Nano-delivery of pyronaridine as an inhibitor of DNA repair for targeted sensitization of head and neck cancer cells to cisplatin

Ahmed Abdelfattah[1,2], Parnian Mehinrad[1], James Donnelly[3], Michael Weinfeld[4,5], Frederick G. West[3], Afsaneh Lavasanifar[1,6*]

1-Faculty of Pharmacy and pharmaceutical science, University of Alberta, Edmonton, AB, Canada.
2-Department of industrial pharmacy, Faculty of Pharmacy, Assiut University, Assiut, Egypt
3-Department of Chemistry, Faculty of Science, University of Alberta, Edmonton, AB, Canada
4-Department of Experimental Oncology, Cross Cancer Institute, Edmonton, AB, Canada
5-Department of Oncology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada
6-Department of chemical engineering, University of Alberta, Edmonton, AB, Canada.
*afsaneh@ualberta.ca

Abstract

Background: Head and neck cancer (HNC) ranks seventh globally, with 660,000 new cases and 325,000 deaths annually. Nonsurgical treatments for HNC mainly include radiation therapy and platinum-based DNA damaging therapies. However, the ability of cancer cells to repair DNA damage can undermine the effectiveness of these treatments. ERCC1-XPF is a key enzyme in removing DNA damage caused by platinum therapies and radiation. Excitingly, our research team has found that the anti-malaria drug pyronaridine (PYD) can effectively hinder the dimerization and activity of ERCC1/XPF. **The purpose** of this study was to investigate the synergistic activity of free versus liposomal formulations of PYD in combination with cisplatin in FaDu cells, *in vitro*. **Methods:** Liposomes were prepared through ethanol injection method followed by external buffer exchange and active loading of PYD in liposomes with an interior acidic pH. Cytotoxicity of cisplatin, PYD, or liposomal PYD as well as PYD formulation with cisplatin combination against FaDu cells was investigated by MTT and colony forming assay. The synergistic effect between the combinations was assessed using Combenefit software. **Results:** The IC_{50} of cisplatin was significantly reduced when combined with 0.5 and 1 μM of PYD in FaDu cells. Furthermore, Combenefit analysis showed a synergistic effect between cisplatin (5-10) μM and PYD (1.2-1.4) μM. This synergy was further enhanced after using liposomal PYD, leading to wider effective concentration ranges and an augmentation in the magnitude of the observed synergy. **Conclusion:** The results showed a synergistic cytotoxicity between PYD and its liposomal formulation with cisplatin against HNC.

Introduction

- The ERCC1/XPF enzyme complex plays a crucial role in repairing DNA damage caused by chemotherapy and radiation. In HNC, ERCC1/XPF is the main enzyme involved in nucleotide excision repair (NER), a process primarily responsible for cisplatin resistance [1,2]. Blocking this complex can potentially make cancer cells more sensitive to therapies that induce DNA damage like cisplatin [2].

- Excitingly, pyronaridine (PYD), an anti-malarial drug, has shown promising potential as an ERCC1/XPF inhibitor in colorectal carcinoma cells (HCT-116), with IC50 range of (0.321 ± 0.022μM) and significantly sensitizes cells towards radiation therapy [3]. Furthermore, Nano-delivery of pyronaridine may further enhance its effectiveness against tumors while sparing healthy tissues [4].

✓ **The objective of this study was to investigate the anti-cancer activity of PYD and Liposomal PYD in HNC models alone or in combination with cisplatin. Meanwhile two lung cancer cell lines (A549 and H1299) were included for comparative analysis with HNC cell line (FaDu).**

Results

1.Liposomes Characterization

Particle size and EE%

Method	Formulation	Size(nm)	PDI	EE (%)
Ethanol injection	Unloaded Liposomes	63.38±1.05	0.107±0.021	
	Liposomal PYD	74.9±1.22	0.103±0.016	98±3.5
Thin film hydration	Unloaded Liposomes	91.17±0.97	0.212±0.005	
	Liposomal PYD	114.8±1.35	0.161±0.002	99.2±4.23

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

In Vitro drug release

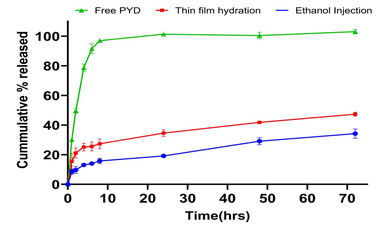


Figure1: Release profile for free PYD Vs Liposomal PYD.

2.Effect of combination on the IC50 of Cisplatin via MTT assay

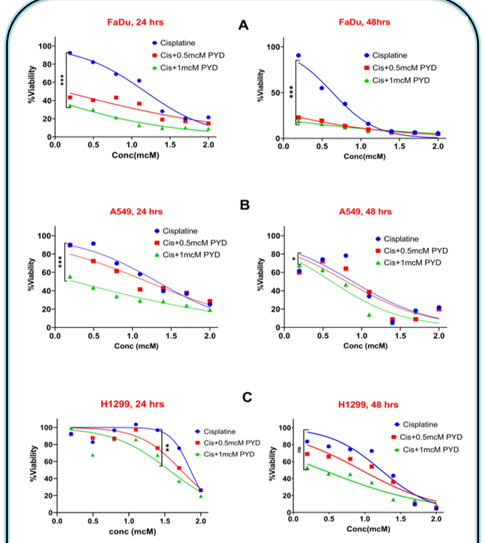


Figure 2: IC50 of cisplatin alone and in combination with PYD in FaDu, A549 and H1299 cell lines after 24 and 48 hrs.

3.Synergy evaluation between Cisplatin and free PYD &Liposomal PYD using Combenefit

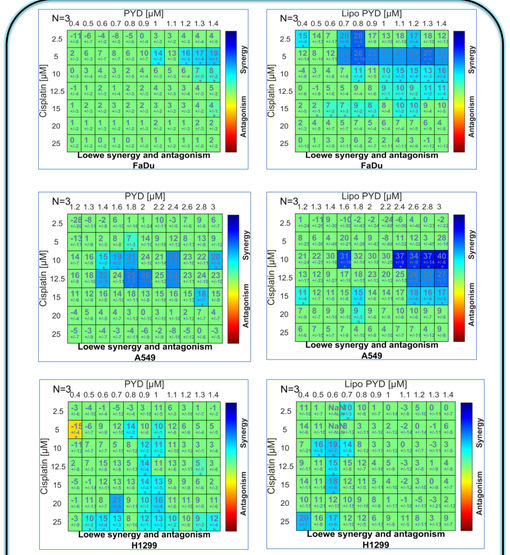


Figure 3: Assessing the possibility of the synergistic effect between PYD &Liposomal PYD and cisplatin(n=3) with FaDu, A549 and H1299

4.Colony formation assay

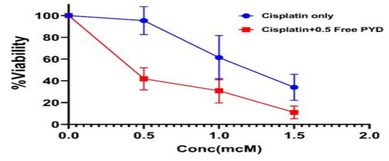


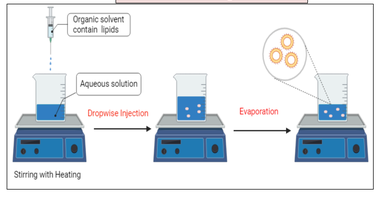
Figure 4: The results of clonogenic assay following combination of cisplatin and PYD against FaDu (n=3).

- **Ethanol injection and thin film hydration produced liposomal formulations with excellent PYD loading (94% and 98%) and ideal particle sizes (75 nm and 115 nm) for passive tumor targeting.**
- **Combining cisplatin with 0.5 and 1 μM of PYD significantly reduced cisplatin's IC50.**
- **Combenefit Analysis showed a synergistic effect between cisplatin (5-10 μM) and PYD (1.2-3 μM), which was further enhanced by using liposomal PYD in both FaDu and A549 cells.**
- **Colony formation assays confirmed a substantial reduction in cell viability after combination of cisplatin with PYD in FaDu cells.**

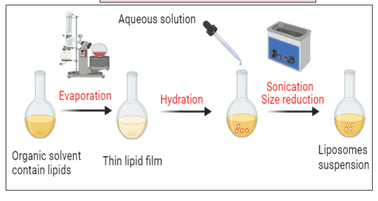
Methods

Liposomes preparation

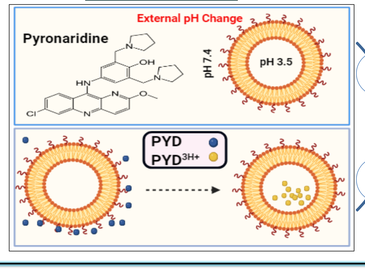
Ethanol injection



Thin film hydration



PYD loading



Characterization

- Particle size analysis
- Encapsulation Efficiency %
- *In vitro* drug release

***In vitro* cytotoxicity of Cisplatin, PYD, liposomal PYD and combination on FaDu(Head and neck cancer cells), H1299&A549 (Lung cancer cell lines)**

1. Effect of combination on the IC50 of Cisplatin via MTT assay

-Concentration Range: Pyronaridine (0.5 and 1) μM, cisplatin (0-60) μM,
-24 and 48 hrs ttt

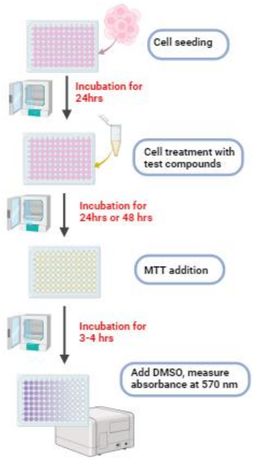
2. *In vitro* evaluation of the synergy between Cisplatin and free PYD &Liposomal PYD using Combenefit

-48 hrs ttt, conc ranges for both drugs
-PYD(0.4-1.4) μM and (1.2-3) μM, Cis (2.5-25) μM

Combenefit: an interactive platform for the analysis and visualization of drug combinations

3. colony formation assay

-48 hrs ttt, conc for both drugs
-PYD(0.5) μM and Cis (0.5, 1 and 1.5) μM



Conclusion

In conclusion, our study identifies a potential synergistic effect between pyronaridine (PYD) and cisplatin in treating head and neck cancer (HNC), especially when PYD is delivered in liposomal form. While these results are promising, further in vivo studies will be conducted to validate these findings and evaluate their clinical applicability.

Acknowledgement

Ahmed Abdelfattah is funded by a scholarship from the Ministry of Higher Education of Arab Republic of Egypt

References

1-C.R.R. Rocha, M.M. Silva, A. Quinet, J.B. Cabral-Neto, C.F.M. Menck: DNA repair pathways and cisplatin resistance: an intimate relationship. Clinics (2018), 73(suppl 1): e478s.
2-McNeil, E. M., Melton, D. W.: DNA repair endonuclease ERCC1-XPF as a novel therapeutic target to overcome chemoresistance in cancer therapy. Nucleic acids research (2012), 40(20): 9990–10004.
3-Jackson N, Alhussan A, Bromma K, Jay D, Donnelly JC, West FG, Lavasanifar A, Weinfeld M, Beckham W, Chithrani DB: Repurposing Antimalarial Pyronaridine as a DNA Repair Inhibitor to Exploit the Full Potential of Gold-Nanoparticle-Mediated Radiation Response. Pharmaceutics (2022), 14(12):2795.
4-Biosca, A., Dirscherl, L., Moles, E., Imperial, S., Fernàndez-Busquets, X.: An ImmunoPEGliposome for Targeted Antimalarial Combination Therapy at the Nanoscale. Pharmaceutics (2019), 11(7): 341.