Cell Death Induced by Phenothiazinas is Mediated by Reactive Oxygen Species Generation and Thiol Oxidation in MDR positive Human Leukemia Cells

Santos, V.M.¹, Paredes-Gamero, E.J.², and Rodrigues, T.¹
¹Centro de Ciências Naturais e Humanas, UFABC, São Paulo-SP, Brazil; ²Depto. de Bioquímica, UNIFESP, São Paulo-SP, Brazil.

INTRODUCTION: Phenothiazines (PTZ) are drugs used in the treatment of schizophrenia that exhibits several other biological properties, including the induction of cell death and the reversal of MDR. In this work it was investigated the involvement of oxidative stress in the PTZ-induced cell death in MDR positive K562-Lucena cells.

MATERIAL AND METHODS: Cell viability was evaluated by the MTT reduction test and trypan blue exclusion assay. It was also performed the annexin V-FITC/PI double-staining flow cytometry and LDH release analysis. The reactive oxygen generation was estimated by cleavage of CM-H₂DCFDA and the protein thiol and glutathione oxidation were measured by using 5,5'-dithiobis-nitrobenzoic acid and o-phthaldialdehyde, respectively.

RESULTS AND DISCUSSION: PTZ derivatives thioridazine (TR), trifluoperazine (TFP) and fluphenazine (FP) incubated for 24h with K562-Lucena cells (1x10⁵) promoted a concentration-dependent decrease of cell viability. The IC₅₀ values ranged from 25 to 27 µM depending on the PTZ derivative structure with TR being the most potent. Differently from K562 cells, flow cytometry analysis showed a PI positive labeling associated to the LDH release indicating the predominance of necrosis. The PTZ-induced cell death in K562-Lucena cells was preceded by the increased of reactive oxygen species generation, protein thiol oxidation, and glutathione depletion.

CONCLUSION: The PTZ-induced cell death in MDR positive K562-Lucena occurs predominantly via necrosis and it is associated to the increase of reactive oxygen species generation and the unbalance of thiol redox homeostasis.

Keywords: K562-Lucena, Necrosis, Oxidative stress, Phenothiazines
Supported by FAPESP, CNPq and CAPES