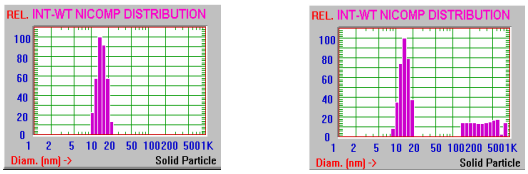
Solubilization of 17-Allylamino-17-demethoxygeldanamycin in Biocompatible and Biodegradable Sterically Stabilized Phospholipid Mixed Micelles - A Novel Nanomedicine for Treatment of Breast Cancer

Prem Swaroop Mohanty[1], Israel Rubinstein[1, 2,4] and Hayat Onyuksel[1, 3]
Department of Biopharmaceutical Sciences[1], Medicine[2], Bioengineering[3]
University of Illinois at Chicago and Jesse Brown Veterans Affairs Medical Center[4], Chicago, IL 60612.

Statement of Purpose: 17-Allylamino-17-demethoxygeldanamycin (17-AAG) is a novel heat shock protein 90 (Hsp90) inhibitor under clinical development for a variety of cancers including breast cancer. 17-AAG is poorly soluble in water (~2µg/ml in 10mM HEPES buffer, pH7.4) and is difficult to formulate. Three formulations of 17-AAG including a DMSO-Egg Phospholipid formulation, a cremophor-based formulation (KOS-953) and an oil-in-water nanoemulsion (CNF1010) are being extensively tested in various stages of Phase I/II clinical trials (1). However all of the above formulations suffer from vehicle associated side effects. Sterically stabilized mixed micelles (SSMM) on the other hand being long circulating nanoparticles should provide targeted delivery. Also it is safer as toxic reagents such as cremophor or DMSO are not used in the SSMM formulation. To begin to develop SSMM as a suitable carrier, here we investigate if sterically stabilized phospholipid mixed micellar system can solubilize sufficient amount of 17-AAG.

Methods: 17-AAG (A.G. Scientific, San Diego, CA) was solubilized into SSMM composed of Distearoyl Phosphatidyl ethanolamine- Polyethylene Glycol 2000 (Lipoid LLC, Newark, NJ) and egg phosphatidyl choline (Lipoid LLC, Newark, NJ) by co-precipitation and rehydration with 10mM HEPES buffer, pH 7.4 (2). Increasing concentrations of 17-AAG ranging from 100-250 µg/ml in 5mM lipids were tested. Excess drug was removed by centrifugation at 13,000g and mean particle size in the supernatant was determined by quasi-elastic light scattering using NICOMP® 380 submicron particle sizer (Particle Sizing Systems, Santa Barbara, CA). Presence of a secondary species (Sterically Stabilized Particles (SSPs)) around 200-500nm was considered to be the end point for reaching the maximum micellar solubilization capacity. The Drug content of the optimum solubilized formulation in SSMM was then determined by Reverse Phase–High Performance Liquid Chromatography (RP-HPLC) (Column- C-18, 4.6mm X 250mm, Mobile phase- Acetonitrile:water (70:30), Flow rate- 1ml/min and Detection wavelength- 330nm).

Results/Discussion: Particle Size analysis of 17-AAG concentrations equal to or below 150µg/ml in 5mM SSMM showed a single peak size distribution around 15-16nm (Fig 1a). At concentration 175µg/ml of 17-AAG, SSPs which we believe are PEGylated lipid coated drug particles were seen intermittently. It was therefore concluded that 175µg/ml was the transition region where SSPs start to form. Higher concentrations of 17-AAG showed consistent SSP formation (Fig 1b). Hence 150µg/ml 17-AAG is the optimum solubilization capacity of 5mM SSMM.



(a) (b)

Figure 1: Representative Intensity weighted Size Distribution of 150µg/ml (a) and 200µg/ml (showing second SSP peak) (b) of 17-AAG in 5mM lipids.

A summary of the particle size analysis of all 17-AAG concentrations tested is shown in Figure 2

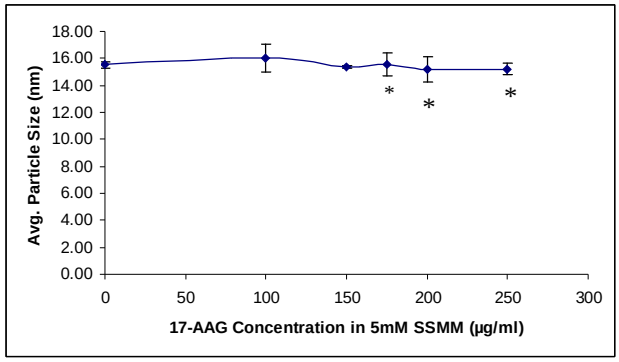


Figure 2: Average Particle Size (Intensity-Weighted) of 17-AAG loaded SSMM (5mM Lipid Concentration), n=3. * denotes presence of SSPs (200-500nm Size) in the system in addition to the micellar peak.

92.4±1.45% of the drug originally incorporated into the system was recovered as determined by RP-HPLC in the final optimal formulation (150µg/ml 17-AAG in 5mM lipids).

Conclusions: The optimum solubility of 17-AAG in 5mM SSMM was determined to be 150µg/ml. This is approximately a 75 fold improvement in the aqueous solubility of the drug. Therefore we will further characterize and develop this formulation for the treatment of breast cancer.

References: 1. Solit DB. Curr Top Med Chem. 2006;6(11):1205-14
2. Krishnadas A. Pharm Res. 2003 ;20(2):297-302

Acknowledgements: This work was supported in part by Department of Defense grant BCRP, No. DAMD 17-02-1-0415, a VA merit review and NIH grant R01 AG024026