Research in Veterinary Science 86 (2009) 533–538

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Heat shock protein 60 expression in heart, liver and kidney of broilers exposed to high temperature

Jianyan Yan, Endong Bao *, Jimian Yu

College of Veterinary Medicine, Nanjing Agricultural University, Weigang 1, 210095, Nanjing, Jiangsu Province, China

ARTICLE INFO

Article history:
Accepted 2 September 2008

Keywords:
Broiler
Heat shock protein 60
Heat stress
Tissue

ABSTRACT

The objective of this study was to investigate the expression and localization of HSP60 in the heart, liver, and kidney of acutely heat-stressed broilers at various stressing times. The plasma creatine kinase (CK) and glutamic pyruvic transaminase (GPT) concentrations statistic increased following heat stress. After 2 h of heat stress, the tissues showed histopathological changes. Hsp60 expressed mainly in the cytoplasm of parenchyma cells heat stress. The intensity of the cytoplasmic staining varied and exhibited an organ-specific distribution pattern. Hsp60 levels in the hearts of heat-stressed chickens gradually increased at 1 h ($p < 0.05$) and peaked ($p < 0.05$) at 5 h; Hsp60 levels in the liver gradually decreased at 3 h ($p < 0.05$); Hsp60 levels in the kidney had no fluctuation. It is suggested that Hsp60 expression is tissue-specific and this may be linked to tissue damage in response to heat stress. The Hsp60 level is distinct in diverse tissues, indicating that Hsp60 may exert its protective effect by a tissue- and time-specific mechanism.

1. Introduction

Heat shock proteins (Hsps) are the most broadly distributed class of proteins known and are also among the most highly conserved in nature. As chaperones, they perform important functions in the folding/unfolding and translocation of proteins as well as in the assembly/disassembly of protein complexes (Zügel and Kaufmann, 1999). Based on their size, Hsps have been classified into six major families: small Hsps, Hsp40, Hsp60, Hsp70, Hsp90 and Hsp110 (Habich and Burkart, 2007).

Heat stress is one of the most challenging environmental conditions affecting commercial poultry, and causes the loss of revenue that ranges into millions of dollars each year (Mahmoud et al., 2003). Chickens have no sweat glands, a rapid metabolism, so they are more sensitive to high ambient temperatures. Heat stress reduced a series of physiological and metabolic changes in chickens such as weight gain, feed efficiency, serum total protein, albumin, triglyceride and uric acid in chickens (Deyhim et al., 1995). Chickens exposed at 32 °C for 2 h caused rectal temperature to rise significantly and the plasma of creatine kinase increasing (Sandercock et al., 2001). Stress responses and liver and heart damage occur in broiler chickens that are acutely exposed to high temperatures (Lin et al., 2006).

A common event of chickens thermoresistance is the high expression of Hsp (Mahmoud et al., 2003; Hagiwara et al., 2007). Many studies have shown the importance of Hsps for the survival of cells under stress conditions. As universal cytoprotective proteins, Hsps may enhance stress tolerance and thereby increase the survival rate of stressed cells (Latchman, 2001). Although stress increased the synthesis of Hsps, some like Hsp60 were also constitutively expressed and played an essential role in protect cells against stress function. Under normal growth conditions, Hsp60 expression changes following thermal challenge or stimulation from a variety of environmental stressors such as heat (Sharma et al., 2006) and water immersion (Kuwabara et al., 1994). Recently evidence exists that Hsp60 contributes to the pathogenesis of atherosclerosis (Bason et al., 2003). Its plasma level was determined in trauma patients, and it was observed that the serum levels of Hsp60 were significantly higher in patients with later developed acute lung injury (Pespeni et al., 2005). Moreover, Hsp60 could activate immunocompetent cells such as macrophages and T-cells (Vabulas et al., 2001). Little is known regarding the variation in Hsp60 levels, in terms of protein expression and tissue damage, in gallinaceous tissues after heat stress. The purpose of this study was to investigate the distribution of Hsp60 in the heart, liver and kidney of heat-stressed broilers and correlate its levels with tissue damage resulting from exposure to high temperatures.

2. Materials and methods

2.1. Animals and experimental design

One-day-old broilers (120) used in this study were male chicks obtained from Nanjing Changjiang Commercial Fowl Company.

* Corresponding author. Tel.: +86 2584395316; fax: +86 2584398669.
E-mail addresses: b_endong@njau.edu.cn, b_endong@hotmail.com (E. Bao).

0034-5288/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2008.09.002

Birds were housed in six big coops (20 birds per coop), and the coops were placed in a controlled climate chamber. The birds were allowed a 4-week period to acclimatize to their new housing and recover from environmental stress. During this period, the broilers were reared under standard conditions and the relative humidity of the chamber was maintained at 60% ± 10%. The birds were maintained on a commercial starter diet and the room temperature was gradually decreased from 40 °C to 35 °C during the first week. The birds diet were changed to a standard finisher diet and the room temperature was gradually decreased to 22 °C ± 1 °C, and this was then maintained by controlled ventilation for the remainder of the experiment. The broilers were vaccinated against Newcastle disease (ND) and infectious bursal disease (IBD) on the 7th and 14th days, respectively. We have undertaken pilot experiments which have suggested that a time scale of 10 h allows investigation of expression of Hsp60 at the beginning of heat stress. At 30 days, the room temperature was suddenly increased from 22 °C ± 1 °C to 37 °C ± 1 °C. The six groups were then subjected to heat stress for one of the following time periods: 0 h (control group), 1 h, 2 h, 3 h, 5 h and 10 h. To regulate the heat, the temperature was monitored at the centre of each coop. During heat stress birds were provided access to a commercial broiler feed and water ad libitum, the relative humidity of the chamber was maintained at 50% ± 5%. The control group was still maintained at thermoneutrality (22 °C ± 1 °C). When the heat stress period was over, birds were humanely killed for sampling. Blood samples (10 mL) were obtained, transferred into blood collection tubes containing heparin anti-coagulant (50 IU mL^{-1}), and immediately chilled on ice. The plasma samples for subsequent enzyme determination were obtained following centrifugation of whole blood at 1500*g* for 5 min and were frozen and stored at −20 °C. The tissues (heart, liver and kidney) for histological analysis and immunohistochemical analyses were fixed in freshly prepared 4% paraformaldehyde in phosphate-buffered saline, pH 7.4, while those tissues from broilers was quickly dissected (approximately 1.0 g) and placed into 1.5 mL tubes. The tubes were frozen in liquid nitrogen, and then stored at −80 °C used for quantifying the Hsp60 levels.

The experimental procedures followed the guidelines of the regional Animal Ethics Committee and were approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University.

2.2. Circulating plasma CK

The activities of plasma creatine kinase (CK) and glutamic pyruvic transaminase (GPT) were assessed using a commercial kit modified for use with a multiwell plate spectrophotometer as previously described by Sun et al. (2007).

2.3. Histopathology

Three 4% paraformaldehyde-fixed and paraffin-embedded tissues were serially sliced into 5-μm sections. One section was routinely stained with haematoxylin and eosin (HE) and examined by light microscopy.

2.4. Immunohistochemistry (IHC)

The paraffin-embedded 5-μm serial sections of the tissues were deparaffinized and subsequently passed through decreasing concentrations of alcohol into water. Endogenous peroxidase activity was quenched in 3% (v/v) hydrogen peroxide (H_2O_2) in methanol for 10 min at room temperature (RT). Unspecific antibody binding was blocked by incubating with normal goat serum (diluted ready-to-use, 85-6643, Zymed Laboratories Inc., South San Francisco, CA, USA) for 30 min at RT. The sections were incubated overnight at 4 °C in a humidified chamber with the primary antibodies (mouse anti-Hsp60 monoclonal antibody, SPA-806, Stressgen Bioreagents Limited Partnership, Victoria, Canada. We have confirmed in this laboratory that it is reactive with Hsp60) diluted 1:100 in phosphate-buffered saline (PBS). For detecting the bound antibodies, the sections were incubated in biotinylated secondary antibody (diluted ready-to-use, 85-6643, Zymed Laboratories Inc., South San Francisco, CA, USA) for 20 min at 37 °C and then incubated in an avidin–biotin–horseradish peroxidase system (diluted ready-to-use, 85-6643, Zymed Laboratories Inc., South San Francisco, CA, USA) for 20 min at 37 °C; this was followed by visualization with the diaminobenzidine (DAB) substrate (diluted ready-to-use, 00-2014, Zymed Laboratories Inc., South San Francisco, CA, USA). After each incubation step, the sections were washed in PBS (pH 7.4). In the control, the primary antibody was replaced with the blocking serum. Nuclear counterstaining was performed using haematoxylin. Finally, the tissue sections were dehydrated, mounted and photographed.

2.5. Hsp60 detection by enzyme-linked immunosorbent assay (ELISA)

The tissue samples were homogenized and completely washed in ice-cold physiological saline. The Hsp60 levels in the heart, liver and kidney of both heat-stressed and control broilers were measured using a commercially available ELISA kit (QRCT-3323113EIA/UTL, goat anti-chicken Hsp60; Adlitteram Diagnostic Laboratories, USA). The assay was performed according to the manufacturer's protocol, and its sensitivity was 0.01 ng/mL. The assay was specific for chicken recombinant inducible Hsp60 and did not cross-react with other Hsps. β-actin was used to control the bias caused by the procedure of protein extraction, and its levels were detected by a commercial available ELISA kit (QRCT-3230311EIA/UTL, goat anti-chicken β-actin; Adlitteram Diagnostic Laboratories, USA). The quantity of Hsp60 in each sample was normalized using the following formula:

$$\text{Relative quantity of Hsp60} = \text{quantity of Hsp60}/\text{quantity of } \beta\text{-actin}$$

2.6. Statistical analysis

Statistical analysis was performed using a one-way ANOVA and the SPSS software (Statistical Package for Social Sciences, Chicago, IL). Data (mean ± SE) were considered to be statistically significant when the value of p was less than 0.05 as determined by the least significant difference (LSD) method.

3. Results

3.1. The level of plasma CK

The plasma CK level in broilers is shown in Fig. 1. The plasma CK activities exhibited a significant increase ($p < 0.01$) after 2 h of heat stress and reached a peak level ($p < 0.01$) after 10 h. The plasma GPT activities increased slightly at the beginning of heat stressing. A significant increase of the plasma GPT activities occurred after 3 h of heat stress ($p < 0.05$) and reached a peak level after 10 h ($p < 0.01$).

3.2. Histopathological and immunohistochemical analyses

Histopathologically, the myocardial fibres had slightly enlarged by various degrees, and expanded intracellular spaces and fine granular particles were seen in the hearts of all heat-stressed chickens (Fig. 2A1). In the liver, the hepatic cords were disorganized, and the hepatic cells were enlarged with vacuolar and gran-