Title: Optimizing drug delivery using arsenic trioxide encapsulated liposomes for increasing the efficacy in treating cervical cancer *in vitro*

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Abstract

Arsenic trioxide (ATO) has been demonstrated to have significant therapeutic effects against acute promyelocytic leukemia and various haematological malignancies. However the lack of success in clinical trials in treating solid tumours due to its toxicity has prevented its further application. In this study, ATO encapsulated liposomes were prepared and investigated for their physico-chemical characteristics, drug loading efficiency and inhibitory activity on cervical cancer cells under different surface charges (neural, negative or positive charges) and sizes (100, 200 and 400nm). Five different liposomal formulations were prepared by conventional lipid film hydration technique. Liposomes of increasing sizes were prepared by extrusion from filters of 100nm, 200nm and 400nm pore sizes respectively. Charged liposomes were prepared in a similar way by incorporating dimethyldioctadecylammonium bromide (DB) and 1-stearoyl-2-hydroxy-*sn*-glycero-3-phospho-sodium salt (DSPG) as cationic and anionic charge carrier lipids for liposomes. The resultant liposomes were stable at 4°C with less than 10% arsenic leakage after a month as determined from inductively coupled plasma optical emission spectroscopy (ICP-OES). Loading efficiency was observed to be independent of tested size range; however neutral liposomes had the highest loading efficiency among the charged liposomes. Cellular uptake and apoptosis studies of these liposomes were evaluated against HPV positive (HeLa) and HPV negative (C33a) cervical cancers and a normal control cell line (CRL 1790) through inductively coupled plasma-mass spectroscopy (ICP-MS), flow cytometry and toxicity studies. Liposomal uptake was found to be independent of sizes but dependent on surface charges and cell lines. Positive liposomes displayed the highest uptake, but the highest toxicity to the cells after their exposure rules them out as suitable candidates as drug carriers. Lower toxicity to the cells was observed when liposomal ATO was used instead of free drug and overall, C33a cells were more susceptible to ATO than HeLa despite a lower arsenic uptake. Cancer cells displayed the significant apoptotic effect and toxicity per unit uptake of ATO as opposed to normal cells when liposomal ATO was employed. In conclusion, neutral 100 nm liposomes were chosen as an effective ATO carrier to cervical cancer cells due to their lower toxicity, higher loading efficiency and high stability.