Effects of stress produced by adrenocorticotropic hormone (ACTH) on lipid peroxidation and some antioxidants in vitamin C treated and nontreated chickens

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Abstract

The aim of this study was to examine the effects of vitamin C on malonaldehyde (MDA) and glutathione (GSH) concentrations, and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity in chickens stressed by adrenocorticotropic hormone (ACTH). Sixty Leghorn chickens (20 weeks old) were randomly allotted to a control and a vitamin C treated group. An isotonic sodium chloride solution was administrated intramuscularly for a period of five days to the control group at a dose of 2.5 mL per chicken per day, and the treatment group received a vitamin C solution (containing 250 mg vitamin C/2.5 mL) intramuscularly for five days. On the fifth day of the experiment the chickens in both groups received 50 IU ACTH intramuscularly. Three hours after ACTH application blood samples were collected to determine the concentrations of MDA and GSH, and activities of SOD and GSH-Px in the blood. The concentration of MDA in the control group increased compared to that in the vitamin C treated group. After the ACTH application the activity of SOD increased in both groups, thought significantly so in the vitamin C treated group. The GSH-Px activity did not differ significantly between the treatments after ACTH application. It was concluded that intramuscularly administrated vitamin C facilitated an adaptation against stress and decreased negative effects of stress in chickens.

Keywords: ACTH, glutathione (GSH), glutathione peroxidase (GSH-Px), malonaldehyde (MDA), superoxide dismutase (SOD), vitamin C

Introduction

Physiological stress is not a disease, but too much stress is unhealthy and counterproductive and can affect many systems and causes a decrease in animal productivity. Some researchers (Siegel, 1971; Gray et al., 1989; Emre et al., 1994; Puvaldopirol & Thaxton, 2000) have reported that stress in birds can be produced experimentally by the injection of adrenocorticotropic hormone (ACTH). Stress is believed to cause many diseases in living beings due to its negative effects in the body. Some of the negative effects are increased biological oxidation, depression of the immune system against infections, reduction of reproduction rate and growth in the organism, metabolic malfunction causing feeding disorders, etc. (Gray et al., 1989; Puvaldopirol & Thaxton, 2000; Whittow, 2000). This situation causes the formation and increase of free radicals in the body and associated negative effects. While radicals affect all the biomolecules in the organism their principal targets are membrane lipids, other lipids, proteins and DNA (Jain et al., 1989; Husveth et al., 2000; Puthpongsiriporn et al., 2001). Free radicals constitute irreversible lipid peroxidation and the most important product of peroxidation is malonaldehyde (MDA).

For the continuation of life it is important to maintain a stable cellular environment. The body develops defence mechanisms to prevent the damage caused by the formation of reactive oxygen species. The substances which react immediately with radicals and prevent the development of the auto-oxidation/peroxidation, are called antioxidants (Soto-Salanova et al., 1993; McKee & Harrison, 1995; Ruiz et al., 2001). By decreasing intracellular disorders and diminishing the effects of stressors, the functions of these compounds include maintaining intracellular stability. Antioxidant defence is carried out in five different stages: the prevention of radical metabolite production, elimination of radical production, the repair of cell damage, inhibiting the chain reactions producing secondary radicals and increasing the endogen antioxidant capacity. Antioxidants can originate from endogenous and exogenous sources and include superoxide dismutase (SOD, E.C. 1.16.1.1), glutathione (GSH), glutathione peroxidase (GSH-Px, E.C. 1.11.1.9) and vitamin C (Aydemir et al., 2000; Puthpongsiriporn et al., 2001; Ruiz et al., 2001; Sahin et al., 2002).

Since chickens can synthesize ascorbic acid (vitamin C), it is not typically added to poultry diets.
(Satterlee et al., 1989), but the metabolic need for ascorbic acid is likely to be exceeded during pathological situations and stress. Therefore, vitamin C is added to poultry diets (McKee & Harrison 1995; Puthpongsiriporn et al., 2001; Sahin et al., 2002). Stress has generally been associated with a decline in production performance. The suppression of adrenocortical steroidogenesis by vitamin C may constitute the primary reason why this vitamin can ameliorate the negative effects of stress (Pardue, 1987; Satterlee et al., 1989).

Although the effects of stress and vitamin C on some antioxidants have been investigated (Satterlee et al., 1989; McKee & Harrison, 1995; Puthpongsiriporn et al., 2001; Sahin et al., 2002) information is limited. Therefore, it would be useful to establish the effects of vitamin C on some antioxidants in chickens stressed by ACTH.

Materials and Methods

In this study, 60 White Leghorn chickens (20 weeks old) were divided into two groups, a control and a vitamin C treated group. Blood samples were taken from both groups before application. An isotonic sodium chloride solution was administrated intramuscularly to the control group at a dose of 2.5 mL per chicken per day for a period of five days. Vitamin C (Redoxon Amp, Roché) was administrated intramuscularly to the experimental group at the same dose (containing 250 mg vitamin C/2.5 mL) and period of time (Emre et al., 1994). Food and water were supplied ad libitum to both groups during the experiment. On the fifth day of the experiment each chicken in both groups received 50 I.U. ACTH (Synackten Depot, Ciba) intramuscularly (Emre et al., 1994; Trout & Mashaly, 1994). Three hours after ACTH application, blood samples were taken to determine the concentrations of MDA and GSH, and activities of SOD and GSH-Px.

Whole blood MDA concentration as indicator of lipid peroxidation was measured, using the method based on thiobarbituric acid (TBA) reactivity, described by Jain et al. (1989). Erythrocyte SOD and GSH-Px activities were estimated in haemolysates, using commercially analytical kits (Randox Laboratory, Ireland). Whole blood GSH concentration was determined, using the method described by Beutler et al. (1963).

Because the initial values of both groups were similar, they were averaged as one initial value. Statistical analysis was done by using Covariance with the covariate the initial measurements. After application, measurements were then compared directly (Steel & Torrie, 1980, SAS, 1985).

Results and Discussion

Under normal conditions, cells are in a steady state, which is maintained by regulatory processes and different reactive oxygen species. Free radicals such as peroxides, singlet oxygen (oxygen free radicals), superoxide, hydroxyl and peroxyl radicals are produced continuously in cells, and could lead to biological damage in cells. Against these free radical attacks, cells have developed different antioxidant systems such as the glutathion redox cycle, superoxide dismutases, catalase, glutathione peroxidases, α-tocopherol and ascorbate (Soto-Salanova et al., 1993; Aydemir et al., 2000; Naziroğlu et al., 2000; Ruiz et al., 2001).

The concentrations of MDA and GSH, and activities of SOD and GSH-Px are presented in Table 1. Lipid peroxidation is a complex process in which the oxidation of polyunsaturated fatty acids of membrane lipids leads to membrane damage and cell death. At the onset of the present study the mean MDA concentration, which is an important sign of lipid peroxidation, was found to be 1.52 nmol/mL in both groups. After the ACTH application it was 4.08 nmol/mL (P < 0.001) in the control group and 1.56 nmol/mL in the vitamin C treated group. This finding supported reports in the literature (Aydemir et al., 2000) that vitamin C is a strong reducer of MDA concentration. Vitamin C can protect the cell membrane and cytosolic component of cells against the damage of oxidants. It can produce its antioxidative effect by removing singlet oxygen hydroxyl, hydroperoxyl, superoxide, lipid peroxyl and lipid alcosil radicals. It has been suggested that lipid peroxidies produced by the oxidation of lipid molecules become soluble in water due to the antioxidative effect of vitamin C (Nikki, 1991). In addition, vitamin C prompts the synthesis of vitamin E from the tocopherol radicals, which do not have antioxidant activities (Levine, 1997), and vitamin E forms the first line of defence against oxidative stress and peroxidation of polyunsaturated fatty acids present in cellular membrane phospholipids.

Super oxide dismutase is an antioxidant enzyme and plays an important role in protecting cells against damage caused by reactive oxygen species. Super oxide dismutase protects the organism against the toxic effects of superoxide radicals by catalysing their desmutation to molecular oxygen and hydrogen peroxide (H2O2) (Forman & Fridovich, 1973). In this study; while the SOD activity after the production of experimental stress by injection was 102.82 u/mL in the control group, it was calculated to be 387.02
u/mL in the group injected with vitamin C. From the result of the current study, it seems that vitamin C utilizes its antioxidant properties by increasing SOD activity to nearly three times its normal level in order to protect cells against negative effects of stress produced by ACTH. Vitamin C protects the cell from the detrimental effects of peroxidation. This finding is in agreement with previous reports (Afanasev et al., 1986; Aydemir et al., 2000). The increased activity of SOD in the experimental group after treatment with vitamin C could be linked to H₂O₂ accumulation and a depletion of intracellular GSH.

### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group (2.5 mL isotonic NaCl)</th>
<th>Vitamin C treated group (250 mg vit. C in 2.5 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean initial values in both groups</td>
<td>After ACTH application</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>1.52 ± 0.4a</td>
<td>4.08 ± 0.9b</td>
</tr>
<tr>
<td>SOD (u/mL)</td>
<td>95.41 ± 9.7a</td>
<td>102.82 ± 9.8a</td>
</tr>
<tr>
<td>GSH (mg/100mL)</td>
<td>75.56 ± 7.2</td>
<td>73.94 ± 8.4</td>
</tr>
<tr>
<td>GSH-Px (u/mL)</td>
<td>34.50 ± 1.85</td>
<td>41.37 ± 4.09</td>
</tr>
</tbody>
</table>

*Means in the same row with different superscripts differ significantly (P < 0.001)*

In order to avoid damage of reactive agents, tissues require sufficient concentrations of biological antioxidants such as GSH and vitamin C, and this plays critical roles in the neutralization oxidants (Avanzo et al., 2001; Hoffman, 2002). In this study, it was determined that GSH concentration in control and vitamin C treated groups decreases slightly after the ACTH application compared with the pre-experiment phase. Glutathione in tissue can be converted into GSSG by GSH-Px during stress produced by ACTH. Vitamin C can be facilitated non-enzymatically by GSH in tissues. This decrease in GSH may not only be a result of vitamin C treatment, but also because of increased tissue susceptibility to the deleterious effects of free radicals, as GSH itself is a powerful antioxidant and can be used in stress. Moreover, vitamin C can react directly with aqueous free radicals such as the hydroxyl and peroxyl radicals by donating one electron and quenching their reactivity.

Glutathione peroxidase together with SOD and catalase protects cells against damage caused by free radicals and hydro- or lipoperoxides (Macpherson, 1994). In this study, the GSH-Px activity increased in the control and vitamin C treated groups after ACTH application, but the increase was not statistically significant. Some reports indicated that the rise in GSH-Px activity may be a response to the need for further enzymatic capacity to deal with the production of H₂O₂ possibly associated with increased SOD activity and plasma vitamin C level (Aydemir et al., 2000; Öztürk-Ürek et al., 2001).

Results of the present study suggested that vitamin C exhibits protection against negative effects of stress induced by ACTH.

### References


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