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Assessing of the role super oxide, nitric oxide and redox metals in apoptotic death of the mammalian cells in culture

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The endogen-produced radicals such as NO and O_{-2} play the crucial part in the regulation both physiology and pathophysiology processes. The relationship between these radicals is determinant in the cytotoxic mechanism of the oxidative stress. The aim of this study was to investigate the interactions between these radicals and cytotoxicity using flow cytometry analysis and fluorescence indicators NO (DAF 2 DA), O_{-2} (dihydroethydine), donors NO (GSNO), redox agents (ascorbic acid AA), H_2O_2 and chelators redox metals (o-phenantrolin). This work was carry out on the three lines cells endotheliocytes ECV-304 (eNOS) and carcinoma cells HeLa-G63 (iNOS) and PC 12(cells of neuroendocrine tumor of rats). Previously, we found that in cultured human endotheliocytes ECV 304 the intracellular levels superoxide O_2 and nitric oxide NO were lower than in carcinoma cells HeLa G-63. Comparative analysis of changes in the intracellular levels of superoxide and NO induced by ascorbic acid revealed a negative correlation between NO and O_{2-} levels, whose strength depended on concentration of the acid. Exposure of the cells to 0.5 and 1 mM AA did not induce the apoptotic death in both cell lines. In contrast, the cell fate dramatically changed at greater AA concentrations starting from 20mM. We showed the potency of AA at high concentrations to induce apoptotic death in tumor cells. The differences in cytotoxicity of AA in high concentrations towards the human carcinoma cells and endotheliocytes were revealed. On the PC12cells was estimating of the governing mechanisms of the cytotoxicity of the oxidative stress and the role of the amyloids in increasing this stress. Using flowcytometric assessment of the cytotoxicity H_2O_2 and fragment β-amyloid (Aβ) peptide (25-35) has been shown the dose-dependent increasing of the quote of the cells with DNA content <2c. Isoeffective consentrations were 1мМ H_2O_2 and 5 мкМ Aβ. The cytotoxicity H_2O_2 and Aβ were accompanig with the increasing of the intracellular level of O_{-2}. The treatment of the cells GSNO (donor of NO) and o-phenantrolin (chelators of Fe ions) significantly decreased the intracellular level of O_{-2} as well as the cytotoxicity H_2O_2 and Aβ . Thus, in direct experiments has been shown the part of amyloids in the increasing of the oxidative stress and participation of the reactive oxide radicals in the cytotoxic effect of the Aβ. The addition argument which confirmed contribution of the oxidative stress in the cytotoxic effect of the Aβ were the similarity of the cellular response on the action of the oxidative agent - H^2O^2 and Aβ

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