LOS-decorated liposomes as stealth delivery system for a Ruthenium anticancer complex

A. Capuozzo[1], F. Acampora[2], A.M. Marzaioli[2], M. Piccolo[1], C. Irace[1], C. De Castro[2], D. Montesarchio[2], L. Paduano[2], R. Santamaria[1]

[1]Dept. of Pharmacy, University Federico II of Naples
[2]Dept. of Chemical Sciences, University Federico II of Naples

In the last decade liposome technology for drug delivery has progressed from conventional vesicles to 'second-generation liposomes' in which a long life-time is obtained by modulating the lipid composition, size, and charge of the vesicle, as well as by surface functionalization strategies [1]. In line with our project [2, 3], we have developed a novel amphiphilic liposome that includes AziRu, a Ruthenium(III) complex inspired to NAMI-A, that proved to be a promising alternative to platinum complexes [4]. The surface decoration includes a partially delipidated lipoligosaccaride (LOS) extracted from *Rhizobium Rubi* bacterium so as to achieve a design similar to Gram-negative bacterial cell wall. For *in vitro* bioscreen, a panel of healthy and cancer cells were treated with a range of concentrations of LOS decorated liposomes. IC_{50} values for Ruthenium free LOS-liposome were always higher than 10^3 μM, suggestive of a virtually null cytotoxicity and of biocompatibility. Conversely, LOS-liposome up-loaded with AziRu exhibited selective cytotoxicity against highly proliferative malignant cells, such as human MCF-7 adenocarcinoma cells. Interestingly, no significant cytotoxicity has been detected on non-cancer control cells. This data is in accordance with the assumption that Ru(III) complexes are converted in the active Ru(II) complexes exclusively in cancer cell environment [5]. Cellular uptake experiments, investigated by fluorescence microscopy, have shown that LOS-liposomes are efficiently and rapidly incorporated. The similar uptake kinetics on both normal and cancer cells, compared to the different bioactivity profiles, provide further evidence of a specifically anticancer action of Ruthenium. Moreover, to evaluate the antigenic skill of LOS decorated liposomes, we investigated macrophages activation by means of nitric oxide production and iNOS induction in J774 murine macrophages. Although fluorescent microphotographs reveal cellular internalization of LOS-decorated liposomes in J774 cells, they showed no evidence of activation responses to environmental stimuli. Taken together, these results show that the LOS-liposome is a good stealth system for delivery of Ru-complex. *Ad hoc* modifications have led to liposomes exploiting the same benefits of the bacterial cell wall, such as stability and life-time, but at the same time devoid of the drawback of being recognized by the immune system, in order to promote an extend blood-circulation time.

1.Gill KK, J Drug Target. 2014; 30:1-10.
2.Mangiapia G, Biomacromolecules. 2013;14(8):2549-60
3. VitielloG., J. Mater. Chem. B 2015; 3:3011--3023
4. Bergamo A, J Inorg Biochem, 2012;106 (1):90-9
5. Brabec V, Drug Resist Updat. 2006;9(3):111-22