

## **MORPHOLOGICAL CHANGES OF INDUCED APOPTOSIS BY STRESS ON THE SECRETORY PATHWAY FROM CARTILAGE OF RAT: PRELIMINARY RESULTS.**

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Apoptosis is a type of cell death involved in ontogenesis and at pathological processes (5, 7). Several stimuli are required to activate apoptosis, which trigger morphological and biochemical changes in the organelles, which leads to the cell disintegration. The later, directs to cytoplasm disruption and fragmentation of the DNA. At present, the most important agents that have been study to induce stress on the secretory pathway including lysosomal involvement are  $H_2O_2$  (1,2,7, 8, 14), sphingosine, ceramide, brefeldin A, etoposide, staurosporin, tunicamicyn and thapsigargin (7,8,13), among others.

Apoptotic changes are link with the activation of a group of enzymes, which follows several pathways (4) such as caspases, calpains (cistein-proteases  $Ca^{++}$  dependent); and the lysosomal enzymes such as cathepsins, lysoaptase (lactoferrin of 78 kDa). (5, 6, 7, 8, 12).

Recently it has been reported the ultrastructural features of chondrocytes cell death that differs from classical apoptosis described by Kerr and Willis (9). The term of chondroptosis has been proposed (13), which main characteristics are: changes on the form of chromatin condensation; increase of Golgi Complex and Endoplasmic Reticulum (ER), the presence of autophagic vacuoles. Probably, one of the differential characteristics of chondroptosis is that it does not depend on phagocytosis, although this process of cellular detritus clearance might be present. Recently, in vitro studies indicated the phagocytic capability of chondrocytes (3).

On the other hand chondroptosis seems to follow the secretory induction pathway and probably it might be related to lysosomal enzymes (7, 14). Noteworthy, it has described a caspase-independent pathway (CICD), which involves some molecules that are related to classic apoptosis. The most common characteristics of caspase-independent cell death are: a partial aberrant condensation of the chromatin, marginalization of the chromatin around the nuclear membrane, no sign of the laddering, contraction of the nucleus, expansion of the perinuclear cisternae. Also, it was described, the presence of cytoplasmic vacuoles and occasionally, but not always, swelling of mitochondrial membranes and their cristae. In addition, darkened cytoplasm, aggregation of ribosomes, damaged cellular surface, annexin-V negative, absence of apoptotic bodies and membrane blebbing were observed (4).

The main objective of this work was the morphological and immunohistochemical observations of the changes caused in cartilages explants (femoro-tibial joint of rat) induced by chemical ( $H_2O_2$ , 50 and 100 mM by 2, 4, 6, 9, and 12 hours) and compare it with the results already reported on OA-induced rat model (11, 12) (Fig.1). The samples were processed for light microscopy using toluidin blue staining on semithin sections.

Light microscopy observations revealed chondrocytes substantial changes when normal cartilage explants were compared with  $H_2O_2$  induced explants (fig. 2).

In conclusion, we found the H<sub>2</sub>O<sub>2</sub> induced cartilage explants displayed similar morphological modifications to those described in OA-induced rat model. We speculate that chondrocytes changes describe here resembles apoptotic cell death.

#### REFERENCES

1. F, Antunes et al, *Biochem. J.* (2001) 356, 549.
2. S, Asada et al, *Inflamm.Res* (2001) 50, 19.
3. E, Castillo, J, Kourí, *Microsc Res Tech* (2004) 64, 269.
4. J, Chipuk et al, *Nature Reviews* (2005) 6, 268.
5. S, Delhalle et al *Ann. N.Y. Acad Sci* (2003) 1010, 1.
6. F, Doonan et al, *J. Neurosci.* (2003) 23,5723.
7. K, Ferri, G, Kroemer, *Nature Cell Biology* (2001) 3, 255.
8. M, Guicciardi et al, *Oncogene* (2004) 23, 2881.
9. J, Kerr et al, *Br J Cancer* (1972) 26, 239.
10. J, Kourí et al, *J Histochem Cytochem* (2002) 50, 1333.
11. J, Kourí et al, *Ultrastructural Pathology* (2002) 26, 33.
12. R, Nixon et al, *Journal Of Alzheimer's Disease* (2001) 3, 97.
13. H, Roach et al, *Apoptosis* (2004) 9,265.
14. V, Stoka et al, *JBC* (2001) 276, 3149.

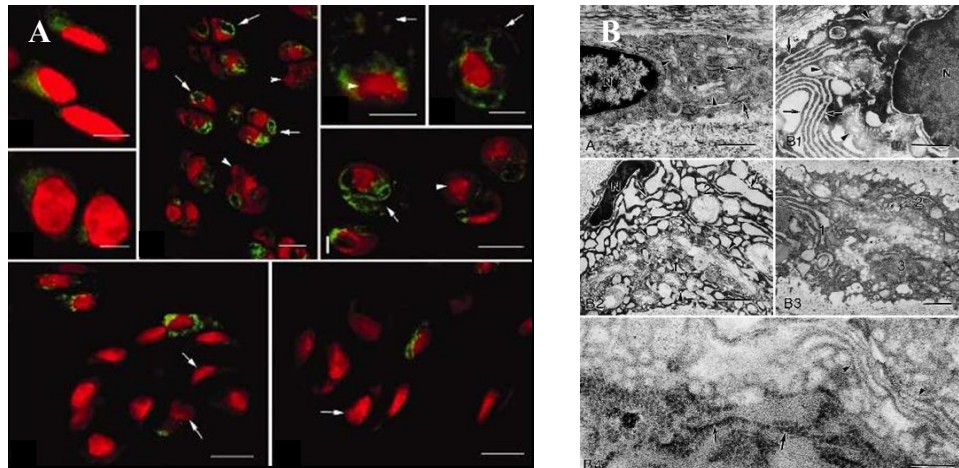


FIGURE 1. A) Immunolocalization of 58-K –FITC Golgi protein (green) from normal and OA-induced rat model. B) Ultrastructural features of Golgi Complex from normal and OA-induced rat model.

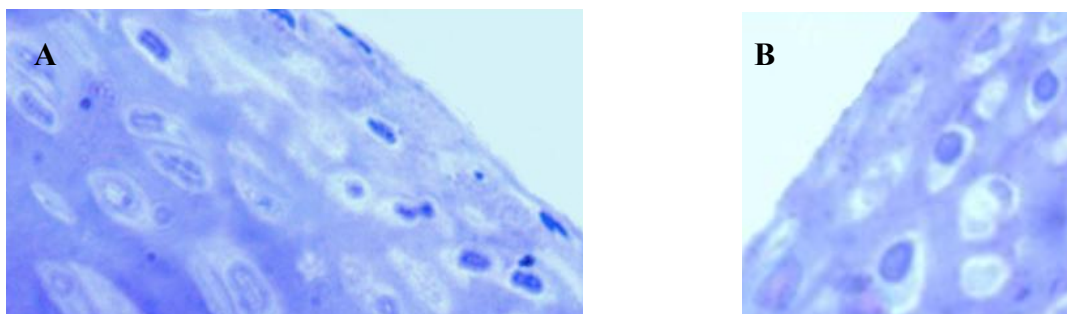


FIGURE 2. A) Semithin sections of normal cartilage B Semithin sections (toluidin blue staining) chondrocytes after induction with H<sub>2</sub>O<sub>2</sub>. Notice that H<sub>2</sub>O<sub>2</sub> induced explants displayed substantial modifications of chondrocytes, where superficial flattened cells (A) became rounded and empty lacunae were observed (B). X63.