Nano-Mesosphere Drug Carriers for Localized Cancer Chemotherapy

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ABSTRACT

The therapeutic activity of most cancer drugs is dose dependant. Systemic toxicity prevents the use of aggressive high dose chemotherapy. There is a great need for more efficient localized and non-systemic drug delivery. Reported here is research devoted to the synthesis, properties, and therapeutic evaluation of drug loaded nano-meso-spheres (meso-MS) designed for administration as aqueous dispersions by local intratumoral (IT) injection. Clinically practical compositions have been synthesized using mitoxantrone (MXN) with biodegradable proteins (i.e. albumin, collagen, casein) or with deoxyribonucleic acid (DNA) as the meso-MS matrix. *In vivo* IT studies in a murine mammary adenocarcinoma demonstrated greatly prolonged survival (>500%) with no systemic toxicity at MS-loaded drug concentrations more than 6x the toxic dose for systemic drug delivery.

Keywords: nano-mesospheres, local drug delivery, intratumoral chemotherapy

INTRODUCTION

The safety and efficacy of conventional intravenous chemotherapy and immunotherapy are severely limited by systemic toxicity. Although significant advances have been made in the fields of cancer diagnosis and microbiology, there have been only modest improvements in the treatment of high mortality cancers. Based on CDC estimates for the past 20-30 years, there has been a slight decline in breast cancer mortality (2%) but more than a 70% increase in lung cancer mortality (>160,000 in 2004) [1,2].

Because the cytotoxic activity of most chemo-therapeutic drugs has been shown to be highly dose-dependant, systemic toxic complications such as myelosupression, mucositis, and cardiomyalgia prevent the use of more effective chemotherapy. There is a consequent need for localized non-systemic drug delivery. Our laboratory has pioneered a therapeutic modality for overcoming this shortcoming of conventional cancer therapy by using direct IT injection of drugs and, with superior results, drug-loaded biodegradable biopolymers in the form of submicron (nano) or <10 micron (meso) particles or spheres. These compositions can perfuse tumors with a superdose of a cytotoxic drug and afford prolonged activity with minimal systemic toxicity. Regression and necrosis of the tumor mass also offers an opportunity for improved outcomes for tissue conserving surgery 1-2 weeks following IT chemotherapy (i.e. neoadjuvant treatment before breast or lung cancer surgery).

A novel syntheses of particulate protein drug carriers was developed which readily yields submicron nanospheres, 1-10 μm mesospheres, or even larger microspheres. Synthesis is by a process of steric stabilization which can produce stable dispersions of aqueous biopolymer solutions as a dispersed phase in an immiscible organic polymer solution (i.e. cellulose acetate in dichloroethane) as the continuous phase. The novel addition of the crosslinking agent (i.e. glutaraldehyde or genipin) via the organic phase results in stable spherical particles which may be washed and collected as a readily dispersable fine

powder. Loading with various drugs (i.e. cytotoxic agents, immunomodulators, or antibiotics) may be achieved during synthesis or by post-loading by adding meso-MS to drug solutions.

For intratumoral drug delivery, nano-meso-sphere formulations appear to be more advantageous than larger microspheres (>10 microns) because the smaller size facilitates dispersion and injection through small gauge needles and affords more complete perfusion of the tumor tissue with adequate tumor tissue fixation.

Previous research has shown that drug release from albumin and casein nano-meso-microspheres is a function of particle size, crosslink density drug-matrix interactions, and rates of drug diffusion and carrier biodegradation. Higher cross-link density leads to slower drug release due to decreased particle swelling and slower biodegradation. Larger drug loaded microspheres (>10 microns) exhibit slower release rates due to the smaller initial surface area. In general, the initial stage of drug release is by drug diffusion after which degradation of the matrix results in a second stage of release accompanied by increasing matrix structure porosity.

There is a rapidly growing body of literature concerning intratumoral drug delivery and we have published a comprehensive review in 2002 [3]. The research reported here emphasizes our recent pre-clinical evaluations of mitoxantrone (MXN) loaded albumin meso-MS in a murine adenocarcinoma with brief mention of the novel use of DNA in meso-MS drug carrier compositions. MXN has been a drug of particular interest in this work. As an anthraquinone derivative, it has a helpful blue chromophore (for tumor perfusion histology and for tracking any diffusion to the draining lymph nodes) [4]. It is similar in antitumor activity to daunorubicin but reportedly less cardiotoxic [5].

IT drug delivery of MXN loaded BSA meso-MS has been studied in a murine Lewis lung carcinoma with significant "cures" (tumor-free survivors) when used in conjunction with surgical resection following IT injections [2,4]. It is also noteworthy that there are now some very favorable clinical reports for IT administration of even free unbound drugs for the treatment of breast cancer [4] and NSC lung cancer with esophageal obstruction [6].

Reported here are the synthesis of DNA meso-MS and the pre-clinical evaluation of MXN loaded albumin meso-MS in a murine mammary adenocarcinoma [7]. In this latter study, IT delivery of free MXN was compared with meso-MS loaded with ~13 wt% MXN and tested at several dose levels.

EXPERIMENTAL METHODS

Nano-Mesosphere Synthesis

MXN-BSA meso-MS of 1-10 micron diameter were prepared by a process previously reported in detail using a steric stabilization procedure in which the continuous organic phase was a 3% solution of cellulose acetate-butyrate [8-10]. The meso-MS were crosslinked via the organic phase with 8% (w/w) glutaraldehyde (GTA) in 1,2-dichloroethane. Crosslink density was controlled by varying the amount of GTA based on the estimate of 0.8 mmole GTA required for reaction with all lysine amino groups per gram of BSA. After aqueous and acetone wash, dried MS were obtained as a free flowing powder of 1-10 micron particles. In-situ loading of MXN to 12.8 wt% drug was readily achieved by the addition of

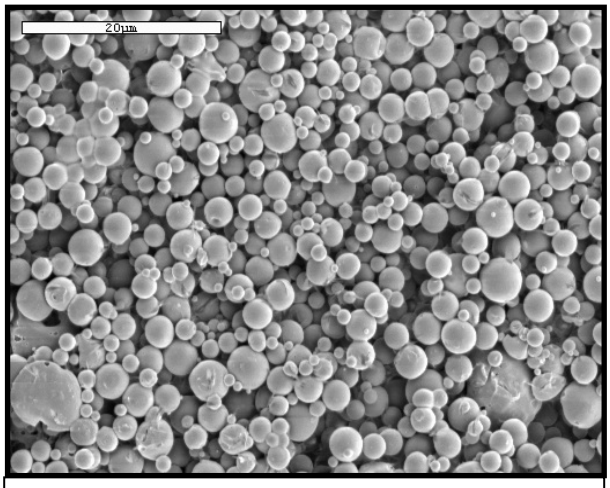

Figure 1 Mesospheres crosslinked with glutaraldehyde.

MXN to the aqueous biopolymer solutions during MS synthesis. MS were water and acetone washed and dried at room temperature The Figure 1 SEM shows a typical meso-MS preparation.

In related studies, casein [10], gelatin, gelatin-poly-L-glutamic acid, and DNA nano-mesospheres have been prepared by a similar procedure. For protein and polysaccharide compositions, another cross-linking agent, genipen (a protein isolated from the gardenia), has also been investigated. Genipen is interesting because it is less toxic than GTA and may therefore prove useful for such possible nano-mesosphere applications as vaccines, gene transfection, and the localized treatment of rheumatoid arthritis. For DNA, ionic crosslinking was also achieved using trivalent metal ions such as chromium (3+) and gadolinium (3+).

In Vivo Intratumoral Mesosphere Studies

In pre-clinical evaluations, the efficacy of MXN loaded albumin meso-MS (12.8 wt% MXN) was compared with free drug at different dose levels in a 16/C murine mammary adenocarcinoma [7]..

Female C3H/HeJ mice (Jackson Laboratories), 12-14 weeks old, were injected subcutaneously with 25 mg of 16/C tumor. Test groups (n=5) were randomized and tumors were treated when they reached 10 mm in the largest dimension (a meta-static stage). Five IT injections, each 0.02 ml, were distributed around and into the tumor to maximize tumor perfusion (total injected volume of 0.10 ml).

For IT injections, meso-MS formulations were dispersed in a vehicle of 0.5% Tween 80 surfactant in normal saline for optimal particle dispersion. The treatment groups in an initial study had drug dosage in the range 4-24 mg/kg for free drug (F4-F24) or 24-48 mg/kg for MXN loaded meso-MS (M24-M48). Results were used to select an appropriate dose range for a second study in which free drug doses (F) of 4, 8, 12 mg/kg and MS doses (M) of 24, 32, 40 mg/kg were compared to controls. Animal weight and tumor size were measured for 60 days after treatment. If tumor mass exceeded 10% of body mass, an animal was euthanized. Animals surviving tumor free 60 days or more after initial IT treatment (>5x the mean survival of controls) were considered “cured”.

RESULTS, DISCUSSION & CONCLUSIONS

Drug loaded nano-spheres and meso-spheres were readily prepared using a steric stabilization process by crosslinking aqueous biopolymer dispersions in a continuous organic polymer solution phase. Albumin meso-MS compositions of 1-10 microns containing >12 wt% of the broad spectrum cancer drug, mitoxantrone (MXN), were evaluated in a murine mammary cancer model.

Doses up to 40 mg/kg were delivered IT in MS with no systemic toxicity and with 80% “cure” of treated animals (Figure 2 – blue M40 line). This dose is more than 6 times the reported intravenous LD_{50} for mitoxantrone (6.6 mg/kg). The increase in median life span (ILS) for non-toxic high dose IT chemotherapy observed in this study was >500% [8]. The survival plot also indicates the greater efficacy of drug-MS compositions compared to best results for IT injection of free drug (Figure 2; 20% survival at day-60 for F4, F8, and F12. These data clearly demonstrate that intratumoral injections of drug loaded mesospheres makes possible the local delivery of extremely high therapeutic doses of cytotoxic drugs with complete tumor regression, greatly prolonged survival, and minimal toxicity. Whether used for debulking lung cancer associated airways obstruction, or for preoperative (neoadjuvant) chemotherapy of breast, lung, or colorectal cancer to improve tissue-conserving surgery outcomes, future clinical use of IT chemotherapy with drug loaded nano-meso-spheres has intriguing therapeutic potential.

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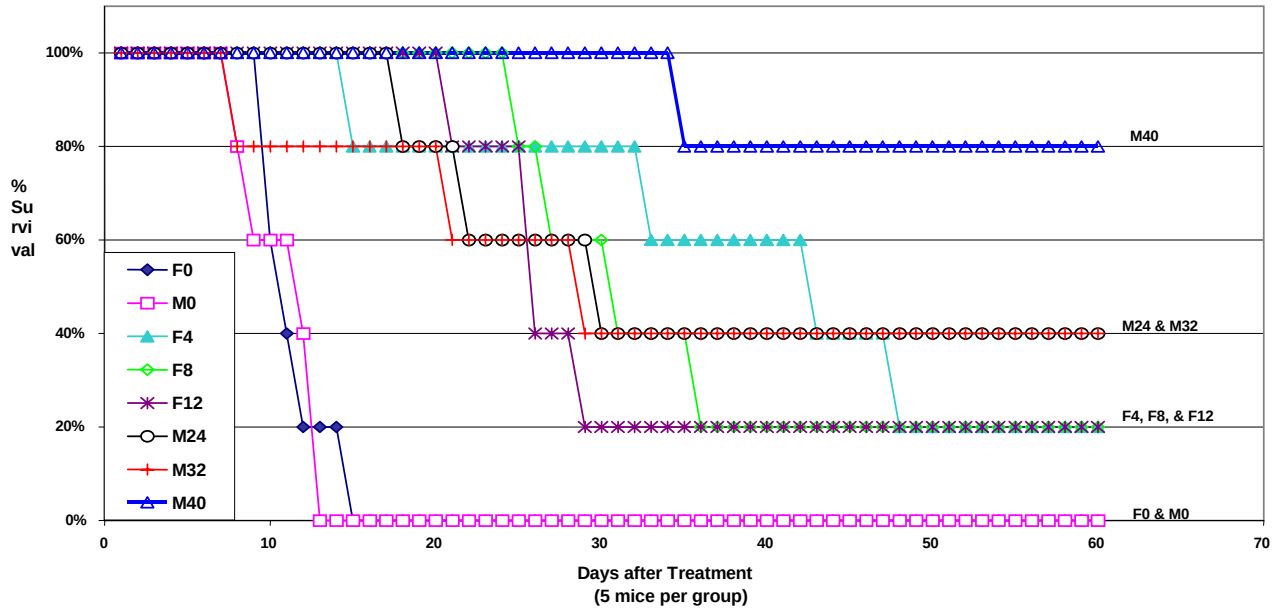

Figure 2 Survival graph for intratumoral therapy with mitoxantrone-loaded mesospheres. (F = Free drug, M = mesosphere-loaded drug, #'s = mitoxantrone dose in mg/kg, and M0 = unloaded BSA mesosphere control.)