NANOEMULSION BASED CHEMOTHERAPY FOR MELANOMA

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Introduction: If timely diagnosed, melanoma can be treated by surgery in early stages, allowing a complete recovery. If neglected or unidentified, melanoma can mature up to IV stage, which is the most critical and in most cases leads to death. After 4years, life expectation in IV stage melanoma is 11,1% and 28,1% in patients treated respectively with standard mono-therapies and immunotherapies. Consequently, the landscape of current treatment has changed rapidly in the last few years. However, current pharmacological therapies are only palliative care. Therefore, the improvement of current chemotherapy is worthy of investigation, including novel strategies for the delivery of new and old drugs. A nanotechnology based poly-chemotherapy is here proposed to treat IV stage melanoma, by loading combinations of drugs within a marketed nanoemulsion for total parenteral nutrition (Intralipid), aiming to personalized nanomedicine. Temozolomide (TMZ, a cytotoxic agent), bevacizumab (BVZ, an anti-VEGF monoclonal antibody) and rapamicyn (an autophagy modulator) were co-loaded in Intralipid. An active lipophilic prodrug of TMZ was employed, while BVZ was lipophilized owing to Hydrophobic Ion Pairing (HIP).

Materials and methods: Drug entrapment efficiency was demonstrated owing to Size Exclusion, Field Flow Fractionation (FFF), Electrophoresis and Transmission Electron Microscopy (TEM). To test the response in vitro of cells to the different drugs and drug combination, the cell viability was evaluated by MTT assay on melanoma cells; both human (M14and PCF-2) and mouse (B16-F10) cells have been employed, in order to allow translation to the animal model. Cell motility was assessed by Boyden chamber assay. The effect on angiogenesis was evaluated in endothelial tube formation assay. In vivo efficacy was assayed in a melanoma mouse model, obtained by B16/BF10 cells implantation in C57/BL6mice. Tumor tissues were evaluated by immuno-hysto-chemical analysis for mytotic index and angiogenesis.

Results: Encouraging entrapment efficiencies for the three co-loaded drugs were obtained after size exclusion of the formulation. In particular entrapment of BVZ, a high molecular weight monoclonal antibody, was assessed by electrophoresis, and confirmed, after fluorescent labelling (FITC-BVZ), through FFF separation, fluorimetry and fluorescence microscopy. In TEM, evidences of association between condensed BVZ and Intralipid droplets were also shown. Drugs loaded in Intralipid exerted larger effects compared to free drugs, when cytotoxicity, migration and angiogenic activity were evaluated. Synergism between the drugs co-loaded in Intralipid (Co-Intralipid) was also demonstrated. These evidences were confirmed by *in vivo* experiments, showing that tumor growth was significantly reduced by Co-Intralipid treatment with respect to free drugs; CD31and Ki-67indicated tube formation inhibition and reduced cell proliferation in the Co-Intralipid treated tumors.

Discussion and conclusion: The superiority of the poly-chemotherapy vs mono-therapy, as well as of Co-Intralipid vs free drugs has been demonstrated, owing to pharmacological synergism and fast cell internalization. Probable reasons for synergism include: overcoming of intrinsic chemoresistance to TMZ (PTEN loss) in melanoma cells and reduced expression of VEGFR in endothelial cells, both due to RAP inhibition of AKT pathway. Taken together our data demonstrate that our new formulation can meet some important issues related to IV stage melanoma treatment, such as lowering of drug therapeutic doses and improving therapeutic efficacy.