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RESEARCH ARTICLE

Manganese enhances the expression of the manganese superoxide dismutase in cultured primary chick embryonic myocardial cells

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Abstract

In the present study, the effect of manganese (Mn) on antioxidant status and the expression of the manganese superoxide dismutase (MnSOD) gene in cultured primary myocardial cells collected from the chick embryos was investigated. The hypothesis that Mn supplementation would enhance the expression of MnSOD in cultured primary myocardial cells of chick embryos was tested. Eggs collected from Mn-depleted Arbor Acres laying breeder hens were incubated for 10 days and then myocardial cells were isolated and cultivated for 8 days. The embryonic myocardial cells on day 6 were treated with Mn in the cell culture medium at different time points when the proportion of cells showing spontaneous contraction was over 95% after the 3-day primary culture. A completely randomized design involving a 3 Mn levels (0, 0.5 and 1.0 mmol L^{-1})×3 incubation time points (12, 24 and 48 h) factorial arrangement of treatments (n=6) was used in the current experiment. The results showed that MnSOD activity and mRNA expression level were induced by Mn and increased with incubation time, which supported the hypothesis that Mn would enhance the expression of the *MnSOD* gene, and thus might protect myocardial cells from oxidative stress during the chick embryonic development.

Keywords: manganese, MnSOD, expressions, cultured primary myocardial cells, chick embryos

1. Introduction

Many cellular processes, such as the embryonic development, involve reduction and oxidation (redox) reactions. These redox processes occur in many forms, from simple electron transfer reactions to radical processes and thiol/disulfide exchanges (Winyard *et al.* 2005). Many researchers have reported that the chick embryos were more susceptible to oxidative stress than the mammalian fetus during the embryonic development (Surai 1999a, b, 2000; Jadhav and Kengar 2016). This is because the embryos obtain oxygen from an air-cell within the egg-space and is therefore in direct contact with the external environment (Starck 1998). As a result, the chick embryos are subjected to high oxidative stress as they grow and must rely on an effective antioxidant system for protection.

Manganese (Mn) is an essential trace element required in small amounts for normal growth and development of the avian embryos (Savage 1968). It is also a crucial component

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of the metalloenzyme, Mn superoxide dismutase (MnSOD). This enzyme is a primary mitochondrial antioxidant enzyme for detoxification of reactive oxygen species (ROS), in particular, superoxide free radicals, generated as by-products of oxidative phosphorylation or under oxidative stress (Oberley and Buettner 1979). The MnSOD catalyzes the conversion of two molecules of superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2) that is further oxidized to water (McCord *et al.* 1971). Cells lacking MnSOD are oxygen intolerant (Fridovich 1995). The MnSOD has been shown to protect cells from oxidative injury (Iwamoto and Takeda 1990; Lindau-Shepard *et al.* 1994; Ishiyama *et al.* 1995; Stephanz *et al.* 1996) to increase resistance to tumor necrosis factor (TNF) (Wong *et al.* 1989) and to protect cells against irradiation (Fridovich 1978; Eastgate *et al.* 1993; Wong 1995; Zhong *et al.* 1996). Other antioxidants, such as glutathione (Arechiga *et al.* 1995) or vitamin E (Cederberg *et al.* 2001), can further reduce the deleterious effects of oxidative damage and support development of embryos as measured during *in vitro* culture. The use of synthetic SOD (SOD mimetic), or increased MnSOD expression by transfection/infection, has also been studied as a method to suppress the malignant phenotype or to protect cells from oxidative stress injury (Wada *et al.* 1994; Kasten *et al.* 1995; Roberfroid and Calderon 1995).

The MnSOD is highly expressed in differentiated organs that contain a large number of mitochondria such as the heart, liver, and kidneys (Marklund 1980). Luo *et al.* (1991, 1992) demonstrated that the heart was the most sensitive target tissue for MnSOD activity compared to other tissues, and the MnSOD activity in heart was a sensitive biomarker for estimating the dietary Mn requirement of broilers. Later *in vivo* studies in our laboratory have also clearly demonstrated that *MnSOD* mRNA and protein expression levels, as well as the enzymatic activity in the broiler heart were all very sensitive in response to dietary Mn supplementation (Li *et al.* 2004, 2005, 2008, 2011; Luo *et al.* 2007). Supplementation of Mn *in vivo* at 15 mg Mn d^{-1} significantly increased lymphocyte MnSOD expression (Davis and Greger 1992), and Mn also induced MnSOD expression in dose- and time-dependent manners in human breast cancer Hs578T (Thongphasuk *et al.* 1999). Our previous study indicated that Mn supplementation elevated *MnSOD* mRNA and protein levels and enzymatic activity in the broiler myocardial cells in dose- and time-dependent manners (Gao *et al.* 2011). These observations highlight the important role of MnSOD in normal heart tissue for protection from oxidative stress. However, embryonic development conditions and physiological characteristics are completely different from those of chicks, and it is not known if the model used by Gao *et al.* (2011) is appropriate for embryonic myocardial cells *in vitro* or whether results from their study are applicable to embryonic myocardial cells. Therefore, we investigated the effect of Mn on the expression of MnSOD in cultured primary chick embryonic myocardial cells *in vitro*. We hypothesized that Mn supplementation would enhance the expression of MnSOD in cultured primary chick embryonic myocardial cells. This enhancement may protect the chick embryos from oxidative damage.

2. Materials and methods

2.1. Animals and diets

All experimental procedures were approved by Animal Welfare Committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences. A total of forty 18-week-old female broiler breeders (Arbor Acres; Huadu Broiler Company, Beijing, China) were randomly allocated to stainless steel cages coated with plastics (2 birds per cage). All broiler breeders were fed a conventional corn-soybean meal diet containing 133 mg Mn kg^{-1} by analysis during adaption from 18 to 29 weeks according to Arbor Acres breeder management guidelines. After the adaptation period, all broiler breeders were fed the corn-soybean meal basal diet with no Mn addition (containing 12.20 mg Mn kg^{-1} by analysis, Table 1) from 30 to 32 wk to deplete the body Mn storage and obtain the low-Mn eggs. This basal diet was formulated to meet or exceed the National Research Council (NRC 1994) requirements for other nutrients except for Mn for laying broiler breeders. During the 32 wk of age, eggs with similar weights ((65±5) g egg^{-1}) were selected and then incubated in the same incubator (9TDJ-A; Lantianjiao Electronic Technology Co., Ltd., Beijing, China) at 37.8°C and a relative humidity of 55 to 60% for 10 days. The Mn contents in the yolks of the eggs collected from the broiler breeders fed the Mn-unsupplemented corn-soybean meal basal diet and the conventional corn-soybean meal diet during the 32 weeks of age were analyzed to be 0.68 and 1.27 μg Mn g^{-1} egg yolk, respectively.

2.2. Isolation and cultivation of primary chick embryonic myocardial cells

Embryonic myocardial cells were acquired from the 10-day-old chick embryos using the methods described by Wu *et al.* (2013). Briefly, the embryonic heart was removed under sterile conditions and blood cells were washed using phosphate-buffered saline (PBS, Cat no. 14190144, Life Technologies, Carlsbad, CA). The myocardial tissue was minced and digested with 0.12% collagenase II (Cat no. 9001-12-1, Life Technologies) for 8 min at 37°C. To collect enough cells, digestion was repeated three times. The digested mixtures were centrifuged at 1 000×g for 4 min