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Effects on Oxidative Stress and Antioxidant Enzyme Activities of Experimentally Induced *Ornithobacterium rhinotracheale* Infection in Broilers

[1]Fulya Benzer and [2]Seval Yilmaz
[1]Veterinary Control and Research Institute, Elazig, Turkey
[2]Department of Biochemistry, Faculty of Veterinary Science, University of Firat, Elazig, Turkey

Abstract: This experiment was conducted to evaluate the effects of *Ornithobacterium rhinotracheale* (ORT) infection on oxidative stress and the activity of antioxidant enzymes in blood, trachea and lung tissues of broilers. Broiler chickens were divided into 2 groups, each consisting of 30 animals. Group 1 was used as control group; Group 2 was used as infection group. The 2nd group was infected by aerosol route with 1 mL of a pure culture of ORT strain B3263/91 (serotype A) containing 3.8×10^8 CFU mL^{-1}. At the end of the experiment blood and tissue samples were collected and biochemical parameters were determined. While, plasma Malondialdehyde (MDA) and Nitric Oxide (NO) levels were increasing at infection group than control group, erythrocyte Catalase (CAT) and Glutathione Peroxidase (GSH-Px) activities were decreasing in accordance with control group. Levels of MDA and NO in trachea tissue increased at infection group more than control group; but activities of CAT and GSH-Px did not change. MDA level and CAT activity increased in lung tissue, however level of NO decreased more than the control group. GSH-Px activity had no change statistically. There are alterations in wide array of oxidants and antioxidants, with balance shifting toward increased oxidative stress in ORT infection. Therapeutic augmentation of the antioxidant defenses might be beneficial.

Key words: *Ornithobacterium rhinotracheale*, broilers, lipid peroxidation, antioxidant, nitric oxide

INTRODUCTION

Ornithobacterium rhinotracheale is a gram-negative bacterium associated with contagious respiratory disease in poultry (Glisson, 1998; Van Empel and Hafez, 1999). Because of the economic losses due to decreased growth, increased mortality, increased condemnation rates, drops in egg production and decreased hatchability, ORT infections are increasingly recognized as a health problem in the poultry industry (Van Veen *et al*., 2000).

Lipopolisaccharide (LPS) is a component of the Gram negative bacterial cell wall that triggers the synthesis and release of cytokines and Nitric Oxide (NO). LPS also, triggers the synthesis of ROS, such as superoxide, in the lung (Zhang *et al*., 2000).

It is known that the airway epithelium contains non-enzymatic low molecular weight antioxidants (Kelly *et al*., 1999), high molecular weight antioxidant enzymes (Kozar *et al*., 2000) and putative unidentified antioxidant system (Cohn *et al*., 1994). In the normal lung, the equilibrium between antioxidants and oxidants is sufficient to maintain the fluids that cover the surface of the airways and fill the extracellular spaces in a highly reduced state. An increase in the concentration of oxidants or a reduction or excessive consumption of antioxidants leads to the loss of this equilibrium in a situation referred to as oxidative stress. In response to stimuli associated with bacterial infection, especially the production of LPSs, macrophages and endothelial cells are activated and express adhesion molecules on their surface, facilitating transmigration of neutrophils from the blood vessels into the alveoli or the airways in general (Vass *et al*., 2003). Activated neutrophils produce a large number of oxidants that usually fall into 2 categories: ROS and Reactive Nitrogen Species (RNS) (Crapo, 2003).

It has been proposed that ROS and RNS, both formed in infected and inflamed tissues, play roles in oxidative stress, which cause damage on biomolecules such as lipids, proteins and nucleic acids, each by different mechanism. NO, potentially toxic gas with free radical properties, is generated from L-arginine by constitutive or inducible Nitric Oxide Synthase (NOS). It is important to note that NO is a modulator of cellular function and can act either as a cellular messenger or, when produced in excess quantities, as pro-oxidant, inducing oxidative stress (Nadeem *et al*., 2003). NO is a free radical that plays an important role in the pathophysiology of pulmonary inflammation. The observation that systemic production

Corresponding Author: Fulya Benzer, Veterinary Control and Research Institute, Elazig, Turkey

of NO is increased in the presence of bacterial infection (Wheeler *et al*., 1997) suggests that NO is a marker of local inflammation in pulmonary infections.

In recent years, numerous studies reported a close relationship between the increased ROS production and incidence or development of pathological processes in the airways, such as bronchial hyperactivity, asthma, respiratory distress syndromes, etc. (Lykens *et al*., 1992; Dohlman *et al*., 1993; Metnitz *et al*., 1999; Quinlan *et al*., 1997; Vachier *et al*., 1994; Plaza *et al*., 1995). The present study, was aimed to investigate the effect of ORT infection on oxidative stress and the activity of antioxidant enzymes in blood, trachea and lung tissues of broilers. In the present study, ORT-induced oxidative stress was assessed by measuring changes in LPO and NO. The status of antioxidant system was assessed by measuring the activities of CAT and GSH-Px.

MATERIALS AND METHODS

Experimental animals: All animal studies were approved by the committee for animal experiments of the Elazig Veterinary Control and Research Institute, according to international regulations. Broiler chickens were divided into 2 groups, each consisting of 30 animals. Group 1 was used as control group; group 2 was used as infection group. The 2nd group was infected by aerosol route with 1 mL of a pure culture of ORT strain B3263/91 (serotype A) containing 3.8×10^8 CFU mL^{-1} (Van Empel *et al*., 1996). Postmortem examination was performed 7 days after challenge. During aerosol challenge, the bacterial culture was administered as a fine spray to the chickens in an isolator, using a commercial paint sprayer; the developed mist was maintained for at least 10 min without air circulation.

Nitric oxide assay: NO measurement is very difficult in biological specimens, because it is easily oxidized to Nitrite (NO_2) and subsequently to Nitrate (NO_3), which serve as index parameters of NO production. Samples were initially deproteinized with NaOH and $ZnSO_4$. Total nitrite ($NO_2 + NO_3$) was measured by spectrophotometer at 545 nm after conversion of NO_2 to NO_3 by assay reactive (Lyall *et al*., 1995). A standard curve was established by a set of serial dilutions of sodium nitrite. Results were expressed as μmol L^{-1} and μmol g^{-1} tissue.

MDA assay: Plasma MDA level was estimated according to the method of Yagi (1984), tissue MDA level was estimated according to the method of Ohkawa *et al*. (1979), which is based on the coupling of MDA with Thiobarbituric Acid (TBA). The organic layer was taken and resulting pink stained TBA-RS was determined in a spectrophotometer at 535 nm. Calibration curve was performed using 1, 1, 3, 3-tetramethoxypropane. The results are reported as nmol mL^{-1} and nmol g^{-1} tissue.

CAT activity assay: The eryrocyte and tissue CAT activities were measured according to the method of Aebi (1984). The principle of the assay is based on the determination of the rate constant, k (rate constant) of hydrogen peroxide decomposition. By measuring the absorbance changes per 30 sec, the rate constant of the enzyme was determined. Activities were expressed as k g^{-1} hemoglobin and k g^{-1} protein.

GSH-Px activity assay: GSH-Px activity was measured by the method of Beutler (1975). Briefly, in the presence of glutathione reductase and Nicotinamid Adenin Dinucleotid Phosphate (NADPH), oxidized glutathione (GSSG) is immediately converted to its reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured. GSH-Px activity was expressed as U g^{-1} hemoglobin and U mg^{-1} protein.

Hemoglobin concentration of erythrocyte lysate was measured by Drabkin's reagent (Tietz, 1986). Protein was measured by the method of Lowry *et al*. (1951) using bovine serum albumin as standard. All results were expressed as the mean± the Standard Error of the Mean (SEM). Statistical analysis was done by t-test, $p<0.05$ were considered significantly different.

RESULTS

As shown in Table 1, while plasma MDA and nitrite levels were increasing at control group than infection group ($p<0.05$), erythrocyte CAT and GSH-Px activities were decreasing in accordance with control group ($p<0.05$). Levels of MDA and NO in trachea tissue increased at infection group more than control group ($p<0.05$); but levels of CAT and GSH-Px did not change ($p>0.05$) (Table 2). MDA level and CAT activity increased in lung tissue ($p<0.05$), however level of NO decreased more than the control group ($p<0.05$). GSH-Px activity had no change statistically ($p>0.05$) (Table 3).

Table 1: MDA, nitrit levels of plasma and CAT and GSH-Px activities of erythrocyte of broilers infected with ORT

Parameters	Control (n = 30)	ORT (n = 30)	p-value
MDA (nmol mL^{-1})	3.06±0.200	4.61±0.510	*
CAT (k g^{-1} Hb)	0.046±0.01	0.029±0.03	*
GSH-Px (U g^{-1} Hb)	3.35±0.230	1.99±0.160	*
Nitrite (μmol L^{-1})	36.85±0.930	40.65±1.100	*

The values represent mean±SD; *Significance was defined as $p<0.05$; - Significance was defined as $p>0.05$

Table 2: MDA, nitrit levels and CAT and GSH-Px activities of trachea tissue of broilers infected with ORT

Parameters	Control (n = 30)	ORT (n = 30)	p-value
MDA (nmol g^{-1} tissue)	0.39±0.04	0.57±0.06	*
CAT (k g^{-1} protein)	11.13±0.69	13.09±0.66	-
GSH-Px (U mg^{-1} protein)	1.05±0.11	1.13±0.13	-
Nitrite (μmol g^{-1} tissue)	190.35±4.20	206.2±4.530	*

The values represent mean±SD; *Significance was defined as $p<0.05$; - Significance was defined as $p>0.05$

Table 3: MDA, nitrit levels and CAT and GSH-Px activities of lung tissue of broilers infected with ORT

Parameters	Control (n = 30)	ORT (n = 30)	p-value
MDA (nmol g^{-1} tissue)	1.40±0.06	1.98±0.10	*
CAT (k g^{-1} protein)	4.28±0.23	6.08±0.31	*
GSH-Px (U mg^{-1} protein)	0.37±0.04	0.44±0.03	-
Nitrite (μmol g^{-1} tissue)	171.76±1.87	158.57±3.73	*

The values represent mean±SD; *Significance was defined as $p<0.05$; - Significance was defined as $p>0.05$

DISCUSSION

In poultry, ORT has been identified as a newly emerging respiratory bacterial pathogen that has caused significant economic losses to the poultry industry (Glisson, 1998). The role of ROS in inducing injury to the lung and other tissues, as a result of infection-induced inflammatory response, has been reported by the other investigators who showed an increased oxidative stress in patients or experimental animals with pneumonia.

Oxidative stress induces the production of highly ROS that are toxic to the cell, particularly the cell membrane, in which these radicals interact with the lipid bilayer and produce lipid peroxides (Kesavulu *et al*., 2001). However, endogenous antioxidant enzymes (SOD, CAT, GSH-Px) are responsible for the detoxification of the deleterious oxygen species (Levy *et al*., 2000). In the cytoplasm, less H_2O_2 is decomposed by GSH-Px than by CAT. CAT is present in peroxisomes and decomposes high concentrations of H_2O_2 (Fujita, 2003).

In the present study, we have shown that broilers infected with ORT have increased oxidative stress, as shown by increased LPO in plasma, trachea and lung tissue. This is accompanied by alterations in plasma and trachea, including increased nitrit levels.

Bacterial LPS or other bacterial by-products have been used to examine the oxidant-antioxidant status of lungs and other organs (Goode and Webstar, 1993; White and Repine, 1985; Goode *et al*., 1995), but we have not found any study on the effects ORT infection on oxidative stress, therefore, we discussed our results with some other bacterial infections such as tuberculosis, asthma, pneumonia. It has been established that tissue injury via oxidative stress-mediated mechanisms occurs only when there is imbalance between the oxidant-antioxidant status (Kinnula *et al*., 1995). The role of ROS in inducing injury to the lung and other tissues, as a result of an infection-induced inflammatory response, has been reported by other investigators who showed an increased oxidative stress in patients or experimental animals with pneumonia (Choi *et al*., 1996; Nowak *et al*., 1996).

Akdogan *et al*. (1999) reported lower erythrocyte SOD and GSH-Px activities in patients with bronchial asthma than controls. They also found lower levels of Glutathione Reductase (GR), CAT and melatonin in patient group but this decline was not statistically significant. There are some studies indicating lower erythrocyte GSH-Px activity in children with bronchial asthma (Denis *et al*., 1993; Powell *et al*., 1994). In another study, Liao *et al*. (2004) found that level of serum reactive oxygen species were higher but level of serum total antioxidant activity were lower in asthmatic children compared to controls.

It was reported by some investigators (Kwiatkowska *et al*., 1999; Lamsal *et al*., 2007) had found higher serum MDA and nitrite levels and lower antioxidants enzyme activities in patients with lung tuberculosis compared to controls. In our study, we found that plasma MDA and nitrite levels were higher and erythrocyte GSH-Px and CAT enzyme activities were lower in infected with ORT compared to controls. GSH-Px and CAT decompose H_2O_2 and inhibit the formation of. OH. Nordstrom *et al*. (1985) and Gupta *et al*. (2004) reported that preventing the formation of. OH protects cells from damage caused by oxidative stress. The reduction in CAT and GSH-Px activities in erythrocytes may be, in part, due to inactivation of these enzymes by high ROS concentrations. Our results are in accordance with other studies in literature (Kwiatkowska *et al*., 1999; Lamsal *et al*., 2007; Akdogan *et al*., 1999; Denis *et al*., 1993; Powel *et al*., 1994; Liao *et al*., 2004).

Frank *et al*. (1996) reported that even minute doses of endotoxin increased lung tissue antioxidants, including CAT, several days after endotoxin exposure in rats. In our study, we found that lung CAT enzyme activity was higher in infected with ORT compared to controls. Our result, is in accordance with Frank *et al*. (1996). The increase in the MDA level in spite of the increased CAT enzyme activity could have been due to the over production of ROSs that exceeded the capacity of the antioxidant enzymes.

NO is an important physiological regulator; however, even at low concentrations, it has been shown to alter respiration (Bolanos *et al*., 1995) and to induce cell death (Burney *et al*., 1997). The balance between physiological regulation and pathological effect is dependent on the relative concentrations of NO and reactive biological targets. The interaction of NO with superoxide results in the formation of peroxynitrite, which may be a critical mediator capable of initiating events that lead to delayed

cell death. As such, inflammatory diseases of the respiratory tract, suh as asthma, Acute Respiratory Distress Syndrome (ARDS) and bronchiectasis, are commonly characterized by increased expression of iNOS within respiratory epithelial and inflammatory-immune cells and markedly elevated local production of NO, presumably as an additional host defense mechanism against bacterial or viral infections (Van der Vliet *et al*., 1999). In the present study, we found that NO levels were higher in trachea in infected group. However, in a number of inflammatory lung diseases, including chronic lung infection, iNOS expression is increased but no elevation of nitrotyrosine, nitrate, nitrite or nitrosothiol concentrations in infected lungs. These findings suggest that chronic infection does not cause increased iNOS activity in the lung, despite increased expression of iNOS (Hopkins *et al*., 2003). In our study, we found that lung nitrit level was lower in infected with ORT compared to control. Decreased nitrit may be due to superoxide/NO formation of peroxynitrite, resulting in lipid peroxidation.

CONCLUSION

The results of this study indicated that lung infection with ORT adversely affected the tissue oxidant-antioxidant status. Such, an imbalance could increase the susceptibility of the lung to inflammation and supplementation with antioxidants should be considered as a part of theraphy.

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