

MORPHOLOGICALS ALTERATIONS INDICATIVE OF APOPTOSIS IN A *IN VIVO* CERVICAL CANCER MODEL AS A RESULT OF THE PHOTODYNAMIC THERAPY.

Luciana Solano Rodríguez (1), Aura Judith Pérez Zapata (1), Oliver P. Scheneider Ehrenberg (2), Ma. Esther Sánchez Espindola (1), Eva Ramón Gallegos (1). (1) Environment Citopathology Laboratory. Department of Morphology. Escuela Nacional de Ciencias Biológicas del IPN. Carpio y Plan de Ayala S/N, Col. Sto. Tomás. México, D.F. C.P. 11340. (2) Ophthalmology Institute of Conde de Valenciana. Ocular Pathology. Chimalpopoca No. 14 Col. Obrera México D.F. Email: lrodriguez@mexico.com

Photodynamic therapy (PDT) is a revolutionary treatment for cancer, citing benefits such as high specificity, minimal invasiveness and good cosmetic outcome. Consist of the administration of non-toxic photosensitizers that are retained in malignant tissues and activated by light delivered at a specific wavelength that is consistent with the absorption spectrum of the sensitizer. This activation results in a production of active molecular species. The cytotoxic properties of photodynamic therapy often result in rapid destruction of tumor cells [1]. Although the exact mechanism by which PDT produces destruction of cells and tissues is still a subject of debate, three principal mechanisms have been suggested: cellular damage of targeting (photodamage by involving the process of apoptosis), vascular damage and immunological response. However, the relative role of each mechanism may be determined by the characteristics of the photosensitizer, the nature of the tumor tissue and its microvasculature, the subcellular and tissue distribution of the photosensitizer, the type and duration of inflammatory and immune responses produced, and, finally, the treatment parameters used [2]. On the basis of experiments *in vitro*[3] we developed an *in vivo* assay using the δ -aminolevulinic acid (ALA) as inductor of protoporphyrin IX (photosensitizer) in a cervical cancer (CeCa) mouse model, in order to study cell death mechanisms for photodynamic treatment. Four groups of mice nu/nu (female 3-4 weeks) and two implants were made in mice in each leg with 1,5 million of HeLa cells by intraepithelial route appearing at 15 days. The mice with a tumor were exposed to 20 mg of ALA/kg body weight by oral route four 1 hour. Then they were exposure at 635 nm wavelength, emitted from a compact diode laser (VISULAS 690s Zeiss). The fluence rate was 300 mW/cm² and each mouse received a total dose of 150 J/cm² with 5 irradiations in intervals of 48 h [4]. Another group of mice was exposed by intraperitoneal route with dexamethasone (apoptosis inductor) to a dose of 0.5mg/20g kg body weight for 6 hours. This group was the positive control, and other group with saline solution at 0.9% was the negative control. The determination of tissue damage in the tumors was made by histology analysis. Mouse were sacrificed immediately, the tumors were removed, fixed in 10% formalin for 24 hours and cut into 3 mm-thick transverse slices. The slices were routinely processed, embedded in paraffin, and serial sections, of 4-6 μ m thickness were cut from it. The section were then stained with hematoxylin and eosin, and observed under a light microscope to find the incidence of damage [5] Other sections of tumor were fixed in 2.5% glutaraldehyde in PBS for two hours, washed in PBS during for 2 times, and immediately postfixated in 1% osmium tetroxide, in the same buffer for two hours. After dehydration in graded ethanol solutions and embedded in epoxy resin and cut into 60-90nm-thick in the ultramicrotome. The sections were examined with a JEOL JEM-1010 transmission electron microscope. After photodynamic treatment cells showed for light microscopy, classic characteristics of apoptosis; as presence of pycnotic nuclei (NP) and karyorrhexis (K) (figure 1C). The positive control (figure 1A) showed scarce cytoplasm remnants (arrows) and cells shrinkage chromosomal fragmentation as well as darkly staining chromatin (arrows) (figure 1C) in comparison with normal nuclei (N) of negative control (figure 1B). By transmission electron microscope structural alteration it was found that characterize the apoptotic death mechanism for example, in the organization of the cytoplasmic membrane, (figures 2A y 2D) the condensed chromatin and aggregated chromatin in the nuclear peripheral, the condensation and posterior fragmentation of the apoptotic chromatin are evident (figures 2A y 2C) and presence of apoptotic bodies (AB) (figure 2C). It is important to note that these images, showed cells early apoptotic, lose plasmatic membrane connection with neighbour cells before the detaching process (figure 1A). Our results indicate that the morphological criteria (apoptotic cell rounding and shrinkage) allow distinguishing the apoptosis as cell death mechanisms of photodynamic treatment. Thus, the morphological analysis under light microscopy and transmission electron microscope constitutes a very important and even decisive tool to identify the specific type of cell death unambiguously.

References

- [1] J.T, Dougherty, et al., J Natl Cancer Inst (1998) 90, 889.
- [2] P.N, Paras. Introduction to Biophotonics (2003) 433.
- [3] E.G, Ramón, et al., Arch Med Research (1999) 30,163.
- [4] V.L, Márquez, et al., Physica Scripta (2005) *in press*.
- [5] G, Clarck, et al., Methods for general tissue (1973) 417.

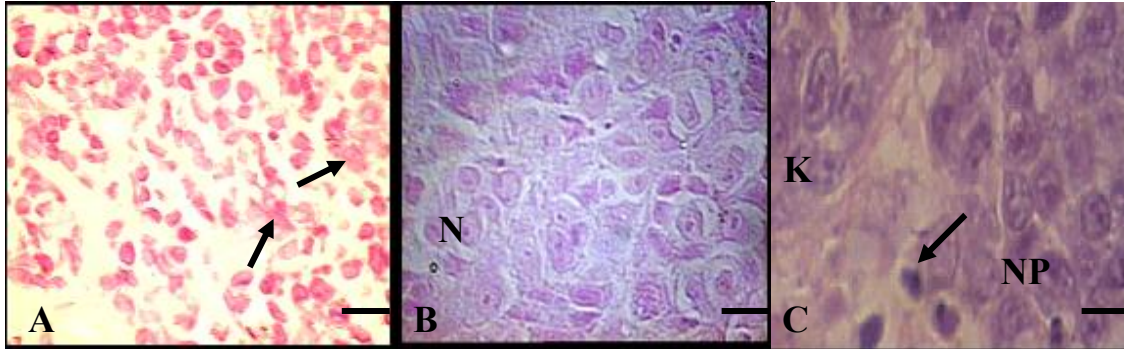


Figure 1. Bars 5 µm in A, B and C. Nucleus (N), Karyorrhexis (K), Pyknotic nuclei (NP).

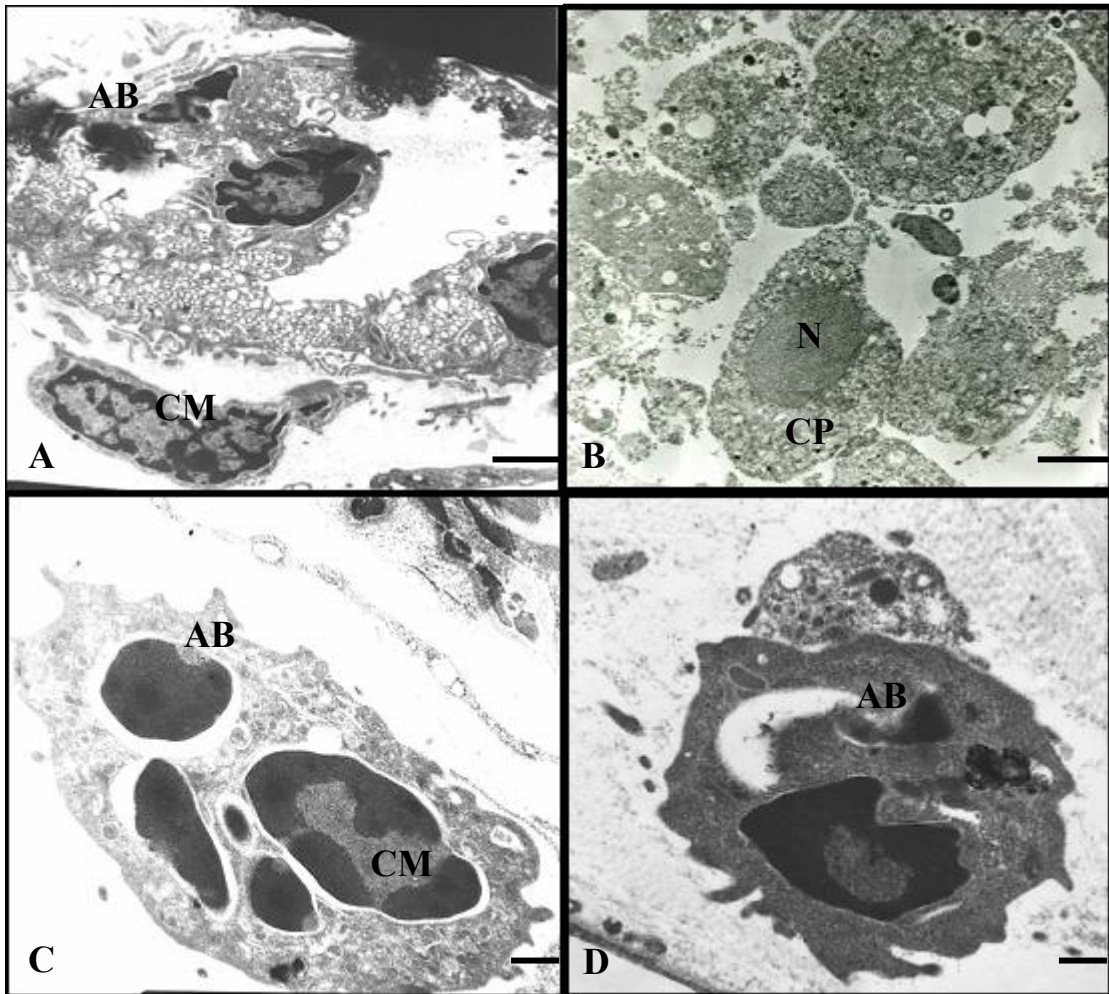


Figure 2. Bars 1 µm in A; bar = 2 µm in B; and bars = 500 nm in C and D. Cell nucleus (N), Mitochondrial (M), Cytoplasmic membrane (CP), Chromatin margins (CM), Apoptotic bodies (AB).