

Full Length Research Paper

Study of malondialdehyde (MDA) content, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in chickens infected with avian infectious bronchitis virus

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This study was carried out to investigate the changes of serum oxidative index in chickens infected with avian infectious bronchitis virus. Eighty (80) 15-day-old chickens were divided into two groups randomly: the control group and the experimental group. Birds in the experimental group were inoculated with infectious bronchitis virus, then the superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities and malondialdehyde (MDA) contents in the serum were detected in each group at days 1, 3, 6, 9, 12 post infection, respectively. Results showed that SOD and GSH-Px activities in the serum of the experiment group decreased after infection ($P < 0.05$ or $P < 0.01$). MDA contents of the experiment group increased after infection, and was significantly higher than those of the control group at days 6 and 9 post infection ($P < 0.01$). The results implied that oxidative damage may regulate the occurrence and development of avian infectious bronchitis.

Key words: Avian infectious bronchitis, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA).

INTRODUCTION

Free radical (FR) refers to the unpaired electronic atoms or atomic group, molecules or ions (Aydin et al., 2006). In the field of biology and medicine, many scholars have conducted extensive and in-depth study on the radical medical science which broadens people's horizons, enabling them to have a new awareness of many illnesses and their causes, the pathogenesis and the physiological or pathological processes and providing a new basis for the treatment of some diseases. So far, almost all the phenomena of life and pathological processes, such as aging, shock, inflammation, auto-

immune diseases, stress and cancer, have been studied from the perspectives of FR (Riccioni and Dorazio, 2005; Sasaki and Joh, 2007; Pérez-Fuentes et al., 2008).

Under disease conditions, oxygen free radical is the most lively and damaging FR which is produced through enzyme and non-enzyme system. It may promote lipid peroxidation of polyunsaturated fatty acids (PUFA) of biomembranes, leading to formation of lipid peroxides such as malondialdehyde (MDA) and hydroxyl, therefore resulting in tissue or cells damage (Ostalowska et al., 2006). The elimination of FR is dependent on the preventive or interrupted regulations of antioxidant defense system (Valdez et al., 2000). Superoxide dismutase (SOD) serves as an important member of the antioxidant system, which removes superoxides and protects cells from damage. Glutathione peroxidase (GSH-Px) protects the structural integrity of cell membrane and function (Drabko et al., 2006). The changes of MDA content, SOD and GSH-Px activity not only reflect the ability of scavenging oxygen FR, but also indirectly reflect the

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Abbreviations: MDA, Malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; FR, free radical; IB, infectious bronchitis; IBV, infectious bronchitis virus.

extents of cell membrane damage attacked by FR (Surapaneni and Venkataramana, 2007; Yuan et al., 2008). Information shows that there is a significant change of FR in viral diseases (He et al., 2007; Zhao et al., 2003). Avian infectious bronchitis (IB) is an acute, highly contagious and primary respiratory infection in chickens (Cavanagh, 2007; Cavanagh and Naqi, 2003) caused by infectious bronchitis virus (IBV), a member of the family Coronaviridae and genus Coronavirus (Ziebuhr et al., 2000). In China, IB is still a major problem and is economically important to the poultry industry due to the high morbidity and production losses associated with the disease (Liu and Kong, 2004; Liu et al., 2006). So far, studies have focused on etiology, epidemiology, diagnosis and control with IB, which is seldom involved in the oxidant-antioxidant status in chickens in the course of the disease. Therefore, detection of SOD, GSH-Px activity and MDA content is done to investigate the possible mechanisms of oxidant stress during IBV infection and their relationship, to provide a scientific basis on the prevention and control of IBV.

MATERIALS AND METHODS

IBV M41 strain was purchased from China Institute of Veterinary Drug Control (Beijing, China). One-day-old Roman male broilers, purchased from JingYu Chicken Farms (Shijiazhuang, China), were housed in climate-controlled rooms at $36 \pm 1^\circ\text{C}$ and kept under 24-h light at the beginning of the pre-trial period. The temperature was gradually reduced to room temperature in the spring and the photoperiod was reduced to 12 h per day, where it was kept constant over the following days. The birds were fed with a commercial starter diet provided by the LIDE Feed Factory of Hebei Province (Baoding, China). All chickens were kept unvaccinated against IBV and reared to 15-day-old for the experiment. SOD, GSH-Px and MDA assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Experimental design and animal treatment

Eighty (80) 15-day-old broilers were randomly divided into two groups of 40 each: The control group and experimental group. Birds in the experimental group were inoculated intranasally with 0.3 ml inoculum containing $10^{6.25}$ EID₅₀/0.1 ml of IBV-M41, respectively. Blood was collected at days 1, 3, 6, 9 and 12 post infection with six chicks from each group randomly, then centrifuged at 3,000 g for 10 min. Serum was stored at -20°C until analysis.

SOD and GSH-Px analysis

Total superoxide dismutase (T-SOD) activity in the serum was determined through xanthine oxidase method. Samples were taken to detect absorbance at 550 nm with a spectrophotometer. The calculated results were expressed by U/ml nitrite unit. GSH-Px activity was measured by dithio-dinitrobenzoic acid method at the absorbance of 412 nm and results were expressed by $\mu\text{mol/L}$ protein. MDA content was measured using thiobarbituric acid (TBA) method at absorbance of 532 nm; results were expressed by nmol/ml protein. Methods and procedures were performed with

assay kits according to the manufacturer's instructions, respectively.

Statistical analysis

All statistical analyses were performed with SPSS 13.0 statistical software, evaluated with one-way ANOVA. Data were expressed as mean \pm SD, $p < 0.05$ was taken as significant, $p < 0.01$ was taken as extremely significant.

RESULTS

Incidence of disease in chickens

Disease was severe in chickens at 48 h post-infection and then spread to the entire groups rapidly. Chicks with IBV infection were not as active as before infection, with clinical signs such as, difficulty in breathing, lethargy, rales, coughing or sneezing with or without nasal discharge. At necropsy, the mucous membrane of trachea or bronchi was inflamed and swelled with various quantity of serous, mucus or cheese-like leakage secretion. The IBV infection was also reflected in depression of growth. No abnormalities were observed in bursa of Fabricius and other organs. Birds in the control group were clinically healthy.

The clinical signs in the experimental group was more significant than in control group ($P < 0.05$), and was obvious in the aspect of coughing or sneezing, lethargy or rales. Morbidity and mortality in the experimental group were significantly higher than the control group ($P < 0.01$) (Table 1).

SOD activities in the serum

SOD activities in the experimental group were lower than that of the control group and there were significant differences at day 6 post infection ($P < 0.01$). At day 9 post infection, the downward trend of serum SOD activity was ceased. Serum SOD activities in the experimental group was lower than those in the control group during the entire trial period (Table 2).

GSH-Px activities in the serum

Serum GSH-Px activities of the experimental group started to decrease after infection. From day 9 onwards, the downward trend of serum GSH-Px activity ceased, but still showed significant differences when compared with the control group ($P < 0.05$). Serum GSH-Px activities in the experimental group was lower than the control group, and showed significant differences at 3, 6, 9 days post infection when compared with control group ($P < 0.01$) (Table 3).

Table 1. The clinical signs of the chickens in each group.

Group	Number	The number and percentage of sign				Morbidity	Mortality
		Coughing or sneezing	Rales	Difficulty in breathing	Lassitude		
Control group	40	0	0	0	2(5%)	5%	0
Experimental group	40	32 (80%)**	16 (40%)**	12 (30%)	34 (85%)**	38 (95%)**	17 (42.5%)**

* P<0.05, ** P<0.01 when compared with control group.

Table 2. The results of SOD activities in serum of chickens (mean±SD).

Group	Number	SOD activity in different day post-infection (U/ml)				
		1	3	6	9	12
Control group	6	280.44±23.31	281.27±12.26	284.85±11.03	291.77±8.56	298.95±9.66
Experimental group	6	276.57±30.81	247.84±22.84**	246.45±17.89**	266.90±35.19	267.18±13.18**

*P<0.05, **P<0.01 when compared with control group.

Table 3. The results of GSH-Px activities in serum of chickens (unit: $\mu\text{mol/L}$).

Group	Number	Day of post-infection				
		1	3	6	9	12
Control group	6	1154.62±27.61	1323.53±96.50	1358.82±20.79	1365.13±21.14	1397.90±37.36
Experimental group	6	1145.80±72.64	1120.58±83.09**	990.76±63.58**	976.89±47.19**	1156.14±35.21**

** P<0.01 when compared with control group.

MDA content in serum

Serum MDA contents in the experimental group increased and peaked at day 6. After that the rising trend slowed down. Serum MDA contents in the experimental group went consistently higher than those in the control group, and showed significant differences at days 6 and 9 post infection. After the resistance (12 days post infection), serum MDA content in the experimental group was still higher, but the difference was not significant ($P>0.05$) (Table 4).

DISCUSSION

Under normal physiological conditions, there maintains a dynamic balance of the formation, usage and removal of FR, and its elimination depends on various antioxidant enzymes. GSH-Px and SOD are the major antioxidant enzymes which can extremely eliminate FR and have anti-oxidative stress function, and they not only prevent the damage of oxygen free radicals, but also have an inter-protective effect, therefore maintaining a healthy balance between oxidants and antioxidants (Aguilar et al., 2007). MDA is the direct products of lipid peroxidation

formed after attacks by radicals on cell membrane of unsaturated fatty acids. MDA content serves as an indicator of the extent of lipid peroxidation and an indirect reflection of the extent of cell damage. MDA may destruct cell membrane structure, cause DNA fragmentation, rearrangement, cross-linking and accelerate apoptosis, and is one of the most important elements of phlegmonosis or tumorigenesis (Surapaneni and Venkataramana, 2007). Therefore, determination of MDA, SOD and GSH-Px can reflect the level of metabolism of oxygen free radicals and the extent of tissue injury during IBV infection. Based on the information contained in this study, we found that antioxidant system changed significantly after IBV infection. Serum SOD and GSH-Px activities in the experimental group were significantly lower than those of the control group. MDA content was significantly increased. The results are consistent with other findings (Guo et al., 2000; Li et al., 1999) and suggest that abnormal changes of cell stable mechanism of oxidation and anti-oxidation occurs in chickens after IBV infection, resulting in increased generation of oxygen free radicals and excessive oxidative damage, which aggravates the cell damage. It is shown that oxygen free radical plays an important role in IBV injury of chickens.

Studies (Giommarelli et al., 2008; Karageuzyan, 2005)

Table 4. The results of MDA contents in serum of chickens (unit: n mol/ml).

Group	Number	Day of post-infection				
		1	3	6	9	12
Control group	6	12.76±0.23	12.39±0.32	11.99±0.24	11.92±0.25	10.79±1.12
Experimental group	6	13.03±0.28	13.26±0.54*	15.26±0.23**	13.78±0.31**	11.48±0.27

*P < 0.05, **P < 0.01 when compared with control group.

have shown that integrity cell and deuto-cell membrane were prone to degradation by FR and its metabolites, and that these processes can be due to the modified protein structure and sensitivity enhancement on protein hydrolysis system within the cells. Goldstein and Merényi (2008) reported that OH changed the primary structure of protein (role of titanium chain of amino acids, etc.), thereby leading to modification of secondary structure or tertiary structure of protein and causing protein peptide chain expansion with the formation of random conformation. This modification exposes original hidden peptide bond and hydrolysis by proteolytic enzyme. Pieri et al. (2003) further pointed out that proline (Pro) and histidine (His) was the major locus of protein fragmentation, during this reaction, Pro and His residues may transform into N-terminal of glutamate (Glu) and aspartate (Asp), and such instability of N-terminal, which was sensitive to aminoacyl tRNA transferase, would transform into the terminal of lysine (Lys) and arginine (Arg), and the ubiquinone degradation system relying on ATP were sensitive to the products and could accelerate its catabolism.

Radicals not only destroy the cell membrane structure, but also attack DNA, making its fracturing, rearrangement and cross-linked one of the important aspect of inflammatory or tumorigenesis, and speeding up normal apoptosis (Djordjevic, 2004). The determination of SOD, GSH-Px activity and MDA content in serum in IBV chickens suggested that a large number of oxygen free radicals accumulate when an imbalance occurs between FR production and detoxification. Such an imbalance decreases the SOD, GSH-Px activities and increases the levels of MDA, and causes a low energy state of anti-oxidation system, then make more lipid peroxidation to be produced than can be processed by the antioxidant defense systems and thus, generate the end product of lipid peroxidation of MDA. Lipid peroxidation not only transforms active oxygen into activity chemical agents, but also creates new radicals and enlarges the role of reactive oxygen through chain or branched-chain reaction, and constantly results in cell membrane and mitochondrial membrane damage, biomembrane degeneration and cellular structure destructure or cell dysfunction; leads to pathological injury and the incidence of diseases in chickens. This study shows that increasing the antioxidant enzyme activity is probably one of the ways for resistance of injury process caused by IBV infection.

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