

## ULTRASTRUCTURE OF ADULT ISOLATED GUINEA-PIG VENTRICULAR CARDIOMYOCYTES EXPOSED TO DIFFERENT FIXERS AND BUFFERS. María Leonor Caldas(1), Yolanda Porras(1), Gustavo Gomez(2), Abdala Javier Chadid (2), Luis Alberto Gomez(2)\*.

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Myocardial injury as well as the protective action of exogenous agents can be studied by morphological changes in cardiac cells<sup>1</sup>. The use of a pure population of isolated cardiomyocytes has the advantage over complete cardiac tissue preparations, as it permits assessment of ultrastructural changes, independent of potential influences of the neighbor tissues<sup>1</sup>. In cardiac cells conservation of some morphological features such as rod-shape structure of myofibrils, smooth cell membrane and conserved mitochondria are indicators of cell viability<sup>2</sup>. In contrast, lost of these morphological features such as round-shape, hypercontraction, disorganization of myofibrils, cell membrane disruption and mitochondria damage are indicators of irreversible cardiac cell injury<sup>1-4</sup>. The consequences of the kind of fixer or buffer on ultrastructure of isolated cardiomyocytes have only been partially addressed<sup>2</sup>.

The aim of this study was to obtain the ultrastructure of fresh adult isolated guinea-pig ventricular cardiomyocytes exposed to different fixer and buffers to reveal the better conditions to study ultrastructural viability features of single adult cardiomyocytes, which is an important criterion to evaluate injury and cardioprotection<sup>1,2</sup>.

This study used calcium-tolerant adult ventricular myocytes that were freshly isolated by enzymatic dissociation<sup>1</sup>. Cell numbers were estimated using a hemocytometer and exclusion of Trypan blue (0,25%). Viable and non-viable cardiomyocytes attached to the tissue culture dishes coated with gelatin (4%) were exposed by 90 minutes and room temperature (21<sup>0</sup>C) to different fixers and buffers (glutaraldehyde 2% with tannic acid 5% or Karnovsky in cacodylate or phosphate 0.1M, pH 7.4 buffers) and processed for transmission electron microscopy (TEM)<sup>3</sup>. Single cells studied here were those classified into the first of the following three phenotypes<sup>1</sup>: (1) rod-shape with clear striations and smooth surface, which excluded trypan blue; (2) square-shaped with striations and regular surface which excluded trypan blue; and (3) round-shaped without clear striations and in contracture, which did exclude trypan blue. The protocols were selected so that the same population of cardiac cell served as its own internal control.

Sample preparation required to increase the number of attached cardiomyocytes to extracellular matrix. This was reached by incubating cardiomyocytes (about two hours) on tissue culture petri dishes previously coated with gelatin (4%) but not with Poly-L-Lysine (100ug/ml). With all fixation conditions we observed rod-shaped cells with clear striations, smooth surface, one or two central nucleus, cytoplasm with glycogen granules between myofibrils, longed and ovoid-mitochondria well defined and disposed in aggregates or in lineal way between myofibrils (Fig.1-2). The mitochondria presented clearly delimited crests disposed in transversal or fingerprint configuration. We observed very well defined Z, I, A and M bands and T tubules. Sarcomeras lengths were  $0.7\pm 0.012\mu\text{m}$  (n=8) and  $1.5\pm 0.015\mu\text{m}$  (n=6) with glutaraldehyde 2% and tannic acid 5% in buffer phosphate (Fig.1) and buffer cacodylate (Fig. 2) respectively. In cardiomyocytes exposed to Karnovsky the sarcomeras length were  $1.6\pm 0.012\mu\text{m}$  (n=8) in buffer phosphate and  $1.5\pm 0.015\mu\text{m}$  (n=10) in cacodylate buffer. Z lines with very good resolution were better observed with tannic acid in cacodylate buffer and osmium tetroxide combination which allowed good preservation and contrast respectively. This ultrastructure features were lost in death cardiomyocytes (Fig. 3 and 4).

The fine structure of isolated guinea-pig ventricular cardiomyocytes described before revealed a good ultrastructural conservation with both glutaraldehyde 2% and tannic acid 5% or Karnovsky fixers. However, the observations suggest that myofibrils are better defined in cacodylate buffer and the mitochondria in phosphate buffer under these conditions *in vitro*. Thus, the conditions described in this study allow a good characterization of some ultrastructural viability features of fresh adult isolated guinea-pig ventricular cardiomyocytes as well as the protective action of exogenous agents on single adult cardiomyocytes.

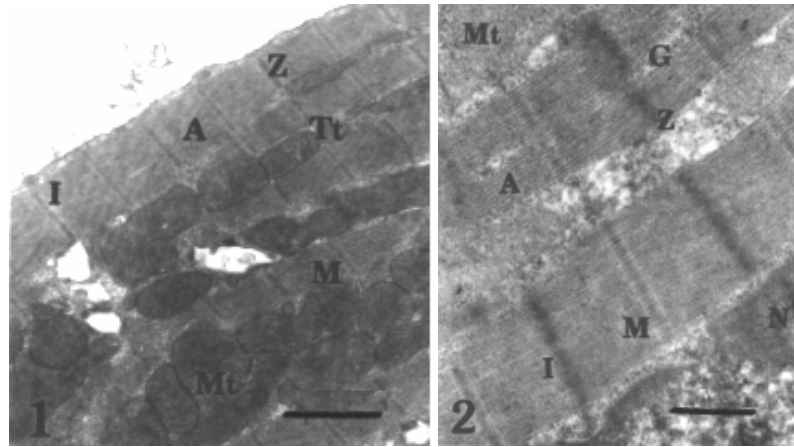


Figure 1. TEM example of a isolated ventricular cardiac cell fixed with glutaraldehyde and tannic acid in phosphate buffer, showing sarcomeras with their Z, I, A and M bands, which have  $0,7\mu\text{m}$  in length and T-tubules (Tt) at level of Z bands. Note better-defined mitochondria (Mt). Bar =  $1\mu\text{m}$ .

Figure 2. TEM example of an isolated ventricular cardiac cell from same cell population of above sample preparation fixed with glutaraldehyde and tannic but in cacodylate buffer, showing sarcomeras with their Z, I, A and M bands, which have  $1.5\pm 0.015\mu\text{m}$  ( $n=10$ ) in length, a nucleus (N), some mitochondria (Mt) and glycogen granules (G) are between myofibrils. Bar =  $0.5\mu\text{m}$ .



Figure 3. TEM example of a isolated ventricular cardiac cell fixed with glutaraldehyde and tannic acid in cacodylate buffer, showing fusion of small mitochondrias (Mt), disrupted plasmatic membrane and hypercontracted sarcomeras (head arrow). Bar =  $0.8\mu\text{m}$ .

Figure 4. TEM example of an isolated ventricular cardiac cell from same cell population of above sample preparation fixed with glutaraldehyde and tannic in cacodylate buffer, showing not very well organized and defined myofibrils (head arrow) and structures similar to blebs (arrow). Bar =  $0.8\mu\text{m}$ .

#### References

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