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Insights on D-aminoacid oxidase - induced cell death in cancer cell lines

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Introduction. D-amino acid oxidase (DAAO) is an enzyme that, when is activated by a D-amino acid, is able to induce cell death in cell lines and primary cultures from tumours that are highly resistant to current therapies, such as exocrine pancreatic cancer, colon cancer and glioblastoma. DAAO-induced cell death is mediated by free radicals and the consequent DNA damage. However in non-tumour cell lines, DAAO has a minimal or no effect as compared with tumour cell lines. We have investigate the mechanism of DAAO-induced cell death in different tumoral cell models.

AIMS. To determinate the mechanism by which DAAO generates cell death in tumours from colon, pancreas and glioblastoma.

Methodology. 2U/ml of DAAO and 1mM of D-Alanine were used in all treatments. The permeability of the plasma membrane was evaluated using a lactate dehydrogenase enzyme assay. Different types of cell death inhibitors were tested: necrostatin, chloroquine, spautine and a general caspase inhibitor. Two calcium chelators have also been used: EGTA (extracellular) and BAPTA/AM (intracellular). The effect of these compounds on death induced by DAAO has been evaluated in viability analysis and cell cycle analysis by flow cytometry. The mitochondrial membrane potential has been studied both by fluorescence microscopy and by flow cytometry.

Results. None of the different types of programmed cell death inhibitors were able to block DAAO-induced cell death in colon and pancreas cell lines, interestingly DAAO-induced cell death was blocked in gliobastoma primary cultures by a general caspase inhibitor. On the other hand, DAAO treatment produced a break on plasma membrane permeability in colon and pancreas cell lines as determined by the lactate dehydrogenase enzyme assay, however, this effect is not observed in glioblastoma primary cultures. The mitochondrial membrane potential has also been study and our results show that it is affected in all cell lines and primary cultures sensitive to the effects of DAAO. Finally, we have determined whether the movement of calcium inside the cell is or not necessary for the cell death induced by DAAO in our cellular models. Our results show that the cDAAO-induced cell death in pancreatic and colon carcinoma cell lines is a necrosis, by the contrary in primary cultures of glioblastoma DAAO-induced cell death seems a classical apoptosis.

Conclusion. DAAO-induced cell death depends of the cellular context, inducing a necrotic mechanism in pancreatic and colon carcinoma and an apoptotic mechanism in glioblastoma.

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