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Full Length Research Paper

Effects of immune synergist of Chinese medicinal herbs on the efficacy of vaccination against classic swine fever

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Two-month-old piglets were fed with 1, 1.5 and 2% immune synergist of Chinese medicinal herbs together with vaccination against classic swine fever. Serum IgG and IgM levels increased more than the control group on day 30 (P<0.05). B and T lymphocyte proliferation in piglets fed with 1.5 and 2% herbal immune synergist markedly increased on day 30 as compared to the control group (P<0.05). Improvement was also observed in T lymphocyte CD3+, CD4+, CD8+ and CD3+/CD8+ levels. Meanwhile, there were significant differences in SOD activities and rate of neutrophil phagocytosis between synergist groups and the control group. These results suggest that herbal immune synergist of Chinese traditional herbs prescription enhances the protective effect of classic swine fever vaccine.

Key words: Immune synergist, Chinese medicinal herbs, classic swine fever vaccine.

INTRODUCTION

Many Chinese medicinal herbs are high efficacious immune synergist, including radix ginseng, astragalus, Szechwan asiabell root, poria mushroom, Chinese angelica and licorice root, which have been proven to possess immune-enhancement effects (Hu, 1997; Liu, 1998). These herbs contain plenty polysaccharides, saponins and flavones that are capable of modulating vertebrate cellular and humeral immunity, and disease resistance (Liu et al., 2006; Gao et al., 2000). Because the prescription is made up of pure Chinese medicinal herbs that are readily available, of low-cost and low side effects (Kong et al., 2004, 2006; Wang et al., 2005; Ung et al., 2007), it has become a research focus recently. However, most studies are centered on single herb; little attention has been paid to compound herbs. We have previously reported the effect of Chinese medicinal herbs synergist on chicken (Wang, et al., 2006). Here, we described its synergistic effects on vaccination against classic swine fever.

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MATERIALS AND METHODS

Animal selection and management

Piglets (n = 60) were provided by Hongdong County Great Locust Tree Ecotech Limited, China. The feed was manufactured by Wuike Feedstuff Scientific Limited, China, according to the formula provided by the research group. Piglets were 50 to 60 days of age with body weight of 20 ± 2 kg. All animals were not previously exposed to any vaccine. Piglets were managed with the same standard of nutrition and procedure. Records and management were performed by designated personnel.

Compound herbs immune synergist

Ginseng (*Panax ginseng* C.A.Mey), milk vetch root (*Astragalus membranaceus* Bge var. mongholicus), Szechwan asiabell root (*Codonopsis tangshen* Oliv), poria mushroom (*Poria cocos* Schw. Wolf), Chinese angelica (*Angelica sinensis* Oliv. Diels), large head atractylodes rhozome (*Atractylodes macrocephala* Koidz), fructus amomi (*Amomum villosum* Lour), pericarpium citri reticulatae (*Citrus reticulata* Blanco), American ginseng (*Panax quinquefolium* L.), and licorice root (*Glycyrrhiza uralensis* Fisch) were used. The herbs were supplied by Taiyuan School of Chinese Traditional Medicine.

Table 1. Effects of herbal immune synergist on serum IgG content.

Group	Time (day)			
	10	20	30	40
A (1.0%)	606.25±43.04[b]	612.17±40.55[b]	618.46±34.95[b]	616.65±44.81[b]
B (1.5%)	617.25±43.04[a]	619.17±40.55[a]	628.66±34.95[a]	626.65±45.11[a]
C (2.0%)	618.02±42.34[a]	619.19±41.35[a]	629.12±33.85[a]	625.35±44.21[a]
D (control)	561.25±43.04[c]	598.17±40.55[c]	609.46±34.95[c]	607.65±44.81[c]

Data presented are means ± SE of 15 replicates. For each column, means with the same letter are not significantly different ($p < 0.05$).

Table 2. Effects of herbal immune synergist on serum IgM content.

Group	Time (day)			
	10	20	30	40
A (1.0%)	320.31±21.45[b]	329.65±27.01[b]	332.87±26.68[b]	330.60±25.36[b]
B (1.5%)	336.54±25.91[a]	339.51±24.91[a]	342.57±25.81[a]	340.55±24.92[a]
C (2.0%)	336.88±26.19[a]	339.89±26.21[a]	343.21±24.92[a]	41.33±23.71[a]
D (control)	309.51±24.42[c]	318.60±25.61[c]	322.58±26.11[c]	319.96±24.61[c]

Data presented are means ± SE of 15 replicates. For each column, means with the same letter are not significantly different ($p < 0.05$).

Vaccine and chemicals

Vaccine was bought from Lanzhou Biological Pharmaceuticals of Zhongmu Industry limited China. Sodium heparin, phytohemagglutinin (PHA), trypan blue, lipopolysaccharide and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were produced by Guangzhou Medical Industry Institute, China. Lymphocyte isolation solution was produced by Chinese Medical Academy Biotech Institute. Aluminum hydroxide gel was supplied by Longkel Biotech Pharmaceuticals, China. Calf serum, antibiotics, 0.8% NH_4Cl solution, Wright's dye and medium were prepared by the authors.

Experimental design

Randomized block design was used in this study. The 60 piglets were randomly assigned to 4 groups (n = 15 in each group). Piglets in the synergist group were treated with classic swine fever vaccine and herbal immune synergist. Starting from the first day of the experiment, piglets in the synergist group were fed, respectively with supplemented 1.0% (A group), 1.5% (B group) and 2.0% (C group) synergist prescription. On day 7, piglets were injected *s.c.* with vaccine, while piglets in the control group (n = 15) were treated with swine fever vaccine alone (D group). The whole experiment lasted for 60 days in which pretreatment lasted for 10 days and the treatment lasted for 50 days. Blood samples were collected from each group at day 10, 20, 30 and 40 after immune synergist treatment.

Assay

Content of serum IgG and IgM was determined by immune transmission turbidity. Peripheral T and B lymphocytes were measured with MTT colorimetric method. Red blood cell (RBC) and hemoglobin (Hb) were measured with general method. Serum GPT, GOT and SOD activity were measured with Automatic biochemical analyzer (TOSHIBA, Tokyo, Japan). Analysis on subpopulation of T lymphocytes was used by Flow Cytometry (Becton Dickinson, Rutherford, NJ, USA). Phagocytotic neutrophil assay was performed as follows:

$$\text{Phagocytosis rate} = \frac{\text{Phagocytotic neutrophil}}{\text{Total neutrophil}} \times 100\%$$

Data analysis

The data were subjected to one-way analyses of variance (ANOVAs) using the statistical package Minitab 15. Where required, Tukey multiple comparison tests were used following ANOVAs to identify significant differences between individual treatments.

RESULTS

Effects of herbal immune synergist on serum IgG and IgM contents

Serum IgG and IgM contents are shown in Tables 1 and 2. There were significant differences in serum IgG and IgM contents on day 10 between the synergist groups and control ($P < 0.05$). On day 30, serum IgG and IgM reached a peak, where average IgG levels in synergist groups were 2.54% higher than that in the control group ($P < 0.05$). Group C had a higher IgG content than group B, but no significant differences ($P > 0.05$).

Effects of herbal immune synergist on peripheral T and B lymphocyte proliferation

Groups B and C had a higher B and T lymphocyte proli-

Table 3. Effects of herbal immune synergist on peripheral B and T lymphocyte proliferation rate (OD).

Group	Lymphocyte	Time (day)			
		10	20	30	40
A (1.0%)	T	0.68±0.32^{b}	0.87±0.53^{c}	1.07±0.61^{b}	1.05±0.43^{c}
	B	0.69±0.41^{c}	0.88±0.56^{b}	1.09±0.60^{c}	1.08±0.46^{c}
B (1.5%)	T	0.81±0.23^{a}	0.98±0.49^{b}	1.18±0.57^{a}	1.13±0.41^{b}
	B	0.79±0.21^{b}	0.99±0.51^{a}	1.17±0.58^{b}	1.15±0.39^{b}
C (2.0%)	T	0.82±0.35^{a}	1.01±0.55^{a}	1.19±0.61^{a}	1.16±0.51^{a}
	B	0.83±0.32^{a}	1.00±0.60^{a}	1.20±0.63^{a}	1.18±0.50^{a}
D (control)	T	0.60±0.25^{c}	0.77±0.51^{d}	0.99±0.52^{c}	0.87±0.38^{d}
	B	0.62±0.31^{d}	0.78±0.48^{c}	0.98±0.54^{d}	0.89±0.37^{d}

Data presented are means ± SE of 15 replicates. For each column, means with the same letter are not significantly different ($p < 0.05$).

Table 4. Effect of herbal immune synergist on T lymphocyte subpopulation on day 40.

Group	Item (X ± SD)			
	CD_3^+	CD_4^+	CD_8^+	CD_3^+ / CD_8^+
A (1.0%)	45.38±0.32^{b}	27.55±0.23^{c}	21.96±0.35^{c}	1.34±0.25^{a}
B (1.5%)	47.87±0.53^{a}	29.76±0.49^{b}	22.86±0.55^{b}	1.35±0.51^{a}
C (2.0%)	47.88±0.61^{a}	29.78±0.57^{a}	22.89±0.61^{a}	1.35±0.52^{a}
D (control)	44.75±0.43^{c}	26.79±0.41^{d}	21.92±0.52^{d}	1.31±0.38^{b}

Data presented are means ± SE of 15 replicates. For each column, means with the same letter are not significantly different ($p < 0.05$).

feration rate (Table 3). From day 10 to 30, proliferation rate showed an increasing tendency. On day 40, the lymphocyte proliferation decreased slightly. On day 30, B lymphocyte proliferation rates in groups B and C showed significant difference ($P < 0.05$), but T lymphocyte proliferation rates in group C were slightly higher than in group B, but there was no significant difference ($P > 0.05$).

Effect of herbal immune synergist on T lymphocyte subpopulation

Table 4 shows the effect of traditional Chinese herbs prescription on subpopulation of T lymphocytes on day 40. The ratio of T lymphocyte subpopulation in the synergist group was markedly increased and improved.

Effects of herbal immune synergist on porcine red blood cell and Hb content

Piglet red blood cell and hemoglobin contents are shown in Table 5. There were no significant differences between synergist groups and the control group ($P > 0.05$).

Effects of herbal immune synergist on serum GPT, GOT and SOD activity

Serum SOD activities in synergist groups were significantly different from that of the control group on days 30 and 40 ($P < 0.05$) (Table 6). GOT and GOT activities were similar between synergist groups and the control group ($P > 0.05$).

Effects of herbal immune synergist on rate of neutrophil phagocytosis

Rate of neutrophil phagocytosis showed a trend of increase in synergist groups, and reached a peak on day 30 (Table 7). Rate of neutrophil phagocytosis of synergist groups was higher than that of the control group ($P < 0.05$), but there was no significant difference between synergist groups B and C ($P > 0.05$). Rate of neutrophil phagocytosis of synergist groups decreased slightly on

Table 5. Effects of herbal immune synergist on porcine red blood cell and Hb content.

Group	Item	Time (day)			
		10	20	30	40
A (1.0%)	RBC	6.68±0.42[a]	6.78±0.33[a]	6.87±0.51[a]	6.75±0.53[a]
	Hb	12.09±0.51[a]	12.58±0.46[a]	12.89±0.50[a]	12.25±0.48[a]
B (1.5%)	RBC	6.81±0.33[a]	6.88±0.29[a]	6.97±0.47[a]	6.90±0.45[a]
	Hb	12.32±0.21[a]	12.99±0.51[a]	13.15±0.58[a]	12.96±0.39[a]
C (2.0%)	RBC	6.83±0.45[a]	6.92±0.56[a]	6.99±0.51[a]	6.95±0.46[a]
	Hb	12.43±0.42[a]	12.68±0.60[a]	13.18±0.82[a]	13.01±0.58[a]
D (control)	RBC	6.59±0.35[a]	6.63±0.46[a]	6.82±0.58[a]	6.80±0.58[a]
	Hb	11.62±0.61[a]	11.88±0.48[a]	12.38±0.46[a]	12.71±0.67[a]

Data presented are means ± SE of 15 replicates. For each column, means with the same letter are not significantly different ($p < 0.05$).

Table 6. Effects of herbal immune synergist on serum GPT, GOT and SOD activity.

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Group	Item	Time (day)			
		10	20	30	40
A (1.0%)	GPT	35.68±0.42[a]	33.12±0.33[a]	31.87±0.51[a]	32.98±0.53[a]
	GOT	36.09±0.51[a]	34.58±0.46[a]	32.89±0.50[a]	33. 05±0.48[a]
	SOD	58.56±0.51[a]	98.55±0.46[a]	102.89±0.50[b]	98. 95±0.48[b]
B (1.5%)	GPT	35.51±0.33[a]	33.08±0.29[a]	30.27±0.47[a]	30.80±0.45[a]
	GOT	36. 32±0.21[a]	32.99±0.51[a]	30.05±0.58[a]	30.96±0.39[a]
	SOD	57. 82±0.21[a]	98.99±0.51[a]	123.15±0.58[a]	118.96±0.39[a]
C (2.0%)	GPT	35.88±0.45[a]	33.92±0.56[a]	31.39±0.51[a]	31.99±0.46[a]
	GOT	36.43±0.42[a]	34.68±0.60[a]	30.18±0.82[a]	30.88±0.58[a]
	SOD	57.43±0.42[a]	97.68±0.60[a]	123.18±0.82[a]	119.79±0.58[a]
D (Control)	GPT	35.59±0.35[a]	34.13±0.46[a]	33.82±0.58[a]	35.80±0.58[a]
	GOT	34.62±0.61[a]	33.18±0.48[a]	32.48±0.46[a]	32.98±0.67[a]
	SOD	57.62±0.61[a]	67.88±0.48[b]	78.38±0.46[c]	70.71±0.67[c]

Data presented are means ± SE of 15 replicates. For each column, means with the same letter are not significantly different ($p < 0.05$). GPT, *Glutamate-pyruvate transaminase; GOT, glutamate-oxaloacetate transaminase; SOD,* superoxide dismutase.

day 40, which was still higher than that of the control group ($P < 0.05$).

DISCUSSION

Animal immunity is closely related to disease resistance. When immune function decreases or certain killing mechanism is damaged, pathogens may invade animals, resulting to clinical symptoms. Up to now, vaccination is still the most effective preventing measure. Although, the strict immune program has been taken in the farms, some infectious diseases are still hard to be controlled

Chinese medicinal herbs has been proven to be possess immune-enhancing properties (Xu et al., 2010, 2011). In addition, Chinese medicinal herbs may have many advantages including extensive availability, lower cost, reliable efficacy, decreased risk of side-effects and toxicity. Therefore, Chinese medicinal herbs or their ingredients have become a hotspot in recent years. The veterinary immune synergist prepared in this study selected herbs containing polysaccharides, flavones and saponins that are capable of activating immune system and stimulating lymphocytes (Jiang et al., 2010; Meng et al., 2011; Perera et al., 2010). In the study, our results demonstrate that supplementation of herbal immune

Table 7. Effects of herbal immune synergist on rate of neutrophil phagocytosis.

Group	Time (day)			
	10	20	30	40
A(1.0%)	0.068±0.21[b]	0.152±0.43[b]	0.216±0.51[b]	0.212±0.43[b]
B(1.5%)	0.074±0.23[a]	0.198±0.42[a]	0.276±0.53[a]	0.274±0.45[a]
C(2.0%)	0.075±0.22[a]	0.199±0.45[a]	0.276±0.47[a]	0.275±0.51[a]
D(blank)	0.057±0.24[c]	0.127±0.3 [c]	0.146±0.55[c]	0.181±0.43[c]

Data presented are means ± SE of 15 replicates. For each column, means with the same letter are not significantly different ($p < 0.05$).

synergist could increase serum IgG and IgM contents upon vaccination against classic swine fever, which is consistent with the report by Kong et al. (2004) and Hu et al. (2004). Herbal immune synergist promotes T and B lymphocyte proliferation and improves T lymphocyte subpopulation composition, which is consistent with the results reported by Chu et al. (2004). On the other hand, there were no significant differences for red blood cell and Hb content between synergist groups and the control group, and there were no significant differences between synergist groups and the control group in GPT and GOT. It indicates that these Chinese medicinal herbs are not toxic to the parenchymal of liver and heart. In addition, SOD activities and rate of neutrophil phagocytosis were markedly increased by herbal immune synergist. Although, 2.0% dose supplementation had a slightly better result than 1.5% dose, we recommend1.5% as the optimal dose.

Vaccine is an important method of animal infectious diseases prevention and control, the synergy and mechanism between the vaccine and immune synergist needs to be studied further. In addition, immune synergist is not effective against all diseases, therefore it is important to continue to develop new immune synergist, and to strengthen its mechanism research. Chinese traditional medicinal herb is a potential candidate as a safe immune synergist for animal infectious diseases prevention and control.

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REFERENCES

Chu YF, Li XR, Hu YL (2004). Effects of Chinese herbal medicinal ingredient on cells mediated immunity in mice. J. Nanjing Agric Uni. 27(1): 97-100.

Gao XX, Fei XF, Wang BX, Zhang J, Gong YJ, Minami M, Nagata T, Ikejima T (2000). Effects of polysaccharides (FI0-b) from mycelium of Ganoderma tsugae on proinflammatory cytokine production by THP-1 cells and human PBMC (I). Acta Pharmacol Sin. 21(12): 1179-1185.

Hu YL (1997). Progress in the study of immunopharmacology of Hu YL, Chinese herbal medicine. Chin J. Immunol. 3: 96-98.

Kong XF, Li XR, Wang DY, Liu JG, Zhang BK, Wang XT (2004). Effects of ten Chinese herbal medicinal ingredients on proliferation and resisting NDV infection of CEF. Acta Veterinaria Et Zootechnica Sinica. 35(3): 301-305 .

Jiang MH, Zhu L, Jiang JG (2010). Immunoregulatory actions of polysaccharides from Chinese herbal medicine. Expert Opin Ther Target.14(12): 1367-1402.

Kong XF, Hu YL, Li XR, Wang DY, Zhang BK, Liu JG, Wang X (2004). Effects of nine Chinese herbal medicinal ingredients on serum HI antibody titer after vaccination of NDV Ⅳ strain vaccine in chicks. Acta Veterinaria Et Zootechnica Sinica. 35(4): 468-472 .

Kong XF, Hu YL, Rui R, Wang DY, Li XR (2004). Effects of Chinese herbal medicinal ingredients on peripheral lymphocyte proliferation and serum antibody titer after vaccination in chicken. Inter Immunopharmacol. 4(7): 975-982.

Kong XF, Hu YL, Yin YL, Wu GY, Rui R, Wang DY, Yang CB (2006). Chinese herbal ingredients are effective immune stimulators for Chickens infected with the Newcastle disease virus. Poult Sci. 85(12): 2169-2175.

Liu J, Henkel T (2002). Traditional Chinese medicine (TCM): are polyphenols and saponins the key ingredients triggering biological activities?. Curr Med Chem. 9(15): 1483-1485.

Liu JX, Wang CY, Bai YM, Fang YP (1998). Influence of Astragalus and other nine natural medicines on Newscastle disease antibody titer (ND-HI), coefficient of the immune organs and gains of chicks. Chin J. Vet. Sci Technol.12: 30-32.

Meng MB, Wen QL, Cui YL, She B, Zhang RM (2011). Meta-analysis: traditional Chinese medicine for improving immune response in patients with unresectable hepatocellular carcinoma after transcatheter arterial chemoembolization. Explore (NY). 7(1):37-43.

Perera PK, Li Y, Peng C, Fang W, Han C (2010). Immunomodulatory activity of a Chinese herbal drug Yi Shen Juan Bi in adjuvant arthritis. Indian J. Pharmacol. 42(2): 65-69.

Ung CY, Li H, Kong CY, Wang JF, Chen YZ (2007). Usefulness of traditionally defined herbal properties for distinguishing prescriptions of traditional Chinese medicine from non-prescription recipes. J. Ethnopharmacol. 3: 21-28.

Wang D, Hu Y, Sun J, Kong X, Zhang B, Liu J (2005). Comparative study on adjuvanticity of compound Chinese herbal medicinal ingredients. Vaccine. 23(28): 3704-3708.0

Wang FC, Fang CG, Zhang YH, Wang HB, Han YC (2006). Effects of Compound Chinese Herbal Immune Synergist on Immunity of Egg laying Chicken. Acta Veterinaria et Zoot echnica Sinica. 37(2): 187-192.

Xu YG, Ma R, Yang XH, Tang XD, Sun SZ (2010). Effect of Yiqi Bushen Granuleon the peripheral natural killer cell and γδ T-cell in the patients with minimal residual leukemia. Chin J. Integr. Med. 16(5): 417-421.

Xu Z, Chen X, Zhong Z, Chen L, Wang Y (2011). Ganoderma lucidum polysaccharides: immunomodulation and potential anti-tumor activities. Am. J. Chin Med. 39(1): 15-27.