Acute Effect of Cadmium on Female Reproduction in Birds

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Abstract: - Cadmium is a well-known environmental toxicant and it has been claimed to induce several negative physiological, productive and reproductive effects on the array of living organisms. In the previous study, we have investigated the in-vivo effects of cadmium on egg production and embryonic development in Japanese quail. In the present study, to clarify the molecular basis of reduced reproductive performance in cadmium-exposed female birds, the acute effect of cadmium on the expression of very-low-density apolipoprotein II (apoVLDL II), a major yolk protein, was examined. Since the disturbance in cellular redox status is one of the most important causes of heavy metal toxicity, the effect of L-ascorbic acid, an antioxidant, was evaluated. In addition, to gain an insight into how does L-ascorbic acid supplementation interfere with cadmium effects on apoVLDL II mRNA expression, oxidative stress markers such as metallothionein and glutathione peroxidase-1 mRNA expression, malonaldehyde production, and catalase activity were studied. Results showed that the apoVLDL II mRNA expression was remarkably suppressed by cadmium administration in a dose-dependent manner, and the increased metallothionein mRNA expression, higher malonaldehyde production, and ovarian follicular atrophy were observed coupled with decreased glutathione peroxidase-1 mRNA expression, body weight and feed intake. The L-ascorbic acid supplementation in feed to cadmium-exposed birds prevented the suppression of apoVLDL II mRNA expression and loss of egg production with maintaining a normal level of all oxidative stress markers measured. These findings strongly suggested that cadmium-induced oxidative stress might be the reason of suppressing yolk protein gene transcription, which can be prevented by the antioxidant supplementation.

Key-Words: Antioxidant, Ascorbic Acid, Bird, Cadmium, Egg, Female Reproduction, Heavy Metal, Oxidative

1 Introduction

Cadmium is a natural and industrially occurring metal-toxicant in the environment that enters into living organisms via mainly food, soil and water. Cadmium has been reported to exhibit reproductive toxicity in birds both at experimental [18] and environmental exposures [6]. We have previously found the reduction of egg production by cadmium in Japanese quail [13]. However, most of the investigations related to the reproductive toxicity of cadmium in birds were done mainly by measuring the rate of egg production and egg quality characteristics as the end-point rather than exploring the molecular clues of cadmium effects that may influence the female reproductive performance.

Very-low-density apolipoprotein II (apoVLDL II) is a major yolk protein in birds. It is also crucial to facilitate the deposition of intact VLDL particles into the growing ova through protecting them from obvious lipolytic degradation by the lipoprotein lipase secreted from granulose cells [15]. As a result, apoVLDL II contributes not only to the total mass of yolk but also plays a key role in the maturation of ovarian follicles that in turn determines the rate of egg production in birds. Due to such importance of apoVLDL II in egg formation process, assessment of its mRNA expression to clarify the molecular causes
of reduced egg production rate in cadmium intoxicated birds will be very important.

2 Problem Formulation

Cadmium is known to cause oxidative stresses by changing the activity of antioxidant enzymes [2] or through the production of reactive oxygen species, a group of free radicals, that usually promote lipid peroxidation, DNA-strand breaks or damage to the DNA repair proteins and finally alter the expression of genes [8,17]. Some investigators have found the amelioration of cadmium toxicity in birds by antioxidants supplementation [7]. L-ascorbic acid, also known as vitamin C and non-essential dietary supplement to the birds, is a well-known antioxidant and has been used by many researchers to recover the heavy metal induced oxidative stress [1]. Other than antioxidants, the amelioration of cadmium toxicity by a metal stress responsive protein, metallothionein, has been reported in rat [4].

The present study has been conducted to assess the effects of cadmium administration alone or in combination with L-ascorbic acid on apoVLDL II gene transcription, egg production and body maintenance of laying Japanese quail. To gain an insight into how L-ascorbic acid supplementation interferes with cadmium effects, the level of some oxidative stress markers, such as glutathione peroxidase-1 mRNA expression, malonaldehyde production, and catalase activity as well as the transcription of metallothionein gene in liver were also evaluated.

3 Problem Solution

3.1 Materials and Methods

3.1.1 Experimental Animals

Laying Japanese quail (Coturnix japonica) of 15-30 week-old were reared on the commercial quail diet (Tokai-Kigyo, Toyohashi, Japan) ad libitum with free access to tap water. The birds were kept in an environmentally controlled room at 25 °C with a photocycle of 14 h light - 10 h dark with light on at 0500 o'clock.

3.1.2 Sample preparation

Liver were collected from the decapitated birds, flash-frozen in liquid nitrogen and stored at -80 °C until RNA extraction. The amount of feed intake and body weight of the birds were recorded at the time of cadmium injection and at the time of sacrifice. The numbers of follicular atrophy were also noted by opening the abdominal cavity of the sacrificed birds.

Total RNA extraction were done exactly in the same way and using the same chemicals and instruments as described in Rahman et al. [14] for the extraction from immature male quail liver. RNA samples exhibiting an A260 to A280 ratio of 1.8 or more were used in the experiments. The mRNA levels of apoVLDL II, metallothionein and glutathione peroxidase-1 were measured by a semi-quantitative rt-PCR described by Uzbekova et al. [20] with the necessary modifications. The primer sets used for amplification were 5´-GGTACAATACAGGAATTGCTATA-3´ (forward) and 5´-AACTAGTGACTAGTTTCGCTCC-3´ (reverse) for apoVLDL II (GenBank Accession No. S82591), 5´-TCAGGACTGCATTTGCTTGCT-3´ (forward) and 5´-TGCGACGAGCGTCTGACGCT-3´ (reverse) for metallothionein [12], 5´-CATGTTGCGCTTGCCGTAGG-3´ (forward) and 5´-CGAGGAACCTTGCCTGCAGAATACCAGG-3´ (reverse) for glutathione peroxidase-1 ([21]; GenBank Accession No. AB371709), and 5´-CCAGGGCTGCTACGGTACCATCCTCTG-3´ (forward) and 5´-CCAGGACATGTGGGATGTTGC-3´ (reverse) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (GenBank Accession No. Z19086).

The PCR profile consisted of an initial denaturation at 94 °C for 1 min, followed by 20 cycles of denaturation at 94°C for 1 min, annealing at 56 °C for 45 s, extension at 72 °C for 1 min for apoVLDL II, 20 cycles of denaturation at 94°C for 1 min, annealing at 59.5 °C for 1 min, extension at 72 °C for 1 min for GAPDH, by 22 cycles of denaturation at 94°C for 1 min, annealing at 64 °C for 30 s, extension at 72 °C for 1 min for metallothionein, and by 35 cycles of denaturation at 94°C for 1 min, annealing at 50 °C for 30 s, extension at 72 °C for 1 min for glutathione peroxidase-1. The PCR products were subjected to electrophoresis in 1% agarose gel stained with ethidium bromide and photographed under ultraviolet illumination. Intensity of the fluorescence was determined using image analysis software (ImageJ, NIH, USA). The result was expressed as the ratio of the target gene signal intensity and that of GAPDH as the standard.

The malonaldehyde production in liver tissues were measured spectrophotometrically according to the procedures of Uchiyama and Miura [19], and catalase activity was determined by the methods of Cohen et al. [5].
3.1.3 Statistics
Data obtained from the replicated experiments were analyzed using one-way analysis of variance followed by Tukey’s multiple-range test to identify the significant difference between groups. Fisher’s exact test was used to determine the differences in hen-day egg production rate among groups. Significant differences were considered at $p < 0.05$.

3.1.4 Results and Discussion
In the time-course study, we had investigated apoVLDL II mRNA levels at 6, 12, 24, 48 and 72 h after single intraperitoneal injection of 3 mg cadmium/kg body weight, which was chosen on the basis of our previous findings [14]. It was found that the cadmium administration lowered the apoVLDL II mRNA levels in liver as the time progress after the cadmium administration. It was found that cadmium/kg body weight, which was chosen on the basis of our previous findings [14]. It was found that the cadmium administration lowered the apoVLDL II mRNA levels at 6, 12, 24, 48 and 72 h after injection and the lowest level was found at 48 h after exposure and later on the level was increasing.

In the next experiment, we had tested the effect of 0.1, 1, 10 and 30 g L-ascorbic acid/kg feed against the cadmium effects on apoVLDL II mRNA level. The levels of metallothionein mRNA in the cadmium injected birds were increased in a linear fashion as the time increased after the cadmium injection. The results showed that 1 g/kg feed supplementation was most effective and the levels of L-ascorbic acid either below or above 1 g/kg feed were unable to overcome the toxicity of cadmium on apoVLDL II mRNA.

Finally, we had investigated the effects of 0.001, 0.01, 0.1, 1 and 3 mg cadmium/kg body weight alone or in combination with the dietary 1 g L-ascorbic acid/kg feed against the mRNA expressions of apoVLDL II, glutathione peroxidase-1 and metallothionein, and on the level of catalase and malonaldehyde in laying quail. The levels of metallothionein mRNA in the cadmium injected birds were increased in a linear fashion as the doses of cadmium increased without L-ascorbic acid supplementation and the 3 mg cadmium injection had significantly uplifted the level over that of control. In contrast, L-ascorbic acid supplementation had prevented this induction in all birds regardless of the doses of cadmium. The reduction of glutathione peroxidase-1 mRNA by the cadmium administration was also prevented by the L-ascorbic acid supplementation. A dose-dependent increase of malonaldehyde level was observed in the cadmium received birds, and the L-ascorbic acid supplementation had minimized this increment irrespective of the doses of cadmium administered.

Compared to control, an L-ascorbic acid-protective decrease in catalase activity was found in the 3 mg cadmium injected bird. The levels of catalase were also found to be significantly increased over the control level at 0.001 and 0.01 mg cadmium exposures in combination with the L-ascorbic acid supplementation.

Compared to control, an L-ascorbic acid-protective reduction in egg production rate, loss of body weight and an increase of follicular atrophy were found in the 3 mg cadmium injected bird.

The antioxidant defense system of the cell is important for the normal function in the body. The lowering of catalase and glutathione peroxidase 1 level along with increased malonaldehyde level at relatively higher cadmium dosed birds had clearly indicated the presence of oxidative stress. The presence of lipid peroxidation along with decreased antioxidant enzymes activity might possibly the results of alterations in the structural component metal of these antioxidant enzymes [3,10]. However, the normal levels of malonaldehyde, apoVLDL II and antioxidant enzymes found in the L-ascorbic acid supplemented and cadmium-exposed laying quail along with regular egg production rate led us to comprehend that cadmium toxicity had reduced the egg production rate by affecting the antioxidant defense system of the bird that was protectable by L-ascorbic acid supplementation. The slightly higher induction of metallothionein by L-ascorbic acid supplementation before cadmium injection and the control of remarkable metallothionein mRNA induction by cadmium after injection in L-ascorbic acid supplemented birds compared to that of non-supplemented counterpart indicated the possibility of cadmium toxicity mitigation in part by L-ascorbic acid supplementation via another way than the antioxidant effects [16].

The positive stimulation of some lower-doses of cadmium administration in combination with L-ascorbic acid supplementation on catalase activity and glutathione peroxidase 1 level in the bird has been coincided with the findings of Jebali et al. [9]. But such stimulation did not able to increase the apoVLDL II mRNA level in the laying quail nor increased the egg production rate significantly. Because, the levels of yolk precursor proteins in laying birds naturally remain higher [11, 22] and might be more vulnerable to drop at any negative physiological stimulation rather than to be induced additively by good physiological conditions.
4 Conclusion

It was concluded that a single intraperitoneal injection of cadmium can induce the remarkable oxidative stress in laying Japanese quail. The suppression of yolk protein gene transcription leading to reduced egg production rate might be resulted from cadmium-induced oxidative stress in the bird that was preventable by L-ascorbic acid supplementation in feed.

References:


