SOLID LIPID NANOPARTICLES CARRYING TEMOZOLOMIDE FOR MELANOMA TREATMENT

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Melanoma is an aggressive cancer with a very poor prognosis and there is a clinical need to develop a systemic therapeutic strategy for the treatment of this tumour. Temozolomide (TMZ) is an alkylating agent used as anticancer drug. It is a small molecule, that can be rapidly absorbed by the gastro-intestinal tract and can pass through the blood-brain barrier, and therefore TMZ can be used in glioblastoma pharmacological adjuvant therapy. For the same reason it is also employed for the treatment of malignant melanoma in order to prevent brain metastasis. TMZ is very stable at acid pH of the stomach, but when at neutral pH, it is hydrolyzed spontaneously to its active metabolite (MTIC), which quickly turns into its reactive form able to generate the DNA damage. This activation of TMZ can lead to drug instability and rapid clearance, thereby causing accumulation of MTIC in non target tissues. Therefore, an increase in TMZ dosage would be necessary to achieve therapeutic effect, also to overcome problems due to tumour cell resistance against the drug. However, derived side effects limit its use in clinical application and lead to the development of new therapeutic approaches, such as drug delivery.

The aim of this work was to study a new system for the delivery of TMZ, based on solid lipid nanoparticles (SLN) that are biocompatible and able to incorporate drugs. TMZ was encapsulated in SLN, as a dodecyl ester derivative (TMZ-C12), since this approach should allow to protect TMZ from the aqueous environment. Subsequently, the effects of TMZ-C12 loaded SLN on the progression of melanoma were evaluated in vitro and in vivo.

TMZ-C12 loaded SLN were obtained through fatty acid coacervation, an approach used to allow a more controlled hydrolysis of TMZ that occurs at neutral pH. SLN have been tested on human lymphocytes in order to assess if they would display a cytotoxic effect, but they showed to be safe and biocompatible, since a decrease in cell viability was not observed after treatment. Subsequently, MTT assay was performed on human (A2058 and M14) and mouse (B16F10) melanoma cells, where TMZ-C12 loaded SLN were able to exert a significant higher inhibition of proliferation (50% at 50 and 25□g/ml; 30% at 10□g/ml; 15% at 5□g/ml) than free TMZ (30%, 15% and 5%, respectively). These results were confirmed by clonogenic assay. Moreover, tube-formation assay was performed on human umbilical vein endothelial cells (HUVEC) after treatment with TMZ-C12 loaded SLN in order to investigate their effect on angiogenesis in vitro. Obtained results showed an inhibition of tube formation exerted by TMZ-C12 loaded SLN at concentration 25, 10 and 1□g/ml, while free TMZ evinced an inhibition only at the concentration 25□g/ml. After having demonstrated TMZ-C12 loaded SLN inhibition effect on cell proliferation and angiogenesis, in vivo analysis was carried out in order to confirm the efficacy of this formulation. C57BL6/J mice were injected with B16F10 cells and ten days after melanoma induction they were treated for two weeks with empty SLN, free TMZ or TMZ-C12 loaded SLN (0.82 micromoles/animal). TMZ-C12 loaded SLN reported great evidence of efficacy, since they

were able to reduce tumor growth and neo-angiogenesis, compared to free TMZ (57% of tumour volume reduction vs 17%; 45% vs 23% of tumour weight reduction), as afterwards demonstrated by histological and immunohistochemical evaluation. Promising obtained results allow to hypothesize that an innovative system based on SLN could be a great strategy for the delivery of TMZ, allowing an increased stability of the drug and thereby its employment in the treatment of aggressive melanoma.

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