CO-DELIVERING OF DOCETAXEL AND CURCUMIN USING POLYMERIC NANOCONSTRUCTS FOR THE TREATMENT OF NEUROBLASTOMA

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INTRODUCTION

Neuroblastoma (NB) is the most common extracranial childhood solid tumor and it accounts for 15% of deaths in pediatric tumor. It is characterized by biological and clinical heterogeneity and, despite aggressive therapies, by adverse outcomes. Also, most approved anticancer drugs are being used at maximally tolerated doses, leading to short- and long-term toxicity [1]. Nanomedicine and combination therapies provide formidable tools to address this disease while minimizing chemotherapy-associated toxicity [2].

SYNTHESIS AND CHARACTERIZATION OF SPNs

Spherical polymeric nanoconstructs (SPNs) for co-delivering Docetaxel (DTXL) and Curcumin (CURC) to NB malignant masses were developed. Both empty and drug loaded particles showed a narrow size distribution (PdI < 0.15) with an average size of about 190 nm (**Fig. 1A**). All the formulations exhibited a biphasic release profile, with almost 90% of the loaded drug being released within the first 24 hours (**Fig. 1B**).

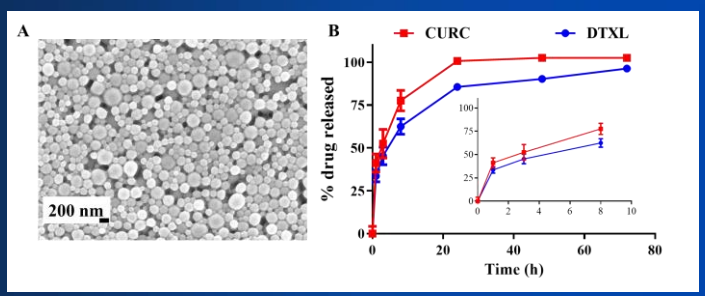
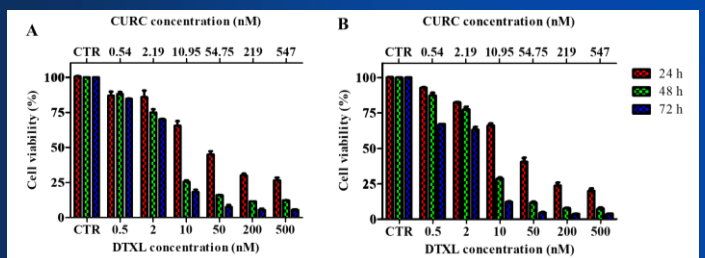


Figure 1. SPNs physico-chemical and biopharmaceutical characterization. A. Representative TEM image of empty SPN; **B**. Release profile of CURC and DTXL from CURC/DTXL-SPNs in PBS, at pH 7.4 and 37±2 °C.

IN VITRO CYTOTOXIC EFFECT OF DTXL/CURC SPNs

The potential cytotoxic effect of free CURC, free DTXL, free CURC/DTXL (1:2 mass ratio), CURC-SPNs, DTXL-SPNs and CURC/DTXL-SPNs (1:2 mass ratio) was assessed on SHSY-5Y LUC$^+$ cells (**Fig. 2**). An analysis of the IC_{50} values showed that the encapsulation of the two drugs, in combination or individually, into SPNs was generally associated with a higher cytotoxic effect. Also, the Combination Index (CI) listed in **Fig. 2C** confirmed the synergism between CURC and DTXL on SHSY-5Y LUC$^+$.



C

	IC $_{50}$ (nM)			CI (average)
Sample	24 h	48 h	72h	72h
DTXL	43.9 ± 8.1	11.2 ± 2.9	6.4 ± 0.9	
CURC	6168 ± 453	4169.5 ± 600.3	2989.5 ± 0.7	
DTXL-SPNs	22.6 ± 5	3.0 ± 0.3	3.2 ± 0.9	
CURC- SPNs	6517 ± 1026.7	2789 ± 158.4	1898.5 ± 215.7	
CURC/DTXL (DTXL)	42.5 ± 6.3	4.8 ± 1.1	3.4 ± 0.04	0.53
CURC/DTXL- SPNs (DTXL)	32.6 ± 13.3	4.9 ± 0.6	2.1 ± 0.1	0.65

Figure 2. *In vitro* cytotoxicity of combination of DTXL and CURC as free or loaded SPNs on SHSY-5Y LUC$^+$ cells. A. and **B.** SHSY-5Y LUC$^+$ cells viability upon incubation with different concentrations of DTXL and CURC as free or loaded SPNs, respectively. **C.** IC_{50} for DTXL, CURC and their combination free or loaded in SPNs on SHSY-5Y LUC$^+$ cells and CI values for their combinations.

IN VIVO THERAPEUTIC EFFICACY OF DTXL/CURC SPNs

After particles efficacy *in vivo* was studied in a mouse NB model. Specifically, omozygous CD1 nu/nu athymic female mice (4 to 6-weeks old) were injected with SHSY-5Y LUC$^+$ cells in the left adrenal gland. Results showed that mice treated with DTXL/CURC –SPNs had a significant increase in life span as compared to untreated mice (control) (p=0.0002), mice treated with CURC-SPNs (p=0.0205), DTXL-SPNs (p=0.0391), and free DTXL (p=0.0054) (**Fig 3**).

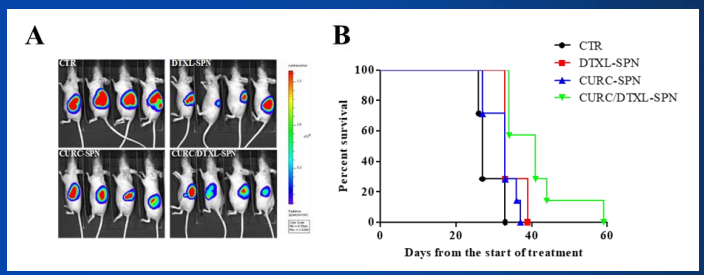


Figure 3. *In vivo* efficacy of DTXL/CURC –SPNs in NB model. A. Representative bioluminescence imaging taken 3 weeks post treatment initiation (24h post 9th administration) for the 3 nanoformulation therapeutic groups (CURC-SPNs; DTXL-SPNs; and DTXL/CURC-SPNs) and untreated control (CTR); **B.** Kaplan-Meyer survival curves with corresponding statistical analysis comparing the nanoformulation therapeutic groups with CTR and CURC/DTXL-SPN with SPN monotherapy.

MRI TUMOR IMAGING AND SPNs BIODISTRIBUTION

Particles biodistribution were investigated in the same animal model by injecting i.v. SPNs labeled with ^{64}Cu, while tumor mass progression was studied using Magnetic Resonance Imaging (MRI) (**Fig 4**). Specifically, at 8 days post tumor cell inoculation, the percentage of injected SPNs normalized by the mass of the organ (%ID/g) was equal to 45 ± 7.1% ID/g in the liver, 26 ± 7% ID/g in the spleen, and 4.9 ± 0.6% ID/g in the kidneys. For the tumor, a SPNs accumulation of 2.3 ± 0.5 % ID/g was measured at day 8 post inoculation.

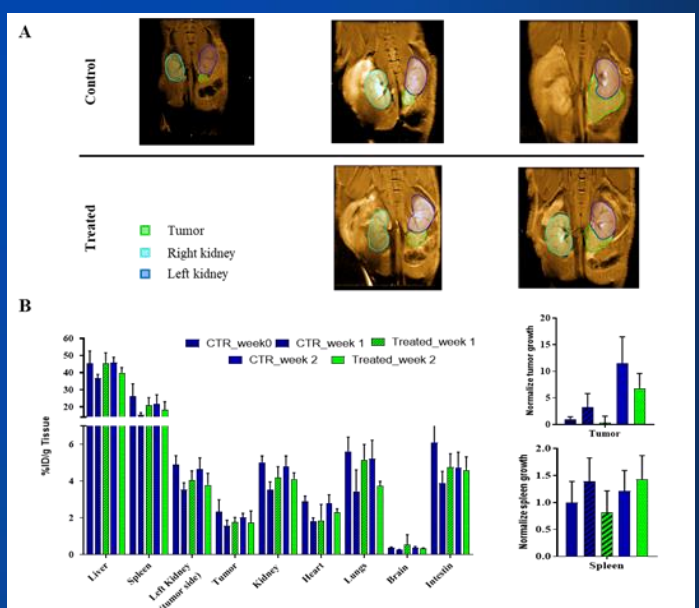


Figure 4. Tumor progression (MRI) and SPN Biodistribution analyses. A. Longitudinal tumor burden by MR imaging analysis (**left**) and ex-vivo weight measurements (**right**) (green contour: tumor; light blue contour: right kidney with tumor; dark blue contour: left kidney with no tumor). **C.** Biodistribution analysis of ^{64}Cu-SPNs at 0, 1 and 2 weeks.

CONCLUSIONS

This work demonstrated that enabling combination therapies via Nanomedicine can modulate NB progression with a significant increase in overall survival.

REFERENCES

1. Luksch, R., et al., *Neuroblastoma (Peripheral neuroblastic tumours).* Critical reviews in oncology/hematology, 2016. **107**: p. 163-181.
2. Pastorino, F., et al., *Overcoming biological barriers in neuroblastoma therapy: the vascular targeting approach with liposomal drug nanocarriers.* Small, 2019. **15**(10): p. 1804591.