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Effect of particle size on the biodistribution, toxicity, and efficacy of drug-loaded polymeric nanoparticles in chemoradiotherapy

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Abstract

Nanoparticle (NP) chemotherapeutics can improve the therapeutic index of chemoradiotherapy (CRT). However, the effect of NP physical properties, such particle size, on CRT is unknown. To address this, we examined the effects of NP size on biodistribution, efficacy and toxicity in CRT. PEG-PLGA NPs (50, 100, 150 nm mean diameters) encapsulating wotrmannin (wtmn) or KU50019 were formulated. These NP formulations were potent radiosensitizers *in vitro* in HT29, SW480, and lovo rectal cancer lines. *In vivo*, the smallest particles avoided hepatic and splenic accumulation while more homogeneously penetrating tumor xenografts than larger particles. However, smaller particles were no more effective *in vivo*. Instead, there was a trend toward enhanced efficacy with medium sized NPs. The smallest KU60019 particles caused more small bowel toxicity than larger particles. Our results showed that particle size significantly affects nanotherapeutics' biodistrubtion and toxicity but does not support the conclusion that smaller particles are better for this clinical application.
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Key words: Nanoparticle; Chemoradiotherapy; Nanoparticle radiosensitization; KU60019; Wortmannin

Chemoradiotherapy (CRT) is a central treatment paradigm in the management of many solid cancers.[1–3] The generation of DNA double-strand breaks is thought to be one of the principle mechanisms of radiation-induced cell death and a number of potent DNA repair inhibitors have been developed. However, there is very little clinical experience combining these with radiation for fear of excess normal tissue toxicity when these drugs are administered systemically. Pre-clinical studies have demonstrated that nanoformulation of radiosensitizing drugs may be an effective way to accomplish this goal.[4–9] A number of potentially radiosensitizing nanotherapeutics have already been formulated and are being tested in the clinic.[10–14] It is worth noting, however, that none of these compounds were specifically engineered for use in CRT. Instead, they were optimized for chemotherapeutic drug delivery and are now being applied to CRT paradigms. The optimum particle characteristics for use in CRT, such as particle size, are currently unknown.

It is generally believed that "stealth" particles in the sub-50 nm range are desirable as drug delivery vehicles because they are more penetrating in tumors.[15–18] However, it is not clear that these characteristics are optimal for use in CRT. Radiation alters both tumor and normal tissue vasculature and it is unknown how this affects the therapeutic index of drug-loaded NPs.[19–23] We hypothesized that highly-penetrating small particles may increase toxicity in irradiated normal tissues and that the optimum therapeutic ratio might be achieved with larger particles (100-150 nm). Understanding the relationship between particle size and therapeutic index will inform the optimal design of NP drug formulations for use in CRT.

We investigated the role of particle size in CRT by generating three populations of polymeric nanoparticles of different mean sizes but similar release kinetics. These particles were approximately 50,

Abbreviations: ACN, acetonitrile; ANOVA, analysis of variance; CBC, complete blood count; CRT, chemoradiotherapy; GI, gastrointestinal; Hb, hemoglobin; HCT, hematocrit; mPEG-PLGA, methoxy-poly(ethylene glycol)-block-poly(lactic-co-glycolic acid); NP, nanoparticle; PLA, poly(D,L-lactide); WBC, white blood cell; wtmn, wortmannin.

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100, and 150 nm in mean diameter. We utilized two DNA repair inhibitors which target distinct DNA repair proteins as model drugs: the DNA-PK inhibitor wortmannin and the ATM inhibitor KU60019 as model drugs. Each NP encapsulated only one drug. We compared the antitumor efficacy of these formulations in three rectal cancer cell lines *in vitro*. We then compared the biodistribution, therapeutic efficacy, and toxicity of the particles when combined with radiation *in vivo* in mice with rectal tumor xenografts.

Methods

Materials

Wortmannin and KU60019 were purchased from Apex Bio (Houston Texas). Methoxy-poly(ethylene glycol)-block-poly(lactic-co-glycolic acid) (mPEG-PLGA) with molecular weights of 2000:15,000 (PEG(2 K):PLGA(15 K)) and 5000:1000 Da (PEG(5 K):PLGA(10 K)) were purchased from Akina (PolySciTech, West Lafayette, IN). Rhodamine B-labeled PLGA with approximate molecular weight 30,000 was purchased from Akina Inc. (PolySciTech, West Lafayette, IN). Poly(D,L-lactide) (PLA), ester terminated with average MW 18000–28,000 was purchased from Sigma–Aldrich (St. Louis, MO). Acetonitrile (HPLC grade) and double distilled water (HPLC grade) were obtained from Sigma–Aldrich (St. Louis, MO).

Preparation of wortmannin or KU60019 nanoparticles

Polymeric nanoparticles encapsulating either wortmannin or KU60019 were generated utilizing a previously described nanoprecipitation method.[6] Briefly, polymers were dissolved in acetonitrile (ACN) (mPEG-PLGA 40 mg/ml, PLA 2 mg/ml). Drugs were also dissolved in ACN (2 mg/ml). Drug-polymer mixtures (1 ml) were then added dropwise to double deionized water (3 ml) over rapid stirring (1000 rpm). The mixture was then constantly stirred under vacuum at room temperature for 3 h to allow self-assembly and evaporation of the organic solvent. Particles were then centrifuged for 15 min at 8000× G in 30 KDa cut-off centrifuge filters (Millipore, Billerica, MA). Particles were washed in 1× phosphate buffered saline (1 ml) followed by repeat centrifugation. After three washes particles were resuspended to desired concentrations in 1× phosphate buffered saline or tissue culture media.

Particle size was adjusted by altering the polymer compositions within the organic solvent mixtures. The smallest particles were obtained by adding 5 mg of 5000:10,000 mPEG-PLGA and 500 μg (10%) KU60019 or wtmn. Intermediate sized particles were generated by adding 5 mg 2000:15,000 mPEG-PLGA, 3 mg PLA, and 800 μg (10%) KU60019 or wtmn. The largest particles were generated by mixing 7 mg 2000:15,000 mPEG-PLGA, 9 mg PLA, and 800 μg (5%) KU60019 or wtmn.

Preparation of Flamma Fluor-labeled nanoparticles

Flamme Fluor (FKR648)-labeled empty PEG-PLGA and PEG-PLGA-PLA particles were prepared *via* nanoprecipitation as above in the presence of 5% wt/wt FKR648-conjugated PLGA (AV 015, Akina Biosciences). FKR648-PLGA was diluted to 1 mg/ml, mixed with the solvent mixtures described above, and added dropwise to double deionized water. The solution was then continuously stirred under a vacuum in the dark for 3 h and then particles were purified and washed 3 times as above. Empty particles were then resuspended in sterile PBS at desired concentrations. Incorporation efficiency was >95% for all particle sizes and was assessed by determining fluorescence intensity from the supernatant after filtration.

Characterization of nanoparticles

Purified particles encapsulating KU60019 or wtmn were characterized by transmission electron microscopy (TEM), dynamic light scattering, and aqueous electrophoresis. TEM images were captured using a Zeiss TEM 910 transmission electron microscope operated at 80 kV (Carl Zeiss Microscopy, LLC, Thornwood, NY) in the microscopy services laboratory core facility at the UNC school of medicine. Prior to TEM imaging, concentrated NP samples were diluted to 5 mg/ml in deionized water. A 5 μL sample of each was mixed with 5 μL 4% uranyl acetate aqueous solution before being added to a 400 mesh carbon-filmed copper grid. Intensity-average diameter (Dh, also known as hydrodynamic diameter) and mean zeta potential (mean ζ) of nanodispersions were determined by dynamic light scattering and an aqueous electrophoresis method using a Zetasizer Nano ZS instrument (Malvern Inc., Warcestershire, UK). All measurements were based on the average of three separate measurements.

Drug loading determination

KU60019 and wtmn loading in polymeric nanoparticles was quantified using a Shimadzu SPD-M20A high-pressure liquid chromatography (HPLC) instrument (Shimadzu, Kyoto, Japan) equipped with a diode array detector at a GP-C18 reverse phase column (pore size =120 Å, 4.6 × 150 mm, Sepax Technology, Newark, DE). A linear gradient from 10% ACN in water to 100% ACN was run over 15 min, followed by 100% ACN for 5 min, and finally 10% ACN for 5 min. Flow rate was 1 mL/min. Wtmn eluted with a retention time of 5.6 min and was read at a wavelength of 250 nm. KU60019 eluted with a retention time of 6.4 min and was detected at a wavelength of 230 nm. For preparation, 100 μL of purified particles was dissolved in 100 μL of ACN, vortexed vigorously, and stored over night at 4 °C to allow complete dissolution of particles. Drug concentrations were determined by generating standard curves from 0 to 100 μM for each drug. Drug loading (wt/wt%) was calculated as (wt drug mg/wt polymer mg) ×100%. Encapsulation efficiency was calculated as (concentration drug in dissolved particles/concentration of drug in initial organic phase solution) ×100%.

In vitro drug release studies

Drug release rates were measured by placing 100 μL of purified particles diluted to 2.5 mg/ml into Slide-A-Lyzer MINI dialysis tubes with a molecular weight cut off of 10 kDa (Pierce, Rockford, IL) and subjected to dialysis against a large excess (4 L) of PBS with gentle stirring at 37 °C. At indicated times, whole samples were removed and dissolved as described above in ACN to allow disruption overnight. Drug concentrations were then determined using HPLC as above. Drug loading and encapsulation efficiency were determined at time 0 (immediately after purification). Drug release half-life ($T_{1/2}$) is defined as the time for half the

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