Anti-tumor efficacy of doxorubicin nanosponges on breast cancer models *in vitro* and *in vivo*

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Doxorubicin (DOX) is an anthracycline widely used in cancer therapy and in particular for breast cancer treatment. Multidrug resistance (MDR) is one of the major unsolved problems regarding several anticancer drugs included DOX. Moreover, although the treatment with DOX appears successful, many important side effects such as cardiotoxicity are connected to the use of the drug and lead to several limitations in clinical application. A system for the delivery of DOX may overcome these disadvantages, since nano-vehicles allow the solubilization of hydrophobic drugs, the reduction of total dose administrated, and the prevention of side effects. Indeed, nano-delivery systems can accumulate in tumors owing to the enhanced permeability and retention effect (EPR). Cyclodextrins (CD) are cyclic Î±-1,4-glucans that can form inclusion complexes to host a wide range of hydrophobic molecules. β-CD nanosponges (BNS) constitute a novel nanosized delivery system composed in a three-dimensional network, being able to incorporate also lipophilic molecules. A large number of CD derivatives have been proposed for drug delivery, and they have improved the bioavailability and increased the aqueous solubility and stability of the hosted drug, as we demonstrated for the delivery of campthotecin in prostate cancer. The aim of this work was to evaluate the effects of a new formulation of DOX (BNS-DOX) in *vitro*, on several cancer cell lines (pancreas, breast, ovarian and melanoma), and *in vivo,* on a transgenic mouse model of breast cancer. It has been shown that BNS-DOX significantly reduced cell proliferation with higher effectiveness than free DOX in all the tested cell lines and also in an ovarian cancer cell line resistant to DOX. Moreover, BNS-DOX was more effective than DOX in reducing the proportion of G1, S and G2 cells and increasing cell death at lower doses in breast cancer cells, as demonstrated by cell cycle, caspase 3 activity and annexin-V positive cells evaluation. The higher antitumor potential of BNS-DOX may be due to its easier internalization in cancer cells, inducing enhanced cellular uptake and cytotoxicity in cancer cell lines (BNS-DOX IC_{50} was 2-57 times higher than DOX IC_{50}, depending on different tested cell lines). For the *in vivo* study BALB-neuT mice have been investigated, since they represent a real model of human HER2 positive breast cancer displaying a histopathologically and transcriptionally course that closely recapitulates many features of human breast carcinogenesis. BNS-DOX used at a dose (2 mg/kg) five time lower than the therapeutic dose of DOX (10 mg/kg), substantially reduced the growth of breast cancer in mice (60% of inhibition), while free DOX was completely inefficient. It has been observed also a reduction in tumor neoangiogenesis and lymphangiogenesis, as evidenced by staining of tumor sections with anti-CD31 and anti-Lyve-1 antibodies, without evident side effects. Biodistribution studies revealed a higher accumulation of BNS-DOX in the tumor site and a lower distribution in the heart tissue compared with free DOX. In conclusion, BNS-DOX revealed their effectiveness at inhibiting tumor progression since they are able to increase the stability of DOX, to display a higher accumulation inside the tumor tissue, due to EPR effect, and to strongly reduce cardiotoxicity. Taken together, our findings demonstrated that BNS may be an efficient strategy for drug delivery in the treatment of a wide panel of cancer cell lines and in particular breast cancer.

Humber CE, et al. Ann Oncol. 2007. 18(3):409-20.
Jeong YI, et al. Arch Pharm Res. 2011. 34(1):159-67.
Hobbs SK, et al. Proc Natl Acad Sci USA. 1998. 95:p. 4607-12.
Trotta F, et al. Expert Opin Drug Deliv. 2014. 11(6):931-41.
Gigliotti CL, et al. J. Biomed. Nanotechnol. 2016. 12: 114-127.
Minelli R, et al. Eur J Pharm Sci.2012 20;47(4): 686-94.
Iezzi M, et al. Translational Animal Models in Drug Discovery and Development. 28: 139-166.