African Journal of Microbiology Research Vol. 4 (11), pp. 1091-1099, 4 June, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2010 Academic Journals

Full Length Research Paper

Protective effects of sucralfate on anti-*H. pylori* VacA IgY *in vivo* and *in vitro*

Zhou Xun[1], Guo Li-yuan[1], Yang Zhibang[1*] and Chen Xiaoping[2]

[1]Chongqing University of Medical Sciences, Chongqing 400016, China.
[2]Guangdong Provincial People's Hospital, Guangzhou 510080, China.

Accepted 26 May, 2010

This study aimed to evaluate the protective effects of sucralfate on immunoglobulin yolk (IgY) and to provide a theory basis for preparing IgY oral preparations which can tolerate acid and pepsin. Engineering bacteria DH5α-vacA-pQE30 were largely induced to express recombinant vacuolate cytotoxin antigen (VacA). VacA antigen was inoculated in Lohman hen and IgY was purified. Different concentrations of sucralfate were added into IgY solution including different concentrations of pepsin at different pH values. IgY solution containing different concentrations of sucralfate underwent seven times of repeated freeze thawing, and then placed at room temperature for 1 d, 4 w. The IgY residual antibody activity (A_R/A_C) of each experiment group was detected by ELISA. *Helicobacter pylori* infected mice were intragastrically administrated with 30% sucralfate and different concentrations of IgY solution and then chronic inflammatory reaction of gastric mucosa was observed to evaluate the protective effects of sucralfate on IgY. At pH = 1.5, 60% sucralfate could maintain 48.9% IgY residual antibody activity. At pH = 2.0, 30% sucralfate could enhance the IgY residual antibody activity to 86.5%, at 50% sucralfate could fully maintain the IgY antibody activity. At pH = 3.0, 30% sucralfate could maintain 87.6% IgY antibody activity, while over 40% sucralfate could almost fully maintain the IgY antibody activity. Under pH 1.5, 3.0 and 0.02 mg/ml pepsin conditions, the residual antibody activities of IgY solution containing 10, 30 and 50% sucralfate were 63.2, 72.4 and 82.2%, respectively. Over 30% sucralfate could enhance the anti-freeze thawing ability of IgY. After one month at room temperature, over 80% antibody activity could still be maintained. Intragastric administration with 0.5 mg IgY plus 30% sucralfate/day could effectively prevent and cure gastric mucosal injure caused by *H. pylori* infection, the effect of which increased eight folds as compared with the group without sucralfate. Over 30% sucralfate can enhance the tolerance of VacA IgY to low pH value and pepsin and enhance the anti-freeze thawing ability, therefore, it is an ideal IgY protective agent.

Key words: *Helicobacter pylori*, vacuolate cytotoxin antigen, immunoglobulin yolk, Sucralfate, antibody activity.

INTRODUCTION

Many studies have demonstrated that *Helicobacter pylori* is one of the common infectious pathogens. About half of the population in the world (more than 3 billion people) underwent *H. pylori* infection. *H. pylori* infection rate is 30 - 50% in children and 90% in adults in developing countries (Granstrom et al., 1997; Salih, 2009).

H. pylori infection is an etiological factor of chronic gastritis and peptic ulcer and also an important risk factor of gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. It has been considered as the first class carcinogenic factor by World Health Organization (International Agency for Research on Cancer, 1994), so there is important clinical significance to prevent and cure *H. pylori* infection. In recent years, many studies have confirmed that oral administration of immunoglobulin yolk (IgY) could effectively prevent and cure the infectious diseases of digestive tract through passive immunity (Mine and Kovacs-Nolan, 2002; Suzuki et al., 2004). However, its application is limited by the

*Corresponding author. E-mail: yyzz1999@foxmail.com. Tel: +8623-68485193.

ecological environment of high level of gastric acid and pepsin in the gastrointestinal tract, especially in stomach. Enhancing IgY stability against gastric acid and pepsin has important significance for the application of oral IgY. As an anti-peptic ulcer agent, sucralfate has been widely applied in the world after the advent in Japan. It has significant adhesiveness and can bind macromolecular substances, such as mucus, glycolipidprotein and other proteins and also bind the mucus on gastric mucosal surface and form compound gel material, thereby playing an important role in mucosal protection (Ochi, 1995).

In our study, we prepared anti-*H. pylori* recombinant VacA specific IgY and added the appropriate proportions of sucralfate into the specific IgY in order to increase its tolerance to the acid and pepsin. Moreover, the protective effects of the specific IgY on *H. pylori* infection was fully played by utilizing the adhesiveness of sucralfate with mucus on the surface of gastric mucosa. The protective effect of sucralfate on the specific IgY was evaluated *in vitro* and intragastrically through comparison of stabilization between sucralfate and carbohydrate.

METHODS AND MATERIALS

Materials

H. pylori VacA toxic fragment recombinant bacteria DH5α-vacA-pQE30 were constructed in our laboratory (Liu et al., 2003) and *H. pylori* standard strain NCTC11637 was preserved in our laboratory. Ni $^{2+}$-NTA affinity chromatography column was purchased from Qiagen. HRP-labeled goat anti-chicken IgG and goat anti-human IgG were purchased from Southern Biotech, Inc. Western blot kit for detecting serum anti-*H. pylori*-specific IgG was purchased from Shanghai YuanGu Science and Technology Development Corporation. LA medium and *H. pylori* selective medium, saturated ammonium sulfate solution, phosphate buffer, ELISA antigen coating solution, enzyme diluent, blocking solution, washing solution, colouring agent and stop solution were prepared in our laboratory. VacA (+) serum was screened from serum of the patients with positive *H. pylori*. Pepsin (1:3000) was purchased from Amresco Inc., Sucralfate (sucralfate suspension) was purchased from Shanghai Xudong Heipu pharmaceutical Co, LTD. Hp urease rapid diagnostic kit was purchased from Fujian Sanqiang biological and chemical Co, LTD. 6 - 8 week-old clean grade Balb/c mice, weighing17 - 20 g and New Zealand rabbits were provided by the animal center of Chongqing Medical University. 25-week-old conventional breeding Lohman hens were provided by Animal Medicine Department of Southwestern University.

Methods

Preparation of recombinant VacA antigen

The recombinant bacteria DH5α-vacA-pQE30 were inoculated into 5 ml LA medium and incubated at 150 r/min at 37˚C overnight. Then the bacteria were inoculated into 100 ml LA medium according to the 1:20 proportion and incubated at 37˚C. After the concentration reached A_{600} = 0.6, 0.5 mmol/L IPTG was used to induce its expression for 4 h. The bacteria were collected and centrifuged at 8500 g at 4˚C for 10 min. The precipitate bacteria were broken by ultrasound and then centrifuged at 8500 g at 4˚C for 40 min. Then, the precipitate was incubated with the inclusions lysate for 2 h at room temperature and then centrifuged at 8500 g at 4˚C for 40 min. The supernatant was collected and dialysis renaturation was performed after Ni2 +-NTA resin affinity chromatography purification. The purity was analyzed with 12% SDS-PAGE. Bradford method was adopted to determine the protein content. The antigenicity of recombinant VacA protein was identified by Western blot (The first antibody was the serum of the patients with VacA (+). The second antibody was HRP-labeled goat anti-human IgG). After VacA antigen immunization in the rabbit, indirect ELISA was adopted to determine the titer of VacA in rabbit serum to identify its immunogenicity. The recombinant was stored at -70˚C for use.

Preparation of Specific IgY against recombinant VacA

The purified recombinant VacA antigen was mixed with the same amount of complete Freund's adjuvant. After fully grinding and emulsifying, the mixture was inoculated in Lohman hens via multi-point chest muscle injection Liu et al. (2003). The initial immunization dose was 100μg/hen, and the second immunization was performed after 15 d with 200 μg/hen. Thereafter, twice strengthened immunization was performed every other month with 300 μg/hen. One week after the last immunization, the eggs were collected. Egg yolk was firstly diluted with water and then IgY was crudely extracted by chloroform organic precipitation method and purified by conventional saturated ammonium sulfate method. Filtration sterilization was performed with 0.22 μm membrane and the purity was analyzed with 12% SDS-PAGE. Bradford method was adopted to determine the protein content and indirect ELISA was adopted to determine the IgY titer. IgY was stored at -20˚C.

In vitro experiment

Protective effects of sucralfate on specific IgY under strong acid condition

Based on the method of Kyong AL (Ohkusa et al., 2003), IgY was diluted with pH 1.5, 2.0 as well as 3.0 of sodium dihydrogen phosphate buffer, respectively. Then 10 - 60% sucralfate (volume percentage) was added to make 1 mg/ml final concentration of IgY. After incubation at 37˚C for 2 h, appropriate amount of 2 mol/L NaOH was added immediately to neutralize the acid until pH value to 7.0 and then A450 value of IgY was measured by ELISA. The pH 7.0 dilution containing 1 mg/ml VacA IgY maintained at 37˚C for 2 h served as the reference. The residual antibody activity was calculated according to the ratio of IgY A_{450} value of the experiment solution to the IgY A_{450} value of the control solution represented by (A_R/A_C%). The protective effects of sucralfate on VacA IgY were evaluated, with the IgY treated with the same condition but without sucralfate as the control. According to the above method, after IgY was diluted with pH 2.0 sodium dihydrogen phosphate buffer, 10 - 60% sucralfate (volume percentage), inulin and sorbitol (weight/volume) were added, respectively, to make 1 mg/ml final concentration of IgY. After incubation at 37˚C for 2 h, the residual activity of IgY was measured. The stabilizing effects of sucralfate, inulin and sorbitol on VacA IgY were compared.

Protective effects of Sucralfate on specific IgY against digestion by pepsin

IgY was diluted with pH 2.0 and pH 3.0 sodium dihydrogen phosphate buffer and then 0.02 mg/ml final concentration of pepsin was added and then 10, 30 and 50 % sucralfate were added, respectively, to make 1 mg/ml final concentration of IgY. The solution was maintained at 37˚C for 4 h and then 2 mol/L NaOH was added immediately to adjust the pH to 7.0. A450 value of IgY was

Table 1. Grouping of the experimental mice and therapy protocol.

Groups		Intragastric administration solution
Infected group	A1	4 mg IgY/day
	A2	4 mg IgY+30% Sucralfate/day
	B1	2 mg IgY/day
	B2	2 mg IgY+30% Sucralfate/day
	C1	0.5 mg IgY/day
	C2	0.5 mg IgY+30% Sucralfate/day
	D	PBS/day
	E	30% Sucralfate/day
Healthy control group	F	PBS/day

examined by ELISA. The pH 7.0 diluted solution containing 1 mg/ml VacA IgY treated with the same condition and without pepsin as well as sucralfate served as the reference. The residual antibody activity was calculated according to the above method. The protective effects of sucralfate on the specific VacA IgY digested by pepsin were evaluated, with the IgY treated with the same condition and pepsin and without sucralfate as the control.

Protective effects of sucralfate on specific IgY under repeated freeze thawing condition

IgY was diluted with pH 7.0 sodium dihydrogen phosphate buffer, and then 10, 30 and 50% sucralfate were added, respectively, to make 1 mg/ml final concentration of IgY. Seven times of repeated freeze thawing were performed at 4˚C per 1 h. The same diluted IgY without freeze thawing and sucralfate treatment served as the reference. A450 value of IgY was examined by ELISA, and the residual antibody activity was calculated according to the above method. The protective effects of sucralfate on VacA IgY under repeated freeze thawing were evaluated, with the IgY treated with the same condition and without sucralfate as the control.

Conserving experiment at room temperature

IgY was diluted with pH 7.4 sodium dihydrogen phosphate buffer and then 30 and 50% sucralfate were added, respectively, to make 1 mg/ml final concentration of IgY. The solution was placed at room temperature for 1, 2 and 3 d and 1, 2, 3 and 4 w. The IgY treated with the same condition and without sucralfate served as the control. The same diluted IgY placed at 4˚C served as the reference. The residual antibody activity was calculated according to the ratio of OD450 value of IgY placed at room temperature to OD450 value of the reference IgY detected by ELISA.

***In vivo* experiment**

Establishment of the H. pylori-infected mouse model

Based on the method of Ohkusa et al. (2003), the recovered *H. pylori* (NCTC11637) bacteria were cultured using microaerophlilic incubation for 3 days with *H. pylori* medium. The bacteria colonies were washed up with pH 7.0 Brucella Broth and made into 1 x 10^9 cfu/ml concentration by turbidimetry. Daily intragastric administration of 0.1 ml (50 mg/ml) azithromycin, 0.25 ml (20 mg/ml) ampicillin and 0.15 ml (4 mg/ml) gentamicin was performed in BALB/c mice for consecutive three days in order to clear other bacteria in mice stomach. After 24 h fasting, 0.2 ml 3% NaHCO3 intragastric administration was performed. After 1 h, the intragastric administrations with 0.3 ml (1 × 10^9 cfu/ml) fresh *H. pylori* bacteria solution and 0.3 ml stroke-physiological saline solution were performed in the mice of the infected group and the healthy control group, respectively. After 4 h, normal diet was recovered. Consecutive 5 times of intragastric administrations were performed every other two days. Four weeks after the last intragastric administration, mouse vena caudalis blood was obtained, and the specific anti-*H. pylori* IgG was examined by Western blot, with the positive serum specific IgG as *H. pylori* infection.

Intragastric protective effects of sucralfate on VacA IgY-treated H. pylori infection

Based on the method of Suzuki et al. (2004) the mice in the infection group with positive VacA IgG in vena caudalis blood were randomly divided into A1, A2, B1, B2, C1, C2, D and E groups (n = 3), and F group was the healthy control group (n = 5). IgY treatment control solution with 0.5, 2 and 4 mg/ml final concentration was prepared with pH 7.0 phosphate buffer. The same solution with 30% Sucralfate/ml was prepared as the treatment experiment solution. The phosphate buffer only containing 30% sucralfate/ml served as the control solution. Intragastric administration of 1 ml experiment solution and the control solution was performed in the infected mice and the healthy control mice, respectively, before eating once per one day for consecutive seven days. The experiment schedule was shown in Table 1. Two weeks after last intragastric administration, the mice were sacrificed by cervical dislocation under anesthesia. Gastric tissue was removed under sterility condition and was cut along the greater curvature. Stomach content was washed with sterile saline.

The gastric tissue was divided longitudinally into three parts. One part was fixed with 10% formalin and paraffin-embedded for HE staining to observe the inflammatory reaction and for Giemsa staining to observe the *H. pylori* colonization in gastric mucosa. The other two parts were used for rapid urease test and *H. pylori* culture. The rapid urease test was performed according to the instruction. Direct smear method was used for *H. pylori* culture: the gastric tissue was placed on *H. pylori* selective medium with the mucosal face downward and the medium was smeared repeatedly and thensectional streak was performed. The colonies were observed after 72 h microaerophlilic incubation. *H. pylori* was identified according to the morphology of the colonies and bacteria, urease test, catalase test and oxidase test. The grading of gastric mucosal chronic inflammation was evaluated based on the new Sidney system (Kyong et al., 2002) visual analogue score program. The four grading of normal, mild, moderate and severe grades were rated as 0, 1, 2 and 3. The score result was analyzed with

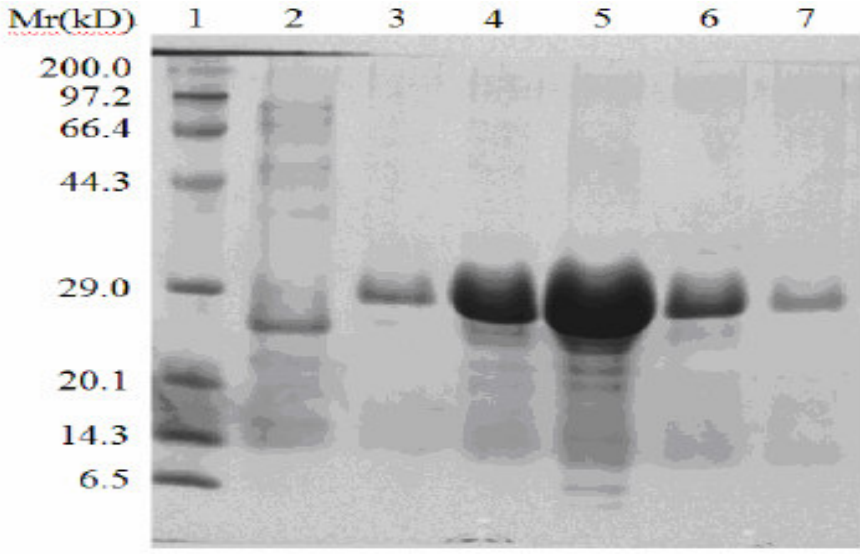

Figure 1. SDS-PAGE analysis on purified recombinant protein. Note: 1. Protein marker, 2. Washings, 3 - 7. Purified recombinant protein.

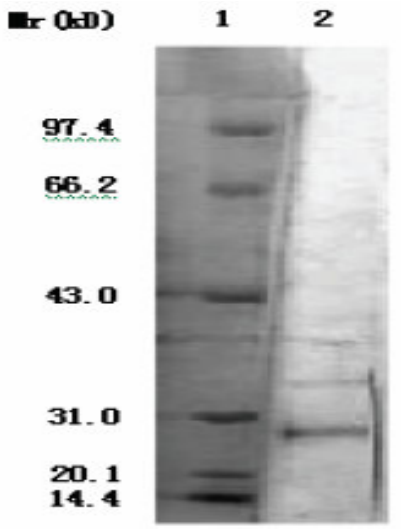

Figure 2. The identification of recombinant protein by Western blot. Note: 1. Protein marker, 2. recombinant protein exhibited with antiserum combined with HRP- labeling antibody.

SPSS10.0 software.

RESULTS

Preparation of the recombinant VacA antigen

The protein purity of the purified recombinant VacA protein was 92.2% detected by SDS-PAGE and gel imaging software analysis (Figure 1). The protein content was 0.376 mg/ml detected by Bradford method. The expected protein band of relative molecular weight 27000 was detected by Western blot (Figure 2). The titer of rabbit serum to VacA antigen was 1:1280 measured by ELISA.

Preparation of the anti-recombinant VacA specific antibody

After inoculation of the recombinant VacA antigen in Lohman hens, the egg yolk was purified and concentrated. The protein content was 6.36 mg/ml detected by Bradford method and the protein was analyzed by SDS-PAGE. The expected protein bands of relative molecular weight 64000 and 25000 were detected, which matched to the IgY heavy chain and light chain (Figure 3). The purity of the protein was 72.0% analyzed by gel scanning image software. The titer of IgY was 1:6400 measured by ELISA.

In vitro experiment

Protective effects of sucralfate on specific IgY under strong acid condition

Under strong acid condition, the antibody activity was significantly increased after adding sucralfate in a dose dependent manner. At pH = 1.5, IgY antibody activity without sucralfate could only be maintained to 31.8%. However, the IgY residual antibody activity was increased to 35.8, 48.9 and 48.9% after adding 30, 50 and 60% sucralfate, respectively. At pH = 2.0, IgY residual antibody activity without sucralfate was 36.1%. However, the IgY residual antibody activity was increased to 86.5% after

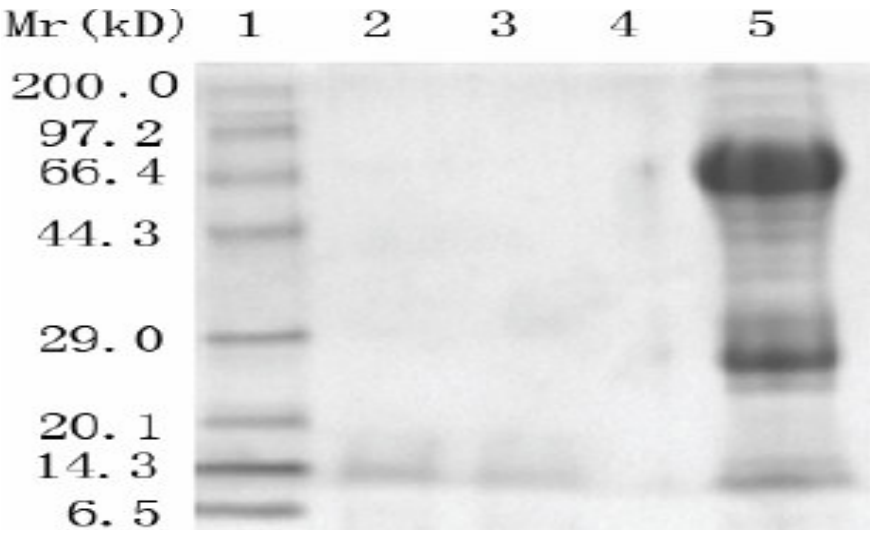

Figure 3. SDS-PAGE analysis on the purified VacA-IgY. **Note:** 1. Protein marker, 5. Purified VacA-IgY.

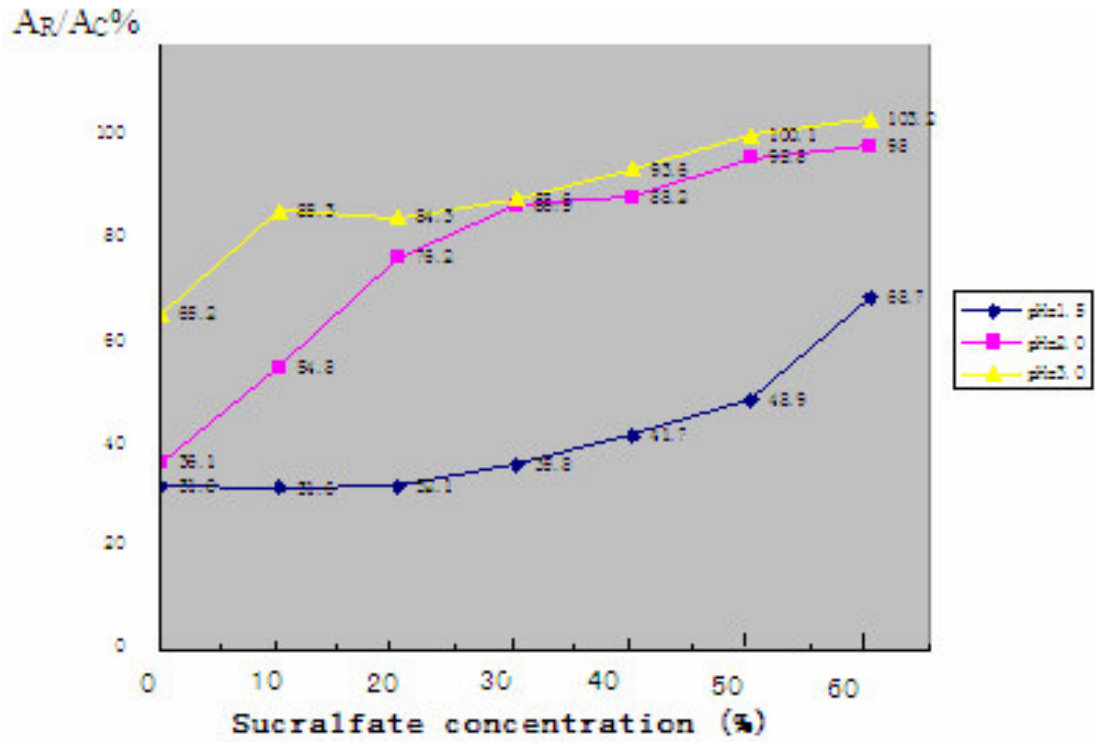

Figure 4. The residue activity of IgY antibody containing 0 - 60% sucralfate at pH1.5, 2.0 and 3.0.

adding 30%sucralfate. 50% Sucralfate could fully maintain IgY antibody activity. At pH = 3.0, IgY antibody activity without sucralfate was 65.2%. However, the IgY residual antibody activity was increased to 87.6% after adding 30% sucralfate. Over 40% Sucralfate could almost fully maintain IgY antibody activity (Figure 4).

At pH = 2.0, the residual antibody activity of IgY in sucralfate group was significantly higher than that of the same concentration of sorbitol group and inulin group.

The residual antibody activity of IgY in sorbitol group and inulin group showed the tendency of firstly increased and then decreased with the increase of their concentrations. After adding over 40% sorbitol or 30% inulin, the residual antibody activity of IgY was decreased in contrary. After adding sucralfate, the residual antibody activity of IgY was increased with the increase of the sucralfate concentration

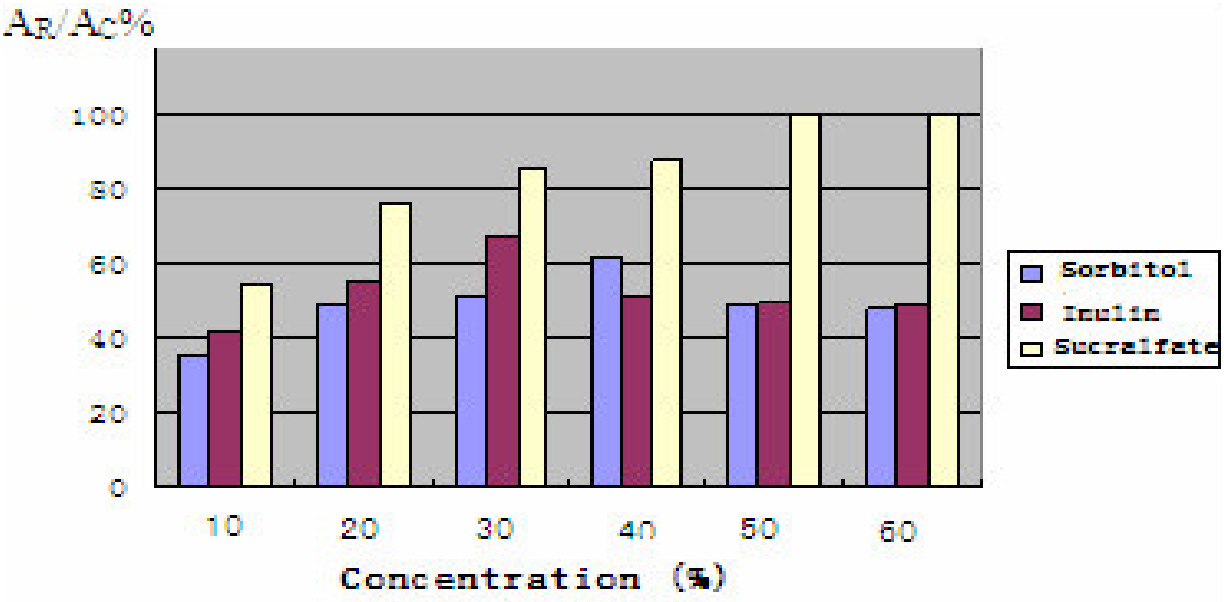

Figure 5. The residue activity of IgY antibody containing 0 - 60% sucralfate, sorbitol and inulin at pH 2.0.

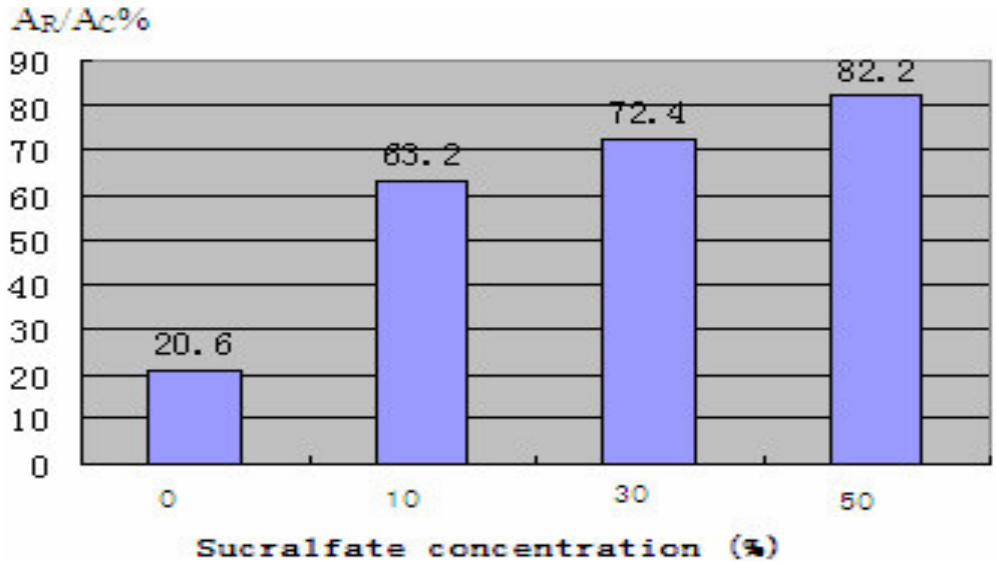

Figure 6. The residue activity of IgY antibody containing 10, 30 and 50% sucralfate, on 0.02 mg/ml pepsine at pH 2.0.

(Figure 5). 10% Sucralfate could maintain 54.8% IgY antibody activity and 50% sucralfate could fully maintain IgY antibody activity.

Protective effects of sucralfate on specific IgY against digestion by pepsin

The residual activity of IgY antibody incubated with sucralfate was increased with the increase of sucralfate concentration at pH = 2.0. Under 0.02 mg/ml concentration of pepsin, the residual activity of IgY antibody without sucralfate was 20.6% and the residual activities of IgY antibody with 10, 30 and 50% sucralfate were 63.2, 72.4 and 82.2%, respectively (Figure 6).

Protective effects of sucralfate on specific IgY after repeated freeze thawing condition

After repeated freeze thawing, the residual activity of IgY antibody without sucralfate reduced to 61.9%. After adding

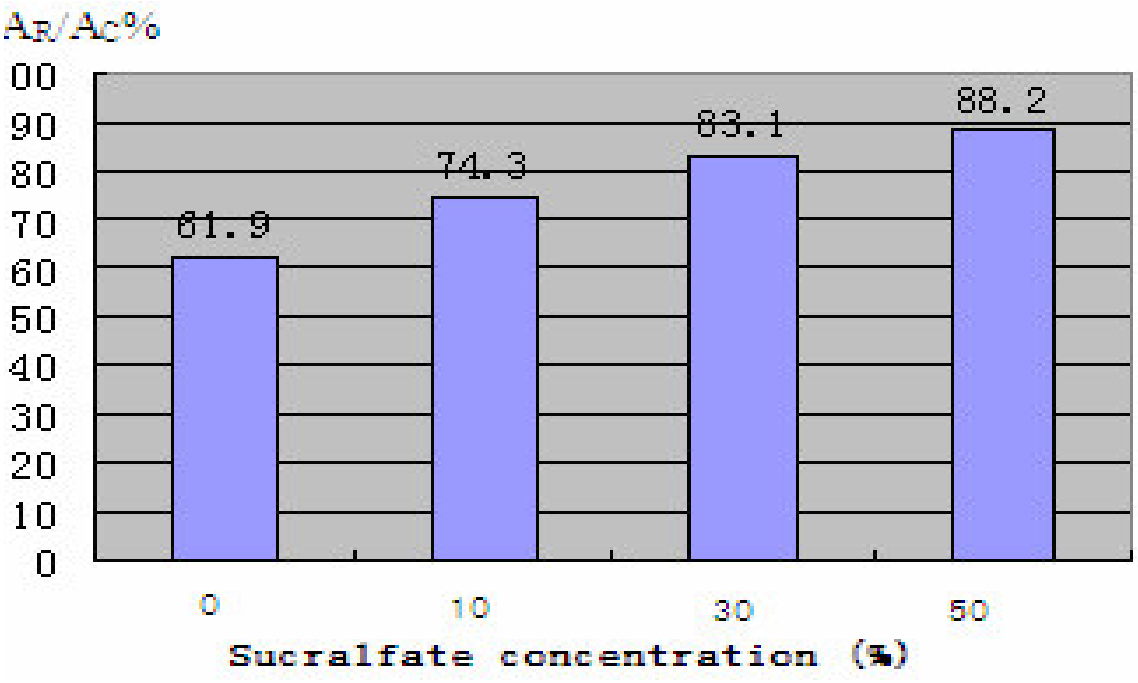

Figure 7. The residue activity of IgY antibody containing 10, 30 and 50% sucralfate after freeze thawing repeatedly.

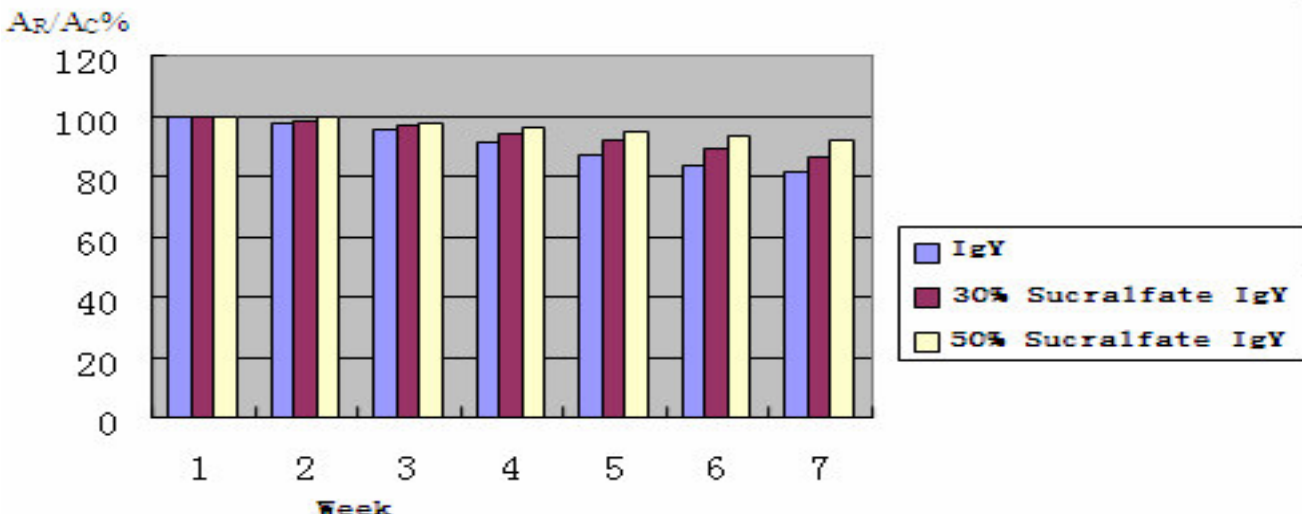

Figure 8. The residue activity of IgY antibody standing at room temperature.

sucralfate, the residual activity of IgY antibody was increased with the increase of sucralfate concentration. 30% sucralfate could enhance the activities of IgY antibody to 83.1 and 50% sucralfate could enhance the activities of IgY antibody to 88.2% (Figure 7).

Conserving experiment at room temperature

The result showed that IgY with 30 and 50% sucralfate and without sucralfate could maintain over 80% antibody activity after 1 month room temperature standing (Figure 8).

In vivo experiment

Establishment of the H. pylori-infected mice model

Among 35 *H pylori*-infected mice, 25 mice showed positive anti-*H. pylori* specific IgG antibody in vena caudalis, with the infection rate of 71.4%.

Table 2. Scores for chronic inflammation and chronic inflammation activity.

Groups	Items for scoring	Average	Median	P value (Mann-Whitney U test)
A1	Neutrophil granule apocyte density	0.67	1	0.043
	Chronic inflammatory cell density	1.67	2	0.034
A2	Neutrophil granule apocyte density	0.33	0	0.043
	Chronic inflammatory cell density	1.00	1	0.025
B1	Neutrophil granule apocyte density	1.33	1	0.068
	Chronic inflammatory cell density	2.33	2	0.114
B2	Neutrophil granule apocyte density	0.67	1	0.034
	Chronic inflammatory cell density	1.00	1	0.037
C1	Neutrophil granule apocyte density	1.67	2	0.099
	Chronic inflammatory cell density	2.67	3	0.317
C2	Neutrophil granule apocyte density	1.00	1	0.034
	Chronic inflammatory cell density	2.00	2	0.025
D	Neutrophil granule apocyte density	2.33	2	0.456
	Chronic inflammatory cell density	2.67	3	0.317
E	Neutrophil granule apocyte density	2.67	3	
	Chronic inflammatory cell density	3.00	3	
F	Neutrophil granule apocyte density	0.00	0	
	Chronic inflammatory cell density	0.00	0	

Intragastric protective effects of sucralfate on VacAIgY-treated H. pylori infection

25 *H. pylori*-infected mice showed positive rapid urease test and positive *H. pylori* culture. *H. pylori* colonization was observed by Giemsa staining. The chronic inflammation status and chronic inflammatory activity score of the gastric fundus and sinus ventriculi in each experiment mice were shown in Table 2. The statistical results showed that there was significant difference in the chronic inflammation status and chronic inflammatory activity score between A1, A2, B2, C2 group and the control group, while there was no significant difference between B1, C1, D groups and the control group. The gastric mucosa in sinus ventriculi of the mice in the healthy control group (F group) was integrity without ulceration, while the gastric mucosa in sinus ventriculi of the mice in the control group (E group) was seriously ulcerated and fell off and the contour disappeared.

DISCUSSION

The main feature of endogastric ecological environment is containing hydrochloric acid and pepsin. Chen et al. (1997) observed the intragastric pH rhythmic changes in normal individuals by 24 h dynamic observation. The baseline of pH value was 1.63 ± 0.34 and it was increased with eating. The intragastric food emptying was the process from eating to the baseline level of pH value. The intragastric pepsin concentration in normal adults was 0.02 mg/ml. The optimum pH of pepsin was 1.5 ~ 2.2 and the optimum temperature was around 37˚C. Therefore, anti-acid and anti-pepsin were necessary for prevention and treatment of *H. pylori* infection.

Kyong et al. (2002) have shown that the IgY activity decreased by 50% under pH < 3 condition. The IgY almost completely deactivated under pH = 2 condition for 4 h. 30% sorbitol could significantly improve the stability of IgY under pH = 3.0 condition and 50% sorbitol could fully maintain the activity of IgY. Shimizu et al. (1993) showed that the IgY residual activity was increased from 60 - 100% with the increase of the proportion of cholesterol to lecithin from 0:1 - 4:1 after yolk antibody was surrounded by liposomes under pH = 2.8 condition. The IgY residual activity was increased from 10 - 60% when the proportion of cholesterol to lecithin from 0:1 - 4:1 under pH = 1.8 condition. The highest IgY residual activities were 73.9

and 38.5%, respectively, under pH = 2.8 or 1.8 and 0.015 mg/ml pepsin condition. Xiao et al. (2007) found that the IgY activity maintained at 61.36 - 74.61% when IgY was packaged with chitosan-alginate microcapsule and placed into simulated gastric fluid.

Our results showed that both sucralfate and many carbohydrates could increase the IgY stability in acidic environments and the protective effect of sucralfate on IgY was significantly superior to carbohydrates and also superior to liposomes-packaged IgY and chitosan-alginate microcapsules-packaged IgY. 30 - 50% sucralfate could effectively protect IgY activity and increase the anti-freeze thawing ability in simulated gastric fluid environment containing 0.02 mg/ml pepsin at pH = 2. The *in vivo* experiment showed that 0.5 mg IgY plus 30% sucralfate/day could effectively prevent gastric mucosal damage caused by *H. pylori* infection and the effect increased eight folds as compared with the group without sucralfate. Sucralfate is a kind of sucrose aluminum sulfate containing 8 sulphates and its molecular mass is 2.08674 ku. It exists as non-crystalline form and presents alkalescence. Its chemical structural formula is $C_{12}H_{30}Al_8O_5lS_{8x}Al(OH)_3yH_20$. Its sulfuric acid sucrose part cross-linked among alkaline aluminum hydroxide molecule and forming stereochemical structural bridge-shaped compound, with marked adhesiveness and negative charges, which can bind a large number of positively charged chemical groups or macromolecular substances, such as mucus, glycolipidprotein and other proteins, peptides, drugs, metal, etc, and also bind the mucus on gastric and duodenal mucosal surface and form compound gel material, thereby playing a role in mucosal protection (Ishimori, 1995; Ochi, 1995). After sucralfate is dissociated by acid, the aluminum hydroxide gel exerts an anti-acid effect, which could not only relieve the strong acid environment, but also eliminate the optimal acidic environment for pepsin. Moreover, sucralfate itself could form complex with pepsin, thereby directly and continuously inhibiting the activity of pepsin. As a protective agent for IgY, sucralfate could not only reduce the damage effect of gastric acid and pepsin on IgY activity, but also exert protective effect on gastric mucosa, therefore, it is an ideal IgY protective agent. The preparation of specific IgY containing sucralfate is expected to make IgY play more effective role in prevention and treatment of *H. pylori.*

REFERENCES

Chen JQ, Xu LW, Zhang Y (1997). Observations of 24-hour Intragastric pH Rhythmic Changes in Normal Individuals. Tianjing. Med.J., 25(11): 668-670.

Granstrom M, Tindberg Y, Blennow M (1997). Seroepidemiology of *Helicobacter pylori* infection in a cohort of children monitored from 6 months to 11 years of age. J. Clin. Microbiol., 35: 468-70

International Agency for Research on Cancer (1994). *Schistosomes,* liver flukes and *Helicobacter pylori*[M]. IARC Monograph on the Evaluation of Carcinggenic Risks to Human.161, Lyon: IARC.

Ishimori A (1995). History of the development of sucralfate. New York:Plenum Medical book Company. pp.35-45.

Kyong AL, Sung KC, Yoon JL (2002). Acid stability of anti–*Helicobacter pylori* IgY in aqueous polyol solution[J]. J Biochem. Mol. Biol., 35(5): 488-493.

Liu M, Yang ZB, Lin SS (2003). Cloning and expressing of vacA gene related segment with vacuolating activity of *Helicobacter pylori*. J. Chongqing Med. Univ., 28(6): 720-723.

Mine Y, Kovacs-Nolan J (2002). Chiken egg yolk antibodies as therapeutics in enteric infections disease, J. Med. Food., 5(3): 159-169.

Salih BA (2009). *Helicobacter pylori* infection in developing countries: the burden for how long. Saudi J Gastroenterol. 15(3): 201-207.

Suzuki H, Nomura S, Masaoka T (2004). Effect of dietary anti-*Helicobacter pylori*-urease immunoglobulin Y on Helicobacter pylori infection. Alimentary Pharmacol. Thera., 20(1): 185-192.

Ochi Kiyoshige (1995). Chemistriy of sucralfate. New York:Pienum Medical book Company. pp. 47-58.

Ohkusa T, Okayasu I, Miwa H (2003). *Helicobacter pylori* infection induces duodenitis and superficial duodenal ulcer in Mongolian gerbils. Gut. 52(6): 797-803.

Shimizu M, Miwa Y, Hashimoto K (1993). Encapsulation of chicken egg yolk immunoglobulin G (IgY) by liposomes. Biosci Biotechnol Biochem. 57(9): 1445-1449.

Xiao YL, Li JJ, TimA MA (2007). Chitosan-Alginate Microcapsules for Oral Delivery of Egg Yolk Immunoglobulin (IgY). Agric. Food Chem. 55(8): 2911-2917.