Development and optimization of nanodelivery of novel inhibitors of ERCC1/XPF for enhanced cancer therapy

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Introduction

- ERCC1/XPF is an enzyme complex consisting of two different proteins that participate in the repair of DNA crosslinks produced by chemotherapeutic agents like Cisplatin and radiation-induced DNA damage (1).
- Blocking the interaction between ERCC1 and XPF through inhibitors can potentially make cancer cells more sensitive to treatments that cause DNA damage (1).
- Excitingly, recent studies have found that a drug clinically used in malaria treatment, i.e., pyronaridine (PYD), is a potent inhibitor of ERCC1/XPF in Human colorectal carcinoma cell line (HCT-116) with IC50 values in the(0.321 ± 0.022µM) range and significantly sensitizes cells towards radiation therapy (2)
- Additionally, nano-delivery of pyronaridine may be used to enhance its effectiveness in tumors while minimizing any negative impact on healthy tissues (3).

Hypothesis

- Platinum-based chemotherapeutics, including cisplatin and carboplatin, are clinically used to treat non-small cell lung cancer (NSCLC) as well as head and neck cancer (HNC).
- We postulate that Pyronaridine (PDY) and its liposomal formulation (LPY) can make NSCLC as well as HNC cells expressing ERCC1/XPF, more susceptible to the effects of platinum-based chemotherapeutics.

Objectives

- To examine the anti-cancer activity of the PYD and LPY in NSCLC and HNC models alone or in combination with cisplatin.

Methods

- Thin film hydration method was used in the preparation of liposomes using three lipids (DSPC, DSPE-PEG, and Cholesterol).
- Drug loading was carried out using a remote loading system (pH gradient- pH of 7.4 outside to 3.5 inside the vesicle).

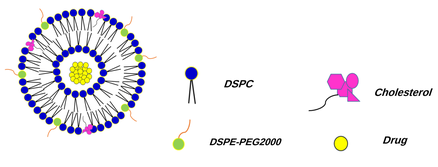

Figure 1: Liposome Structure

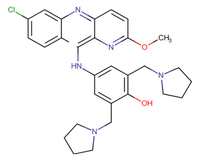

Figure 2: Pyronaridine structure

Methods

- 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 cytotoxic activity of free PYD was assessed in combination with cisplatin by MTT assay against FaDu (HNC) H1299 and A549 (NSCLC). The possibility of a synergistic effect was assessed using the Combenefit software.
- Future MTT assay will be carried out for the liposomal PYD for comparison with free drug.

Results

Table 1: physicochemical characters of the liposomes (n=3)

Formulation	Size (nm)	PDI	Encapsulation Efficiency (%)
Liposomal PYD	114.8±1.352	0.161±0.002	99.2±4.23
Empty liposomes	91.17±0.977	0.212±0.005	

- **Encapsulation efficiency (EE%)** = The amount of encapsulated drug/ The initial amount of added drug X 100

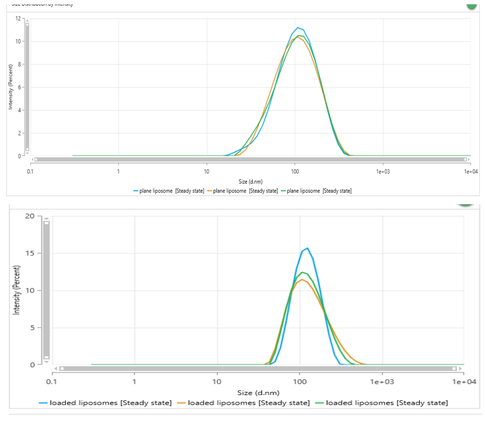

Figure 3: DLS of plain and loaded liposomes

- The liposomal formulation of pyronaridine showed an average diameter of 115 nm, which is suitable for passive tumor targeting.
- Liposomal formulations showed excellent loading of pyronaridine.

Results

Table 2: IC_{50} of cisplatin alone and in combination with PYD in FaDu cells

TTT time	Cisplatin	Cis+0.5µM of PYD	Cis+1µM of PYD
24 hrs	18.19	6.257	6.022
48 hrs	5.622	1.711	0.5394

Concentration Range: Pyronaridine (0.5 and 1) µM, cisplatin (0-60) µM

- The combination of PYD with cisplatin resulted in a decrease in the IC50 value of cisplatin which suggests a potential synergistic effect between the two compounds

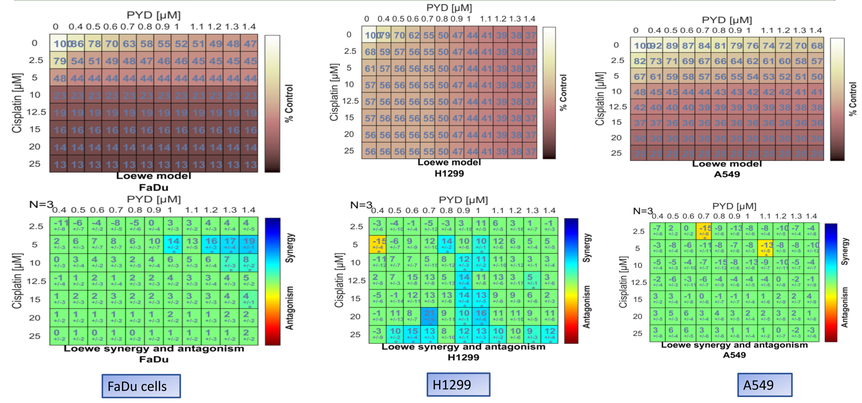

Figure 4: Assessing the possibility of the synergistic effect between Pyronaridine and cisplatin(n=3) with FaDu, H1299 and A549

- Combination of cisplatin (2.5-25) µM with pyronaridine (0.4-1.4) µM showed some extent of synergistic and additive anticancer effect against FaDu and H1299 cells
- In A549, no synergistic effect was observed when PYD (0.4-1.4) µM is combined with cisplatin (2.5-25).
- Future work will be carried out using a higher conc range of PYD for confirmation of these effects and comparison with liposomal formulation of PYD

Conclusion

- The preliminary results indicate a potential for pyronaridine and its liposomal formulation in chemo-sensitization of FaDu and H1299 cells to cisplatin.

Acknowledgement

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References

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