Camptothecin in nanosponges as new therapeutic delivery agent in prostate cancer: *in vitro* and *in vivo evaluation*

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Camptothecin is a pentacyclic quinoline alkaloid that exerts a potent DNA Topoisomerase I inhibitory effect with potent antitumoral activity in haematological and solid tumors. However, it did not reach the clinical use because of its poor solubility and high degradation rate. We have previously shown that camptothecin encapsulated in β-cyclodextrin nanosponges (CN-CPT) can overcome CPT chemical disadvantages and ameliorate the antitumoral efficacy in vitro. The aim of the present study was to examine the impact of CN-CPT on the ability of human prostate cancer cell to complete key steps in the metastatic process, including invasion and angiogenesis. PC-3 and DU145 cells were treated with CN-CPT and their adhesion to human umbilical vein endothelial cells (HUVEC) was quantified by a computerized micro-imaging system. Cell migration was assessed by the scratch 'wound-healing' assay and the Boyden chamber invasion assay. Phosphorylation of STAT3 was evaluated by Western blot. The antitumor activity of CN-CPT was assessed in vivo by PC-3 xenografting in SCID/beige mice. The effect on angiogenesis was assessed using the tubulogenesis and the sprouting assays in HUVEC. CN-CPT treatment impaired the metastatic phenotypes of prostate cancer lines, by significantly reducing adhesion and migration. The anti-adhesive effect was concentration and time-dependent, exerted on both the cancer cells used and additive, treating either HUVEC or PC-3 cells. Inhibition of STAT3 phosphorylation was also observed. This suggests that CN-CPT interferes with critical cell functions associated with the metastatic cascade. CN-CPT had also direct effects on the ability of HUVEC to induce angiogenic activity as assessed by the tubulogenesis and the sprouting assays. PC3-xenograft SCID/beige mice was inoculated with PC-3 cell and treated two times a week with 2,5 mg/Kg CPT and CPT or with the same volume of PBS, starting when the tumor diameter reached 2 mm. Analysis of the tumor dimension showed that treatment with CN-CPT strikingly delayed the tumor growth compared to that detected in the control or in the CPT treated mice, yet without apparent toxic effects. Histological analysis performed at day 52 showed higher Ki-67 staining, marking proliferating cells, in tumors of PBS- and CPT-treated mice than in CN-CPT-treated mice. To further demonstrate the effect of CN-CPT on angiogenesis, we evaluated the presence of blood vessels on tumors. CD31 staining showed that tumors of PBS- and free CPT-treated mice display area of vascularisation, whilst no vessels were detected on tumor of mice treated with CN-CPT. Taken together, these results support the use of β-cyclodextrin nanosponges nanotechnology to deliver anticancer drugs for the treatment of prostate cancers.