



Vitamin C: An immunomodulator that attenuates anaphylactic reactions to soybean glycinin hypersensitivity in a swine model

Peng Sun^a, Defa Li^{a,b,*}, Bing Dong^a, Shiyan Qiao^a, Xi Ma^a, Xin Chen^c

^a National Key Laboratory of Animal Nutrition, China Agricultural University, No. 2 Yuanmingyuan West Road, Haidian District, Beijing 100094, PR China

^b National Feed Engineering Technology Research Center, Beijing 100094, PR China

^c The Second Affiliated Hospital of Chinese PLA General Hospital, Beijing 100091, PR China

ARTICLE INFO

Article history:

Received 7 April 2008

Received in revised form 30 May 2008

Accepted 6 August 2008

Keywords:

Soybean glycinin allergy

Piglets

Vitamin C

Histamine content

IgE

Cytokines

ABSTRACT

To date, there is insufficient satisfactory therapeutic intervention for food-induced allergies caused by glycinin, the main storage globulin found in soybean protein. The purpose of this study was to evaluate the possible attenuation effects of oral supplementation with vitamin C on soybean glycinin-induced hypersensitivity using a swine model. Ten day old piglets that were intraperitoneally sensitised with purified soybean glycinin were orally challenged with a diet containing 4% glycinin. Vitamin C was administered starting one day prior to sensitisation and continuing everyday thereafter. Results showed that unsupplemented soybean glycinin sensitised piglets presented an increase in serum IgE and histamine release as well as occurrence of diarrhoea, but reduced performance. Vitamin C supplementation attenuated these indicators by increasing interferon- γ whilst decreasing interleukin-4 in the cultured primary splenocytes, which suppresses the Th2-type immune response. These results suggest that a mega-dose of vitamin C can be used to prevent soybean allergies.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Soybeans are widely used as a high-quality protein in the food and feed industries due to their well-balanced amino acid composition (Friedman & Brandon, 2001). However, glycinin, the main storage globulin in soybean protein, has long been recognised as a source of dietary allergens for humans (Burks, Brooks, & Sampson, 1988; Foucard & Yman, 1999). Glycinin is composed of five subunits, and each contains an acidic and a basic polypeptide linked by a disulphide bond. The acidic chain of the G1 subunit has an immunoglobulin E-binding region (Beardslee, Zeece, Sarath, & Markwell, 2000), which may increase serum glycinin-specific immunoglobulin E antibody in soybean allergic subjects (Burks et al., 1988; Shibasaki, Suzuki, Tajima, Nemoto, & Kuroume, 1980; Sun, Li, Li, Dong, & Wang, 2008). This may cause a series of allergic reactions resulting in damage to the intestinal morphology, immune function disorders, growth depression, and diarrhoea (Dréau, Lallès, Philouze-Romé, Toullec, & Salmon, 1994; Guo, Piao, Cao, Ou, & Li, 2008; Li et al., 1990).

Clinically, an effective method of preventing soybean allergy is to simply avoid the offending food (Nieuwenhuizen & Lopata,

2005). However, in practice this is difficult to achieve because of the widespread use of soybean in various food products. Novel approaches to treat IgE-mediated food allergies have been tested such as anti-IgE, probiotics and food allergy vaccines, but these have potential adverse side effects, which hamper their feasibility (Nowak-Węgrzyn, 2003). With the ever-increasing occurrence of food allergies it is urgent to find safer and more effective immunomodulators to attenuate allergic diseases.

One such immunomodulator is the antioxidant vitamin C, which functions as an electron donor to protect the body from radicals and pollutants (Iqbal, Khan, & Khattak, 2004). To date, mega-doses of vitamin C have shown promise as an effective influence in the treating and preventing of allergic rhinitis (Thornhill & Kelly, 2000), diabetes (Anderson et al., 2006), heart disease (Liu et al., 2002) and cancer (Enwonwu & Meeks, 1995). It is postulated that exogenous vitamin C can lower blood levels of histamine (Clemenson, 1980), and thus help to reduce the severity of allergic reactions. Although the beneficial effect of vitamin C remains controversial, which may cause diarrhoea and abdominal distention in humans (Thornhill & Kelly, 2000), doctors have recommended patients to consume even higher doses of vitamin C at the level of grams per day (Johnston, 1999). However, this has not been proven conclusively and the underlying mechanism through which vitamin C functions is still unknown.

The objective of this study was to investigate whether orally administered vitamin C can attenuate soybean glycinin allergy

* Corresponding author. Address: National Key Laboratory of Animal Nutrition, China Agricultural University, No. 2 Yuanmingyuan West Road, Haidian District, Beijing 100094, PR China. Tel.: +86 10 6273 3588; fax: +86 10 6273 3688.

E-mail address: defali@public2.bta.net.cn (D. Li).

using an experimental piglet model for glycinin anaphylaxis established by feeding purified glycinin. The advantage of this model is that it eliminates interference from other potential allergic components in soybean meal or whole soybean protein extract.

2. Materials and methods

2.1. Purified soybean glycinin

Purified glycinin samples containing more than 85% glycinin were kindly provided by Prof. Shuntang Guo of the Food Institute at China Agricultural University (Patent No. 200410029589.4, China).

2.2. Animals

All animals used in this experiment were maintained according to the principles of the China Agricultural University Animal Care and Use Committee. Eighteen crossbred (large white $\times$ landrace) barrows were weaned at 10 days of age and had an average weight of 4.36 ± 0.29 kg.

Each piglet was individually housed in a 0.7×1.1 m stainless steel metabolic crate equipped with a plastic-coated, totally slatted floor. The pigs were housed in a mechanically ventilated nursery room with the lighting was set on a cycle of 12 h light: 12 h dark, the temperature was maintained at $26\text{--}28^\circ\text{C}$ and the relative humidity was set at 50–60%.

The pigs were raised on a liquid diet (fed three times daily) of skim milk with gradual adaptation to a corn-based starter diet (Table 1). On day 21, the pigs were completely removed from the liquid diet and thereafter received the starter diet ad libitum.

2.3. Experimental diets

Two corn-based starter diets were formulated (Table 1). The control diet, devoid of soybean protein, contained skim milk, fish-meal, whey powder, casein and spray dried porcine plasma as protein sources. The challenge diet was similar to the control diet but

contained 4% soybean glycinin added at the expense of casein. Both diets were formulated to provide similar levels of crude protein (22.9%), lysine (1.6%) and energy (DE, 14.4 MJ/kg). As noted above, the control diet was gradually introduced to the piglets during the period when they received a liquid diet (10–21 days). Thereafter, pigs received only the control diet throughout the trial with the exception of the challenge period when pigs in groups 2 and 3 received the challenge diet (days 24 and 38 only).

2.4. Experimental protocol

The eighteen, 10-day old piglets were randomly assigned to one of three treatments. Piglets in groups 2 and 3 were sensitised on days 1, 2 and 3 with an intraperitoneal injection of 1.0 mg purified glycinin dissolved in 0.15 mol/l phosphate-buffered saline (PBS, pH 7.4) with subsequent booster injections on days 10 and 17. Piglets in group 1 (control) were injected with PBS intraperitoneally.

Piglets in group 2 were treated with 1.0 g of vitamin C (Tiger Biotechnology Company, Anhui Province, China; 93% ascorbic acid) starting one day prior to sensitisation (day 0) and continuing everyday thereafter (day 38). On days 24 and 38 only, piglets in groups 2 and 3 were fed the challenge diet in place of the control. Feed and water were available ad libitum. Piglets were weighed at the start and end of the trial (days 0 and 38) in order to calculate average daily gain.

Diarrhoea incidence was recorded as described in our previous study (Sun et al., 2008). Faecal consistency was visually assessed at 8:00 each morning and diarrhoea incidence was calculated according to the faecal consistency. Faecal consistency was graded as: 0, solid; 1, semi-solid; 2, semi-liquid and 3, liquid. The occurrence of diarrhoea was defined as production of faeces at level 2, semi-liquid or level 3, liquid for two continuous days. Diarrhoea incidence (%) was defined as the number of pigs on a treatment with diarrhoea $\times$ diarrhoea days / (6×38) $\times$ 100%.

A 7 ml sample of blood was obtained from each piglet by vena cava puncture using a 9 ml clot activator tube (Greiner Bio-One GmbH, Kremsmunster, Austria). The samples were taken on days 10, 24 and 38 with the day 10 sample being taken 3 h after boosting whilst the days 24 and 38 samples were taken 3 h after feeding the challenge diet and the serum was isolated and stored at -70°C for later analysis.

Immediately after blood sampling on day 38, all piglets were killed with an intracardial injection of sodium pentobarbital (50 mg/kg body wt) and jugular exsanguination. The peritoneal cavity was opened, and the spleen and the small intestine were removed. The duodenum (5 cm away from the pyloric junction), the jejunum (the middle part of the whole jejunum) and the ileum (5 cm anterior to the ileocaecal junction) were quickly isolated and flushed with ice-cold saline to remove the digesta. The intestinal tissue was then stored in liquid nitrogen until needed for analysis.

2.5. Determination of serum IgE antibody levels

Total serum IgE antibody was determined by using a swine IgE ELISA kit (RapidBio Lab., Calabasas, CA) according to the manufacturer's instructions. Glycinin-specific IgE level was determined using an indirect ELISA as described by Helm et al. (2002) with a slight modification. Briefly, 96-well microtiter plates (Canda Jet Bio-Chemicals Int., Guangzhou, China) were coated with 5 $\mu\text{g}/\text{ml}$ glycinin in carbonate buffer ($\text{NaHCO}_3/\text{Na}_2\text{CO}_3$, pH 9.6). Then appropriately diluted swine serum was added and incubated with biotinylated anti-pig IgE and horseradish peroxidase conjugated (RapidBio Lab., Calabasas, CA). After being washed five times, tetramethylbenzidine was added to measure the absorbance at 450 nm and the data were expressed as optical density (OD) units.

Table 1
Ingredient composition and nutrient levels of the diets (as-fed basis)

Ingredient composition (%)	Dietary treatment	
	Control diet	Challenge diet
Corn	58.8	58.8
Skimmed milk powder	9.5	9.5
Whey powder	10.0	10.0
Casein	8.0	4.0
Soybean glycinin	0.0	4.0
Spray dried porcine plasma	5.0	5.0
Fish meal	6.0	6.0
Limestone	0.5	0.5
Calcium phosphate	1.0	1.0
Salt	0.2	0.2
Vitamin–mineral premix ^a	1.0	1.0
<i>Chemical analysis^b</i>		
Crude protein (%)	22.87	22.91
Lysine (%)	1.67	1.57
Calcium (%)	1.06	1.03
Phosphorus (%)	0.80	0.76
Digestible energy (MJ/kg) ^c	14.37	14.43

^a Premix provided per kilogram of complete diet: vitamin A, 10,000 IU; vitamin D₃, 1500 IU; vitamin E, 30 IU; vitamin K₃, 2.5 mg; vitamin B₁, 1.5 mg; vitamin B₂, 10 mg; vitamin B₆, 10 mg; vitamin B₁₂, 0.05 mg; folic acid, 1 mg; biotin, 0.5 mg; niacin, 30 mg; pantothenic acid, 20 mg; Cu, 20 mg; Fe, 100 mg; Zn, 110 mg; Mn, 40 mg; Se, 0.3 mg; and I, 0.5 mg.

^b Analysed value.

^c Calculated value.

2.6. Spleen cell culture

The spleen was aseptically removed from slaughtered piglets immediately after blood collection. The spleen tissue was finely chopped with scissors into fragments of 0.5–2.0 mm³, minced by syringe and washed twice with RPMI 1640 containing 10% foetal bovine serum (HyClone Laboratories Inc. Logan UT). After erythrocyte lysis, splenocytes were re-suspended in complete medium (RPMI 1640 supplemented with 10% foetal bovine serum, 2 mM L-glutamine, 25 mM Hepes, 100 IU/ml penicillin, 100 mg/ml streptomycin) and incubated at a density of 2×10^6 cells/well in the presence or absence of 100 µg/ml glycinin for 72 h at 37 °C and 5% CO₂. Concanavalin A (Sigma, St. Louis, MO) was served as the positive stimuli. The supernatant was harvested and stored at –70 °C for cytokine assay.

2.7. Analysis of cytokine levels by ELISA

Concentrations of interleukin-4 and interferon-γ in the cultured splenocyte supernatant were determined using the swine enzyme-linked immunosorbent assay kit (ELISA kit; Biosource, Camarillo, CA) following the instructions of the manufacturer.

2.8. Preparation and sensitisation of pig intestinal mast cells

A sample of the jejunum segment of the intestine was obtained from the control pigs in group 1 for histamine release analysis. Piglet intestinal mast cells were isolated and cultured as described by He, Gaça, and Walls (1998). Purified mast cells were re-suspended in complete Hank's balanced salt solution containing 1.8 mM CaCl₂ and 0.5 mM MgCl₂. The mast cell suspension (1×10^6 cell/ml) was incubated in medium which was supplemented with piglet sensitised serum (groups 2 and 3) or normal piglet serum (group 1) in 24-well microplates (Canda Jet Bio-Chemicals Int., Guangzhou, China) for 2 h. Thereafter, glycinin was added to the suspension (final concentration of 100 µg/ml) and cultured for another hour. The reaction was terminated by placing the plates on ice. The histamine content of both the cell pellet and the supernatant were measured. Residual histamine in the cell pellet was released by disrupting the cells in perchloric acid and centrifuging at 400g for 5 min at 4 °C. The histamine release ratio was calculated by the following equation: histamine release ratio (%) = {supernatant histamine/(supernatant histamine + cell pellet histamine)} × 100.

2.9. Measurement of histamine levels with HPLC

The histamine content in the small intestine, supernatant and cell pellet were measured by high performance liquid chromatography (Waters Corporation, Milford, MA), as described by Liu et al. (2006). Briefly, after being filtered by a 0.45 µm dialyser the sample was applied to a reversed-phase column (symmetry, C₁₈, 5 µm, 4.6 × 150 mm, Waters Corporation, Dublin, Ireland), which was eluted using a gradient elution program at a flow rate of 1 ml/min. Histamine in the eluate then reacted with o-phthalaldehyde at 40 °C, and fluorescence was detected at 340 nm excitation and 450 nm emission. A standard sample of histamine was supplied by Sigma Company (St. Louis, MO). A data integrator (Waters Tm 600, Waters Corporation, Dublin, Ireland) was used to analyse the data.

2.10. Statistical analysis

Differences in the occurrence of diarrhoea amongst the treatment groups were tested by Chi-square contingency test. All other data were analysed using the analysis of variance (ANOVA) procedure

of SAS system (version 8.2, SAS Institute Inc., Cary, NC, USA). A *P*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Performance and diarrhoea

Data for performance and the occurrence of diarrhoea are shown in Table 2. Piglets sensitised with glycinin had lower average daily gain ($P < 0.05$) and feed conversion ($P < 0.01$) compared with the control, although average daily feed intake was not influenced ($P = 0.21$). Supplementation with 1.0 g vitamin C increased the weight gain to 229 g/d. Furthermore, the occurrence of diarrhoea was increased significantly in the piglets from the unsupplemented challenged pigs compared with those from the 1.0 g vitamin C and control groups ($P < 0.05$).

3.2. Total serum IgE and glycinin-specific IgE levels

To explore the possible effects of vitamin C on soybean glycinin allergy in sensitised pigs, total IgE and glycinin-specific IgE levels in the piglet serum were determined three times after the initial sensitisation. As presented in Fig. 1, the total serum IgE and glycinin-specific IgE concentrations were increased on day 24 and significantly elevated on day 38 ($P < 0.05$) in the glycinin sham-treated group compared with the control. However, the glycinin-specific IgE level was dramatically lowered on day 24 and significantly lowered on day 38 ($P < 0.05$) in the 1.0 g/d vitamin C-treated group compared with the sham-treated group.

3.3. Cytokine levels in the cultured splenocyte supernatant

To determine whether orally administered vitamin C treatment altered Th1/Th2 cytokine levels in response to soybean glycinin stimulation, interleukin-4 and interferon-γ levels were measured in the cultured splenocyte supernatant. The results showed that interferon-γ levels were significantly higher, whilst interleukin-4 levels were drastically lower in the control and 1.0 g vitamin C-treated groups compared with the sham group ($P < 0.05$).

Table 2

Effects of vitamin C on performance^a and diarrhoea^a, cytokine concentrations^a in cultured splenocyte supernatant, histamine content^a in the small intestine, and histamine release ratio in glycinin sensitised and control piglets^b

Item	Control	Glycinin sensitisation		SEM ^c	P value
		1.0 g vitamin C	No vitamin C		
<i>Performance</i>					
ADG (g/d)	249a	229ab	180b	16.76	0.04
ADFI (g/d)	323	306	264	22.85	0.21
F/G	1.30b	1.34b	1.47a	0.03	<0.01
<i>Occurrence of diarrhoea (%)</i>					
	0	0	17.6	–	<0.05 ^d
<i>Cytokine concentrations in cultured splenocyte supernatant (pg/ml)</i>					
Interferon-γ	312.7a	296.8a	191.2b	28.89	0.02
Interleukin-4	31.1b	34.0b	69.0a	8.69	0.02
<i>Histamine content in the small intestine (μg/g)</i>					
Duodenum	20.3	17.4	12.3	2.70	0.16
Jejunum	23.0a	21.4a	14.8b	1.70	0.01
Ileum	17.8a	17.6a	11.7b	1.09	<0.01
<i>Histamine release ratio (%)</i>					
	32.6b	40.9b	63.1a	4.22	<0.01

^a Each value is the mean of data from six piglets (average initial BW of 4.36 ± 0.29 kg) per group.

^b Mean values with different superscripts are different at the *P* value indicated.

^c Standard error of the mean.

^d Significantly different by Chi-square contingency test.

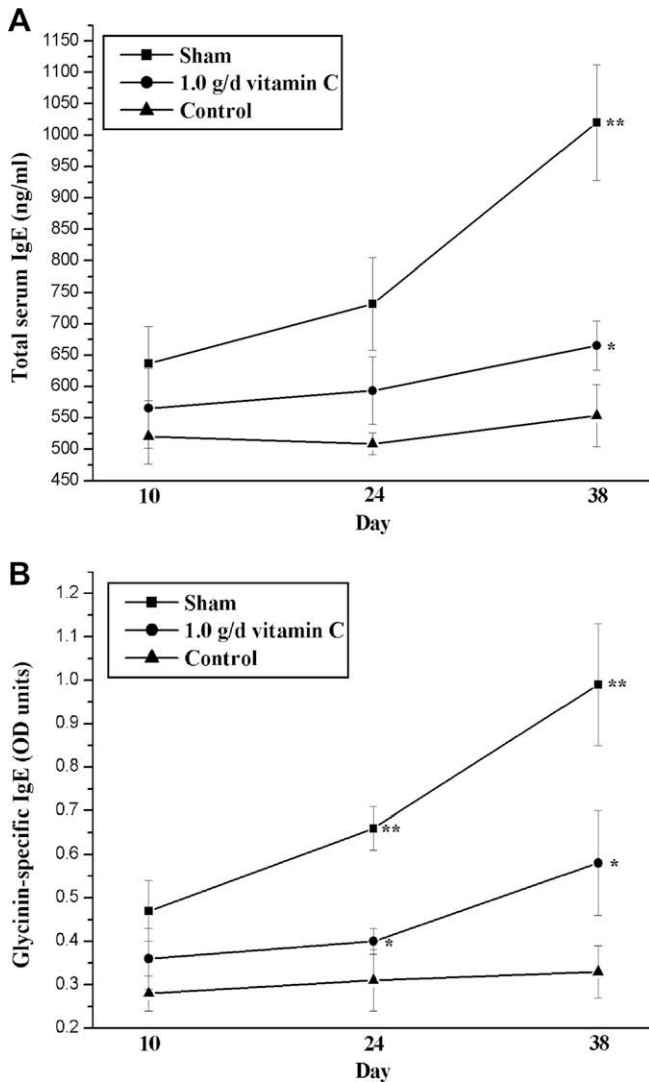


Fig. 1. Total serum IgE (A) and glycinin-specific IgE (B) levels in the piglets on days 10, 24 and 38 after initial sensitisation. Each data point is presented as the groups mean $\pm$ SEM of six piglets per group. ** $P < 0.05$ vs. control; * $P < 0.05$ vs. sham.

3.4. Histamine release ratio in incubated mast cells in vitro

Compared with the sham-treated group, supplementation with 1.0 g/d vitamin C suppressed histamine release from the mast cells as indicated by a lower release ratio ($P < 0.01$) (Table 2).

3.5. Histamine content in the small intestine

The histamine contents in the jejunum ($P = 0.02$) and ileum ($P < 0.01$) of the sham-treated piglets were significantly lower than the control (Table 2). Treatment with 1.0 g/d of vitamin C significantly increased the histamine content compared to the sham-treated group. But the levels of histamine in the duodenum were unaffected by treatments.

4. Discussion

Many studies have been conducted using young pigs as an animal model to investigate the allergenicity of soybean proteins (including glycinin) that often cause disorders of immune function and reduced performance (Dr  au et al., 1994; Li et al., 1990). This study evaluated the effects of oral vitamin C supplementation on

soybean glycinin-induced food allergy using an experimental swine model. The results may provide new hope for the prevention of soybean allergy.

Previous studies have shown that soybean allergy is associated with increased serum IgE antibodies (Burks et al., 1988; Shibasaki et al., 1980), and glycinin is the major trigger for the majority of soybean-induced hypersensitivity. The clinical symptoms in soybean sensitive individuals range from gastrointestinal distress to life-threatening asthma and death (Foucard & Yman, 1999). It is elucidated that food allergy is IgE-mediated and characterised by type I allergic reactions, which are due to the release of inflammatory mediators such as histamine by mast cells, which play an important role in inducing the hypersensitive disorders (Helm et al., 2002; Sun et al., 2008; van Halteren, van der Cammen, Biewenga, Savelkoul, & Kraal, 1997).

In this study, reduced performance with transient diarrhoea was observed in glycinin sensitised piglets. Furthermore, the total serum IgE and glycinin-specific IgE levels increased, which was associated with high histamine release in the piglets from the sham-treated group, indicating a successful animal model had been established.

A traditional method for treating allergic responses caused by accidental exposure to offending food is the use of anti-histamine medicine (Thornhill & Kelly, 2000). Compared with traditional anti-histamine treatment, vitamin C presents a number of advantages for alleviating food allergic reactions because it is an essential nutrient for human survival and readily available as a constituent of many fruits and vegetables. In addition, it is virtually free of adverse side effects (Thornhill & Kelly, 2000) and nontoxic since excessive vitamin C is simply excreted in the urine. As a powerful antioxidant, it can protect the body from damage by free radicals (Iqbal et al., 2004).

Recent reports show that vitamin C presents some anti-inflammatory effects, which are helpful in the treatment of asthma (Hatch, 1995), a common symptom that occurs in hypersensitivity. In a preliminary study conducted in Italy, researchers found that young children with asthma experienced significantly less wheezing when they consumed a diet with sufficient fruits rich in vitamin C (Forastiere et al., 2000). Furthermore, consumption of vitamin C can lower blood levels of histamine (Clemeston, 1980) and reduce the natural inflammatory response in nasal passages (Fortner, Danziger, Rabinowitz, & Nelson, 1982), which exhibit some anti-allergic activity.

The results of this study showed that vitamin C could suppress the production of serum IgE antibody, inhibit the activation of the cultured intestinal mast cells after glycinin sensitisation, and subsequently reduce histamine release in glycinin sensitised piglets. These results implied that the anti-histamine effect might not function through the simple reaction between histamine and vitamin C. There might be some other mediators involved in the suppression.

The analysis for cytokines in the cultured splenocyte supernatant showed that piglets with vitamin C supplementation had higher levels of interferon- γ but lower levels of interleukin-4 compared with those in the sham-treated group, which suggested that the mechanism behind the anti-histamine effect might be mediated by the reversing bias of Th1/Th2 cells differentiated from T cells in the allergic subjects. Similar results were reported by Noh et al. (2005) who found that exogenously administered mega-dose of vitamin C shifted immunity in Balb/c mouse toward Th1 when vitamin C was administered during T cell activation.

It has been well documented that food-induced hypersensitivity is a predominantly Th2-type immune response (van Wijk & Knippels, 2007), in which Th2-type representative cytokine interleukin-4 plays a key role in allergic inflammation by favoring production of total IgE and antigen-specific IgE antibodies (Prescott & Hogan, 2006). The large quantity of IgE antibodies generated in

allergic individuals immediately bind to the corresponding receptor FcεRI on the surface of the mast cells in the gastrointestinal tract and other tissues, which provoke the mast cells to degranulate and release active mediators, such as histamine, leading to typical hypersensitivity (Metcalf, Baram, & Mekori, 1997; Sun et al., 2008). In this study, however, this trend was suppressed by vitamin C which inhibited a dominance of T cells switching to the T helper-2 phenotype. Vitamin C promotes the production of interferon- γ , which functions reciprocally on the induction of IgE responses and histamine release thus alleviating the inflammation in vivo (Snapper & Paul, 1987). These findings supported the evidence of a reduction in serum IgE and histamine release in the small intestine but a promotion of growth in piglets consuming vitamin C even when challenged with glycinin.

In conclusion, this study demonstrated that oral supplementation of vitamin C encourages the synthesis of interferon- γ , which in turn suppresses the production of interleukin-4, resulting in a decrease of total and glycinin-specific serum IgE levels as well as histamine release, thus helping to attenuate IgE-mediated soybean glycinin-induced hypersensitivity and promote growth and health without any adverse symptoms. Our results suggest that vitamin C is a relatively safe immunomodulator that can be used in prevention of soybean allergy.

Acknowledgements

The investigation was financially supported by the National Natural Science Foundation of PR China (Nos. 30430520 and 30121004) and by the National Basic Research Program (2004 CB 117503). We thank Dr. Guoyao Wu, Texas A&M University, and Dr. Phil Thacker, University of Saskatchewan, for excellent assistance in editing this manuscript.

References

- Anderson, R. A., Evans, L. M., Ellis, G. R., Khan, N., Morrist, K., Jackson, S. K., et al. (2006). Prolonged deterioration of endothelial dysfunction in response to postprandial lipaemia is attenuated by vitamin C in type 2 diabetes. *Diabetic Medicine*, 23, 258–264.
- Beardslee, T. A., Zeece, M. G., Sarath, G., & Markwell, J. P. (2000). Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. *International Archives of Allergy Immunology*, 123, 299–307.
- Burks, A. W., Brooks, J. R., & Sampson, H. A. (1988). Allergenicity of major component proteins of soybean determined by enzyme linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. *Journal of Allergy and Clinical Immunology*, 81, 1135–1142.
- Clemeston, C. A. B. (1980). Histamine and ascorbic acid in human blood. *Journal of Nutrition*, 110, 662–668.
- Dréau, D., Lallès, J. P., Philouze-Romé, V., Toullec, R., & Salmon, H. (1994). Local and systemic immune responses to soybean protein ingestion in early-weaned pigs. *Journal of Animal Science*, 72, 2090–2098.
- Enwonwu, C. O., & Meeks, V. I. (1995). Bionutrition and oral cancer in humans. *Critical Reviews in Oral Biology and Medicine*, 6, 5–17.
- Forastiere, F., Pistelli, R., Sestini, P., Fortes, C., Renzoni, E., Rusconi, F., et al. (2000). Consumption of fresh fruit rich in vitamin C and wheezing symptoms in children. *Thorax*, 55, 283–288.
- Fortner, B. R., Danziger, R. E., Rabinowitz, P. S., & Nelson, H. S. (1982). The effect of ascorbic acid on cutaneous and nasal response to histamine and allergen. *Journal of Allergy and Clinical Immunology*, 69, 484–488.
- Foucard, T., & Yman, I. M. (1999). A study on severe food reactions in Sweden – Is soy protein an underestimated cause of food anaphylaxis? *Allergy*, 54, 261–265.
- Friedman, M., & Brandon, D. L. (2001). Nutritional and health benefits of soy proteins. *Journal of Agricultural and Food Chemistry*, 49, 1069–1077.
- Guo, P. F., Piao, X. S., Cao, Y. H., Ou, D. Y., & Li, D. F. (2008). Recombinant soybean protein β -conglycinin α' -subunit expression and induced hypersensitivity reaction in rats. *International Archives of Allergy Immunology*, 145, 102–110.
- Hatch, E. G. (1995). Asthma, inhaled oxidants and dietary antioxidants. *American Journal of Clinical Nutrition*, 61, 625S–630S.
- He, S., Gaça, M. D. A., & Walls, A. F. (1998). A role for tryptase in the activation of human mast cells: Modulation of histamine release by tryptase and inhibitors of tryptase. *Journal of Pharmacology and Experimental Therapeutics*, 286, 289–297.
- Helm, R. M., Furuta, G. T., Stanley, J. S., Ye, J. H., Cockrell, G., Connaughton, C., et al. (2002). A neonatal swine model for peanut allergy. *Journal of Allergy and Clinical Immunology*, 109, 136–142.
- Iqbal, K., Khan, A., & Khattak, M. M. A. K. (2004). Biological significance of ascorbic acid (vitamin C) in human health – A review. *Pakistan Journal of Nutrition*, 3, 5–13.
- Johnston, C. S. (1999). Biomarkers for establishing a tolerable upper intake level for vitamin C. *Nutrition Reviews*, 57, 71–77.
- Li, D. F., Nelssen, J. L., Reddy, P. G., Blecha, F., Hancock, J. D., Allee, G. L., et al. (1990). Transient hypersensitivity to soybean meal in the early-weaned pig. *Journal of Animal Science*, 68, 1790–1799.
- Liu, Y. H., Piao, X. S., Ou, D. Y., Cao, Y. H., Huang, D. S., & Li, D. F. (2006). Effects of particle size and physical form of diets on mast cell numbers, histamine, and stem cell factor concentration in the small intestine of broiler chickens. *Poultry Science*, 85, 2149–2155.
- Liu, L., Zhao, S. P., Gao, M., Zhou, Q. C., Li, Y. L., & Xia, B. (2002). Vitamin C preserves endothelial function in patients with coronary heart disease after a high-fat meal. *Clinical Cardiology*, 25, 219–224.
- Metcalf, D. D., Baram, D., & Mekori, Y. A. (1997). Mast cells. *Physiological Reviews*, 77, 1033–1079.
- Nieuwenhuizen, N. E., & Lopata, A. L. (2005). Fighting food allergy current approaches. *Annals of the New York Academy of Sciences*, 1056, 30–45.
- Noh, K., Lim, H., Moon, S., Kang, J. S., Lee, W. J., Lee, D., et al. (2005). Mega-dose vitamin C modulates T cell functions in Balb/c mice only when administered during T cell activation. *Immunology Letters*, 98, 63–72.
- Nowak-Węgrzyn, A. (2003). Future approaches to food allergy. *Pediatrics*, 111, 1672–1680.
- Prescott, V. E., & Hogan, S. P. (2006). Genetically modified plants and food hypersensitivity diseases: Usage and implication of experimental models for risk assessment. *Pharmacology and Therapeutics*, 111, 374–383.
- Shibasaki, M., Suzuki, S., Tajima, S., Nemoto, H., & Kuroume, T. (1980). Allergenicity of major component proteins of soybeans. *International Archives of Allergy and Applied Immunology*, 61, 441–448.
- Snapper, C. M., & Paul, W. E. (1987). Interferon- γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science*.
- Sun, P., Li, D., Li, Z., Dong, B., & Wang, F. (2008). Effects of glycinin on IgE-mediated increase of mast cell numbers and histamine release in the small intestine. *Journal of Nutritional Biochemistry*, 19, 627–633.
- Thornhill, S. M., & Kelly, A. M. (2000). Natural treatment of perennial allergic rhinitis. *Alternative Medicine Review*, 5, 448–454.
- van Halteren, A. G. S., van der Cammen, M. J. F., Biewenga, J., Savelkoul, H. F. J., & Kraal, G. (1997). IgE and mast cell responses on intestinal allergen exposure: A murine model to study the onset of food allergy. *Journal of Allergy and Clinical Immunology*, 99, 94–99.
- van Wijk, F., & Knippels, L. (2007). Initiating mechanisms of food allergy: Oral tolerance versus allergic sensitization. *Biomedicine and Pharmacotherapy*, 61, 8–20.