Superior Anti-tumor Activity From A Gemcitabine Prodrug Incorporated Into Nanoparticles

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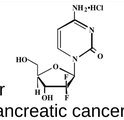
Introduction

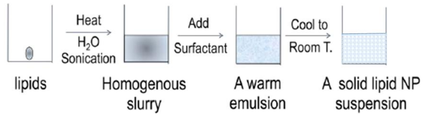
Cancer is one of the leading causes of death in the United States. Chemotherapy remains an important cancer treatment modality, and superior chemotherapeutic drugs are constantly being sought. Gemcitabine, manufactured by Eli Lilly & Company, is an anti-tumor chemotherapeutic drug, and is the first line of defense in treating pancreatic cancer, as well as, combination therapy for non-small cell lung, ovarian, and metastatic breast cancers. Patients with cancer, specifically pancreatic carcinoma, have a high mortality rate and exhibit poor anti-tumor activity while on gemcitabine. The anti-tumor activity of gemcitabine shows needed improvement due to its short half-life and low specificity in patients. The objective of current study was to synthesize a lipophilic gemcitabine prodrug and package it into Nanoparticles (NPs). Delivering gemcitabine in NPs may improve its systemic half-life, and has the potential to be targeted to specific tissues.



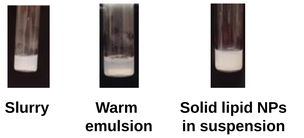
GEMZAR (gemcitabine)

Methodology

Engineering of lipid based NPs were prepared from emulsions in a step wise manner as illustrated in *Scheme 1*. The size of the NPs was measured by photon correlation spectroscopy (PCS) using a Coutler N4 Plus submicron Particle Sizer. Size and morphology was also observed using a transmission electron microscopy (TEM).

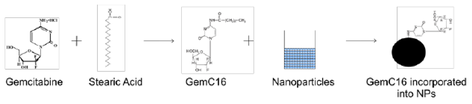


Scheme 1. Formation of NPs



Scheme 2 Formation of NPs (Actual vials)

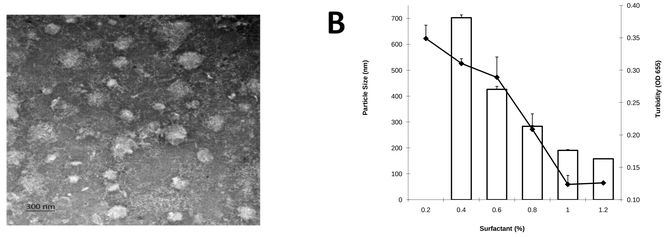
Synthesis of the gemcitabine prodrug (GemC16) was achieved by coupling a fatty acid onto the amine group of gemcitabine. Described briefly in *Scheme 3*, synthesis and incorporation of GemC16 into NPs was performed. GemC16 synthesis was confirmed by thin layer chromatography (TLC) and nuclear magnetic resonance (NMR).Incorporation of GemC16 into NPs was confirmed using gel permeation chromatography (GPC, Sepharose 4B).



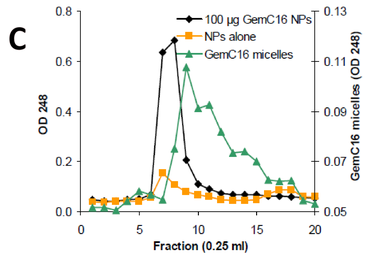
Scheme 3 GemC16 incorporation into NPs

Cytotoxcity of GemC16-NP was first evaluated *in vitro* using TC-1, PanCO2, and BxPC3 cell lines. The efficacy of the GemC16-NPs was evaluated in a mouse tumor model.

Results



Figures A. B:. **A.** A TEM of blank NPs showed an average diameter of 180 nm, and were spherical in shape. **B.** As surfactant was added to the homogenous emulsion in a step wise manner, the particle size and turbidity (OD655) decreased. The addition of surfactant to a concentration of 1% (v/v) resulted in particles in ~175 nm in diameter; further, increasing the concentration of surfactant did not result in significantly smaller particles or lower the turbidity. Data reported are mean S.D.



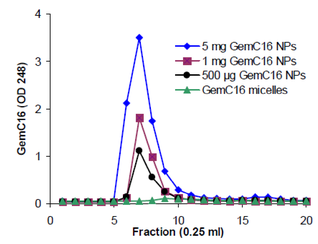


Figure C & D:. **C.** GPC demonstrated that NPs can be separated from unincorporated GemC16 in micelles.NPs were detected by measuring the absorbance at 269 nm. **D.** The chromatograms of the different ratios of incorporated GemC16 in NPs and free GemC16. In C and D, data reported were the mean values from three independent determinations. In all cases, fractions 6 - 8 corresponded to GemC16 loaded NPs that could be reasonably separated from free GemC16 in micelles. A maximum concentration of 5 mg/mL of GemC16 in NPs was observed.

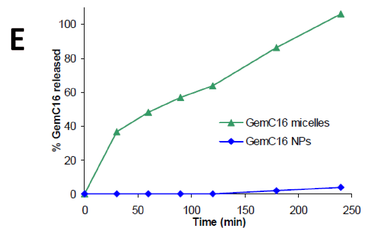
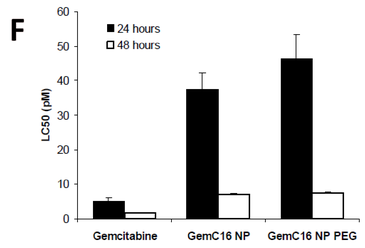


Figure E: The release of GemC16 from NPs. GemC16 released slowly from NPs (~18% within 40 hours), where GemC16 loaded into micelles quickly diffused out of the membrane. The diffusion medium was 0.5% SDS in PBS (pH 7.4).



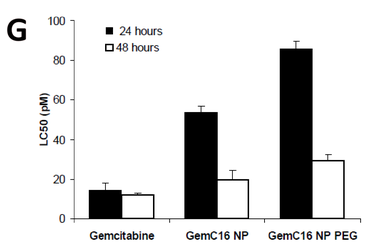


Figure F & G: F. Cytotoxcity of the GemC16 NPs to TC-1 cells *in vitro*. Toxicity assay shows free gemcitabine is more toxic than GemC16 NPs, with and without PEG. Two time points were taken, 24 and 48 hours, plotted against the 50% lethal concentration (LC50). **G.** Cytotoxcity of the GemC16 NPs to PanC02 cells *in vitro.* Free gemcitabine was more toxic than GemC16 NPs. Incorporation of PEG decreased the toxicity at both time points (24, 48 hr). Data reported are mean S.D. (n = 3).

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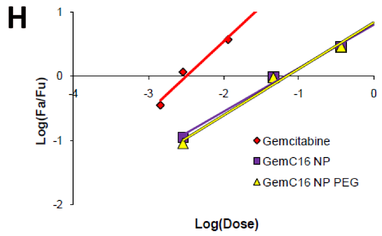
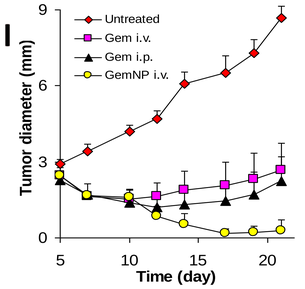


Figure H: Cytotoxcity of the GemC16 NPs to mouse pancreatic cells (BxPC3) *in vitro*. Native gemcitabine was more toxic than GemC16 loaded NPs. The toxicity of GemC16 NPs with and without PEG exhibited similar toxicity. The LC50 is determined by taking the log of the value where the regression crosses the x-axis.



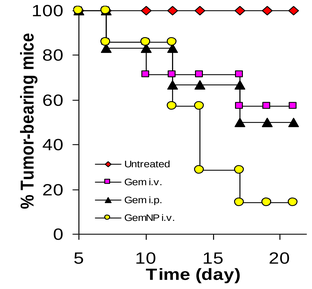


Figure I & J:. **I**. The anti-tumor activity of GemC16 NPs in a mouse tumor model. Mice were implanted with TC-1 tumors subcutaneously on day 0 and administered one dose of GemC16 NPs intravenously on day 4.. The tumors of untreated mice grew steadily, while mice given one injection of free gemcitabine either i.v or i.p showed an initial decrease in tumor size, followed by growth. However, mice administered with GemC16 NPs showed significant tumor regression. Data reported are mean S.D. **J.** The percent tumor bearing mice graph shows untreated mice exhibited tumors throughout the study; whereas mice treated with GemC16 NP showed significant tumor regression.

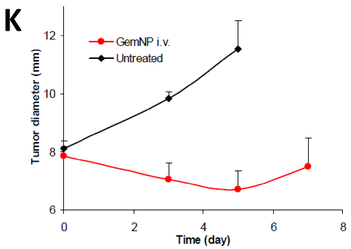


Figure K: The anti-tumor activity of GemC16 NPs in an advanced tumor study. Mice treated with GemC16 NP showed a decrease in tumor diameter following an i.v injection. Untreated mice exhibited advanced tumor growth throughout the study. Data reported are mean S.D.

Conclusions

GemC16 loaded NPs had an average diameter of ~200 nm. GemC16 was successfully incorporated into lipid based NPs at a maximum concentration of 5 mg/ ml. GemC16 in NPs were toxic to TC-1, PanC02, and BxPC3 tumor cell lines *in vitro*. GemC16 NP exhibited superior anti-tumor activity in mice tumor studies as compared with mice treated with gemcitabine. NPs was an effective delivery platform for GemC16 to tumor bearing mice and showed positive anti-tumor activity.

Future Research

1. A biodistribution study will be performed to analyze GemC16 NP circulation within the body and localization within organ systems
2. A pharmacokinetics experiment will be performed to calculate appropriate half-life of GemC16 drug concentration *in vivo*
3. A n efficacy study of GemC16 NP against human pancreatic cancer cell line

Acknowledgements

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