Phenothiazine-Induced Cell Death in HTC Hepatoma Cells Are Associated to Mitochondrial Dysfunction

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Phenothiazines (PTZ) are anti-schizophrenic drugs that present other interesting but unrelated properties. Recent studies showed that PTZ exhibit pro-apoptotic activity, although their cytotoxic mechanisms remain unclear. In this study, we investigated the effects of five structure-related PTZ derivates chlorpromazine (CPZ), fluphenazine (FP), thioridazine (TR), trifluprom azine (TFPZ), and trifluoperazine (TFP) on the viability of HTC (hepatoma tissue culture) cells, exploring possible mechanisms of action. All PTZ derivatives were able to decrease the viability of HTC cells in a concentration-dependent manner. Cytotoxicity increased in the following order: TR > TFP > FP > TFPZ > CPZ, with the IC_{50} values ranging from 46.7 to 102 µM. Flow cytometry analysis showed the occurrence of the necrotic and apoptotic cell death. The addition of PTZ to digitonin-treated cells promoted an immediate dissipation of the mitochondrial transmembrane potential ($\Delta\Psi$). Besides, PTZ induced a time-dependent oxidation of protein thiol groups and reduced glutathione. It was also observed LDH release suggesting the occurrence of a permeabilization of plasma membrane. Interestingly, the pre-incubation of the cells with Ca^{2+} chelators potentiate the cytotoxic effects of PTZ and the cathepsin inhibitor E-64 had no effect. Our results showed that PTZ were able to induce cell death in cultured HTC hepatoma cells and the structure-activity analysis revealed that the piperidinic derivatives presented the major cytotoxicity followed by piperazinic and aliphatic derivatives. The $\Delta\Psi$ dissipation and –SH oxidation point to the involvement of mitochondrial permeability transition in the PTZ-induced cell death in these cells, apparently without participation of lysosomal permeabilization. The effects of Ca^{2+} chelators are under investigation in our lab. Supported by FAPESP, CNPq and FAEP-UMC.