Digoxin-loaded polymeric nanoparticles as an immunogenic cell death inducer in cancer cells

Nicholas Orlandini[1], Alessandra Invidia[2], Clara Baldari[3], Ilaria E. Palamà[2], Giuseppe Gigli[2,3], Gabriele Maiorano[2]

[1] Department of Innovation Engineering, University of Salento, Monteroni Street, 73100 Lecce, Italy
[2] Nanotechnology Institute, CNR-NANOTEC, Monteroni Street, Lecce 73100, Italy
[3] Department of Experimental Medicine, University of Salento, Monteroni Street, 73100 Lecce, Italy

nicholas.orlandini@unisalento.it
gabriele.maiorano@cnr.it

In recent years, cancer immunotherapy has revolutionized cancer treatment to activate and sustain specific immune responses against tumours. However, its effectiveness remains limited, particularly in cold tumours, which are characterised by poor immune cell infiltration and a compromised antigen presentation mechanism [1].
In this frame, the induction of immunogenic cell death (ICD) in cancer cells reveals a powerful approach to improve immunotherapeutic outcomes. To achieve this goal, in this study, we developed polymeric nanoparticles (pNPs) for the controlled delivery in cancer cells of digoxin, a cardiac glycoside able to induce ICD, thus triggering the release of tumor-associated antigens (TAAs), neoantigens (TANs), and damage-associated molecular patterns (DAMPs) [2,3].
In particular, we formulated poly(caprolactone) (PCL)-based nanoparticles encapsulating digoxin starting from the synthesis of PCL with controlled molecular weight through an organo-catalyzed ROP strategy. Using water as an initiator, the PCL obtained was characterized by a terminal carboxyl group that improved the stability in water of the prepared digoxin-loaded PCL NPs, thus avoiding surfactant-based approaches.
The produced digoxin-loaded pNPs showed a diameter of around 200 nm, with a high degree of monodispersion and a negative surface charge. The encapsulation efficiency was found to be 80%, as measured by HPLC. *In vitro* experiments with human glioma and breast cancer cell lines revealed that digoxin-pNPs effectively delivered the ICD inducer, thus showing significant antitumor activity, with time-dependent cellular uptake and controlled drug release.
Preliminary investigations of ICD markers focused on two main DAMPs: high mobility group box 1 (HMGB-1) and calreticulin, a calcium-binding ER-resident protein. Confocal microscopy investigations were carried out to study the effects of drug-loaded pNPs compared to free drug upon HMGB-1 release and calreticulin localization, revealing that nanoparticle formulation led to a more controlled and sustained ICD induction, as compared to free drug. However, further investigations are underway to deeply assess the spatial and temporal dynamics of ICD induction by qualitative and quantitative investigation of DAMP release after administration of digoxin-loaded pNPs.
In conclusion, the development of polymeric nanoparticle-based delivery systems for digoxin-induced ICD could represent a promising approach to modulate a robust ICD in cancer cells with an improvement of immunotherapeutic outcomes. This strategy not only enhances drug stability, bioavailability, and bioactivity thanks to controlled delivery, but also exemplifies the potential of drug repositioning in oncology through nanotechnologies.

Acknowledgements

TecnoMed Puglia; EU funding PNRR Projects n. CN00000041-CN3-RNA; LSH-TA-PNC-E3-2022-23683269; PNRR-MR1-2022-12376725, PRIN-PNRR-2022-P2022EPK9B; Project “Biotecnologia, Bioinformatica e Sviluppo farmaceutico” POS2014-2020, Tract4, Azione4.1-Cod.T4-AN-01.

References

[1] Calvillo-Rodríguez, K. M., Lorenzo-Anota, H. Y., Rodríguez-Padilla, C., Martínez-Torres, A. C., & Scott-Algara, D., Frontiers in Immunology, (2023), 14, 1294434.
[2] Sun, H., & Zhong, Z., Biomacromolecules, (2024), 26(1), 33-42.
[3] Galluzzi, L., Guilbaud, E., Schmidt, D., Kroemer, G., & Marincola, F. M., Nature Reviews Drug Discovery, (2024), 1-16.