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Critical effects of inorganic phosphate at threshold concentrations on cultured aortic valve interstitial cells. Macroautophagocytosis versus procalcific cell degeneration

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The conventional threshold values ascribed to inorganic phosphate concentration ([Pi]) in diagnosing normophosphatemia range between 0.8mM and 1.45mM to 2.0mM [Pi].

In cultures mimicking metastatic calcification ([Pi]=3.0mM) a major role was found to be played by [Pi] (Pi-cultures) in priming a procalcific cell degeneration of bovine aortic valve interstitial cells (bAVICs), with mineralization enhancing subsequent to superstimulation with bacterial lipopolysaccharide (LPS) plus conditioned medium from cultured LPS-stimulated macrophages (Pi-LPS-CM-cultures) [1]. Here, bAVIC primary cultures were carried out which contained different [Pi] (0.4, 0.6, and 1.3mM in added solutions, i.e. 0.8, 1.3, and 2.0mM in final cultures), so including border concentrations on respect to hypophosphatemic- and hyperphosphatemic-like conditions. At 0.8mM and 1.3 [Pi] and for each incubation time (3, 9, 15, 21, and 28 days), bAVICs from Pi-cultures and Pi-LPS-CM-cultures shared common ultrastructural features showing prominent macroautophagocytosis to occur, consistently with the immunohistochemical detection of the specific marker of mature autophagosomes MAP1-LC3A. Neither cell death signs nor appearance of calcific nodules were observed. At 2.0 [Pi], most bAVICs were affected by degenerative fragmentation as described for severe metastatic-like calcifcation, i.e. the appearence of phthalocianin-positive material outcropping at cell surface, acting as hydroxyapatite nucleator and being source of real calcospherulae. Quantitative spectrophotometric estimation of calcium amounts and alkaline phosphatase activity were consistent with the ultrastructural data, with (i) similar values for Pi-LPS-CM-cultures versus Pi-cultures and control cultures, at 0.8 and 1.3mM [Pi], and (ii) significantly higher values for Pi-LPS-CM-cultures versus Pi-cultures and these latter versus controls, at 2.0mM [Pi]. Restriction of immunopositivity to caspase-8 to very few cells and complete immunonegativity to annexin-V suggested apoptosis to be a negligible epiphenomenon. In conclusion, the propensity of bAVICs to undergo procalcific degeneration resulted to correlate with [Pi] in such a manner that a differential discrimination of this parameter within the conventional normophosphatemic range is suggested for a proper evaluation of the risk for dystrophic valve calcification. Moreover, bacterial-derived inflammation seems to be regarded as an effective trigger for the higher normophosphatemic [Pi].

References

[1] Bonetti A, Della Mora A, Contin M, Tubaro F, Marchini M, Ortolani F. (2012). Anat Rec 295: 1117-1127.

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