Repurposing Pyronaridine as a novel inhibitor of heterodimeric ERCC1-XPF DNA endonuclease complex for targeted sensitization of colorectal cancer to platinum-based chemotherapeutics

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Introduction

- ERCC1/XPF is a heterodimeric enzyme complex with endonuclease activity that participates in the repair of DNA inter-and intra-strand crosslinks produced by chemotherapeutic agents such as Carboplatin and the repair of DNA damage induced by ionizing radiation.
- Inhibitors of ERCC1/XPF dimerization that can disrupt the interactions between ERCC1 and XPF are expected to sensitize cancer cells to genotoxic therapeutics.
- We have made an exciting discovery that a drug clinically used in malaria treatment, i.e., pyronaridine (PY), is a potent inhibitor of a DNA repair enzyme known as ERCC1/XPF with Kd (150 ± 10nM) and IC50 values in the(0.321 ± 0.022µM) range.
- Our previous studies have shown that 2 µM of Py will disrupt the interaction between ERCC1/XPF in A549 (lung cancer) cells. So, we decided to further investigate the effect of PY on other cancer cell lines.
- Nano-delivery of pyronaridine can be used to enhance the chemo-sensitizing activity of this compound in the tumor while sparing normal tissues from this effect.

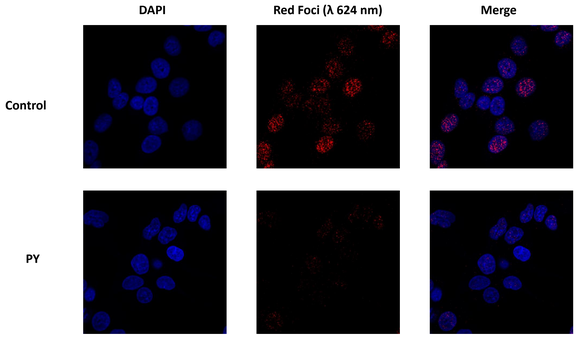

Figure 1: (PLA) Representative PLA images of A549 cells exposed to 2 µM of pyronaridine (PY) or the equivalent amount of DMSO vehicle (1 µl/ml, Control). Images were obtained at 40X magnification. ERCC1-XPF complexes appear as red dots, and cellular nuclei are shown in blue after DAPI staining.

To confirm that PY was able to disrupt the interaction between subunits in the ERCC1-XPF heterodimer in cells, we performed PLA using A549 lung cancer cells exposed to 2 µM PY or DMSO vehicle for 24 h.

In the presence of vehicle (0.2% DMSO) alone, we observed an average of 85.6 ± 16.1 foci per cell, whereas in the presence of PY, an average of 5.4 ± 3.1 foci per cell was detected.

Hypothesis

Liposomal formulations of Pyronaridine (LPY) can act as novel inhibitors of ERCC1-XPF heterodimerization, sensitizing colorectal cancer cells to the effects of platinum-based chemotherapeutics.

Objectives

1. To develop and optimize liposomal formulation (Lpy) of Py for passive tumor targeting.
2. To examine the anti-cancer activity of the free and encapsulated drug in colorectal cancer models in combination with platinum drugs.

Methods

- Liposomal delivery system was made of three lipids (DSPC, DSPE-PEG, and Cholesterol), and the preparation method was thin lipid formation.
- Py was loaded in the Lpy by remote loading using a pH gradient system (pH of 7.4 outside to 3.5 inside the vesicle).
- The unloaded drug is separated from the loaded one by ultracentrifuge (150000X g,2.5 h, 4◦C)

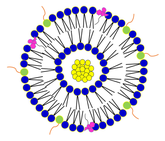

Figure 2: Liposome Structure

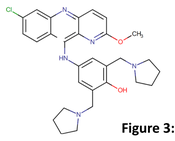

Figure 3: Pyronaridine structure

- The size (Z average diameter) of the liposomes was assessed with Dynamic light scattering (DLA) using Zetasizer Nano Malvern, UK.
- Encapsulation efficiency and drug content were assessed after disruption of liposome by 4% SDS using UV spectroscopy at 424 nm.
- The vitro drug release was assessed by placing the formulations in the dialysis bags (MWt 4kDa) and immersing them in release media consisting of PBS and 4% BSA. The samples were taken from the inside of bags at different time points during a 72h period.
- The cytotoxic activity of loaded and free Py was assessed in combination with platinum-based drugs (carboplatin and oxaliplatin) by MTT assay against the HCT116 cell line. The possibility of a synergistic effect was assessed using the Combenefit software.

Results

Formulation	Size (nm)	PDI	Loading content (w/w%)	Encapsulation Efficiency (%)
Drug + Liposome	82.32±0.33	0.1±0.008	15.2±0.76	99.2±4.23
Free Liposome	106.9±0.25	0.2±0.07		

Table 1: physicochemical characters of the liposomes (n=3)
Loading Content= Amount of encapsulated drug/ The total amount of phospholipids X 100
Encapsulation efficiency (EE%) = The amount of encapsulated drug/ The initial amount of added drug X 100

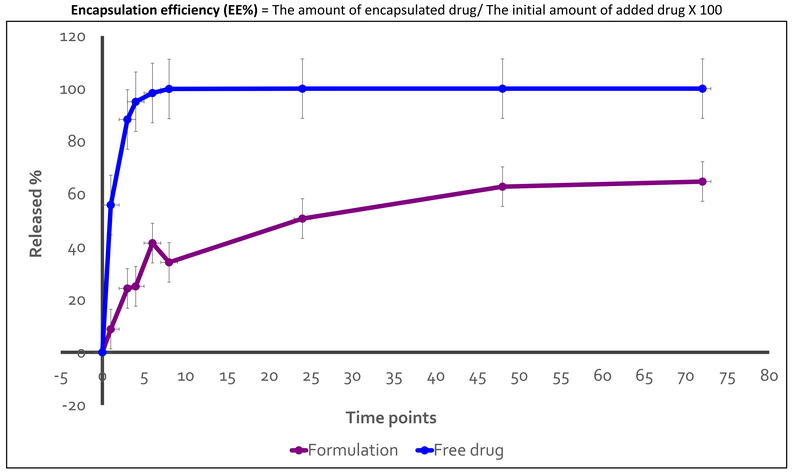

Figure: The in vitro release profile of free versus encapsulated pyronaridine at set time points during 72h test.
Release media: PBS+4% BSA

- The liposomal formulation of pyronaridine showed an average diameter of 82 nm, which is suitable for passive tumor targeting.
- Liposomal formulations showed excellent loading of pyronaridine and slowed its release in a biologically relevant media.

Results

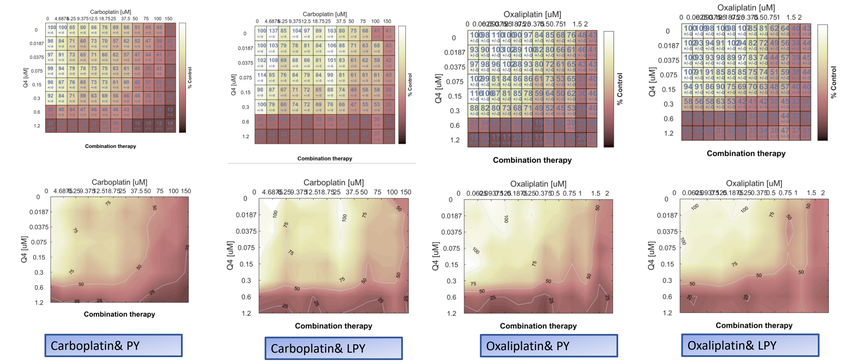

Figure: Dose-response graphs, presenting the effect of combination therapy
Concentration Range: Pyronaridine (0-1.2) µM, Carboplatin (0-150) µM, Oxaliplatin (0-2) µM

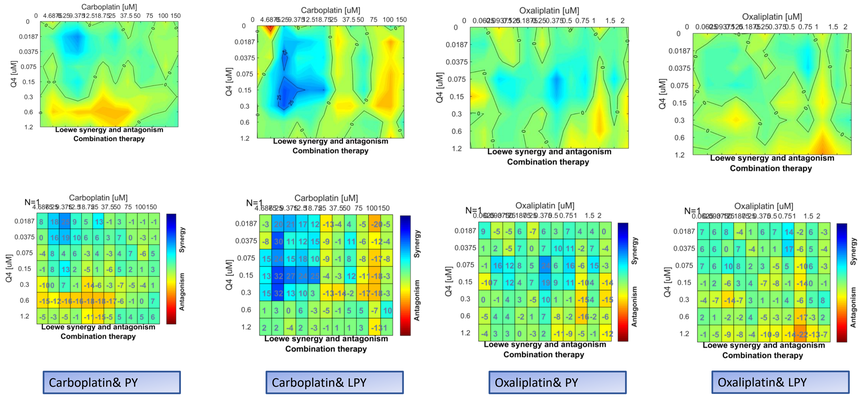

Figure: Assessing the possibility of the synergistic effect between Pyronaridine and platinum-based drugs (n=3)
Concentration Range: Pyronaridine (0-1.2) µM, Carboplatin (0-150) µM, Oxaliplatin (0-2) µM

- In combination with carboplatin, the liposomal formulation of pyronaridine showed a synergistic anticancer effect against HCT116 cells at a (0.075-0.5) µM concentration range for Py and (25-100) µM for carboplatin.
- In combination with Py (0.075-0.15) µM and 0.375 µM oxaliplatin, synergistic activity is seen.

Conclusion

The preliminary results indicate a potential for pyronaridine and its liposomal formulation in chemo-sensitization of CRC to platinum chemotherapeutics.

Acknowledgement

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FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

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