Development of Targeted Polymer-Drug Conjugates for Ovarian Cancer Chemotherapy

Omotola D. Ogundipe, Simeon K. Adesina (R.Ph., Ph.D.), Department of Pharmaceutical Sciences, College of Pharmacy, Howard University, Washington DC 20059 USA

INTRODUCTION

Ovarian cancer (OC) is the most lethal of gynecological cancers.[1] Limitations of existing OC chemotherapeutics are chemoresistance and non-specific toxicity to normal cells.[2] One approach to addressing these limitations is by developing anticancer drugs as polymer-drug conjugates (PDCs). PDCs can be used to passively target cancer by the enhanced permeability and retention (EPR) effect (Fig.1), actively target overexpressed enzymes in cancer, and selectively release higher loads of cytotoxic drugs in the tumor microenvironment.[3]

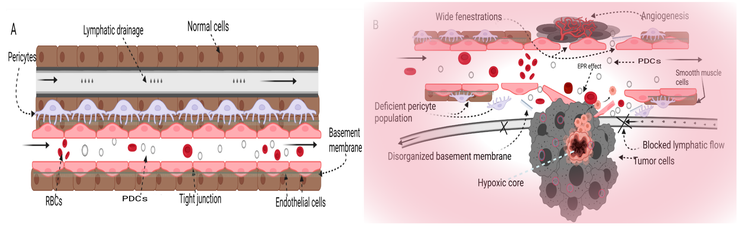

Figure 1. Comparison of the microenvironment of (A) healthy and (B) tumor tissues.

The objective of this study is to synthesize and characterize targeted, linear hydrophilic PDCs for the treatment of OC treatment using doxorubicin as the model drug.

METHODS

A α-,ω-bis-azide-terminated, linear hydrophilic ethylenediaminetetraacetic acid derivative (azidoPEG-EDTA) was coupled to glycylphenylalanylleucylglycine (GFLG) tetrapeptide by solid phase peptide synthesis to obtain azidoPEG-GFLG-EDTA intermediate for the polymer backbone. GFLG is a specific substrate for cathepsin-B, an enzyme that is overexpressed in ovarian cancer and is widely reported for selective drug release in the tumor microenvironment.[4] A doxorubicin-coupled intermediate, Dox-EDTA, was synthesized by reacting doxorubicin hydrochloride with the synthesized azidoPEG-GFLG-EDTA using isobutyl 1,2-dihydro-2-isobutoxy-1-quinolinecarboxylate as the coupling agent. End-to-end joining of Dox-EDTA was done using strain-promoted alkyne-azide cycloaddition-mediated polymerization. The synthesized compounds were analyzed using HPLC, mass spectrometry, proton NMR, and FT-IR spectroscopy.

RESULTS

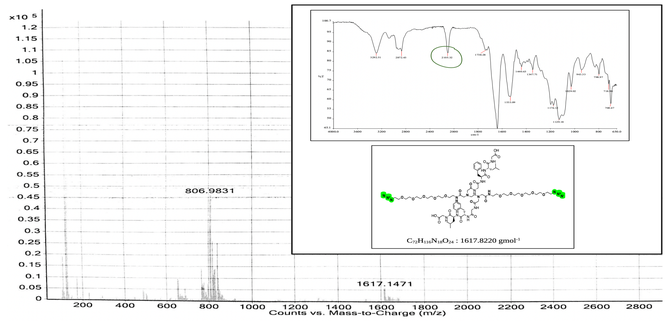

Figure 2. ESI-MS spectrum of azidoPEG-GFLG-EDTA (*mass calculated: 1617.8220; m/z observed: 1617.1471 [M⁺] and 806.9831 [M/2]⁺*) with overlaid chemical structure and FT-IR spectrum. The terminal azide groups of azidoPEG-EDTA remains intact after coupling with GFLG.

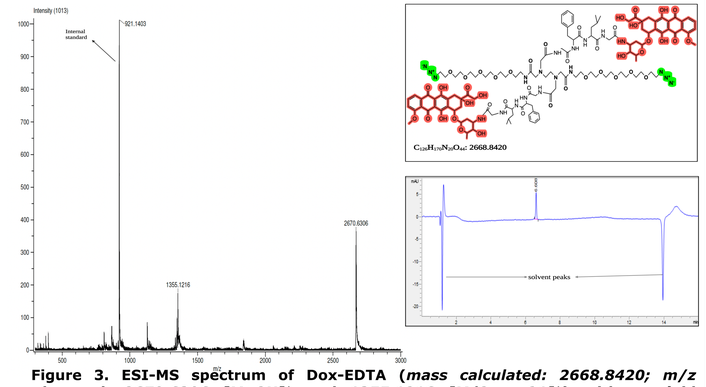

Figure 3. ESI-MS spectrum of Dox-EDTA (*mass calculated: 2668.8420; m/z observed: 2670.6306 [M+2H]⁺ and 1355.1216 [M/2 + 21]⁺*) with overlaid chemical structure and analytical HPLC spectrum at 480 nm. Purity of the compound is confirmed by the single peak seen at retention time 6.608 minutes.

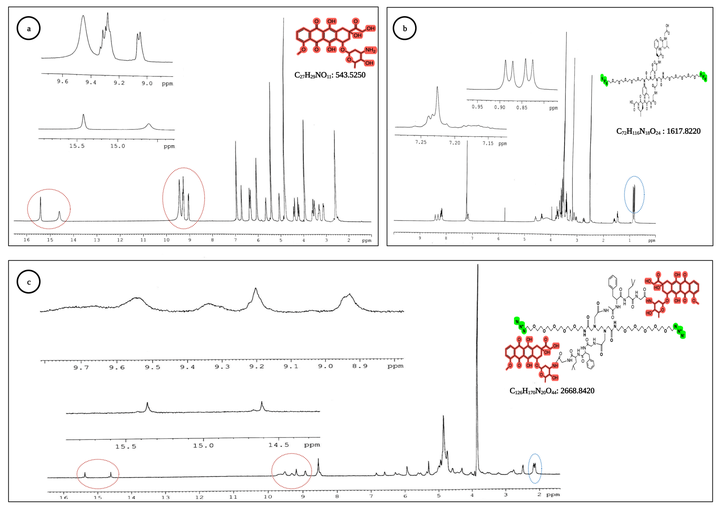

Figure 4. Proton NMR spectra of doxorubicin HCl (a), azidoPEG-GFLG-EDTA (b), and Dox-EDTA (c) in *d*-DMSO at 400MHz. Presence of the characteristic peaks (9.2 – 9.5 ppm; 14.6 ppm; 15.4 ppm) of doxorubicin in (C) indicates the successful coupling of doxorubicin to azidoPEG-GFLG-EDTA.

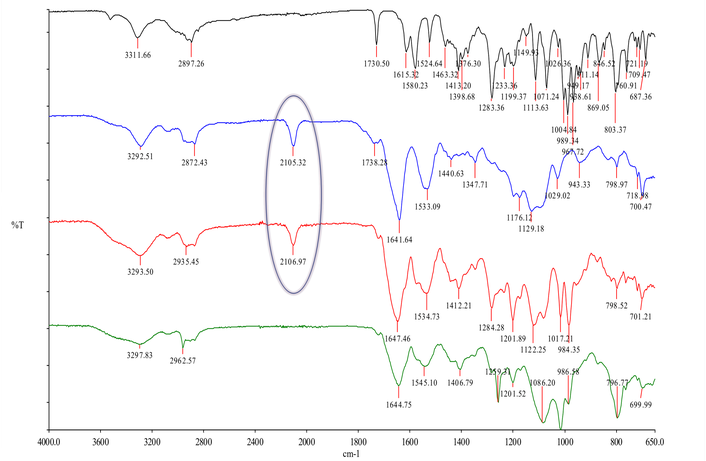

Figure 5. Overlay of the FT-IR spectra of doxorubicin HCl (black), azidoPEG-GFLG-EDTA (blue), Dox-EDTA (red), and polymerized Dox-EDTA (green). The disappearance of the azide stretch at ~ 2106cm $^{-1}$ in the polymerized Dox-EDTA (green) suggests that polymerization has occurred.

CONCLUSION

A linear hydrophilic doxorubicin-coupled polymer was synthesized for the development of PDCs designed to target and treat OC. Specific cathepsin B cleavage of the PDCs will allow selective drug release in the tumor microenvironment, thereby minimizing off-target toxicity to healthy cells.

REFERENCES

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