (40~45%) controlled room. Light : dark cycle was 12 hr : 12 hr and food (Samyang, Korea) and water were supplied free to access. All ani-

Table 1. Body weight gains after oral dose of PGB-2

Group		Intervals		
		Day 0[a]~Day 7	Day 7~Day 13	Day 0~Day 14
Male	G0M	9.24 ± 0.52	1.26 ± 1.19	7.52 ± 1.32
	G1M	8.80 ± 1.86	1.90 ± 0.38	8.64 ± 1.54
	G2M	9.54 ± 1.05	2.24 ± 0.86	9.52 ± 1.27*
	G3M	10.10 ± 1.03	2.08 ± 0.88*	9.72 ± 1.57*
	G4M	9.44 ± 0.93	1.18 ± 1.94	8.00 ± 1.66
	G5M	9.22 ± 1.54	2.70 ± 1.29*	9.32 ± 2.40
Female	G0F	6.32 ± 1.75	1.18 ± 0.79	5.72 ± 1.63
	G1F	5.84 ± 2.29	0.20 ± 0.87	4.50 ± 2.15
	G2F	5.50 ± 1.59	0.80 ± 1.50	4.70 ± 1.89
	G3F	6.22 ± 0.89	2.12 ± 1.00	5.66 ± 1.71
	G4F	5.24 ± 0.49	1.60 ± 0.64	4.64 ± 0.83
	G5F	4.56 ± 0.69	1.50 ± 1.25	4.76 ± 1.04

Values are expressed as mean ± S.D., g (n = 5), G0: vehicle control, G1: 2000 mg/kg-dosing group, G2: 1000 mg/kg-dosing group, G3: 500 mg/kg-dosing group; G4: 250 mg/kg-dosing group, G5: 125 mg/kg-dosing group.
[a]Day of dosing, *$p < 0.05$ compared to that of vehicle control by MWW test.

Table 2. Changes on the absolute organ weights after oral dose of PGB-2

Group	Principal organs											
	Lung	Heart	Thymus	Kidney L	Adrenal gland L	Spleen	Testis-ovary L	Liver	Pancreas S	Brain	Epididymis L/Uterus	Lymph node L[a]
G0M	0.202 ± 0.015	0.162 ± 0.014	0.052 ± 0.005	0.308 ± 0.051	0.008 ± 0.003	0.094 ± 0.014	0.114 ± 0.020	1.519 ± 0.074	0.193 ± 0.025	0.492 ± 0.028	0.036 ± 0.004	0.043 ± 0.030
G1M	0.208 ± 0.020	0.166 ± 0.014	0.058 ± 0.005	0.362 ± 0.045	0.007 ± 0.002	0.112 ± 0.027	0.119 ± 0.017	1.564 ± 0.183	0.206 ± 0.027	0.493 ± 0.015	0.036 ± 0.004	0.029 ± 0.010
G2M	0.207 ± 0.014	0.176 ± 0.006	0.042 ± 0.016	0.342 ± 0.051	0.009 ± 0.002	0.115 ± 0.018	0.128 ± 0.004	1.545 ± 0.099	0.184 ± 0.038	0.486 ± 0.021	0.037 ± 0.005	0.038 ± 0.009
G3M	0.209 ± 0.014	0.170 ± 0.018	0.053 ± 0.015	0.362 ± 0.033	0.009 ± 0.003	0.099 ± 0.016	0.118 ± 0.013	1.632 ± 0.106	0.192 ± 0.028	0.485 ± 0.022	0.039 ± 0.004	0.030 ± 0.010
G4M	0.201 ± 0.006	0.180 ± 0.014	0.053 ± 0.010	0.321 ± 0.028	0.007 ± 0.002	0.103 ± 0.011	0.123 ± 0.015	1.525 ± 0.108	0.190 ± 0.034	0.477 ± 0.021	0.041 ± 0.004	0.024 ± 0.006
G5M	0.208 ± 0.012	0.178 ± 0.014	0.054 ± 0.012	0.332 ± 0.031	0.007 ± 0.003	0.095 ± 0.009	0.119 ± 0.017	1.574 ± 0.181	0.184 ± 0.019	0.490 ± 0.013	0.039 ± 0.004	0.023 ± 0.009
G0F	0.186 ± 0.011	0.143 ± 0.012	0.067 ± 0.018	0.201 ± 0.020	0.008 ± 0.001	0.102 ± 0.013	0.030 ± 0.004	1.189 ± 0.066	0.200 ± 0.021	0.483 ± 0.011	0.173 ± 0.072	0.025 ± 0.009
G1F	0.178 ± 0.003	0.137 ± 0.009	0.057 ± 0.023	0.196 ± 0.023	0.009 ± 0.002	0.097 ± 0.027	0.034 ± 0.017	1.116 ± 0.170	0.180 ± 0.022	0.483 ± 0.021	0.137 ± 0.053	0.023 ± 0.009
G2F	0.178 ± 0.006	0.139 ± 0.011	0.049 ± 0.010	0.203 ± 0.024	0.006 ± 0.001	0.105 ± 0.024	0.029 ± 0.011	1.168 ± 0.091	0.175 ± 0.031	0.487 ± 0.024	0.124 ± 0.023	0.019 ± 0.006
G3F	0.175 ± 0.008	0.140 ± 0.009	0.057 ± 0.015	0.213 ± 0.026	0.009 ± 0.002	0.102 ± 0.015	0.036 ± 0.014	1.188 ± 0.217	0.181 ± 0.018	0.474 ± 0.022	0.146 ± 0.076	0.019 ± 0.006
G4F	0.178 ± 0.011	0.139 ± 0.007	0.061 ± 0.011	0.202 ± 0.007	0.008 ± 0.002	0.119 ± 0.024	0.038 ± 0.010	1.207 ± 0.085	0.183 ± 0.024	0.467 ± 0.026	0.125 ± 0.044	0.024 ± 0.005
G5F	0.178 ± 0.006	0.144 ± 0.029	0.057 ± 0.010	0.198 ± 0.020	0.009 ± 0.002	0.101 ± 0.023	0.042 ± 0.023	1.085 ± 0.187	0.190 ± 0.024	0.465 ± 0.029	0.146 ± 0.050	0.021 ± 0.009

Values are expressed as mean ± S.D., g (n = 5), G0: vehicle control, G1: 2000 mg/kg-dosing group, G2: 1000 mg/kg-dosing group, G3: 500 mg/kg-dosing group; G4: 250 mg/kg-dosing group, G5: 125 mg/kg-dosing group, M: male, F: female, L: left sides, S: splenic lobes.
[a]Popliteal lymph node.

ing and terminal necropsy. Animals were marked by picric acid. The experimental protocols were conducted in accordance with the Korea Food and Drug Administration (KFDA) guidelines.

Purification of PGB-2, grouping and dosing. The strain *Citrobacter* sp. BL-4 was cultivated in pH-stat fed-batch culture in a 5 l jar fermenter (KoBiotech Co., Korea) containing 3 l of a liquid medium composed of 1.5% (w/v) sodium acetate, 0.1% $(NH_4)_2SO_4$, 0.1% yeast extract, 2.0% $MgSO_4 \cdot 7H_2O$, 0.5% NaCl, and 0.05% trace elements, at 30°C, 5000rpm, and 0.3 volume of air added to liquid volume per minute for 96 h. Acetic acid solution was fed intermittently to maintain the pH at 8.0. showed as previously (Kim *et al.*, 2006). The extracellular polymer excreted from the *Citrobacter* sp. BL-4 was mixed with three volume of absolute ethanol and precipitated at 4°C for 12 h (Yu *et al.*, 2004). The precipitant was then treated twice with 2 M NaOH at 121°C for 10 min to remove the residual protein and then neutralized with 2 M HCl to near neutrality. The recovered biopolymer was lysophilized after dialysis against distilled water (Kim *et al.*, 2006). PGB-2 is white powder and stored in a refrigerator at -20°C to protect from light and degeneration. PGB-2 was well solublized upto 100 mg/ml concentrations in distilled water and observed as clear light yellow solution. The test article was orally administered once at a dosage volume of 20 ml/kg with distilled water as vehicle.

The animals were distributed into 12 groups (5 mice per group) upon receipt. The highest dosage level was 2000 mg/kg according to the recommendation of Korean Food and Drug Administration (KFDA) (2005) and Organization for Economic Co-Operation and Development (OECD) (2001) guidelines, and 1000, 500, 250 and 125 mg/kg was selected using common ratio 2. In addition, a vehicle-treated control group was added. Animal was orally dosed once using a sonde attached to a syringe of 1ml after overnight fasting (about 18hr, water was not restricted). Food and water were restricted further for about 3 hours after dosing.

Observation of clinical signs. All abnormal clinical signs were recorded before and after treatment at least

Table 3. Changes on the relative organ weights after oral dose of PGB-2

Group	Principal organs											
	Lung	Heart	Thymus	Kidney L	Adrenal gland L	Spleen	Testis-ovary L	Liver	Pancreas S	Brain	Epididymis L/Uterus	Lymph node L[a]
G0M	0.527 ± 0.050	0.422 ± 0.035	0.134 ± 0.011	0.803 ± 0.129	0.021 ± 0.008	0.244 ± 0.031	0.295 ± 0.044	3.962 ± 1.520	0.524 ± 0.074	1.284 ± 0.099	0.095 ± 0.007	0.110 ± 0.074
G1M	0.522 ± 0.043	0.428 ± 0.036	0.152 ± 0.019	0.933 ± 0.101	0.019 ± 0.004	0.269 ± 0.046	0.340 ± 0.046	3.965 ± 0.159	0.518 ± 0.086	1.252 ± 0.093	0.093 ± 0.011	0.077 ± 0.022
G2M	0.506 ± 0.036	0.439 ± 0.010	0.102 ± 0.041	0.854 ± 0.109	0.020 ± 0.001	0.290 ± 0.041	0.317 ± 0.012	3.820 ± 0.205	0.463 ± 0.091	1.197 ± 0.067	0.091 ± 0.012	0.095 ± 0.019
G3M	0.517 ± 0.053	0.422 ± 0.048	0.135 ± 0.031	0.886 ± 0.110	0.022 ± 0.005	0.256 ± 0.033	0.288 ± 0.044	3.969 ± 0.081	0.489 ± 0.051	1.190 ± 0.100	0.094 ± 0.009	0.078 ± 0.015
G4M	0.504 ± 0.041	0.451 ± 0.036	0.138 ± 0.022	0.821 ± 0.113	0.017 ± 0.005	0.258 ± 0.032	0.320 ± 0.025	3.840 ± 0.282	0.473 ± 0.071	1.203 ± 0.124	0.105 ± 0.008	0.063 ± 0.017
G5M	0.529 ± 0.050	0.457 ± 0.021	0.147 ± 0.023	0.858 ± 0.149	0.018 ± 0.005	0.240 ± 0.008	0.305 ± 0.035	3.942 ± 0.142	0.468 ± 0.066	1.238 ± 0.087	0.101 ± 0.004	0.064 ± 0.015
G0F	0.594 ± 0.020	0.455 ± 0.039	0.212 ± 0.048	0.644 ± 0.079	0.024 ± 0.003	0.326 ± 0.044	0.096 ± 0.013	3.790 ± 0.141	0.638 ± 0.035	1.545 ± 0.117	0.552 ± 0.234	0.078 ± 0.024
G1F	0.596 ± 0.040	0.459 ± 0.031	0.204 ± 0.057	0.674 ± 0.051	0.029 ± 0.005	0.344 ± 0.055	0.123 ± 0.043	3.663 ± 0.289	0.577 ± 0.083	1.605 ± 0.085	0.467 ± 0.165	0.082 ± 0.015
G2F	0.585 ± 0.024	0.457 ± 0.033	0.170 ± 0.016	0.647 ± 0.036	0.021 ± 0.004	0.356 ± 0.074	0.101 ± 0.032	3.835 ± 0.194	0.562 ± 0.088	1.584 ± 0.111	0.400 ± 0.071	0.062 ± 0.018
G3F	0.568 ± 0.084	0.456 ± 0.052	0.199 ± 0.037	0.713 ± 0.086	0.031 ± 0.009	0.344 ± 0.030	0.131 ± 0.021*	3.773 ± 0.325	0.593 ± 0.044	1.535 ± 0.195	0.499 ± 0.189	0.065 ± 0.013
G4F	0.572 ± 0.044	0.453 ± 0.020	0.204 ± 0.035	0.644 ± 0.038	0.028 ± 0.005	0.362 ± 0.048	0.113 ± 0.020	3.881 ± 0.176	0.599 ± 0.054	1.488 ± 0.146	0.357 ± 0.059	0.076 ± 0.013
G5F	0.579 ± 0.024	0.478 ± 0.085	0.177 ± 0.016	0.638 ± 0.076	0.028 ± 0.006	0.315 ± 0.050	0.125 ± 0.065	3.519 ± 0.395	0.624 ± 0.104	1.509 ± 0.183	0.440 ± 0.131	0.067 ± 0.027

Values are expressed as mean ± S.D., % (n = 5); G0: vehicle control, G1: 2000 mg/kg-dosing group, G2: 1000 mg/kg-dosing group, G3: 500 mg/kg-dosing group; G4: 250 mg/kg-dosing group, G5: 125 mg/kg-dosing group, M: male, F: female, L: left sides, S: splenic lobes.

[a]Popliteal lymph node, *$p < 0.05$ compared to that of vehicle control by MWW test.

twice a day based on the functional observational battery test (Irwin, 1968; Dourish, 1987).

Body weight changes. Body weights were measured at the day of dosing (Day 0) immediately before treatment, 1, 2, 7, 13 and 14 days after dosing. In addition, to reduce the erratum originated from individual body weight differences of animals at initial dosing, body weigh gains during Day 0~Day 7, Day 7~Day 13 and Day 0~Day 14 was also calculated based on measured body weight at each points.

Necropsy. All unschedully died animals were grossly observed immediately after death and all survived animals were subjected to terminal necropsy. Animals were asphyxiated by carbon dioxide and gross necropsy was performed at 14 days after treatment.

Specific organs such as lung, heart, kidney, spleen, testis, liver, pancreas, epididymis, popliteal lymph node, ovary, brain, and uterus were grossly observed.

Organ weight measurement. The absolute organ weight was measured and then relative organ weight (% of body weight) was calculated for the following organs of all experimental animals when they were sacrificed.

Measured organs: Lung, Heart, Kidney (left), Spleen, Testis (left), Liver, Pancreas (splenic lobes), Epididymis (left), Popliteal lymph node (left), Ovary (left), Brain, and Uterus.

Histopathology. Principle organs listed below were sampled at terminal necropsy, and fixed in 10% NBF (neutral buffered formalin). After 18 hrs of fixation, paraffin embedding was conducted and 3~4 μm sections were prepared by routine histological methods. Representative sections of each specified organs were stained with Hematoxylin & eosin for light microscopical examination.

Table 4. Necropsy findings after oral dose of PGB-2

Group	Male						Female					
	G0M	G1M	G2M	G3M	G4M	G5M	G0F	G1F	G2F	G3F	G4F	G5F
Lung												
Normal	3/5	3/5	2/5	3/5	2/5	4/5	4/5	4/5	4/5	3/5	3/5	5/5
Red spots	2/5	2/5	3/5	2/5	3/5	1/5	1/5	1/5	1/5	2/5	2/5	0/5
Heart												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Thymus												
Normal	3/5	4/5	2/5	4/5	4/5	4/5	4/5	3/5	4/5	4/5	5/5	5/5
Atrophy	1/5	1/5	3/5	1/5	1/5	1/5	1/5	2/5	1/5	1/5	0/5	0/5
Hemorrhage	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Kidney												
Normal	5/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Cyst	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Adrenal gland												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Spleen												
Normal	2/5	3/5	4/5	4/5	3/5	2/5	4/5	3/5	4/5	4/5	5/5	3/5
Reduced size	3/5	2/5	1/5	1/5	2/5	3/5	1/5	2/5	1/5	1/5	0/5	2/5
Testis/Ovary												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Liver												
Normal	5/5	3/5	5/5	4/5	4/5	4/5	5/5	4/5	5/5	5/5	5/5	5/5
White foci	0/5	2/5	0/5	1/5	1/5	1/5	0/5	1/5	0/5	0/5	0/5	0/5
Pancreas												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Brain												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Epididymis/Uterus												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Lymph node[a]												
Normal	5/5	3/5	3/5	4/5	4/5	4/5	3/5	4/5	4/5	3/5	3/5	4/5
Enlarged	0/5	2/5	2/5	1/5	1/5	1/5	2/5	1/5	1/5	2/5	2/5	1/5

Observed animals/total observed animals (n = 5), G0: vehicle control, G1: 2000 mg/kg-dosing group, G2: 1000 mg/kg-dosing group, G3: 500 mg/kg-dosing group; G4: 250 mg/kg-dosing group, G5: 125 mg/kg-dosing group.
[a]Bilateral popliteal lymph node.

Specific organs sampled: Lung, Heart, Kidney (left), Spleen, Testis (left), Liver, Pancreas (splenic lobes), Epididymis (left), Popliteal lymph node (left), Ovary (left), Brain, and Uterus.

Statistical analyses. Changes of body weights were analyzed by Mann-Whitney-Wilcoxon test (MWW test) compared to those of vehicle controls. LD_{50} was calculated by Probit method. Statistical analyses were conducted using SPSS for Windows (Release 6.1.3., SPSS Inc., USA). In addition, degree of clinical signs, gross and histopathological findings were subdivided into 3 degrees: 3 + Severe, 2 + moderate, 1 + slight.

RESULTS

Mortalities and clinical signs. No unscheduled or PGB-2-treatment related mortalities and clinical signs were detected in all dose levels tested in this study.

Changes on body weights and gains. No meaningful changes on body weight gains were detected in all dosing groups tested compared to that of vehicle control except for significantly ($p < 0.05$) increases of body weight gains during the whole experimental periods (day 0~14) of PGB-2 500 and 1000 mg/kg-dosing male groups (Table 1).

Changes on the organ weight. Absolute and relative organ weights of 12 principal organs were observed. Although there was no significant difference among treatment and control groups, 500 mg/kg-dosed female group showed increase of ovary weight (Tables 2, 3).

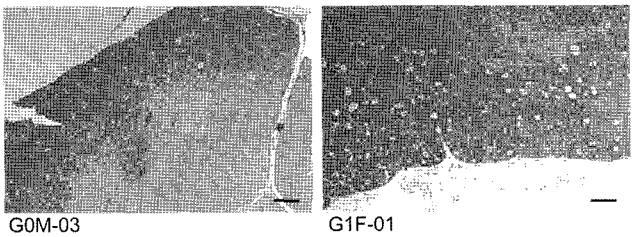

Fig. 2. Histopathological changes detected in the thymus. Note that depletion of thymocytes in the cortex were randomly detected dispersed throughout all the testing groups including vehicle control. They did not show any dose dependency. All Hematoxylin & Eosin stain; Scale bars = 100 μm.

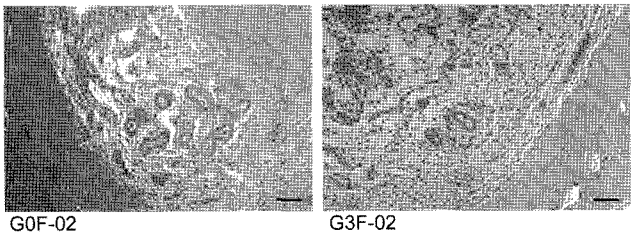

Fig. 3. Histopathological changes detected in the uterus. Note that various degrees of edematous changes were detected in all testing groups including vehicle control. Any dose dependency were not shown in histopathological changes and considered as usual changes with estrus cycle. All Hematoxylin & Eosin stain; Scale bars = 100 μm.

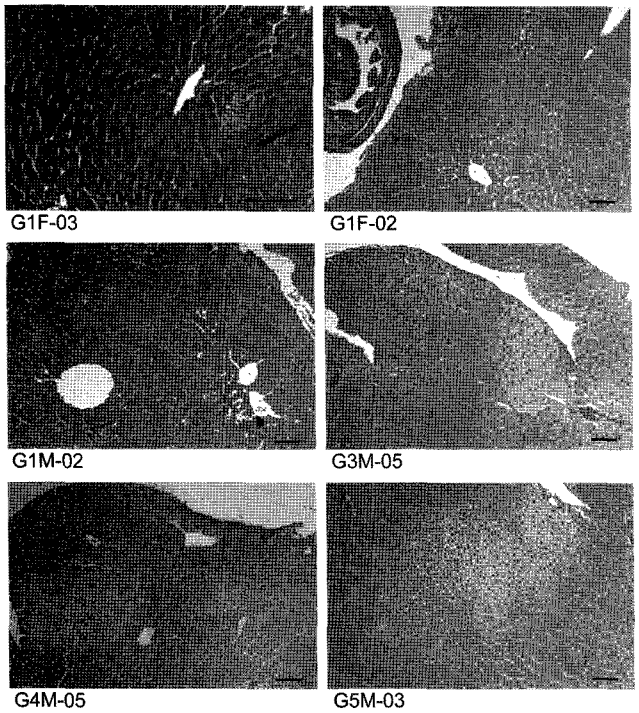

Fig. 1. Histopathological changes observed in the liver. Note mononuclear inflammatory cell infiltrations with necrosis or fatty changes were observed throughout the whole PGB-2-dosing groups not in vehicle control. All Hematoxylin & Eosin stain; Scale bars = 100 μm.

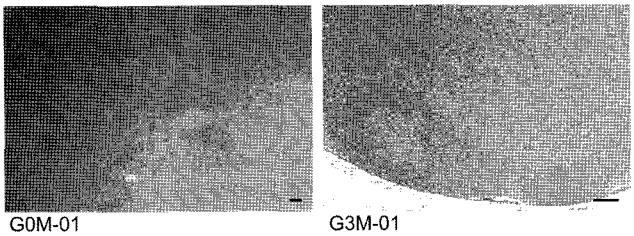

Fig. 4. Histopathological changes detected in the popliteal lymph node. Note that hyperplasia of lymphoid cells in the cortex and the change was observed in all testing groups including vehicle control. All Hematoxylin & Eosin stain; Scale bars = 100 μm.

Table 5. Histopathological findings after oral dose of PGB-2

Group	Male						Female					
	G0M	G1M	G2M	G3M	G4M	G5M	G0F	G1F	G2F	G3F	G4F	G5F
Lung												
Normal	2/5	4/5	3/5	2/5	4/5	3/5	3/5	3/5	4/5	4/5	2/5	3/5
Hypertrophy-hemorrhage[a]	3/5	1/5	2/5	3/5	1/5	2/5	2/5	2/5	1/5	1/5	3/5	2/5
Heart												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Thymus												
Normal	4/5	5/5	5/5	5/5	4/5	5/5	5/5	4/5	5/5	5/5	5/5	5/5
Depletion of lymphoid cells	1/5	0/5	0/5	0/5	1/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5
Kidney												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Adrenal gland left												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	4/5	5/5	5/5	5/5
Hemorrhage	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
Spleen												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Testis/Ovary left												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Liver												
Normal	5/5	3/5	5/5	3/5	4/5	4/5	4/5	4/5	5/5	5/5	5/5	5/5
IF-NE[b]	0/5	1/5	0/5	1/5	0/5	0/5	1/5	1/5	0/5	0/5	0/5	0/5
Fatty changes	0/5	0/5	0/5	1/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Pancreas splenic												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Brain												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Epididymis left/Uterus												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	4/5	5/5	5/5	4/5	4/5	5/5
Edematous changes	--	--	--	--	--	--	1/5	0/5	0/5	1/5	1/5	0/5
Lymph node[c]												
Normal	3/5	5/5	4/5	3/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Lymphoid hyperplasia	2/5	0/5	1/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Observed animals/total observed animals (n = 5); G0: vehicle control, G1: 2000 mg/kg-dosing group, G2: 1000 mg/kg-dosing group, G3: 500 mg/kg-dosing group; G4: 250 mg/kg-dosing group, G5: 125 mg/kg-dosing group.
[a]Hypertrophy-hemorrhage: hypertrophy of lung alveolus wall with focal hemorrhage.
[b]IF-NE: Focal inflammatory cell infiltration with necrosis.
[c]Bilateral popliteal lymph node.

Necropsy findings. Some animals in male treatment groups showed white foci scattered on the surface of liver. One female in the G1 group showed the same change (Table 4).

Histopathological findings. White foci found in the liver were composed of inflammatory cells with hepatocytes necrosis and fatty change of hepatocytes (Fig. 1). Depletion of lymphoid cells in the cortex of thymus (Fig. 2), hemorrhage of adrenal glands, edematous changes in the uterus (Fig. 3), hypertrophy of lung alveolus wall with focal hemorrhage, and hyperplasia of lymphoid cells in the popliteal lymph nodes (Fig. 4, Table 5) were observed in animals showed gross lesions.

DISCUSSION

In the present study, we investigate the acute toxicity of PGB-2 with single oral dose, a novel polyglucosamine polymer in female and male mice as part of the safety test. In order to calculate 50% lethal dose (LD_{50}), test article was orally administered once to male and female ICR mice at dose levels of 2000, 1000, 500, 250, 125 and 0 (control) ml/kg (body wt.). Mortality and body weight changes, clinical signs were monitored for 14 days after single treatment according to KFDA (2005) and OECD (2001).

In results, we could not find any mortalities, clinical signs, changes in the body weight, and gross findings except for white foci in the liver. In addition, no PGB-2-treatment related abnormal changes in the organ weight and histopathology of principle organs except for some accidental findings and atypical signs of liver. White liver foci were confirmed as focal infiltration of inflammatory cells. The results obtained in this study suggest that the PGB-2 is relatively safe in mice but the potential hepa-

totoxicity could not be excluded.

Significant ($p < 0.05$) increases in body weight or gains observed in 1000 and 500 mg/kg-treated male groups compared to those of vehicle control groups were considered as no meaningful changes since no dose dependency was observed. These changes were restricted to PGB-2 1000 mg/kg-teated male group only.

Significant ($p < 0.05$) increases of relative ovary weight detected in 500 mg/kg-dosing female group was also considered as no PGB-2-treatment related abnormal changes. They also did not show any dose dependency and the ovary weights in the mice are generally changed with estrus cycles (Pineda, 1989). These changes were restricted to the some animals in 500 mg/kg-dosing female group only.

Congestion spots in lung, atrophy and/or hemorrhage of thymus, cyst in kidney, spleen atrophy and hypertrophy of popliteal lymph node detected in the present study as gross findings, and as depletion of lymphoid cells in the cortex of thymus, hemorrhage of adrenal gland, hypertrophy of lung alveolus wall with focal hemorrhages, edematous changes in the uterus, and hyperplasia of lymphoid cells in the popliteal lymph node detected as histopathological findings were considered as accidental findings and they were not considered as PGB-2-treat abnormal findings because they were restricted in some dosing groups and in some case and also they were observed in vehicle control. In addition, gross finding data and histopathological data did not show clear dose dependency and most of them were rarely observed in normal mice (Lee *et al.*, 2005, 2006). The edematous changes of uterus are general signs related to the estrus cycles (Banks, 1986). The atypical white foci detected in some PGB-2 dosing groups were revealed as focal infiltration of inflammatory cells with necrosis or fatty changes and these changes on the liver were considered as PGB-2 treatment related abnormal signs because they were restricted to PGB-2-dosing groups.

In our experiment, no obvious acute toxicity was observed after a single oral exposure upto 2000 mg/kg PGB-2 except for liver white foci composed of inflammatory cells with hepatocytes necrosis and fatty change of hepatocytes. Therefore, the LD_{50} of PGB-2 in mice was considered as over 2000 mg/kg in both male and female but the possibility of hepatotoxicity could not be excluded.

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