Journal of Animal and Veterinary Advances 12 (5): 660-663, 2013
ISSN: 1680-5593

Effect of Chinese Medicine Effective Component Prescription of Chickens Immune Organ CD4+, CD8+ After IBDV Infected

Guisheng Gao, Qiumei Shi, Guangping Gao, Yanying Zhang,
Jianzhong Xu and Ping Shen
Hebei Key Laboratory of Preventive Veterinary,
Hebei Normal University of Science and Technology, Changli, Hebei, China

Abstract: To elucidate the pharmaceutical mechanism about the prevention and treatment effects of Zihuangsan, so as to provide theoretical basis on animal production. The 240, 1 day old chickens were acclimatized for 6 days and then randomly divided into groups A, B, C, D, E and F, 40 birds in each group. The birds in group A, B and C were administered with three dose such as high, middle and low of Chinese Medicine Prescription-Zihuangsan. The chickens in groups D, E and F were given with the same volume of Normal Saline (NS). The birds in groups A, B, C, D and E were infected with IBDV by eye drop, group F as normal control by using NS instead of IBDV and Zihuangsan. The 3 days after IBDV infected, 0.5 mL yolk antibody per bird in group D was given intramuscularly. Result show Zihuangsan could significantly reduce the chicken's mortality and increase the development of positive expression of CD4+, CD8+ T in immune organs such as thymus and intestinal. Zihuangsan could regulate the immune deficiency caused by IBDV. The best dosage of Zihuangsan is middle dose.

Key words: Chinese medicine effective component, IBDV, immunomodulator, CD4+, CD8+

INTRODUCTION

The main active constituents of Echinacea is polysaccharides glucoprotein and caffeic acid derivatives which can enhance the organism resistance to pathogen infection by stimulating the immune system. Polysaccharides and saponin of Mongolian Milkvetch Root which are considered to be in two compounds with biological activity can stimulate the immune and antiviral role. Chicken Infectious Bursal Disease (IBD) which caused by virus is an acute infectious disease and hazards in young chickens (Nique, 1999). IBDV can make B cells degeneration and necrosis that carry SIgM sign (Hirai *et al*., 1981) and lead to serious long-term immunosuppression (Sharma *et al*., 2000).

CD4 and CD8 molecules as the auxiliary receptors of the TCR participate in antigen recognition of presentation on MHC molecules. CD4+T can recognize antigen by MHCII type molecules presentation (Zhou, 2007), the number of CD4 T to some extent reflects B cell proliferation and activation degree. But the adhesion function of molecular CD8 is to combine α3 structural domain on MHCI molecules. This combination stabilizes Cytotoxic T cells (CTL) and presenting antigen of MHC class I molecule attachment of target cells, to play the role of the damage to the target cells. Clearly, the CD4 and CD8 molecules are closely related with chicken's immune defenses.

Chinese Medicines Section Zihuangsan, researchers designed be used to artificially infected with IBDV chicks that based on active ingredients of traditional Chinese medicine. By determining the number of immune organs such as chickens in the thymus, small intestinal CD4+, CD8+ change can reflect Zihuangsan on the treatment of chickens infected with IBDV, so researchers can elucidate the pharmaceutical mechanism about the prevention and treatment effects of Zihuangsan and provide theoretical basis on animal production.

MATERIALS AND METHODS

Medicines and reagents: Echinacea extract (TYE080718): cichoric acid = 2.0%, polyphenol = 4.0%; Mongolian Milkvetch root extract (TYA080531): Astragaloside = 10%, astragalin 50%. Shipped from Xian Tianyi bio-technology limited company; Echinacea: These extracts proportionally misce bene, distilled water dissolve into high (90 mg mL kg^{-1}), medium (45 mg mL kg^{-1}), low (4.5 mg mL kg^{-1}) dose.

Corresponding Author: Qiumei Shi, Hebei Key Laboratory of Preventive Veterinary,
Hebei Normal University of Science and Technology, Changli, Hebei, China

IBDV (AV7-BC6/85), IBDantigens, antibody was purchased from China Veterinary Drugs Monitor, SPF 1day fertilized eggs, bought from Beijing Meiliyawei laboratory animal technology limited company; IBDantibody (Veterinary drugs words (2006) 160022064), Luoyang pulaike bio-engineering limited company; Rat anti-chicken CD4+ and CD8+ monoclonal antibody, Southern Biotechnology Inc product; SP-9002 Immunohistochemistry kits, Beijing Zhongshan Golden Bridge bio-technology limited company; DAB Coloration kit, Wuhan boshide bio-engineering limited company.

Main instrumenttation: YD-1900 type freezing microtome, Motic 3.0 Pathologic Image Analysis System.

IBD pathological model checking: The 240, 1 day old chickens were acclimatized for 6 days and then randomly divided into groups A, B, C, D, E and F. The 4 repeat for every group, repeat 10 chickens each, for a trial period of 7 weeks. The chickens in group A, B and C were administered with three dose such as high, middle and low of Chinese Medicine Prescription-Zihuangsan. The chickens in groups D, E and F were given with the same volume of Normal Saline (NS). The birds in groups A, B, C, D and E were infected with IBDV by eye drop, group F as normal control by using NS instead of IBDV and Zihuangsan. The 3 days after IBDV infected, 0.5 mL yolk antibody per bird in group D was given intramuscularly. After the conteracting toxic substances, researchers observe clinical symptomse of the conteracting toxic substances group chickens. After 72 h, randomly selected groups of chickens, autopsy, observe pathological changes of pathological changes in of bursal lesion and pectoralis muscles, leg muscle bleed as the standard and statistics mortality rate.

Determination the number of CD4+ and CD8+T cells in the thymus, intestine: The 120 chickens of 14 days is divided into three groups A, B and C. The chickens in group A were administered with a middle dose of Chinese Medicine Prescription-Zihuangsan. The chickens in group A and B were administered with physiological saline, Continuing 7 days and group C isolation rearing. The 21 days the chickens in group A and B were conteracted toxic substances 0.1 mL. On the 24, 28, 35, 42 days old Harveste chicks thymus and intestinal, prepare frozen section, immunohistochemical staining, observe the distribution of slices in the CD4+, CD8+T. Each slice random extract of 5 visual fields and use Motic 3.0 pathologic image analysis system CD4+, CD8+ positive cells factor, area equation (Hu *et al*., 2007):

$$\text{Positive cell factor, area (\%)} = \frac{\text{Positive cell area}}{\text{Total field of view area}}$$

Consequence

Bursa of Fabricius pathological model checking: The 21 days old chicks infected with IBDV after 72 h you can check out chicken body of IBDV for immuno electrophoretic (Fig. 1a); infected with IBDV of the dead chicken leg muscle bleed, bursa 2~3 times more than normal, bleeding spots sporadically (Fig. 1b); control groups of chicken bursa no lesion (Fig. 1c).

Zihuangsan on impact of IBD morbidity and mortality in chickens: Mortality of the drugs group A (6.67%), B (4.44%), C (4.44%) and D (6.67%) very patency levels below E (22.22%), $p<0.01$. D group of egg-yolk antibodies and drugs group A difference not patency ($p>0.05$) but group B and C mortality rate is smaller than group D, patency difference ($p<0.05$).

CD4+, CD8+T positive cell factor, area changes in the thymus: CD4+ and CD8+T morphological characteristics: toroid, brown-yellow dye. The group of Zihuangsan (Fig. 2a and b). Positive cells patency more conteracting toxic substances group (Fig. 2c and d). The chickens in group A number CD4+ and CD8+T cells in the thymus compared with the group of B and C, CD4+T number of significantly more than the control group, patency difference ($p<0.05$), CD8+T number compared with the other two groups, there are patency differences ($p<0.05$) (Table 1, Fig. 2a-d).

Positive cell factor, area of CD4+, CD8+T in the small intestine: In the intestinal mucosa, CD4+T are mainly

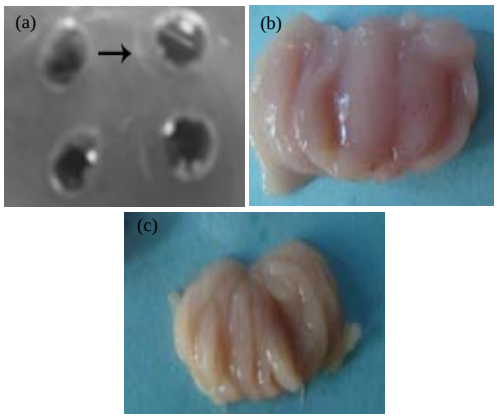

Fig. 1: a) CIE test IBDV of the infection group, the marker was the CIE precipitation lines; b) the infection of bursal; c) the normal bursal infection group, the marker was the CIE precipitation lines

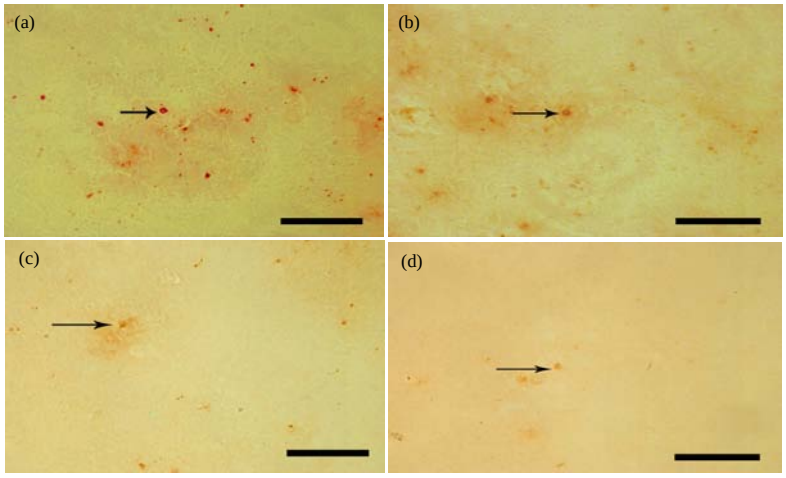

Fig. 2: a, b) CD4+ CD8+ in thymus of Zihuangsan (24 days); c, d) E2:CD4+ CD8+ in thymus of IBDV-infected (24 days); the bar shows 50 μm

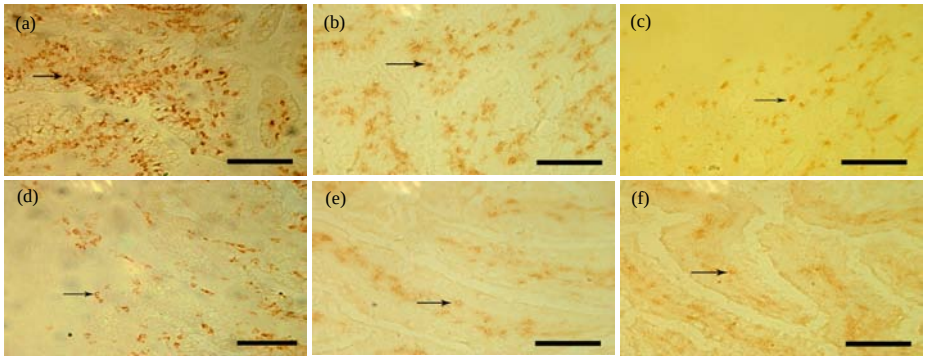

Fig. 3: a, b) CD4+ CD8+ in small intestine of Zihuangsan (24 days); c, d) B2:CD4+ CD8+ in small intestine of IBDV-infected (24 days); e, f) CD4+ CD8+ in small intestine of no treatment (24 days); the bar shows 50 μm

Table 1: CD4+ and CD8+ in thymus ($\bar{X}$ + SD)

Items	Groups	24 days	28 days	35 days	42 days
CD4+	A	63.79±7.74^{a}	70.53±6.87	70.94±7.30	73.27±6.77^{a}
	B	58.44±4.35^{b}	69.17±4.56	70.14±5.02	69.60±4.80^{b}
	C	61.27±4.34^{a}	68.08±3.98	68.32±4.00	70.31±4.26^{b}
CD8+	A	59.88±6.54^{a}	69.08±6.09	65.86±6.78	67.53±5.74
	B	54.99±3.58^{b}	68.57±4.72	67.09±4.35	68.08±4.48
	C	58.10±3.65^{b}	67.17±4.10	66.49±4.23	67.59±4.51

Values with different lowercase and capital letters in the same column are significantly different at 0.05 and 0.01 levels

Table 2: CD4+ and CD8+ in small intestine ($\bar{X}$ + SD)

Items	Groups	24 days	28 days	35 days	42 days
CD4+	A	43.03±5.97^{a}	40.63±6.75^{a}	47.76±4.84^{a}	57.81±7.01
	B	35.55±4.58^{b}	47.34±4.35^{b}	48.71±3.03^{a}	57.08±4.88
	C	36.22±3.25^{b}	40.07±4.39^{a}	50.26±3.99^{b}	58.65±2.68
CD8+	A	35.10±4.56^{a}	39.07±3.17	47.48±3.54	54.02±4.22^{a}
	B	30.00±3.29^{b}	39.06±4.19	48.07±4.46	48.38±4.19^{b}
	C	34.49±4.50^{a}	39.14±3.78	47.20±3.09	55.07±3.91^{a}

Values with different lowercase and capital letters in the same column are significantly different at 0.05 and 0.01 levels

located in the lamina propria and CD8+T are mainly located in the intestinal epithelium and the lamina propria. From the 24-42 days old, the number CD4+ and CD8+T within each group of chicken small intestine there is an increasing trend. From Fig. 3c and d by IBDV infection, the marked decline in group B T cells in the intestinal mucosa which CD8+ less than the number of CD4+ number. After 24 days old blocks vaccinated IBDV 3 days, group A CD4+, CD8+T number in chicken small intestine compared with control group B patency differences. But 28 days old chicken group B CD4 T increased significantly compared with other groups while CD8 and there is no significant increase. The age from 35-42 days old, CD4+T, CD8+T number of differences between the groups are shrinking (Table 2, Fig. 3a-f).

DISCUSSION

Research has shown that CD4 molecule express in about 70% of thymocyte cell, 15% spleen cell and 40%

periphery lymphocytes but in bursal cells of expression is <1% (Chan *et al*., 1988); the CD8 molecule of chicken express in about 80% thymocytes cell, 50% spleen cell 15% periphery lymphocytes and express in bone marrow and bursa cells is <1% (Tregaskes *et al*., 1995). But there has also been reported that in bursa in chickens was found in a <10% of T-lymphocytes and can be transferred and will not translate into no mark of T-lymphocyte cells (Gobel *et al*., 2001).

CONCLUSION

The test results display that when the Zihuangsan is applied, CD4+T and CD8+T lymphocytes positive cell factor, area of thymus, intestine epithelium lamina propria patency increase ($p<0.05$). That is, patency increase in the number CD4+T, CD8+T. T-B intercellular interactions, CD4+T maturation differentiation Th2 cell provides for the B lymphocyte activation and differentiation of the second signal, to further promote proliferation and activation of B-lymphocytes. CD8+T lymphocyte cell is effector cell about immune reaction. The number of CD8+T lymphocytes' increasing indicates that chickens IBDV may be to drive the CD8T cell activation and differentiation antigen and activation of CD8+T can kill marked with specific antigen of target cell. To some extent, so Zihuangsan may reduce or block the proliferation of IBDV in the body. When the Zihuangsan is applied, the number of CD4+T increase in IBD chicken, promote the proliferation and activation of B-lymphocytes and within a certain range, humoral immunity recover. The increasing of CD8+T make cell-mediated immunity to further strengthen. Zihuangsan on immune regulation of IBD in chicken specific functions process has yet to be further studied, confirms.

ACKNOWLEDGEMENTS

This research was supported by Ministry of Science and Technology in China 2012GB2A200045, Postdoctorate Foundation of China No.: 20100470565, Technology support project of Hebei Province in China No.: 12220408D, 1282041D, Technology support project of Shijiazhuang City in Hebei Province No.: 08150132A and Scientific Research Innovation Team of Hebei Normal University of Science and Technology No.: CXTD201201. This research is supported by the colleges and universities of Hebei Province Science and Technology Research Program (ZH2011244).

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