J. Toxicol. Pub. Health
Vol. 21, No. 4, pp. 361-365 (2005)

Official Journal of
Korean Society of Toxicology
Available online at http://www.toxmut.or.kr

Single Oral Dose Toxicity Studies of Polycan, β-Glucan Originated from Aureobasidium in Mice

Hyeung-Sik Lee[1], Kun-Ju Yang[2], Hyun-Dong Shin[2], Bok-Ryeon Park[2], Chang-Woo Son[2], Hee-Jeong Jang[2], Dong-Chan Park[3], Young-Mi Jung[3] and Sae-Kwang Ku[4]

[1]Department of Herbal Biotechnology, Daegu Haany University, Gyeongsan, 712-715
[2]Glucan Corp. Research Institute, Marine Biotechnology Center 221, Busan 617-763
[3]ENZ BIO Co., Kyungpook National University, Daegu 702-701
[4]Pharmacology & Toxicology Lab., Central Research Laboratories, Dong Wha Pharm. Ind. Co. Anyang 430-017, Korea

Received October 19, 2005; Accepted November 9, 2005

ABSTRACT. This study was conducted to obtain the acute information of the oral dose toxicity of Polycan - originated from *Aureobasidium pullulans* SM-2001 (half of the dry material is -1,3/1,6-glucans), a UV induced mutant of *A. pullulans*, having various pharmacological effects, in male and female mice. In order to calculate 50% lethal dose (LD_{50}), approximate LD and target organs, test article was administered twice by oral gavage to male and female ICR mice at total 1000, 500 and 250 mg/kg. The mortality and changes on body weight, clinical signs and gross observation were monitored during 14 days after dosing. As the results, we could not find any mortalities, clinical signs, changes in the body weight and gross findings. The results obtained in this study suggest that the Polycan is non-toxic in mice and is therefore likely to be safe for clinical use. The LD_{50} and approximate LD in mice after single oral dose of Polycan were considered over 1000 mg/kg, respectively.

Keywords: Polycan, β-Glucan, Single oral dose toxicity, Mice.

INTRODUCTION

β-Glucan is a fiber-type complex sugar (polysaccharide) derived from the cell wall of baker's yeast, oat and barley fiber, and many medicinal mushrooms, such as maitake. The primary uses of β-glucan are to enhance the immune system (Czop, 1986; Estrada *et al.*, 1997), to lower blood cholesterol levels (Lia *et al.*, 1995; Bell *et al.*, 1999) and to treat tumor (Gu *et al.*, 2005; Yan *et al.*, 2005). In addition, like other sources of soluble fiber, β-glucan is, according to preliminary studies, helpful in reducing the elevation in blood sugar levels that typically follow a meal. β-Glucan produces this effect by delaying gastric emptying so that dietary sugar is absorbed more gradually, as well as by possibly increasing the tissue sensitivity to insulin. These effects suggest possible benefit in blood sugar control in people with diabetes (Braaten *et al.*, 1994; Bourdon *et al.*, 1999).

The preclinical safety tests for human trails of β-glucan have been studied in various types having different origins, and β-glucan has been showed any toxicological evidences and no drug-drug interactions (Chihara, 1983; Feletti *et al.*, 1992; Spicer *et al.*, 1999; Delaney *et al.*, 2003). Therefore, β-glucan has been used as ingredients of food, cosmetics and various types of drugs with direct use as drugs (Bais *et al.*, 2005; Douwes, 2005; Plat and Mensink, 2005). However, the potential safety of β-glucans has still not been fully understood because they showed somewhat different characteristics according to origin (Seo *et al.*, 2002). In addition, the preclinical safety study about β-glucans originate from fungi are seldom except for *Candida albicans* (Feletti *et al.*, 1992).

Polycan™ is purified from *Aureobasidium pullulans* SM-2001 (half of the dry material is -1,3/1,6-glucans), a UV induced mutant of *Aureobasidium pullulans* and they showed somewhat different characteristics from other β-glucan derived from other origins (Seo *et al.*, 2002). Until now, there are no preclinical studies about Polycan even if they have somewhat different characteristics.

Correspondence to: Sae-Kwang Ku, Pharmacology & Toxicology Lab., Central Research Laboratories, Dong Wha Pharm. Ind. Co., Anyang, 430-017, Kyunggido, Korea
E-mail: skku@iris.dwcrc.co.kr

The objective of the present study, therefore, was to obtain the primary safety information about Polycan, newly purified β-glucan from *Aureobasidium pullulans* SM-2001 and further clarify their safety for clinical use.

MATERIALS AND METHODS

Animals and husbandry

Each of twenty female and male ICR mice (6-wk old upon receipt, Charles River, Japan) was used after acclimatization for 19 days. Animals were allocated five per polycarbonate cage in a temperature (20~25°C) and humidity (30~35%) controlled room. Light : dark cycle was 12 hr : 12 hr and feed (Samyang, Korea) and water were supplied free to access. All animals were overnight fasted before dosing and terminal necropsy. Animals were marked by picric acid.

Test articles and formulation

Polycan (Glucan Corp. Ltd., Korea) is brownish-sticky but homogenous solution. Polycan was stored in a refrigerator at 4°C to protect from light and degeneration. The test article was orally administered twice by oral gavage to male and female ICR mice at total 1000, 500 and 250 mg/kg.

Groupings and dosing

The animals were distributed into eight groups 5 mice per group upon receipt. The fixed highest dosage level was 1000 mg/kg - the highest concentration of Polycan in vehicle (distilled water) was 2.5%, according to the recommendation of KFDA Guidelines (1999-61), and 500 and 250 mg/kg was selected using common ratio 2. In addition, a vehicle control group was added as listed in Table 1. Animal was dosed twice by oral gavage (4 hr interval in one day; Day 0) after overnight fasting (about 18 hr, water was not restricted). Feed and water were restricted further for about 3 hours after dosing.

Table 1. Experimental design used in this study

Group	Sex	No. of animals	Animal No.	Total Dose (mg/kg)
G0M*	Male	5	G0M-01~G0M-05	0
G1M	Male	5	G1M-01~G1M-05	1000
G2M	Male	5	G2M-01~G2M-05	500
G3M	Male	5	G3M-01~G3M-05	250
G0F*	Female	5	G0F-01~G0F-05	0
G1F	Female	5	G1F-01~G1F-05	1000
G2F	Female	5	G2F-01~G2F-05	500
G3F	Female	5	G3F-01~G3F-05	250

* Vehicle control; distilled water 20 ml/kg as vehicle in this study; All test articles in vehicle were dosed twice in one day by oral gavage.

Observation of clinical signs

All abnormal clinical signs were recorded before and after dosing at least twice a day.

Changes of body weights

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 13 was also calculated based on measured body weight at each points.

Necropsy

All unscheduled died animals were grossly observed immediately after finding them and all survived animals were subjected to terminal necropsy. Animals were asphyxiated by ethyl ether (Duksan pure chemical Co., Ltd., Korea) and gross necropsy was performed in all animals at Day 14 after overnight fasting (about 18 hr, water was not restricted).

Statistical analyses

Changes of body weights were analyzed by Mann-Whitney U-Wilcoxon Rank Sum W test (MW test) compared to those of Vehicle control. LD_{50} was calculated by Probit method. Statistical analyses were conducted using SPSS for Windows (Release 6.1.3., SPSS Inc., USA).

RESULTS

Mortalities

No unscheduled mortalities were detected in all dose levels tested in this study. At terminal, all of animals (5/5; 100%) were survived in all dose levels tested including vehicle control (Table 2). Therefore, the LD_{50} and approximate LD in mice after single oral dose of Polycan were calculated over 1000 mg/kg, respectively in both male and female.

Clinical signs

In this study, no Polycan-treatment related abnormal clinical signs were observed during observation periods regardless of male and female mice except for some accidental findings such as salivation, anorexia and depress (Table 3).

Changes of body weights

A significantly ($p < 0.05$) increase in body weight gain

Table 2. Mortalities observed in male and female mice after twice in one day oral gavage of Polycan

Group ID		Day after dosing													Total[b]
		0[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	
Male	G0M	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5 (0%)
	G1M	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5 (0%)
	G2M	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5 (0%)
	G3M	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5 (0%)
Female	G0F	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5 (0%)
	G1F	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5 (0%)
	G2F	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5 (0%)
	G3F	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5 (0%)

Number of died animals; [a]Dosing day; [b]total mortalities during 14 days of observation periods, died animals/total observed animals (n = 5) (percentages).

Table 3. Clinical signs observed in male and female mice after twice in one day oral gavage of Polycan

Group ID		Clinical signs			
		Normal	Salivation	Hair loss	Depress
Male	G0M	2/5 (40%)[a]	1/5 (20%)	2/5 (40%)	1/5 (20%)
	G1M	2/5 (40%)	1/5 (20%)	1/5 (20%)	1/5 (20%)
	G2M	3/5 (60%)	1/5 (20%)	1/5 (20%)	1/5 (20%)
	G3M	3/5 (60%)	2/5 (40%)	0/5 (0%)	1/5 (20%)
Female	G0F	2/5 (40%)	2/5 (40%)	2/5 (40%)	1/5 (20%)
	G1F	3/5 (60%)	1/5 (20%)	1/5 (20%)	0/5 (0%)
	G2F	2/5 (40%)	2/5 (40%)	1/5 (20%)	0/5 (0%)
	G3F	3/5 (60%)	2/5 (40%)	1/5 (20%)	1/5 (20%)

[a]Observed animals/total observed animals (n = 5) (percentages).

Table 4. Body weight gains in male and female mice after twice in one day oral gavage of Polycan

Group ID		Interval		
		Day 0[a]~Day 7	Day 7~Day 13	Day 0~Day 13
Male	G0M	3.34 ± 1.03	2.24 ± 0.67	5.58 ± 0.95
	G1M	4.38 ± 1.72	2.60 ± 0.73	6.98 ± 2.20
	G2M	5.52 ± 0.48*	2.60 ± 0.44	8.12 ± 0.56*
	G3M	6.10 ± 1.14*	3.38 ± 0.73**	9.48 ± 1.68**
Female	G0F	2.90 ± 0.53	2.86 ± 1.69	5.76 ± 2.13
	G1F	2.98 ± 1.61	1.94 ± 0.65	4.92 ± 1.55
	G2F	3.94 ± 0.87	1.68 ± 1.05	5.62 ± 1.38
	G3F	2.98 ± 0.97	1.64 ± 1.06	4.62 ± 1.43

Values are expressed as mean ± S.D., g (n = 5); [a]Day of dosing; * $p < 0.01$ compared to that of G0M by MW test; ** $p < 0.05$ compared to that of G0M by MW test.

was detected in 250 mg/kg-dosing male group compared to that of vehicle control at Day 13 and Day 14, respectively. In addition, significantly ($p < 0.01$ or $p < 0.05$) increases in body weight gains were detected in 500 and 250 mg/kg-dosing male groups compared to that of vehicle control. However, no meaningful changes on body weight and gains were detected in female groups compared to that of vehicle control in all dose levels tested (Table 4).

Necropsy findings

No Polycan-treatment related abnormal gross findings were observed during observation periods regardless of male and female mice except for some accidental findings such as cyst formation of kidney, atrophy and/or hypertrophy of spleen, atrophy of thymus or hypertrophy of popliteal lymph nodes (Table 5).

DISCUSSION

In the present study, we investigated the acute toxicity of single oral dose with Polycan, a newly purified β-glucan from *Aureobasidium pullulans* SM-2001 to mice as a part of the development. In order to calculate 50% lethal dose (LD_{50}), approximate LD and target organs, test article was administered twice by oral gavage to male and female ICR mice at total 1000, 500 and 250 mg/kg in the present study. As the results, we could not find any mortalities, clinical signs, changes in the body weight and gross findings quite similar to other types of β-glucan isolated from different origins (Chihara, 1983; Feletti *et al.*, 1992; Spicer *et al.*, 1999; Delaney *et al.*, 2003).

In KFDA Guidelines (1999-61), the recommended highest dose of test materials were 2 g/kg or the maxi-

Table 5. Necropsy findings observed in mice after twice in one day oral gavage of Polycan

Group ID[b]	MALE				FEMALE			
	G0	G1M	G2M	G3M	G0F	G1F	G2F	G3F
Lung								
Normal	5/5[a]	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Liver								
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Heart								
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Kidney								
Normal	4/5	4/5	4/5	4/5	4/5	5/5	5/5	4/5
Cyst	1/5	1/5	1/5	1/5	1/5	0/5	0/5	1/5
Spleen								
Normal	3/5	3/5	4/5	4/5	4/5	5/5	5/5	5/5
Atrophy	1/5	2/5	1/5	1/5	1/5	0/5	0/5	0/5
Hypertrophy	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Testis or Ovary								
Normal	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
Thymus								
Normal	3/5	4/5	4/5	5/5	4/5	3/5	5/5	4/5
Atrophy	2/5	1/5	1/5	0/5	1/5	2/5	0/5	1/5
Lymph node[b]								
Normal	5/5	5/5	5/5	5/5	4/5	4/5	3/5	3/5
Hypertrophy	0/5	0/5	0/5	0/5	1/5	1/5	2/5	2/5
Others								
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

[a]Observed animals/total observed animals (n = 5); [b]bilateral popliteal lymph node.

mum solubility, and they also recommended that in case of acute toxicity in mice, the dosage volume was below 20 ml/kg. In the present study, the highest dose of Polycan was selected as 1000 mg/kg base on the solubility in distilled water - the highest concentration in distill water was 2.5%, and 500 and 250 mg/kg was selected using common ratio 2 according to the recommendation of KFDA Guidelines (1999-61), and Polycan was dose twice a day. Therefore, total doses of Polycan used in this study were 1000, 500 and 250 mg/kg/20 ml, respectively.

Although the Hodge and Sterner (1949) classify as non toxic materials those LD_{50} were 5000~15000 mg/kg and the materials those LD_{50} were 500~5000 mg/kg also classified as relatively low toxic (Class III) by US Environmental Protection Agency (OPPTS 870.100, 1998), recently Notified Guidelines by Korean Food and Drug Administration (1999-61) recommended that the highest oral dose of test materials was 2 g/kg or the possible dose base on the solubility. In the present study, the LD_{50} and approximate LD in mice after single oral dose of Polycan were considered over 1000 mg/kg, respectively in both male and female.

Salivation, hair loss and/or depress signs detected in the present study were considered as accidental findings because these abnormal signs were detected in all dose levels tested including vehicle controls, and cyst formation of kidney, atrophy and/or hypertrophy of spleen, atrophy of thymus or hypertrophy of popliteal lymph nodes were detected as accidental findings in all dosing groups tested including vehicle controls. These increasing tendencies on the body weight gains detected in male groups did not considered as toxicological findings because the one of main functions of β-glucan generally have been known to enhance the immune system (Czop, 1986; Estrada *et al.*, 1997). In generally, enhanced animals their immune system, they showed relatively good growth patterns (Duarte *et al.*, 2000; Pinzone Fox *et al.*, 2005).

From these results, oral gavage of Polycan caused no serious toxic effect to the male and female mice upto 1000 mg/kg - the highest dosage tested in this study. Therefore, Polycan was considered that it has relatively favorable toxicological profiles similar to other types of β-glucan having different origins (Chihara, 1983; Feletti *et al.*, 1992; Spicer *et al.*, 1999; Delaney *et al.*, 2003).

REFERENCES

Bell, S., Goldman, V.M., Bistrian, B.R., Arnold, A.H., Ostroff,

G. and Forse, R.A. (1999): Effect of beta-glucan from oats and yeast on serum lipids. *Crit. Rev. Food Sci. Nutr.*, **39**, 189-202.

Bais, D., Trevisan, A., Lapasin, R., Partal, P. and Gallegos, C. (2005): Rheological characterization of polysaccharide-surfactant matrices for cosmetic O/W emulsions. *J. Colloid Interface Sci.*, **290**, 546-556.

Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., Backus, R., Richter, D., Knuckles, B. and Schneeman, B.O. (1999): Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with beta-glucan. *Am. J. Clin. Nutr.*, **69**, 55-63.

Braaten, J.T., Scott, F.W., Wood, P.J., Riedel, K.D., Wolynetz, M.S., Brule, D. and Collins, M.W. (1994): High beta-glucan oat bran and oat gum reduce postprandial blood glucose and insulin in subjects with and without type 2 diabetes. *Diabet. Med.*, **11**, 312-318.

Chihara, G. (1983): Preclinical evaluation of lentinan in animal models. *Adv. Exp. Med. Biol.*, **166**, 189-197.

Czop, J.K. (1986): The role of beta-glucan receptors on blood and tissue leukocytes in phagocytosis and metabolic activation. *Pathol. Immunopathol. Res.*, **5**, 286-296.

Delaney, B., Carlson, T., Zheng, G.H., Hess, R., Knutson, N., Frazer, S., Ostergren, K., van Zijverden, M., Knippels, L., Jonker, D. and Penninks, A. (2003): Repeated dose oral toxicological evaluation of concentrated barley beta-glucan in CD-1 mice including a recovery phase. *Food Chem. Toxicol.*, **41**, 1089-1102.

Douwes, J. (2005): (1-->3)-Beta-D-glucans and respiratory health: a review of the scientific evidence. *Indoor Air*, **15**, 160-169.

Duarte, C.G., dos Santos, G.L., Azzolini, A.E. and de Assis Pandochi, A.I. (2000): The effect of the antithyroid drug propylthiouracil on the alternative pathway of complement in rats. *Int. J. Immunopharmacol.*, **22**, 25-33.

Estrada, A., Yun, C.H., Van Kessel, A., Li, B., Hauta, S. and Laarveld, B. (1997): Immunomodulatory activities of oat beta-glucan *in vitro* and *in vivo*. *Microbiol. Immunol.*, **41**, 991-998.

Feletti, F., De Bernardi di Valserra, M., Contos, S., Mattaboni, P. and Germogli, R. (1992): Chronic toxicity study on a new glucan extracted from *Candida albicans* in rats. *Arzneimittelforschung*, **42**, 1363-1367.

Gu, Y.H., Takagi, Y., Nakamura, T., Hasegawa, T., Suzuki, I., Oshima, M., Tawaraya, H. and Niwano, Y. (2005): Enhancement of radioprotection and anti-tumor immunity by yeast-derived beta-glucan in mice. *J. Med. Food*, **8**, 154-158.

Hodge, H.C. and Sterner, J.H. (1949): Tabulation of toxicity classes. *Am. Ind. Hyg. Q.*, **10**, 93.

Korea Food and Drug Administration (1999): Testing Guidelines for Safety Evaluation of Drugs (Notification No. 1999-61, issued by the Korea Food and Drug Administration on December 22, 1999).

Lia, A., Hallmans, G., Sandberg, A.S., Sundberg, B., Aman, P. and Andersson, H. (1995): Oat beta-glucan increases bile acid excretion and a fiber-rich barley fraction increases cholesterol excretion in ileostomy subjects. *Am. J. Clin. Nutr.*, **62**, 1245-1251.

Pinzone Fox, M.L., Sastry, M.K., Parenti, D.M. and Simon, G.L. (2005): Plasma leptin concentration increases early during highly active antiretroviral therapy for acquired immunodeficiency syndrome, independent of body weight. *J. Endocrinol. Invest.*, **28**, 1-3.

Plat, J. and Mensink, R.P. (2005): Food components and immune function. *Curr. Opin. Lipidol.*, **16**, 31-37.

Seo, H.P., Kim, J.M., Shin, H.D., Kim, T.K., Chang, H.J., Park, B.R. and Lee J.W. (2002): Production of -1,3/1,6-glucan by *Aureobasidium pullulans* SM-2001. *Korean J. Bitechnol. Bioeng.*, **17**, 376-380.

Spicer, E.J., Goldenthal, E.I. and Ikeda, T. (1999): A toxicological assessment of curdlan. *Food Chem. Toxicol.*, **37**, 455-479.

US Environmental Protection Agency (1998): Health Effects Test Guidelines OPPTS 870.100, Acute Toxicity Testing Background. US EPA August, Washington, USA.

Yan, J., Allendorf, D.J. and Brandley, B. (2005): Yeast whole glucan particle (WGP) beta-glucan in conjunction with antitumour monoclonal antibodies to treat cancer. *Expert Opin. Biol. Ther.*, **5**, 691-702.