Combination Therapy for Treating Angiogenesis by Targeting Downstream Signaling Pathways (HIF-1 & mTOR)

Bhuvana S. Doddapaneni¹, Adam WG. Alani¹,

¹Oregon State University College of Pharmacy, Corvallis, OR, 97331, USA
doddapab@onid.orst.edu

ABSTRACT SUMMARY
The effect of two antiangiogenic drugs loaded in a single nanocarrier was evaluated in vitro. Everolimus (EVR) and Chetomin (CHE) were loaded into Poly (ethylene glycol)-block-poly (D,L-lactic acid) (PEG-b-PLA) micelle individually and in combination at clinically relevant concentrations. The antiangiogenic effect of these nanocarriers was evaluated in an angiogenesis in vitro model.

INTRODUCTION
The use of antiangiogenic drugs for cancer treatment was heralded as an improvement over conventional chemotherapy due to its presumptive inability to lose therapeutic activity as a result of tumor-acquired resistance over time. Unfortunately, recent clinical experience has demonstrated that acquired resistance to antiangiogenic therapeutic strategies is possible since many patients whose tumors initially respond to drugs such as bevacizumab (a monoclonal antibody against VEGF), sorafenib, or sunitinib (tyrosine kinase inhibitors targeting VEGF receptors and PDGF receptors) become nonresponsive, often within months of therapy initiation¹. One approach to overcome this resistance is the implementation of co-targeting strategies, where multiple mechanisms of drug action, as in the case of EVR and CHE loaded in single nanocarrier that can target neovascular angiogenic endothelial cells within the cancer tissue.

Mammalian target of rapamycin (mTOR) inhibitor EVR was shown to have antiangiogenic effects in tumor tissues². EVR targets mTORC1 which is an essential pathway for cell proliferation and angiogenesis in cancer tissue. Hypoxia in cancer/endothelial cells is a major stimulus that produces Hypoxia Inducible Factor, HIF-1α which in turn triggers the HIF-1α signaling pathway. EVR plays a role in suppressing the increase in HIF-1α³. CHE, another small molecule, disrupts the HIF-1α pathway by preventing the binding of HIF-1α with its co-activator p300³. Therefore, the combination of EVR and CHE can potentially be synergistic in inhibiting angiogenesis in cancer tissue while also mitigating any resistance.

PEG-b-PLA micelles provide an excellent delivery system that can produce a one-two punch approach to inhibit the angiogenesis process in cancer tissue. Our objective is to develop a new nanocarrier for the combination EVR and CHE and evaluate its application for antiangiogenic therapy for cancer treatment.

EXPERIMENTAL METHODS
PEG₂₀₀₀-b-PLA₁₈₀₀ (15 mg) and CHE (0.5 mg) were dissolved in 0.5 mL of acetonitrile. The organic solvent was removed using a rotary evaporator at 40 °C. After complete evaporation of the organic solvent, the resulting polymeric film was reconstituted with 0.5 mL double-distilled water. Finally, polymeric micelles were centrifuged at 7,500 rpm for 3 minutes and filtered through 0.45μm filter. A similar method was followed for EVR (0.5 mg) incorporation into PEG₄₀₀₀-b-PLA₂₂₀₀ (15 mg) micelles. Combination micelles were also prepared following the EVR micelle and the loading ratio for CHE:EVR was 1:1. Micelles were characterized for size and drug entrapment efficiency by DLS and HPLC methods respectively.

Cell proliferation studies were performed on Human Umbilical Vein Endothelial Cells (HUVECs). The cells were seeded into 96 well plates at 5,000 cells/well and were incubated at 37 °C for 24 h. The cells were treated with different concentrations of blank micelles,
drug(s) in DMSO, individual drug loaded micelles and combination micelles followed by incubation for 48 h. Cell viability was determined by treatment with Cell Titer Blue® reagent and after one hour of incubation at 37 °C, fluorescence (560Ex/590Em) signal was measured. GraphPad prism software was utilized to generate dose response curves and IC\textsubscript{50} values were computed. Migration and tube formation assays are still in progress.

**RESULTS AND DISCUSSION**

The average diameter of the CHE loaded PEG-b-PLA micelles was 27.32 ± 1.12 nm and that of EVR micelles was 28.10 ± 0.14 nm. The poly dispersity index of CHE and EVR micelles was 0.139 ± 0.024 and 0.153 ± 0.002 respectively. Entrapment efficiencies up to 93.5 ± 1.91% and 83.8 ± 1.72% was be achieved for CHE and EVR loaded micelles respectively. The same loading efficiencies were achieved for the combination micelles. HUVEC proliferation was inhibited by CHE and EVR in DMSO with IC\textsubscript{50} values of 161 ± 38.56nM and 1.6-2.4nM. Micelles loaded with CHE and EVR had IC\textsubscript{50} values of 56.85 ± 12.4nM and 0.8 ± 0.4nM respectively. Combination micelles of CHE and EVR (1:1 ratio) were found to be synergistic. Similar kind of response was observed when the cells were treated with free drugs (CHE & EVR) simultaneously. Work is in progress for the migration and tube formation assays and data will be presented at the conference.

**CONCLUSION**

These results suggest that EVR and CHE loaded in a single nanocarrier showed strong antiangiogenic effect in in vitro model.

**REFERENCES**

(1) Bocci, G.; Loupakis, F. *Medical Hypotheses* 2012, 78, 646.


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