ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 3 (3): 238-241, 2004

Does Hsp70 Play a Protective Role in Tibial Dyschondroplasia?

Gesiane Ribeiro[1], Juliana C. Bellam[1], Marcos Macari[2], Jesus A. Ferro[1] and João Martins Pizauro Júnior[1]
[1]Departamento de Tecnologia, Faculdade de Ciências Agrárias e Veterinárias - UNESP, 14884-900, Jaboticabal, SP, Brazil
[2]Departamento de Morfologia, Faculdade de Ciências Agrárias e Veterinárias - UNESP, 14884.900, Jaboticabal, SP, Brazil
E-mail: pizauro@fcav.unesp.br

Abstract: Hsp70 was found in both normal and tibial dyschondroplasia-affected chickens suggesting that Hsp70 is expressed constitutively in the growth plate. Although the Hsp 70 content does not change significantly among the groups with lesion's degree I to III, a decrease in the amount of total protein in the tibial growth plate of chickens with tibial dyschondroplasia was observed. There was a significant difference in the levels of Hsp70 in the growth plate of the tibia ($P<0.001$) between lesion of intermediary degree and lesion of severe degree. The enhancement of compressive forces in the articular cartilage might induce the chondrocytes to express induced heat-shock protein (Hsp) 70, as a defense mechanism in bone cellular survival up to score levels III. The possibility that Hsp70 is able, depending lesion's severity, to protect and block the chondrocyte death up to score levels III, but it fails on severe lesions (degree IV), is discussed.

Key words: Apoptosis, chondrocytes, Hsp70, tibial dyschondroplasia

Introduction

Avian tibial dyschondroplasia (TD) is a disorder characterized by a defective endochondral bone formation in the cartilage of the growth plate in fast growing poultry (Orth and Cook, 1994). The disease is characterized by the accumulation of an avascular and unmineralized mass in which the cells apparently show a deviation of their normal developmental pattern from proliferative to hypertrophy phase (Praul *et al*., 1997; Pines *et al*., 1998) lacking the replacement of cartilage by trabecular bone. It has been suggested that the later stage of calcification is absent due to a disruption of the physiological cascade events necessary for chondrocytes maturation (Praul *et al*., 2000; Farquharson and Jefferies, 2000; Jefferies *et al*., 2000). This deviation from normal events leads to the accumulation of a prehypertrophic condrocytes layer that may trigger the development of tibial dyschondroplasia (Praul *et al*., 2000). Despite this possibility seems attractive, the mechanism(s) by which several factors can trigger the development of the lesion remains to be clarified.

A number of biochemical and immunohistochemical evidences revealed that a wide variety of cells can trigger the expression of Hsp70 as a protection to chemical and physical stressors (Ohtsuka and Hata, 2000; Kaarniranta *et al*., 2001).

Heat shock proteins (Hsps) play a protective role as molecular chaperones helping the cells to adapt to rapid changes in their environment and, also, play an essential role as molecular chaperones under normal conditions (Morimoto *et al*., 1994). Many conditions increasing the expression of Hsps have been found (Welch, 1993; Alexandrov, 1994). High levels of heat shock proteins accumulated both in the chondocytes of the tibial growth plate cartilage from young rats (Vanmuylder *et al*., 1997) and in condrocytes exposed to stress by high continuous hydrostatic pressure (Kaarnirata *et al*., 1998; 2001, 2003). However, it is not clear whether the Hsps might play a protective role in cellular function and survival in cartilage diseases, such as tibial dyschondroplasia.

This study was undertaken to investigate the relationship between the levels of Hsp70 and the severity of tibial dyschondroplasia in broiler chickens.

Materials and Methods

Chicks were kindly provided by Agroceres Ross® and were reared until 6 weeks of age. The bird's tibia epiphyseal growth plates were inspected *in vivo* by using an X-ray Lexiscop and classified into four degrees according to the extension of the dyschondroplasia. Degree I: lesion absence (normal); degree II: small and shallow lesion, occupying up to 25% of the epiphysis area; degree III: intermediate lesion, occupying from 25 to 50% of the epiphysis area and deeper than the previous one; degree IV: severe lesion, occupying an area higher than 50% and extending in such a way that the lesion is up to 35mm deep in direction to the metaphyses. The inspection of the femur did not reveal lesions in this bone. For each lesion's degree (treatments) ten birds (replication) were used and the lesion's degree were confirmed by visual inspection after the broilers were slaughtered.

Extraction and quantification of Hsp 70: Epiphyseal

growth plates from tibia and femur bones were dissected, cut into slices 0.1-0.2 mm thick and 1g of tissue was homogenized with 10 mL lysis buffer (20 mM Tris-HCl, pH 7.5; 9 g/L NaCl; 2 mM β-mercaptoethanol) using an ultra-turrax homogenizer, at 20,000 rpm for 3 times (30 seconds each), with intervals of 30 seconds in ice-bath. Cell lysates were centrifuged at 31,000Xg for 30 minutes at 4 °C. The protein concentration of the supernatant was determined by the procedure of Hartree (1972), using bovine serum albumin as standard. Hsp70 quantification was carried out by Western blotting as described by Givisiez *et al.* (1999).

Statistical analysis: The data of total protein and Hsp70 content were submitted to analysis of variance in a completely randomized design and differences between means were verified by Tukey's test (P<0.05).

Table 1: Total protein (mg. g^{-1} tissue) in the epiphyseal growth plates of normal and TD-affected chickens

Tibia lesion's degree (Treatments)	Total protein (mg.g^{-1} tissue)	
	Femur	Tibia
I	27.87^{ns}	26.52^{a}
II	25.40^{ns}	24.14^{b}
III	23.00^{ns}	19.40^{c}
IV	25.34^{ns}	18.12^{c}
CV (%)	18.62	7.87

No lesions were found in the femur for all TD treatments. Different letters in the columns indicate difference by Tukey's test (P<0.05). I: absence of lesion; II: small lesion; III: intermediate lesion; IV: severe lesion

Table 2: Hsp70 content (ng Hsp70. g^{-1} tissue) in the epiphyseal growth plates of normal and TD-affected chickens

Tibia lesion's degree (Treatments)	Hsp70	
	Femur	Tibia
I	9.25^{ns}	8.40^{ab}
II	9.75^{ns}	8.22^{ab}
III	9.56^{ns}	10.25^{a}
IV	8.94^{ns}	6.49^{b}
CV (%)	20.58	20.61

No lesion was found in the femur for all TD treatments. Different letters indicate difference by Tukey's test (P<0.05). I: absence of lesion; II: small lesion; III: intermediate lesion; IV: severe lesion

Results

The relative amount of protein was investigated in the epiphyseal growth plates from the tibia and femur of normal and affected chickens. There was no difference in the amount of total protein (mg/g) in the femur's growth plate (Table 1). On the other hand, there was a significant difference (P<0.05) among the treatments in the case of the tibia's growth plate. The amount of total protein decreases significantly as the lesion gets more severe, i.e., from TD degree I (health) to TD degree III (intermediary lesion), but there was no difference (P>0.05) between TD degree III and TD degree IV (lesion of severe degree).

There was no difference in Hsp 70 content between normal and femural dyschondroplasia-affected chickens (Table 2). Since the Hsp 70 content for both normal and femural dyschondroplasia-affected chickens does not change significantly, this amount might represent the constitutive form of Hsp70 found in this tissue.

There was a significant difference in the levels of Hsp70 in the growth plate of the tibia (P<0.001) when compared with the treatments showing lesion of intermediary degree (degree 3) and lesion of severe degree (degree 4). No significant effect was observed among the groups with lesion's degree 1, 2 and 3.

Discussion

The decrease in the amount of total protein (Table 1), is in agreement with several studies that have shown that the protein level decreases both with the progression of the lesion and under cellular stress (Theodorakis and Morimoto, 1987; Lammi *et al.*, 1994; Orth and Cook, 1994; Pines *et al.*, 1998; Kaarinata *et al.*, 1998). Another point that should be emphasized is that in the center of the lesion, an inadequate vascular supplementation of nutrients and oxygen to the chondrocytes will occur (Praul *et al.*, 2000; Hargest *et al.*, 1985), resulting in a low protein synthesis due to dead or dying chondrocytes (Orth and Cook, 1994).

The amount of Hsp70 in the femur epiphyseal growth plate did not change, irrespective the severity of TD (Table 2). No significant (P>0.05) effect also was observed among the groups with lesion's degree I to III (Table 2). The amount Hsp70 detected in the normal chickens suggests that this quantity represent the constitutive form. Hsp70 is synthesized in the epiphyseal growth plates from tibia and femur of normal and affected chickens (Table 2). Similar results were obtained by Otusuka *et al.* (1996), who verified that Hsp70 is synthesized in chondrocytes under stress conditions or not. In addition, it has been proposed that Hsp70 is expressed constitutively in nonstress conditions with a molecular chaperone activity, as well as playing a protecting role in the terminal differentiation of chondrocytes (Otsuka *et al.*, 1996; Trichilis and Wroblewski, 1997). The maintenance in the levels of Hsp70 until lesion's degree III (Table 2) suggests that the stabilization of Hsp70 mRNA molecules may be important in cellular protection under pathological conditions (Kaarniranta *et al.*, 1998).

The significant difference (P<0.001) of the levels of Hsp70 in the growth plate of tibia between intermediary degree (degree III) and lesion of severe may be interpreted as resulting from the accumulation of prehypertrofic chondrocytes which lost the capacity to differentiate, calcify (Berry *et al.*, 1996; Farquharson *et al.*, 1995) and synthesize high levels of Hsp70, in the tibial discondroplasia with severe lesion. Thus, the increased progressively in the amount of hsp70 from normal to TD affected chickens, could only occur by enhance hsp70 expression in different lesions degrees. This assumption is supported by the fact that according to Takashi *et al.* (1997), a positive correlation was seen between the expression of Hsp70 and the severity of the human osteoarthritis, suggesting that Hsp70 could have a critical role in the protection of the cellular stress. It has been proposed that Hsp70 can also promote hypertrophy and calcification, interrupting protein synthesis in the chondrocytes (Otsuka *et al.*, 1996). In addition, Neri *et al.* (1992) have suggested that Hsps can participate in other events involved in the calcification process. There are evidences that the Hsps are expressed differently during bone development, specifically during the endochondral ossification process (Loones and Morange, 1998).

Tibial dyschondroplasia is a disease characterized by a failure of endochondral ossification in the growth plate of growing chicken that leads to the development of nonmineralized cartilage. The severity of the pathology and size of the affected area in the growth plate varies widely. It has been suggested shown that, in the large lesion, the abnormal and nonuniform longitudinal bone growth might lead to an increased tibial plateau angle (Lynch *et al.*, 1992; Farquharson and Jeferreis, 2000). This alteration modify the mechanical properties of the growth plate and compressive forces in the articular cartilage of this tissue would occur (Farquharson and Jeferreis, 2000). As a consequence, stress on growth plate cells may induce the expression of the Hsp70 as a defense mechanism. The increase in Hsp70 levels could prevent and inhibit the apoptosis of the chondrocytes. This assumption is supported by fact that Hsp70 is accumulated in condrocytes exposed to high hydrostatic pressure ([9,14] Kaarniranta *et al.*, 1998). It has been also shown that high levels of Hsp70 could inhibit apoptosis by preventing the release of cytochrome C from the mitochondria and/or blocking the activation of the proenzyme caspase-3 (Mosser *et al.*, 2000). However, the protective role of Hsp70 against TD remains to be clarified. The findings that the Hsp70 can modulate apoptosis in the chondrocytes (Vanmuylder *et al.*, 1997) suggest that Hsp70 has an important role in regulating cellular death in chondrocytes and in the mechanisms inducing pathologic conditions such as TD.

References

Alexandrov, V.Ya., 1994. Function: aspects of cell response to heat shock. Int. Rev. Cytol, 148: 171-227.

Berry, J.L., C. Farquharson, C.C. Whitehead and E.B. Mawer, 1996. Growth plate chondrocyte vitamin D receptor number and affinity are reduced in avian tibial dyschondroplastic lesions. Bone, 19: 197-203.

Farquharson, C. and D. Jefferies, 2000. Chondrocytes and longitudinal bone growth: the development of tibial dyschondroplasia. Poult. Sci., 79: 994-1004.

Farquharson, C., J.L. Berry, E.B. Mawer, E. Seawright and C.C. Whitehead, 1995. Regulators of chondrocyte differentiation in tibial dyschondroplasia: an *in vivo* and in vitro study. Bone, 17: 279-86.

Givisiez, P.E., J.A. Ferro, M.I. Ferro, S.N. Kronka, E. Decuypere and M. Macari, 1999. Hepatic concentration of heat shock protein 70 kD (Hsp70) in broilers subjected to different thermal treatments. Br. Poult. Sci., 40: 292-6.

Hargest, T.E., C.V. Gay and R.M. Leach, 1985. Avian tibial dyschondroplasia III. Electron probe analysis. Am. J. Pathol., 119: 199-209.

Hartree, E.F., 1972. Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal. Biochem., 48: 422-7.

Jefferies, D., B. Houston, D. Lester, C.C. Withe head, B.H. Thorp, M. Botman and C. Farquharson, 2000. Expression patterns of chondrocyte genes cloned by differential display in tibial dyschondroplasia. Biochim. Biophys. Acta.,1501: 180-8.

Kaarniranta, K., C.I. Holmberg, M.J. Lammi, J.E. Eriksson, L. Sistonen and H.J. Helminen, 2001. Primary chondrocytes resist hydrostatic pressure-induced stress while primary synovial cells and fibroblasts show modified Hsp70 response. Osteoarthritis Cartilage, 9: 7-13.

Kaarniranta, K., M. Elo, R. Sironen, M.J. Lammi, M.B. Goldring, J.E. Eriksoson, L. Sistonen and H.J. Helminen, 1998. Hsp70 accumulation in chondrocytes cells exposed to high continuous hydrostatic pressure coincides with mRNA stabilization rather than transcriptional activation. Proc. Natl. acad. Sci., 95: 2319-2324.

Kaarniranta, K., M.A. Elo, R.K. Sironen, H.M. Karjalainen, H.J. Helminen and M.J. Lammi, 2003. Stress responses of mammalian cells to high hydrostatic pressure. Biorheology., 40: 87-92.

Lammi, M.J., R.I. Inkinen, J.J. Parkkinen, M. Jortikka, T.P. Hakkinen, L.O. Nelimarkka, H.T. Javerlainen and M. Tammi, 1994. Biochem. J., 304: 723-730.

Loones, M.T. and M. Morange, 1998. Hsp and chaperone distribution during endochondral bone development in mouse embryo. Cell Stress Chaperones, 3: 237-44.

Lynch, M.M., B.H. Thorp and C.C. Withe head, 1992. Avian tibial dyschondroplasia as a cause of bone deformity. Avian Pathol., 21: 275-285.

Morimoto, R.I., A. Tissieres and C. Georgopoulos, 1994. The biology of heat shock proteins and molecular chaperones. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Mosser, D.D., A.W. Caro, L. Bougert, A.B. Merin, M.Y. Sherman, M.I. Morimoto and B. Massie, 2000. The chaperone function of Hsp70 is required for protection against stress-induced apoptosis. Mol. and Cell. Biol., 20: 7146-7159.

Neri, M., F. Descalzi-Cancedda and R. Cancedda, 1992. Heat shock response in cultured chick embryo chondrocytes. Osteonectin is a secreted heat shock protein. Eur. J. Biochem., 205: 259-274.

Ohtsuka, K. and M. Hata, 2000. Molecular chaperone function of mammalian Hso7- and Hsp40- a review. Int. J. Hyperthermia, 16: 231-245.

Orth, M.W. and M.E. Cook, 1994. Avian tibial dyschondroplasia: a morphological and biochemical review of the growth plate lesion and its causes. Vet. Pathol., 31: 403-4.

Otsuka, G., T. Kubo, J. Iamanishi and Y. Hirasawa, 1996. Expression of heath-shock-proteins in the differentiation process of chondrocytes. Nippon Geka Hokan, 65: 39-48.

Pines, M., O. Knopov, S. Genina, L.C. Hurwitz, Gerstenfield and R.M. Leach, 1998. Development of avian tibial dyschondroplasia: Gene expression and protein synthesis. Calc. Tissue Int., 63: 521-527.

Praul, C.A., C.V. GAY and R.M. Leach, Jr., 1997. Chondrocytes of the tibial dyschondroplastic lesion are apoptotic. Int. J. Dev. Biol., 41: 621-6.

Praul, C.A., B.C. Ford, C.V. Gay, M. Pines and R.M. Leach, 2000. Gene expression and tibial dyschondroplasia. Poult. Sci., 79: 1009-13.

Takahashi, K., 1997. Hydrostatic pressure influences mRNA expression of transforming growth factor-beta 1 and heat shock protein 70 in chondrocyte-like cell line. J. Orthop. Res., 15: 150-8.

Theodorakis, N.G. and R.I. Morimoto, 1987. Posttranscriptional regulation of Hsp70 expression in humam cells: effects of heat shock, inhibition of protein synthesis and adenovirus infection on translation and mRNA stability. Mol. Cell. Biol., 7: 4357-4368.

Trichilis A. and J. Wroblewski, 1997. Expression of p53 and Hsp70 in relation to apoptosis during Meckel's cartilage development in the mouse. Anat. Embryol., (Berl) 196: 107-13.

Vanmuylder, N., L. Evrard and N. Dourov, 1997. Strong expression of heat shock proteins in growth plate cartilage, na immunohistochemical study of Hsp28, Hsp70 and Hsp110. Anat. Embryol., 195: 359-62.

Welch, W.J., 1993. How cells respond to stress. Sci. Am., 268: 34-41.