Increased Efficacy of Doxorubicin Delivery with Phytosterol Nanoassemblies

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Statement of Purpose: The biological activity and toxicity of low molecular weight anticancer drugs such as doxorubicin (DOX) depend on the physicochemical properties that contribute to their pharmacokinetics, biodistribution, cellular retention, and bioavailability. Anthracyline drugs such as DOX kill tumor cells via apoptotic pathways by inhibiting DNA topoisomerase during DNA replication in S-phase of the cell cycle.[1] However, due to its short half-life, rapid distribution and excretion, DOX has relatively low bioavailability. Thus it would be beneficial if new drug delivery materials with relatively low toxicity and higher stability could be utilized for encapsulation of DOX to increase its bioavailability. In this work, we utilized the plant-based steroid 24-Epibrassinolide (EPIB) for the preparation of nanocarriers of DOX. EPIB mimics the structure of cholesterol (a plasma membrane component), which can self order into liquid crystalline assemblies. Thus far many biomaterials containing the cholesterol moiety have been examined as anchoring materials for cell attachment and targeting.[2] Here we report the efficacy of EPIB–DOX nanoassemblies as in the killing of human cervical cancer (Hela) cells.

Methods: Assemblies of EPIB were grown at varying concentrations ranging from 1 to 10 mg/mL in DMSO. The solutions were vortexed for 10 minutes and then allowed to assemble at room temperature for 2 to 3 weeks. Growth was monitored using dynamic light scattering analysis. For encapsulating DOX, samples grown at 5mg/mL for 3 weeks were utilized. To 500 µl of the EPIB assemblies, 200 µl of DOX (stock concentration at 2 mg/mL) was used. Samples were incubated overnight at 4°C and then washed into distilled water by centrifugation. The morphologies of the assemblies formed were characterized using TEM. Confirmation of DOX incorporation was carried out using uv-vis and FTIR spectroscopy. To examine the effect of the assemblies on Hela cells, *in vitro* cytotoxicity (WST-1 cell proliferation assays) and apoptosis (scored by FITC-annexin V binding to the plasma membrane) assays were performed.

Results / Discussion: EPIB belongs to the family of brassinosteroids involved in plant regulation.[3] Structurally it is composed of a hydrophobic steroidal moiety containing one lactone ring and four hydroxyl groups. Hydroxyl groups promote hydrogen-bonding between the EPIB units, allowing formation of assemblies. We found that nanospheres in the range of 50 to 100 nm were formed, dependent upon the concentration and the growth period. DOX incorporation was confirmed by absorbance spectroscopy. A characteristic peak at 485 nm was observed for the encapsulated assemblies (slightly blue shifted) compared to DOX solution (490 nm). This shift is likely due to binding interactions between EPIB and DOX. The encapsulation efficiency was found to be 82%. We next incubated the materials with Hela cells to determine their efficacy as anticancer agents. Cells were treated with DOX encapsulated EPIB for 24 hrs. DOX, EPIB and DMSO (controls) were also compared. In the light microscope, we found that DOX loaded EPIB assemblies were more potent compared to DOX in killing cells (Fig. 1). Cytotoxicity studies revealed 13% killing in DMSO; 5% with EPIB, 26% with DOX and 89% with DOX loaded EPIB. Much of this cell death was found to be a result of apoptosis as determined by the binding of FITC-labeled annexin V in parallel experiments. We found that 7% of the cells were apoptotic when treated with DMSO, 12% with EPIB, 33% with DOX and 56% with EPIB-DOX.

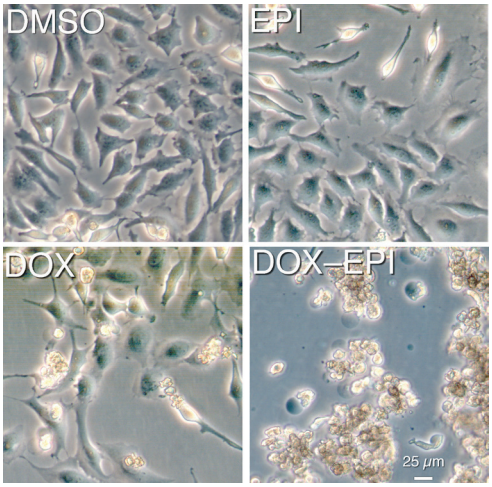

Fig. 1. Phase contrast images of Hela cells incubated for 24 hrs with DMSO (solvent control), EPIB, DOX and DOX encapsulated in EPIB. Bar = 25 µm.

Conclusions: EPIB assemblies were formed and utilized for encapsulation of DOX. The materials were cytotoxic to Hela cells compared to controls. Such assemblies may have potential applications for targeted drug delivery.

References:

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