Donepezil exerts cytotoxic effect on glioma cells

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Donepezil is a highly selective reversible inhibitor of acetylcholinesterase (AChE) that has been used to treat Alzheimer's disease (AD) due to its neuroprotective effects. There are a few studies that show its cytotoxic effect on cancer cells, but the mode of its action is poorly understood. In the present study, we investigated the mechanisms of potential cytotoxic effect of donepezil on C6 (rat glioma) and U251 (human glioma) cell lines. The viability rate of C6 and U251 cell lines was determined with the Cristal violet and MTT assay after 24h. Morphological changes were followed using light microscopy. Production of the reactive oxygen species (ROS), caspases activity, externalization of phosphatidylserine and the presence of the acid cytoplasmic vesicles were measured by Flow cytometry using specific fluorochromes (DHR, apostat, annexin-propidium iodide, and Acridine orange, respectively). In order to silence autophagy, U251 cells were transfected shRNA targeting human LC3II and AMPKα 1/2 genes. Donepezil decreased viability of both cell lines in dose dependent maner. When applied in its IC50 concentration donepezil triggered oxidative stress which has led to the caspase activation and the increased number of double-positive Ann/PI cells indicating the induction of apoptosis. In addition, donepezil induced autophagy since it increased the presence of the acid cytoplasmic vesicles (quantified as an increase of the orange-FL3/FL1 fluorescence, compared to the control). However, the production of ROS was decreased in AMPK and LC3II knockout cells, pointing out that oxidative stress triggers autophagy in U251 cell line. Based on the given results, it could be concluded that donepezil exerts its cytotoxic effect by inducing oxidative stress that causes both, apoptotic and autophagic cell death of C6 and U251 cells.