Effect of Dietary Genetically Modified β-Carotene Biofortified Rice on Immune in Rats

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ABSTRACT

This study aims to examine the effect of Genetically Modified β-Carotene Biofortified Rice rice developed by simultaneous expression technology in NAAS on biological immunity. Accordingly, this study added Genetically Modified β-Carotene Biofortified Rice 25, 50% and general rice 50% as control group into diet and provided rats with the prescribed feeds and then measured the contents of immunoglobulin and cytokine in blood. As a result, male and female IgM, IgE, male IgG1, female IgG2a and TNF-a, IL5 and IL12 showed no significant difference; male IgG2a tended to decrease dependently on the combined concentration of Genetically Modified β-Carotene Biofortified Rice; female IgG1 showed significance with control group, but its association with diet was not found. The higher the dietary mixing ratio, the more the male and female IFN-a and female IL-4 contents, regardless of rice variety, and it was found that female IL6 content decreased significantly, but its association with diet was not found. The risk of beta carotene-enriched rice into environment and human body has not been reported yet. The digestion of Genetically Modified β-Carotene Biofortified Rice can be seen as "safe" as this test result showed no big difference between general rice and Genetically Modified β-Carotene Biofortified Rice, and its usability is full of suggestions.

(Key words : β-Carotene biofortified rice, Immunogloblin, Cytokine, GMO)

INTRODUCTION

GM crops have advantages of production increase and harvesting high-quality crops. It is, however, still controversial over the biosafety of GMO on human and environments. Since the enactment of 'LMO law' (The trans-boundary Movements of Living Modified Organisms Act), a strict validation of the safety of GMO on human and environments has been required for the extensive utilization of GM crops (Moseley BE, 2002).

'GM β-carotene biofortified rice', which has yellow color, has been developed to produce carotenoids. The first generation of GM β-carotene biofortified rice was developed via genetic recombination by inserting phytoene synthase(PSY) gene from Narcissus and phytone desaturase(CtrI) gene from a bacteria, Erwinia uredovora. The second generation was developed by inserting corn PSY gene to contain high carotenoid content. The Philippine International Rice Research Institute (IRRI) reported that golden rice would be released into the India market for commercial plantation in 2013 (Ye *et al.*, 2000; Paine *et al.*, 2005).

The national academy of agricultural science of the rural development administration has developed GM β-carotene biofortified rice using multiple gene co-expression technology (Ha SH, 2009), and reported that unlike the removal of beta-carotene contained in the aleurone cells of brown riceduring milling process (Sautter *et al.*, 2006), beta-carotene with the similar content was biosynthesized in the endosperm of the rice that underwent milling (Ha SH, 2009; Lee *et al.*, 2010).

There are a few preliminary studies on the effect of β-carotene biofortified rice on human, animals, and environments. Lee *et al.*, (2010) conducted a study on the validation of agricultural features, reporting that no significant difference in the prevention of rice leaf blast and rice sheath blight was found. They also conducted a study to analyze nutritional composition and beta-carotene amount. Park *et al.*, (2011) reported that no toxicity caused by diet was observed when orally administered to rats. They also reported that the antigenicity of the proteins expressed from GM β-carotene biofortified rice was negative for an adult intake amount of 500 g/day, and suggested that further studies should be required for validating the safety of GM β-carotene

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biofortified rice.

GM crops that have been internationally developed include organisms biofortified with agricultural features such as herbicide-resistance and insect-resistance, and GMO biofortified with a specific nutrient such as β-carotene and flavonoid. In Korea, 16 cultivars and 48 species such as insect-resistant and herbicide-resistant crops have been developed by the rural development administration and other organizations, and test cultivation and safety study have been in progress (Woo *et al.*, 2006). Until now, GMO such as corn, canola, potato, bean, and sugar beet have been approved in Korea (Park *et al.*, 2008).

Due to concerns about health impact such as allergenicty caused by immunosuppression, it is very important to verify the safety of GM crops from a perspective of the insertion of a new gene (Reis *et al.*, 2006). CP4EPSPS and PAT are typical proteins with herbicide-resistance that are expressed in GMO. They are resistant to glyphosate and glufosinate, but no validation of side effects including allergy was conducted for domestically approved GMO (Park *et al.*, 2008).

In 2008, Park *et al.*, (2011) reported that no significance of the allergic reaction of GM foods was found as the experimental concentration shown in a previous assessment of the allergenicty of GM crops was lower than the protein consumption concentration of customers.

It has been reported that the determination of the administering dose of the assessment of allergenicity using GM crops was incorrect in a previous study on the assessment of allergenicity using GM crops including the minimum intake amount of allergen that causes allergy (Taylor *et al.*, 2002).

It is difficult to confirm that allergy occurs by GM food intake. Thus, serum screening that detects the presence of specific IgE is used as a supportive method to assess the safety of allergenicity.

Although the existence of a specific IgE to the inserted protein of GM foods does not necessarily mean the occurrence of allerigic reaction, it can be used as an intermediate stage to assess the safety of allergenicity in the case of inserted protein without dietary experience (Lee *et al.*, 2009; Metcalfe DD, 2005; Goodman *et al.*, 2005; Spok *et al.*, 2005; Goodman *et al.*, 2008).

No study has reported the risk of GM β-carotene biofortified rice on environments and human to date. However, the reaction of the body due to the insertion of various genes may vary depending on individuals (Ye *et al.*, 2000). Furthermore, it can not be ruled out that the mixture of proteins might stimulate or deteriorate allergy (Beroni & Marsan, 2005).

Few studies have been conducted to investigate the safety of GM crops and to investigate responses of various subjects who were sensitive to the same allergen (Lee *et al.*, 2009). Furthermore, as no study has been conducted to investigate intake duration and amount, various studies on the safety of GM crops are required.

Accordingly, this study was conducted to investigate the effect of GM β-carotene biofortified rice intake on immune system in rats by analyzing the content of immunogloblin and cytokine after preparing for the mixtures of GM β-carotene biofortified rice with feeds at doses of adult intake and 2-fold higher intake, and then repeatedly admisntering them to the rats for 90 days.

MATERIALS AND METHODS

Materials and Feed

GM β-carotene biofortified rice used in the study was provided by the department of biosafety, national academy of agricultural science, rural development administration. Rakdong rice was used as a control. The composition of the feed of each group is described in Table 1.

Study Animals

Specific pathogen free(SPF) Sprague-Dawley rat aged 5 weeks were purchased from Hallrim experimental animal institute. After one-week adaptation for check-

Table 1. Composition of diet

Ingredients (%)		Control	G1	G2	G3
No. of animals	Male	10	10	10	10
	Female	10	10	10	10
Golden rice[a]		0	0	25	50
Mild rice[b]		0	50	25	0
Feed		100	50	50	50
Total		100	100	100	100

[a] Mild rice : Nak-Dong rice.
[b] Golden rice : β-carotene biofortified rice.

ing the health status of the rats, 10 male and 10 female rats were assigned to each group using randomized block design, and then fed with the prepared feeds for 90 days (13 weeks). The amount of feed and water consumption, and the weight of the rats were measured once a week. They were also measured before administration and on the day of autopsy. The housing condition was as follows: temperature 23±2℃, humidity 55±5% ventilation 10～15/hr, lighting 12 hours, and illuminance 200～300 Lux, and drinking water was freely available. The blood sample was collected from the saphenons vein under anesthesia with CO_2 and 12-hour fasting condition after the completion of administration. It was centrifuged at 3,000 rpm for 15 min to separate the serum. The separated serum was used for the analysis of immunogloblins and cytokines.

Analysis of the Blood Sample

The blood sample collected from the saphenons vein was analyzed for WBC and LYMPH using a hematology analyzer (Baker system 9118 Hematology Analyzer. Biochem Immunosystems Inc., U.S.).

Analysis of Immunogloblin and Cytokine

Immunoglobulins (IgE, IgG1, IgG2a, IgM) were measured using Millipore's MILLIPLEX Rat Immunoglobulin Isotyping Panel(RGAMMAG-302K, Millipore Corp., USA). cytokines (IFN-r, TNF-a, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12) were measured using Millipore' MILLIPLEX MAP RAT CYTOKINE / CHEMOKINE KIT (RCYTO-80K, Millipore Corp., USA) Luminex 200 System(Luminex, USA) (Merck Millipore Coporation, USA).

Statistical Analysis

All data were denoted with Mean±SD. A student *t*-test was conducted to test difference between the groups.

RESULTS

Weight and Feed Intake Amount

When the body weight gain of each group was compared, the body weight gain was higher in the test group than in the control group, but was statistically insignificant (Fig. 1). No clinical symptom caused by toxicity was found. In addition, no significant difference in food and water intake was found between the groups for both male and female rats.

Analysis of Blood Sample

The results of analyzing the blood sample of the rats fed with vitamin A biofortified rice are presented in Table 2 and 3. The white blood cell (WBC) count was

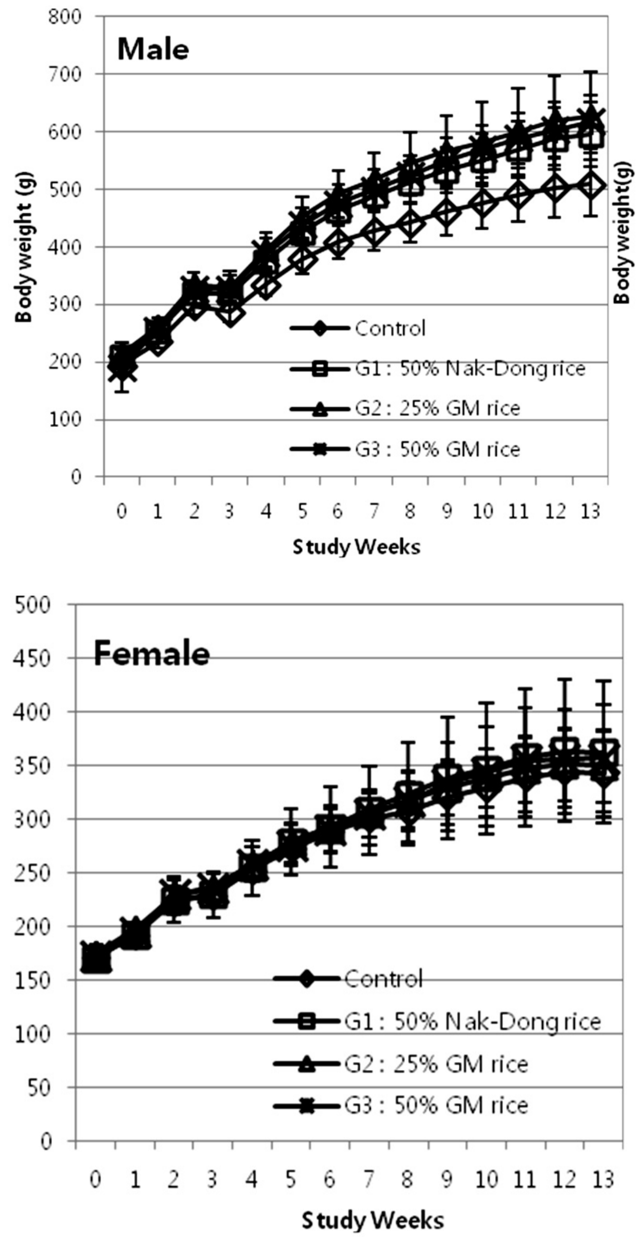

Fig. 1. Body weight of rats for 13 weeks. G1 : 50% Mild rice, G2 : 25% golden rice, G3 : 50% golden rice, Mild rice : Nak-Dong rice, golden rice : β-carotene biofortified rice. Values are presented as mean±SD(g).

shown to have decreased in the male rats fed with GM β-carotene biofortified rice 50%. Meanwhile, the WBC count was shown to have decreased in the female rats fed with GM β-carotene biofortified rice 50%, whereas % GRAN and LYMPH were shown to have increased. However, no statistical significance was found.

Measurement of the Contents of Immunoglobulin and Cytokine

The results of measuring the blood immunogloblin

Table 2. Hematology values for male rats

Parameter	Control	G1	G2	G3
WBC (THSN/CU MM)	9.60±2.56	10.17±3.22	9.97±5.04	9.99±1.36*
% LYMPH (%)	7.30±1.73	9.53±5.08	10.41±5.42	8.16±1.69
% MID (%)	87.69±5.58	82.69±7.43	90.37±2.52	86.00±6.53
% GRAN (%)	8.01±2.53	10.13±2.75	6.61±1.51	9.45±3.17
LYMPH (THSN/CU MM)	4.30±3.20	7.18±4.88	2.96±2.14	4.55±3.56
MID (THSN/CU MM)	8.40±2.40	8.33±2.30	9.06±4.55	8.59±1.25
GRAN (THSN/CU MM)	0.77±0.30	1.04±0.48	0.67±0.40	0.95±0.37

G1 : 50% Mild rice, G2 : 25% Golden rice, G3 : 50% Golden rice.
Mild rice : Nak-Dong rice, Golden rice : β-carotene biofortified rice.
Values are presented as mean±SD(g).
* $p<0.05$; Significantly different from control.
** $p<0.01$; Significantly different from control.
*** $p<0.001$; Significantly different from control.

Table 3. Hematology values for female rats

Parameter	Control	G1	G2	G3
WBC (THSN/CU MM)	6.82±2.30	6.17±2.12	7.30±3.35	5.11±1.48*
% LYMPH (%)	7.84±4.39	6.16±0.48	6.32±0.99	6.70±0.41
% MID (%)	81.70±5.98	81.84±6.70	87.40±6.39	76.39±5.40
% GRAN (%)	9.23±2.62	9.77±3.06	7.01±3.03	11.14±2.98*
LYMPH (THSN/CU MM)	9.07±3.60	8.39±3.84	5.59±3.43	12.47±2.47***
MID (THSN/CU MM)	5.57±1.87	5.12±2.05	6.83±3.09	3.93±1.29
GRAN (THSN/CU MM)	0.65±0.31	0.58±0.17	0.50±0.21	0.53±0.17

G1 : 50% Mild rice, G2 : 25% Golden rice, G3 : 50% Golden rice.
Mild rice : Nak-Dong rice, Golden rice : β-carotene biofortified rice.
Values are presented as mean±SD(g).
* $p<0.05$; Significantly different from control.
** $p<0.01$; Significantly different from control.
*** $p<0.001$; Significantly different from control.

concentration in the rats fed with GM β-carotene biofortified rice are presented in Table 4 and 5. The IgM concentration was shown to be 2,301.48～2,771.91 ng/ml, and 2,415.22～3,078.18 ng/ml for the male and female rats, respectively. The IgE concentration was shown to be 26.86～27.28 ng/ml, and 30.29～32.92 ng/ ml for the male and female rats, respectively. No significant difference was found. In addition, no significant difference in the male IgG1 and female IgG2a concentrations was found. The IgG2a concentration was shown to have decreased dependently from the mixture ratio of the GM β-carotene biofortified rice in the male rats. A significant difference in the IgG1 concentration was found the female test groups and the control groups. As no influence caused by mixed βcarotene, however, was found, it is unlikely that GM β-carotene biofortified rice feeding and it mixture ratio affect the immunoglobulin concentration.

The results of measuring the concentration of cytokines (TNF-α, IFN-r, IL1b. IL2, IL4, IL5, IL6, IL10, IL-12) are presented in Table 6 and 7. The IL1b, IL2, and IL10 concentrations were shown to be significantly lower in the male and female rats. The IL4 and IL6 concentrations were shown to be significantly lower in the male rats. The TNF-α, IL5, and IL12 concentrations were shown to be different in the male ad female rats between the groups, but were statically insignificant. The IFN-α concentration was shown to have increased in the male and female rats as the rice mixture ratio increased, which was independent from rice type ($p>0.001$).

Table 4. Immunogloblin values for male rats

	Control	G1	G2	G3
IgE(ng/ml)	26.86±6.45	26.88±3.98	27.11±6.24	27.28±4.74
IgG1(ng/ml)	364.26±78.92	339.29±99.54	261.35±74.08	376.08±88.28
IgG2a(ng/ml)	1,645.63±341.76	1,831.95±500.59	1,714.51±375.59	1,627.73±330.13
IgM(ng/ml)	2,771.91±651.69	2,509.14±521.02	2,481.07±347.19	2,301.48±282.77

G1 : 50% Mild rice, G2 : 25% golden rice, G3 : 50% golden rice.
Mild rice : Nak-Dong rice, golden rice : β-carotene biofortified rice.
Values are presented as mean±SD(g).
* $p<0.05$; Significantly different from control.
** $p<0.01$; Significantly different from control.
*** $p<0.001$; Significantly different from control.

Table 5. Immunogloblin values for female rats

	Control	G1	G2	G3
IgE(ng/ml)	32.92±7.64	32.88±6.19	30.29±6.04	32.14±5.13
IgG1(ng/ml)	461.32±183.38	299.89±193.08	324.76±133.63	379.69±116.13
IgG2a(ng/ml)	3,076.08±887.78	3,255.04±503.81	2,588.76±534.62	3,046.41±885.18
IgM(ng/ml)	3,075.73±709.49	2,415.22±629.24*	2,886.12±619.42	3,078.18±711.43

G1 : 50% Mild rice, G2 : 25% golden rice, G3 : 50% golden rice.
Mild rice : Nak-Dong rice, golden rice : β-carotene biofortified rice.
Values are presented as mean±SD(g).
* $p<0.05$; Significantly different from control.
** $p<0.01$; Significantly different from control.
*** $p<0.001$; Significantly different from control.

This tendency was also observed in the female rats for IL4 concentration ($p>0.01$). The IL6 was shown to have significantly decreased in the female rats as the rice mixture ratio increased, but no correlation with diet was found.

DISCUSSION

This study was conducted to investigate the effect of the GM β-carotene biofortified rice that has been developed using co-expression technology by national academy of agricultural science on immune system in rats fed with the feeds prepared by mixing GM β-carotene biofortified rice 25 and 50% for the test group, and common rice 50% for the control group, respectively.

The body weight gain was higher in the test group than in the control group, but was statistically insignificant No clinical symptom caused by toxicity was found. Kim *et al.*,(2008) reported that when rats were fed with the mixture of GM rice that expresses Arabidopsis calcium transporter and a feed, no significant difference in body weight gain was found between the groups. Lee *et al.*, (2004) reported that when old rats were fed with herbicide-resistant rice, no change in food intake amount or body weight was found. The aforementioned studies showed that feeding with GM crops did not affect body weight gain and food intake amount, which was consistent with the result of this study showing that GM β-carotene biofortified rice feeding did not affect body weight gain and food intake amount.

WBCs increase when immune function decreases (Nieman & Nehlsen-Cannarella, 1992). Kim *et al.*,(2008) reported that no significant difference in the WBC count was found in rats fed with GM rice that expresses Arabidopsis calcium transporter. Based on the result of this study showed that no significant difference in the WBC count was found between the test group and the control group, the result indirectly indicates that GM β-carotene biofortified rice does not affect immunosuppression.

Homeostasis and immune cell response vary according to the type of stimuli (LaPerriere *et al,* 1994; Ndon *et al*, 1992). Immunoglobulins(Ig) are glycoproteins that are synthesized in B cells and plasma cells that play an important role in the immune system (Roitt IM, 1993), of which an increase in IgM increases resistance for preventing inflammation, an increase in IgG has an inverse correlation with a morbidity of respiratory infection (Nieman DC, 1994), and IgE is involved in chronic inflammatory reaction, and skin responses such as eczema and flare (Kim *et al.*, 2008). In addition, if immune function decreases, WBC and inflammatory proteins such as TNF-α and cytokines increase (Kim *et al.*, 2008). As a result of measuring the blood immunoglobulin and cytokine concentrations, no significant difference in the male and female IgM and IgE concentrations, male IgG1 concentration, female IgG2a, TNF-a, IL5, and IL12 concentrations were found among the groups. The male IgG2a concentration was shown to have decreased dependently from the mixture ratio of GM β-carotene biofortified rice. A significant difference in the female IgG1 concentration was found between the test group and the control group, but no correlation with diet was found. The male and female IFN-a concentrations and the female IL-4 concentration were shown to have increased as the dietary mixture ratio increased independently from rice type. Meanwhile, the female IL6 concentration was shown to have significantly decreased, but no correlation with diet was found.

Batista *et al.* reported that no significant difference in IgE concentration was found between GM and non-Gm crops in a study on GM corns (Batista *et al.*, 2005), and Kim *et al.*, also reported the same result in a study on the IgE reaction of non-GM crops (Kim *et al.*, 2006), which were consistent with the result of this study. Thus, until now, no risk of GM β-carotene biofortified rice in human and environments has been reported. In addition, as the result of this study showed that no significant difference was found between the GM β-carotene biofortified rice and common rice, it can be concluded that GM β-carotene biofortified rice intake is safe.

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