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The disruption of mitochondrial metabolism and ion homeostasis in chicken hearts exposed to manganese

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HIGHLIGHTS

- Mn exposure induced the increase of serum CK and cTnT; abnormal electrocardiogram and ultra-structure, and apoptosis in heart of chicken.
- Mn exposure induced the disruption of mitochondrial metabolism in heart.
- Mn exposure induced the alteration of ion homeostasis in heart including Ca, Fe, Cu, Zn.

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ABSTRACT

Exposure to high levels of manganese (Mn) can result in cardiotoxicity in animals. However, little is known about the effect of excess Mn on poultry hearts. The aim of this study was to investigate the effect of dietary Mn on chicken cardiac injuries and the possible mechanisms of this process. In the present study, 400 fifty-day-old Hy-line brown cocks were randomly divided into four groups, and were fed either a commercial diet (containing 100 mg/kg Mn) or a Mn-supplemented diet containing 600 mg/kg, 900 mg/kg, or 1800 mg/kg Mn for 30, 60 or 90 days, respectively. Next, we examined several biomarkers of cardiac injury, including biochemical blood serum analyses, electrocardiogram assays, histological analyses, ultra-structural assays and apoptosis assays. To investigate the possible mechanisms of Mn-induced cardiotoxicity, we examined the effect of $MnCl_2$ on mitochondrial function and metal ion homeostasis. We found that subchronic $MnCl_2$ exposure induced damage in chicken hearts. Further investigations indicated that possible mechanisms for Mn-induced chicken cardiac injury included the disruption of mitochondrial metabolism and the alteration of ion homeostasis.

1. Introduction

Manganese (Mn) is an essential trace element that is required for normal biological activities and a multitude of enzymatic reactions. Mn plays an important role in the regulation of reproduction and carbohydrate metabolism, the formation of connective tissues, bone marrow and lipids, and the maintenance of neurological tissues (Park and Park, 2010). It is also an essential component of key enzymes such as glutamine synthetase, arginase, phosphoenolpyruvate decarboxylase, and mitochondrial superoxide dismutase (Carl et al., 1993; Finley and Davis, 1999; Stohs and Bagchi, 1995; Takeda, 2003). However, exposure to excess amounts of Mn has long been known to lead to a progressive neurological disorder similar to Parkinson's disease (Aschner et al., 2007; Au et al., 2008). In addition, Mn exposure has been shown to result in the accumulation of Mn in the brain (Liccione and Maines, 1988) and liver (Shukla and Chandra, 1987), which induces hepatotoxicity and/or neurotoxicity. With the increasing usage of Mn products exist including fireworks, dry-cell batteries, fertilizers, paints, medical imaging agents, pharmaceutical products, cosmetics and gasoline additives, the health risks of Mn exposure have been increasing dramatically (Park and Park, 2010; Zayed et al., 1994). In recent years, the number of studies on Mn intoxication have also increased, and the predominant focus of these studies has been the effect of Mn on the nervous tissues. However, the effect of Mn on the cardiovascular system has received less attention. Although cardiac injury is not characteristic of chronic Mn intoxication, Mn exposure can induce ventricular fibrillation and depress contractile function in the rat and inhibit spontaneous electrophysiological activity in the guinea pig (Li and Zhou, 1987; Sui et al., 1990; Wolf and Baum, 1983; Zeng et al., 1990). Nevertheless, Mn toxicity has not been shown to be toxic to the hearts of whole animals or humans (Aschner et al., 2005), and it remains to be determined if chicken hearts are affected by Mn exposure.

The mechanisms by which Mn exerts cellular toxicity are poorly understood but are thought to include the disruption of

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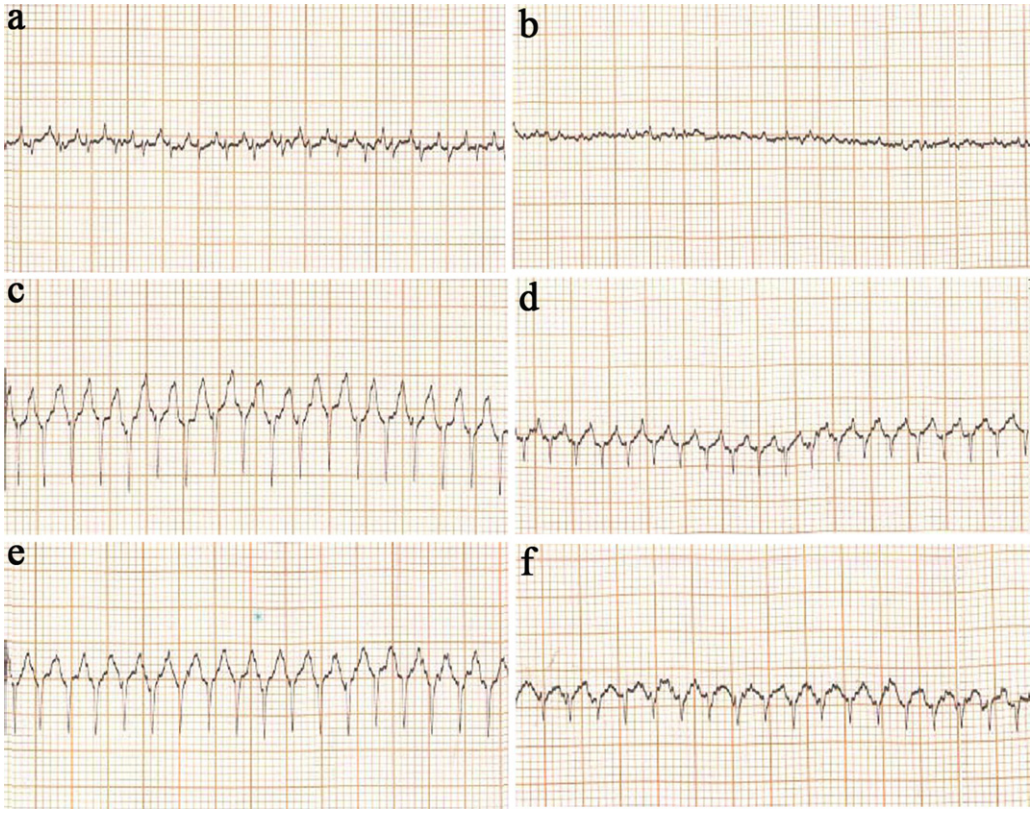

Fig. 1. Chicken ECGs. (a) The ECGs of the control group were assayed by lead I at 90 days. (b) The ECGs of the H group were assayed by lead I at 90 days. (c) The ECGs of the control group were assayed by lead II at 90 days. (d) The ECGs of the H group were assayed by lead II at 90 days. (e) The ECGs of the control group were assayed by lead III at 90 days. (f) The ECGs of the H group were assayed by lead III at 90 days.

mitochondrial metabolism (Montes et al., 2001) and the alteration of ion homeostasis (Kwik-Uribe and Smith, 2006), both of which may also cause disorders in cardiac function (Chen et al., 2012; Gunter et al., 2010; Nojiri et al., 2006) and have primarily been studied in Mn-induced hepatotoxicity and neurotoxicity (Dobson et al., 2004; Hazell and Butterworth, 1999; Lebda et al., 2012; Milatovic et al., 2011). Whether a similar mechanism occurs in Mn-induced cardiotoxicity is unclear, and the effect of Mn exposure on chicken hearts has not been studied. Therefore, this study aims to (1) investigate whether Mn exposure induces cardiac injury in poultry and (2) examine the possible mechanisms of this process.

2. Materials and methods

2.1. Poultry and experimental design

All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. Before conducting the present study, we performed acute toxicity experiments to confirm the median lethal dose (LD50) of $MnCl_2$ for cocks and found that the LD50 was 1122.35 mg/kg. Next, four hundred 50-day-old male Hy-line brown cocks were divided randomly into four groups. The doses used in this study correspond to one-fifteenth, one-tenth, and one-fifth of the LD50 for cocks. The control group was fed a baseline diet containing 100 mg/kg Mn. The low-dose group (L group) was fed with a Mn-supplemented diet containing 600 mg/kg Mn, the middle-dose group (M group) was fed with a Mn-supplemented diet containing 900 mg/kg Mn, and the high-dose group (H group) was fed a Mn-supplemented diet containing 1800 mg/kg Mn. The poultry were maintained at the Laboratory Animal Center of the College of Veterinary Medicine at the Northeast Agricultural University in China. Food and water were provided ad libitum. On the 30th, 60th, and 90th days of the experiment, 30 cocks in each group were randomly selected and euthanized with sodium pentobarbital. The cardiac tissues were quickly removed, blotted, and rinsed with ice-cold, sterile, deionized water, frozen immediately in liquid nitrogen, and stored at −80 °C until required.

2.2. Serum CK and cTnT activity

Within 2 h after blood collection into serum separator tubes, the serum was obtained by centrifugation at 6000 × *g* for 6 min. The serum creatine kinase (CK) activity was analyzed as previously described (Yen et al., 1996). The activity of troponin T (cTnT) was determined using a commercial kit (Roche Co. Ltd., China) according to the manufacturer's instructions, and the results were reported as ng/mL.

2.3. ECG assay

Electrocardiogram (ECG) recordings were taken from standard leads I, II, III. The electrocardiographic graph paper was used as the ECG record, with 1 mm of distance between every two horizontal or vertical lines. The electrocardiograph (Japan ECG-6251) was set at a sensitivity of 10 mm/mV, a gain reduction of 1, and a chart drive speed of 25 mm/s. The electrical axis of the heart was calculated according to the QRS resultant wave in leads II and III. The P, R, S, T voltages were calculated by determining the amplitude of the P, R, S, T waves in lead II. In addition, the R–R and P–S intervals were calculated according to the intervals on the graph paper.

2.4. ATPase activity assay

The activities of the Na^+–K^+-ATPase, the Ca^{2+}-ATPase, and the Mg^{2+}-ATPase were determined using the appropriate assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturers' instructions. The activities of the Na^+–K^+-ATPase, the Ca^{2+}-ATPase and the Mg^{2+}-ATPase were measured by quantifying the inorganic phosphorus (Pi) production from the conversion of ATP to ADP at 636, 636, and 660 nm, respectively, using the molybdenum blue spectrophotometric method and were expressed as U/mg protein.

2.5. Histological analysis

After necropsy, the cardiac tissue specimens were rapidly fixed in 10% neutral-buffered formalin solution for at least 24 h. The fixed specimens were processed using the conventional paraffin-embedding technique. From the prepared paraffin blocks, 5 mm-thick sections were obtained and stained with hematoxylin and eosin (H&E) for light microscopic examination.