ANTITUMORAL ACTIVITY OF NOVEL 8-OXYQUINOLINATE-PLATINUM(II)-LOADED NANOSTRUCTURED LIPID CARRIERS TARGETED WITH RIBOFLAVIN

Boztepe, Tugce[1]; Islan, Germán A.[1]; Gambaro, Rocio C.[2]; Castro, Guillermo R.[3,4]; León, Ignacio E.[5,6]

[1] Laboratorio de Nanobiomateriales, CINDEFI- Universidad Nacional de La Plata-CONICET (CCT La Plata), Argentina.
[2] Instituto de Genética Veterinaria (IGEVET, UNLP-CONICET La Plata), Facultad de Ciencias Veterinarias Universidad Nacional de La Plata (UNLP), La Plata, Argentina.
[3] Max Planck Laboratory for Structural Biology, Chemistry and Molecular Biophysics of Rosario (MPLbioR, UNR-MPIbpC), Partner Laboratory of the Max Planck Institute for Biophysical Chemistry (MPIbpC, MPG), Centro de Estudios Interdisciplinarios (CEI), Universidad Nacional de Rosario, Maipú 1065, S2000 Rosario, Santa Fe, Argentina.
[4] Nanomedicine Research Unit (Nanomed), Center for Natural and Human Sciences (CCNH), Universidade Federal do ABC (UFABC), Santo André, SP, Brazil.
[5] CEQUINOR (UNLP, CCT-CONICET La Plata, Asociado a CIC), Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata. Blvd. 120 N° 1465, La Plata 1900, Argentina
[6] Cátedra de Fisiopatología, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata. 47 y 115, La Plata 1900, Argentina.

Introduction

- As anticancer agents, platinum-based compounds have been utilized in the treatment of various types of cancer including colorectal, bladder, cervical, ovarian, head and neck, lung, and testicular cancer. They showed their efficiency in clinical applications, however, long-term use can develop drug resistance and dose-limiting toxicity [1].
- Drug delivery systems are designed to overcome the drawbacks of anticancer agents by increasing their cytotoxic effect by accumulating in the tumor area without creating damage in healthy tissues [2].
- Nanostructured lipid carriers (NLC) are one of the most promising systems for drug delivery as they have a high capacity to load drugs and also, increase their solubility, especially for the hydrophobic molecules [2].
- Riboflavin transporters displayed up-regulation in several tumor types including breast, esophagus, melanoma, and colon carcinoma cells. So, riboflavin (RFV) could be a potential targeting molecule for active drug delivery [3].

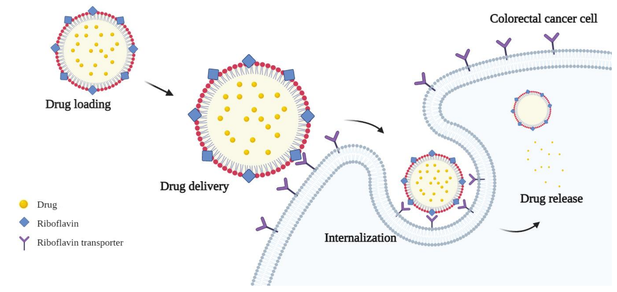

Figure 1. Scheme of RFV-targeted tumor-specific drug delivery.

Objectives

- Design, synthesize and characterize the RFV-functionalized 8-QO-Pt loaded NLC.
- Evaluate the antitumoral activity in the HT-29 colon carcinoma cell line.
- Determine the safety profile with the hemotoxicity assay.

Methods

- The drug 8-Oxyquinolinate-platinum(II) (8-QO-Pt) loaded, RFV-targeted NLC of myristyl myristate (MM) was synthesized by ultrasonication.
- Three different NLC formulations were designed with different ratios of RFV added to the lipidic or the aqueous phases.
- Cell viability, cellular uptake, and apoptosis assays in HT-29 cells and hemotoxicity analysis of the nanoparticles were carried out.

Formulations	RFV phase (mg)	
	Aqueous	Lipid
R_1-8-QO-Pt-NLC	10.0	-
R_2-8-QO-Pt-NLC	-	10.0
R_3-8-QO-Pt-NLC	5.0	5.0

Table 1. The amount of RFV in the formulations.

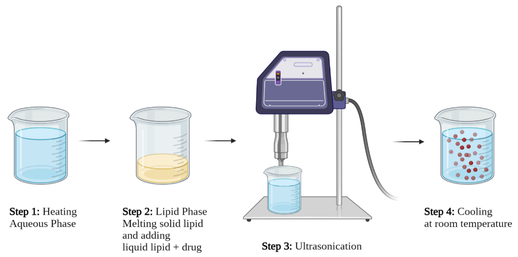

Figure 2. Ultrasonication technique.

Results

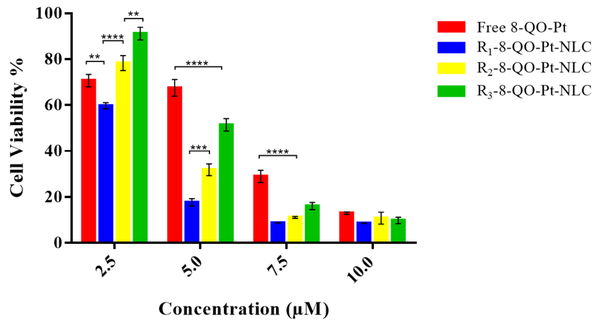

Figure 3. Cell cytotoxicity of the free 8-QO-Pt compound and 8-QO-Pt loaded (+) formulations of NLC/RFV against HT-29 cell line for 24 h.

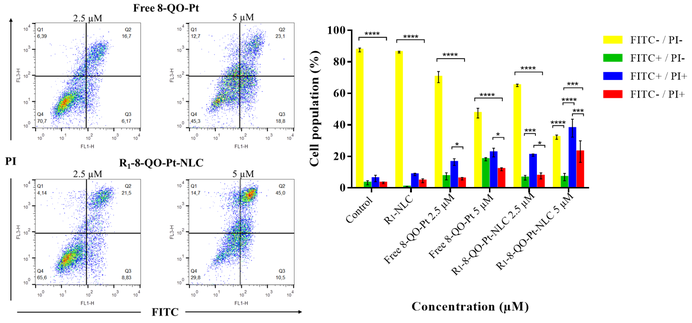

Figure 5. Apoptosis effect of free 8-QO-Pt compound and R1-8-QO-Pt-NLC.

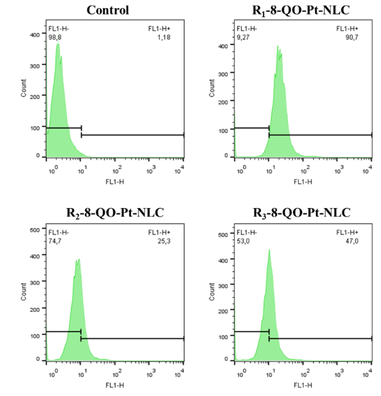

Figure 4. Cellular uptake of DiOC18-loaded nanoparticles of NLC/RFV in HT-29 cells measured by flow cytometry.

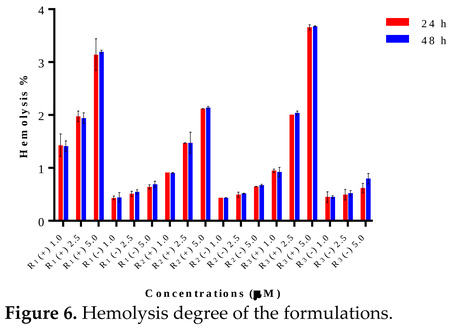

Figure 6. Hemolysis degree of the formulations.

Results

- All the RFV-targeted 8-QO-Pt-loaded formulations demonstrated a higher cytotoxic effect than the free 8-QO-Pt compound.
- R1-8-QO-Pt-NLC exhibited the highest cellular uptake (89.5%) in comparison to R2-8-QO-Pt-NLC (25.5%) and R3-8-QO-Pt-NLC (47.7%).
- Free 8-QO-Pt compound and R1-8-QO-Pt-NLC induced 16.7% and 21.5% (at 2.5 µM) and 23.1% and 45.0% (at 5 µM) of cells in late apoptosis (FITC+/PI+), respectively.
- The R1-8-QO-Pt-NLC formulation showed hemolysis around 3.0%, R2-8-QO-Pt-NLC around 2.0%, while R3-8-QO-Pt-NLC around 3.7% at the concentration of 5.0 µM.

Conclusions

- RFV ligand-targeted NLC nanoparticles have been revealed as potential tumor-specific drug delivery systems.
- The antitumor effect of the R1-8-QO-Pt-NLC system was superior to the free 8-QO-Pt compound and the other tested nanosystems.
- In comparison to the free drug, the apoptosis assay displayed that active targeting with RFV resulted in an elevated antiproliferative effect due to cancer cell selectivity.
- All the formulations are in an acceptable and low range of hemotoxicity.

References

1. L. Galluzzi, L. Senovilla, I. Vitale, J. Michels, I. Martins, O. Kepp, M. Castedo, G. Kroemer, Oncogene. 31 (2012) 1869–1883.
2. T. Boztepe, G.R. Castro, I.E. León, Int. J. Pharm. 605 (2021) 120788.
3. V. Tutino, M.L. Defrancesco, M. Tolomeo, V. De Nunzio, D. Lorusso, D. Paleni, M.G. Caruso, M. Notarnicola, M. Barile, Anticancer Res. 38 (2018) 2659–2667.

Acknowledgments

This work was supported by UNLP (X053, X701), CONICET (PIP 2051), ANPCyT (PICT 2019-2322, PICT 2017-2251, PICT2016- 4597).

tugceboztepe@gmail.com