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Effect of thermal stress on HSP70 expression in dermal fibroblast of zebu (Tharparkar) and crossbred (Karan-Fries) cattle

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ABSTRACT

The present studies were conducted to investigate the difference response of dermal fibroblasts to heat stress in Tharparkar and Karan-Fries cattle. Skin is the most important environmental interface providing a protective envelope to animals. In skin, dermal fibroblasts are the most regular cell constituent of dermis that is crucial for temperature homeostasis. The study aimed to examine the reactive oxygen species (ROS) formation, cytotoxicity (%) and heat shock protein 70 (HSP70) genes expression in dermal fibroblast of Tharparkar and Karan-Fries cattle and to assess whether resistance of dermal fibroblast to heat stress is breed specific. Dermal fibroblasts from ear pinna of Tharparkar and Karan-Fries cattle were exposed at 25 °C, 37 °C, 40 °C and 44 °C for 3 h to measure the ROS, cytotoxicity (%) and HSP 70 (HSPA1A, HSPA2 and HSPA8) genes' expression. The results showed that ROS formation at low temperature (25 °C) decreased in both breeds as compared to control (37 °C) and the differences were significant ($P<0.0001$). Heat stress at 40 °C did not increase ROS formation significantly in Tharparkar but increased significantly ($P<0.001$) in Karan-Fries cattle. The overall cytotoxicity (%) was also found to be significantly different ($P<0.001$) between Tharparkar and Karan-Fries cattle, and on exposure to different temperatures ($P<0.001$). The cytotoxicity (%) in dermal fibroblast cells of Karan-fries cows was more than Tharparkar. The expression studies indicated that all HSP70 genes (HSPA8, HSPA1A and HSPA2) were up-regulated at different temperatures in both breeds. In Tharparkar, the relative mRNA expression of HSPA8 gene was higher but HSPA1A and HSPA2 genes were low as compared to Karan-Fries cattle. At 40 and 44 °C, the relative expressions of inducible HSP 70 genes (HSPA1A and HSPA2) were higher in Karan-Fries than Tharparkar. In summary, dermal fibroblast resistance to heat shock differed between breeds. Dermal fibroblasts of Tharparkar were observed to be more heat tolerant than crossbred Karan-Fries cattle. The study concludes that zebu cattle (Tharparkar) dermal fibroblasts are more adapted to tropical climatic condition than crossbreed cattle (Karan-Fries). Differences exist in dermal fibroblasts of heat adapted and non-adapted cattle.

1. Introduction

Heat stress causes significant loss to animal production systems in most tropical countries (Hansen, 2009). High ambient temperatures both directly and indirectly affect production and health of farm animals (Gaughan et al., 2009). The thermal balance is affected by different climatic elements (ambient temperature, relative humidity, solar radiation, air movement, and precipitation), animal factors (rate of metabolism, moisture loss) and thermoregulatory mechanisms of the animals (Collier et al., 2008). High environmental temperature challenges the homeostatic system and stimulates excessive production of free radicals (Bernabucci et al., 2002) which causes cellular damage.

Heat stress also initiates a complex program of gene expression and biochemical adaptive responses (Fujita, 1999). Heat shock proteins (HSPs) involved in these responses are highly conserved and these molecular chaperones encompass several families, play important physiological roles and help cope with heat stress (Parsell and Lindquist, 1993). HSPs function as molecular chaperones in restoring cellular homeostasis and promoting cell survival (Collier et al., 2008). HSPs have been considered to play crucial roles in environmental stress tolerance and in thermal adaptation (Sorensen et al., 2003). Several studies in bovine, mice and human cells give evidence that constitutive elevation of the inducible HSPs levels in gene and protein expression provides cytoprotection upon thermal stress (Collier et al., 2006). Among HSPs family

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HSP70 transcription is increased by heat shock as well as other stress stimuli such as oxidative stress, ischemia, inflammation, or aging (Favatier et al., 1997) and can be an indicator of stress in cells (Sonna et al., 2002). Over expression of HSP70 confers thermotolerance in bovine, equine, ovine and chicken lymphocytes (Guerriero and Raynes, 1990). However, continuous temperature rise does not protect cellular damage due to an imbalance between various physiological and cellular functions (Patir and Upadhyay, 2010).

Among members of the HSP family, HSP70i (namely, HSPA1A and HSPA2) is the most temperature sensitive and induced by various physiological stressors, pathological stressors, and environmental stressors (Beckham et al., 2004). The expression of HSP70i is strictly stress inducible and can only be detected following a significant stress upon the cell or organisms (Satio et al., 2004). HSPA1A and HSPA2 play a crucial role in guiding conformational status of the proteins during folding and translocation (Arya et al., 2007). In the hot environmental niche, greater amount of constitutive HSP70 (HSPA8) is found during non-stress conditions. The HSPA8 assists in the day to day cell functions of protein folding and unfolding, prevention of polypeptide aggregation, disassembly of large protein complexes, and aid in the translocation of proteins between cellular compartments (Gething, 1997).

The skin being the largest organ of body protects the internal body structures from a hostile external environment from varied natural conditions (pollution, temperature, humidity and radiation). Skin also helps in maintaining water and heat loss from the body (Kierszenbaum, 2002). Skin is composed of three layers: epidermis, dermis and hypodermis. In skin, dermal fibroblasts are the most regular cell constituent of dermis (Fanny, 2004), which represent a heterogeneous population of cells defined according to their location within the dermis. Although dermal fibroblast cells constitute a major portion of skin, the information on fibroblasts and its response to heat stress, particularly on the molecular behavior of dermal fibroblast in livestock, is meager. An important understanding on physiological role of skin will help in modulating heat tolerance and improve functions under different heat load conditions. The present research will help in pin pointing the role of dermal fibroblasts in thermotolerance. Therefore, present studies were conducted to know the effect of thermal stress on skin of zebu (Tharparkar) and crossbred (Karan-Fries) cattle. Improving our knowledge on molecular mechanisms of acclimation may contribute to the development and adoption of procedures that may help in improving health and productive efficiency of animals living in hot environments.

2. Materials and methods

All the chemicals and media were purchased from Hyclone Laboratories, Inc., Logan, UT, USA, unless otherwise indicated. The chemicals required for counting of the cell number i.e. Trypan blue and Thiazolyl Blue Tetrazolium Bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). All the chemicals for the western blotting and immunostaining were purchased from Thermo Scientific, Rockford, IL, USA, unless otherwise indicated. Primary antibodies: monoclonal anti-HSP70 antibody produced in mouse was purchased from Sigma Chemical Co., St. Louis, MO, USA. FITC-labeled bovine anti-mouse IgG secondary antibodies were purchased from Santa Cruz Biotechnology, Inc., USA. Disposable cell culture grade plasticware was purchased from Nunc, Roskilde, Denmark, whereas the 0.22-μm filters were purchased from Millipore Corp., Bedford, MA, USA.

2.1. Experimental animals

Twenty heifers (10 each of Tharparkar and Karan-Fries cattle) ranging from 1 to 2 year age group were selected from the herd of National Dairy Research Institute (NDRI), Karnal, for experiments. All these animals were maintained under general management practices followed for heifers at the institute. At the time of the actual experiment, all the animals were clinically healthy and free from any physical or anatomical abnormalities.

2.2. Ethical permission

The experiment was approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the article number 13 of the CPCSEA rules laid down by Government of India. Norms regarding the ethical treatment of animals during the whole operation were strictly followed.

2.3. Isolation, culture and thermal treatment of Tharparkar and Karan-Fries dermal fibroblast

Skin biopsy specimens were aseptically taken from the ear pinna of healthy heifers in the sterile DPBS and transported to the laboratory for further processing. The tissues were washed thoroughly with Ca^{++} and Mg^{++} free DPBS and transferred into a cell culture dish containing 2 ml culture medium (DMEM/F-12 supplemented with 10% FBS, 1% gentamicin solution and 2.5 μg/ml amphotericin B). The tissue was minced into small pieces (about 1 mm in size) using a sterile surgical blade. Again, tissues were washed five times in the culture medium, and seeded into 60 mm tissue culture dishes without the addition of any medium (the small amount of medium sticking to the tissue was sufficient to nourish them till their attachment). The dishes were incubated for 4–8 h in a CO_2 incubator and observed at different intervals so as not to allow them to dry out completely. Upon attachment of tissues, sufficient culture medium was added to the dishes and incubated in a 5% CO_2 incubator at 37 °C. Tissue explants were regularly observed for proliferation of fibroblasts and were removed aseptically when a sufficient number of cells had proliferated and formed a monolayer on the cell culture dishes. After reaching 70–80% confluence, the fibroblast cells were sub-cultured by partial trypsinization. The cells were subjected to 5–6 continuous passages for selection of homogeneous population of dermal fibroblast. The dermal fibroblasts were routinely evaluated for sterility by growing them in antibiotic free media. The cells were also tested for incidence of mycoplasma contamination using Myco Alert Mycoplasma detection kit (Lonza, USA). For thermal treatment, the dermal fibroblasts were divided into the following four groups. Group 1 (control): carried out at 37 °C. The dermal fibroblasts of the experimental groups were exposed to a temperature of 25 °C (Group 2), 40 °C (Group 3) and 44 °C (Group 4) for 3 h.

2.4. Quantitative real-time PCR

Total RNA from dermal fibroblast cells was prepared using RNeasy Mini Kit (Qiagen India Pvt. Ltd.) according to the manufacturer's protocol. RNA integrity was assessed in 1.5% agarose gel electrophoresis by observing rRNA bands corresponding to 28S and 18S. Possible genomic DNA contamination in RNA preparation was removed by using the RNase-Free DNase Set (Qiagen India Pvt. Ltd.) according to the manufacturer's protocol. Purity of the RNA was checked in a UV spectrometer with the ratio of OD at λ_{260} and λ_{280} being >1.8. For each sample, about 200 ng of total RNA was used for cDNA synthesis using Revert Aid First strand cDNA synthesis kit (Fermentas, USA) by reverse transcription PCR