Molecular hybrids for NO-photodelivery

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Nitric oxide (NO) is an endogenous messenger involved in many physiological and pathophysiological processes. In cancer biology, low levels (pM-nM) of NO promote cancer growth, while high levels (µM) reduce cancer progression. The photogeneration of NO achieved using NO photodonors (NOPDs), namely, compounds able to release NO under the action of the visible light, has received a great attention as potential new anticancer therapy. Another relevant issue in anticancer therapy is the targeting of mitochondria. Low levels of NO in these organelles up-regulate cellular respiration and stimulate mitochondrial biogenesis. On the contrary, higher levels of NO induce toxicity through the inhibition of crucial mitochondrial enzymes, the production of reactive nitrogen (RNS) and oxygen (ROS) species and consequently, mitochondrial apoptosis. The aim of my Ph.D. project was the development of new hybrid molecules able to accumulate into mitochondria and release NO upon blue light irradiation. Thus, NOPDs were linked to vectors that display high tropism for these organelles, as Rhodamine B and alkytriphenylphosphonium chains of variable length.[1,2] Although these compounds were able to release enough NO to perform an anticancer activity, with the purpose of further increasing the antitumoral efficacy, a novel NO-photoresponsive polymeric platform as an enhancer of doxorubicin delivery was developed. An amphiphilic block-copolymers has been designed and synthesized as the drug carrier, via friendly Ring-Opening Polymerization (ROP), and thoroughly characterized. As monomers lactide and an in-house prepared cyclic carbonate bearing a boc-protected amine, named tert-butyl (2-oxo-1,3-dioxan-5-yl) carbamate (referred as CT) were used, while as macroinitiator methoxy-polyethylene glycol with molecular weight 5000Da (mPEG5000). The amine groups present in this polymeric system were deprotected and activated on-demand and enabled the introduction of a variety of drugs. Namely, two grafted-polymers were produced via N,N'-disuccinimidyl carbonate (DSC) chemistry, coupling doxorubicin and NOPD separately, and then formulated in nanoparticles by the means of solvent displacement technique. Comparing free doxorubicin, DOXO-nanoparticles and the mixed micelles (formed by the coprecipitation of NOPD-polymer and DOXO-polymer) both in dark (normal lab conditions) and light (under blue light irradiation) conditions, a remarkable enhancement in killing activity against lung, intestine, and skin cancer cell lines was observed when the mixed micelles were tested. The intra-cell colocalization study of each nanoparticle formulation was also performed by exploiting the intrinsic fluorescence in the red range of cyanine-5 previously coupled with the same polymeric platform and then co-self-assembled in the same nanoparticles. The cell-uptake assay has highlighted that all formulations were, to some degree, trafficking to the lysosomes during the endocytosis with an absence of cyanine-5 signals in nuclei. The next step will be to clarify if the enhanced anticancer activity is derived by the co-administration of two cytotoxic molecules (NO and doxorubicin) or is given to a synergic effect between the DOXO-polymer and NOPD-polymer.

[1] Sodano F., Gazzano E., Fraix A., Rolando B., Lazzarato L., Russo M., Riganti C., Fruttero R., Gasco A., Sortino S. *ChemMedChem.* **2018**, *1*, 87-96.
[2] Sodano F., Rolando B., Spyrakis F., Failla M., Gazzano E., Lazzarato L., Riganti C., Fruttero R., Gasco A., Sortino S. *ChemMedChem.* **2018**, *12*, 1238-45.