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D-aminoacid oxidase nanoparticles induced cell death in cancer cell lines.

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Introduction. D-amino acid oxidase (DAAO) from *Rhodotorula gracilis* is an enzyme able to catalyze D-amino acids producing H_2O_2 as a side molecule. Hydrogen peroxide is a potential generator of reactive oxygen species (ROS), which produce oxidative stress in cells. The main advantage of this enzyme is that its substrate, D-amino acids, is not present in cell proteins, which will allow to regulate its activity by D-aminoacid addition. DAAO could be immobilized in nanoparticles to facilitate its specific vehiculation towards the tumour. We have used DAAO tag with a Clyt-A domain, which has high affinity for choline and its analogues, such as diethylaminoethanol (DEAE).

Objectives. The main aim is to combine nanotechnology with an enzymatic therapy, based on the use of DAAO, to treat tumours that are resistant to current treatments.

Methodology. The Clyt-DAAO protein has been obtained from transformed *E. coli* cells. In all cell lines, the concentration used for the treatments was 2U/ml of DAAO and 1mM of D-Alanine. DAAO has been used both free and immobilized in magnetic nanoparticles (200nm) coated with starch and functionalized with DEAE. Cell viability assays and flow cytometry analysis of cell cycle distribution have been performed to study antiproliferative capacity in cell lines and primary cultures from highly chemo-resistant tumours (pancreatic carcinoma, colon carcinoma and glioblastoma). The antiproliferative effect of DAAO has also been evaluated on non-tumour cells (pancreatic and fibroblasts). The levels of endogenous free radicals have been measured with 2',7'-Dichlorofluorescein diacetate (DCF-DA) and DNA damage has been measured through the histone H2A.X phosphorylation.

Results. Free DAAO, as well as immobilized in magnetic nanoparticles, is able to induce cell death in most cell lines and primary cultures tested, although some of them have shown total or partial resistant. In contrast, in the non-tumour cell lines the effect of DAAO is much lower. In tumour cell lines, a minimal exposure to activated DAAO (15 minutes) followed by 24 h incubation in the absence of the enzyme, is able to evoke the effects of longer exposure to the enzyme. The first effects observed, is a cell cycle block in G2/M phase, later on the blocked cells move to the sub G1 phase of cell cycle indicating that they are in a cellular death procedure. DAAO immobilized in magnetic nanoparticles shows a more powerfull effect probably due to a higher stability at 37ºC than free DAAO. Finally, cell death generated by DAAO is mediated by free radicals and DNA damage.

Conclusion. Combination of an enzyme therapy with DAAO immobilized in nanoparticles may be an efficient alternative to the conventional treatments against highly resistant tumours.

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