Design and *in vitro* study of etoposide loaded lipid nanomedicines for neuroblastoma treatment

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BACKGROUND

Neuroblastoma is the **most frequent pediatric extracranial solid tumor**. Patient's **outcome** is strongly related with **heterogeneity** and **complex tumor biology**.[1,2] **Chemotherapy** emerges as the **last opportunity** for children with poor prognosis but can be extremely toxic. Etoposide is a podophyllotoxin derivative given to neuroblastoma patients that often presents acute and late toxicity.[3] Nanotechnology has been widely studied in cancer treatment with the aim of improving the therapeutic index of chemotherapeutic drugs.[4] **Lipid nanosystems** in particular are known to have **low toxicity** and **avoid the use of organic solvents**.[5]

METHODS

1. Development of etoposide-lipid nanomedicines(ETP-NP)

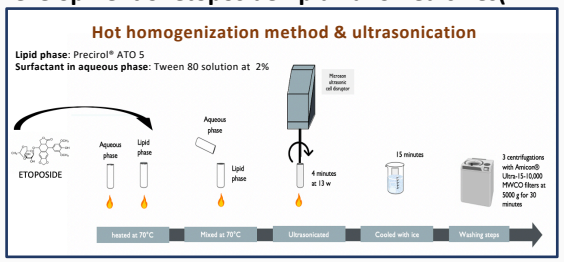

Figure 1. Diagram of the hot homogenization and ultrasonication process.

2. *In vitro* characterization

I. Physicochemical characterization

By dynamic light scattering → Polydispersity index (PDI), size and Z potential (Zpot)

II. Drug content λ=285nm

UV-vis

Calibration curve ranging from **30 to 170 µg/ml**

UHPLC-UV

Column: stabilized at 50°C.
Gradient elution(A/B: Methanol/Water)
Flow rate: 0.5 ml/min.
Autosampler set at 4°C
Run time: 2.0 min.
injection volume: 2 µl (partial loop mode)
Calibration curve ranging from **2.4 µg/ml to 150 µg/ml**

III. Cell viability studies(MTS)

CELL LINES

SH-SY5Y
CHLA-90
NBL-S
SK-N-SH
SK-N-BE(2)
SK-N-BE(2)C
NGP-A

MYCN status(-)→
MYCN status(+)→

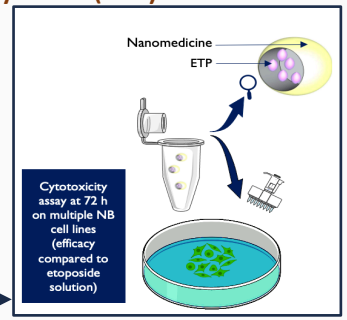

Figure 2. Comparative cell viability study on multiple NB cell lines with MTS assay at 72h

OBJECTIVE

The objective of this work is to **design** and **characterize** etoposide-loaded lipid nanomedicines with the aim **of improving therapeutics in neuroblastoma** management.

RESULTS

Table 1. Physicochemical characteristics of the developed nanomedicines (data n ≥ 3, data: mean ± SD) and quantification of etoposide within lipid nanomedicines s by UV-vis method and UHPLC-UV method (data n ≥ 2, data: mean ± SD)

DRUG CONTENT				PHYSICOCHEMICAL CHARACTERIZATION		
UV-VIS		UHPLC-UV				
Drug loading	EE	Drug loading	EE	Size	PDI	Zpot
4.58 ± 0.60 µg/mg	89.23 ± 4.58 %	4.26 ± 0.18 µg/mg	85.66 ± 2.53 %	105 ± 3	0.19 ± 0.01	-19.9 ± 4.2

Cell viability studies after 72 hours of treatment

Table 2. IC_{50} values of etoposide in solution and etoposide-loaded lipid nanomedicines on different neuroblastoma cell lines after 72 h of treatment (data n ≥ 3, data: mean ± SD)

	SK-N-BE(2)	SH-SY5Y	SK-N-BE(2)C	NGP-A	SK-N-SH	CHLA-90	NBL-S
ETP SOLUTION	0.752 ± 0.125 µM	1.202 ± 0.297 µM	0.505 ± 0.141 µM	0.206 ± 0.133 µM	0.199 ± 0.054 µM	18.29 ± 4.021 µM	0.047 ± 0.015 µM
ETP NP	0.328 ± 0,150 µM	0.246 ± 0.042 µM	0.558 ± 0.140 µM	0.310 ± 0.014 µM	0.164 ± 0.040 µM	7.892 ± 2.362 µM	0.042 ± 0.037 µM

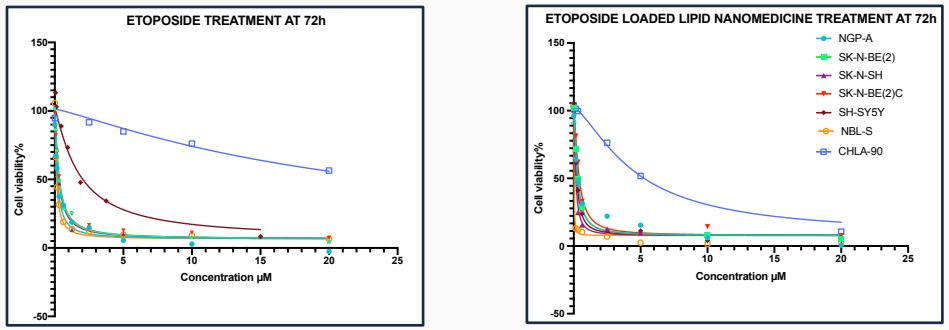

Figure 3. Cell viability assays. Cells were exposed to etoposide(ETP) and etoposide-loaded nanomedicines (ETP-NP) treatments for 72 hours.

CONCLUSIONS

- ETP-NP were successfully developed (homogeneous distribution, adequate size and surface charge) suggesting that the formulation is physiochemically adequate.
- Drug loading study revealed high EE with UHPLC-UV method and UV-vis method indicating that lipid nanoparticles and the chosen technique are a suitable option for etoposide's quantification within lipid nanomedicines.
- UHPLC-UV enables to have wider calibration curve range, better reproducibility and is more appropriate in terms of quality to quantify etoposide in these nanosystems.
- Cytotoxicity assay revealed similar efficacy for etoposide solution and ETP-NP suggesting that the encapsulation process did not affect etoposide's antitumor efficacy.
- ETP-NP enhance efficacy of etoposide in CHLA-90, SH-SY5Y and SK-N-BE(2) cell lines suggesting that ETP-NP could overcome drug resistance.

In-depth *in vitro* and *in vivo* tests will be performed to evaluate the therapeutic potential of ETP-NP in neuroblastoma.

ACKNOWLEDGEMENTS

This study was supported by grants from the Children with Neuroblastoma Association (NEN, Nico contra el Cancer).

REFERENCES