Published in final edited form as:

Nanomedicine. 2016 October ; 12(7): 1951–1959. doi:10.1016/j.nano.2016.04.013.

Improving paclitaxel pharmacokinetics by using tumor-specific mesoporous silica nanoparticles with intraperitoneal delivery

Qiang Fu, PhD, Derek Hargrove, BS, and Xiuling Lu, PhD*

Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT, 06269, USA

Abstract

Mesoporous silica nanoparticles (MSNs) containing paclitaxel for intraperitoneal (i.p.) delivery were developed to exploit the tumor specific accumulation of these nanocarriers after i.p. injection and the slow release of paclitaxel from the MSNs. A 3.5-fold increase in tumor cellular drug uptake was observed for the paclitaxel-loaded MSNs compared with free paclitaxel. An in vivo study using xenograft mice bearing peritoneal human pancreatic carcinoma MIA PaCa-2 demonstrated that the MSN-paclitaxel formulation, compared to free paclitaxel, exhibited a 3.2-fold increase in peritoneal cavity residence time, slower absorption into the systemic circulation with one third systemic exposure, but a 6.5-fold increase in peritoneal tumor accumulation. Tissue distribution imaging showed significantly greater accumulation of fluorescent MSNs in tumor tissues compared to other peritoneal tissues. In conclusion, intraperitoneal administration of drug-containing MSNs was effective at reducing systemic exposure and increasing the peritoneal tumor accumulation of paclitaxel.

Keywords

Mesoporous silica nanoparticles; Intraperitoneal therapy; Peritoneal cancer; Paclitaxel; Pharmacokinetics

Effective drug delivery to solid tumors remains a challenge for treating aggressive peritoneal cancers, especially in late stages with peritoneal metastasis. Intraperitoneal (IP) chemotherapy provides the advantage of exposing tumors located in the peritoneal cavity to high drug concentrations based on spatial proximity.¹ IP therapy has been safely used in cancer patients achieving 20- to 1000- fold higher drug concentrations in the IP space compared with that measured in plasma after intravenous (IV) administration.^{2,3} It has been shown that the intraperitoneal administration of paclitaxel and cisplatin improved the clinical outcome of patients suffering from ovarian cancer.^{4–7} The results of six randomized trials showed that IP therapy yielded, on average, a 21% decrease in mortality rate and a 12-month longer overall survival time. Clinical trials of IP paclitaxel were first conducted using paclitaxel solubilized in a 50:50 Cremophor EL/ethanol mixture which was the first formulation approved by the U.S. Food and Drug Administration. However, Cremophor EL (polyoxyethylated castor oil) was reported to be responsible for significant side effects.⁸ Much effort has been devoted into seeking alternatives to this formulation. Recently,

*Corresponding author. xiuling.lu@uconn.edu (X. Lu).

Nanotax[®] (sterile nanoparticulate paclitaxel powder for suspension, CritiTech, Inc., Lawrence, KS) was developed for IP delivery of paclitaxel particles (mean particle size 600–700 nm) without the need for toxic solvents such as Cremophor EL. Their recent Phase I clinical trial demonstrated that IP administration of Nanotax[®] particles provides higher and prolonged peritoneal paclitaxel levels with minimal systemic exposure and reduced toxicity compared to IV paclitaxel administration.⁹ IP therapy has not been widely accepted despite the positive clinical results, due to catheter-related complications, the high rate of grade 3/4 toxicities and the poor tolerance of the regimen. An implantable, nonresorbable IP micro-device was developed to release a chemotherapeutic agent to the peritoneal cavity at a constant rate.¹⁰ Preclinical studies showed reduced toxicity by the controlled drug release from this micro-device with similar efficacy outcomes to frequent IP bolus doses. Current efforts related to IP drug delivery are mainly to provide a depot for sustained release of chemotherapeutic agents in the peritoneal tumor environment, thus increasing local drug levels while reducing systemic exposure. However, drug accumulation in the tumors was not significantly increased to improve the treatment efficacy. Therefore, an IP delivery system that is tumor-specific and also provides sustained drug release would be extremely beneficial for safe and effective chemotherapy treating peritoneal cancers.

We reported the use of mesoporous silica nanoparticles (MSNs) for intraperitoneal delivery of a radioisotope as a potential treatment for ovarian cancer metastasis.¹¹ Predominant tumor accumulation of the radioisotope after IP injection of the isotope-loaded MSNs was observed. It is likely that the MSNs preferably accumulated in tumor tissues as in vitro studies demonstrated minimal release of the isotope from the MSNs, and that almost no radioactivity was detected in blood in the in vivo studies. MSNs have been studied as a drug delivery system for intravenous administration in light of their attractive properties including: large surface area with high loading capacity, tunable pore size, controllable particle size and morphology, functional surfaces that can be modified, and ease of large scale synthesis and stability.^{12–16} The safety of MSNs have been assessed and reported to be biocompatible and biodegradable with low systematic toxicity in vivo.^{12,17} Mice administered 1 mg of MSNs by i.p. injection twice weekly for 2 months exhibited no unusual responses or behaviors.¹⁸ Chemotherapeutic agents such as paclitaxel have been loaded onto MSNs, replacing the need to use solvents that are often toxic to healthy tissues.^{19,20} The pore sizes of MSNs may be manipulated to control drug release rate.¹⁹ However, most of the reported administrations of drug-loaded MSNs have been by intravenous injection with fast clearance; subsequent biodistribution has not been extensively studied.

In this study, we advanced an MSN drug delivery system for the intraperitoneal delivery paclitaxel taking advantage of the unique tumor-specific accumulation of MSNs and the sustained release of paclitaxel from MSNs. The improved pharmacokinetic profile of MSN-based paclitaxel compared with IV and IP delivery of the drug in its free form is reported. This strategy significantly increases the drug level in peritoneal tumors in a pancreatic metastasis mouse model.

1. Methods

1.1. Materials

Paclitaxel (PTX), cephalomannine, rhodamine, mesoporous silica type MCM-41, tetraethyl orthosilicate (TEOS), trypsin-EDTA solution, Triton X-100, and Dulbecco's modified Eagle's medium (DMEM) were purchased from Sigma (St. Louis, MO, USA). 1,1'-Diocadecyl-3, 3, 3',3'-tetramethylindotricarbo-cyanine iodide (DiR), Fetal Bovine Serum (FBS), and penicillin-streptomycin solution were purchased from Biotium (Invitrogen, USA). All other chemicals were of analytical grade.

1.2. Synthesis of mesoporous silica nanoparticles

MSNs were synthesized as described previously.¹¹ Briefly cetyltrimethylammonium bromide (CTAB; 0.2 g) was dissolved in 100 mL of distilled water to which 0.7 mL of 2 M sodium hydroxide had been added. The mixture was heated to 80 °C and stirred vigorously. Once the temperature of the CTAB solution had stabilized, 1 mL of TEOS was added dropwise for 1 minute. After heating and stirring for 6 hours, the solution was cooled to room temperature and the particles were collected and washed three times with methanol using centrifugation at 14,000 g for 10 minutes and allowed to dry at room temperature overnight. To remove the surfactants from the pores of the particles, MSNs were added into a solution of methanol (50 mL) and hydrochloric acid (37.5%, 1 mL) and stirred for 24 hours. The particles were then centrifuged at 14,000 g for 10 minutes and washed thoroughly to remove the surfactants.

1.3. Analysis of paclitaxel by HPLC

The chromatographic system consisted of a pump (LC-20AT), an automatic injector (SIL-20A) and a UV detector (SPD-20A) (Shimadzu Scientific Instruments, Tokyo, Japan). Cellular, plasma, ascites and tumor concentrations of paclitaxel were determined by an HPLC assay described below. Cephalomannine was used as an internal standard. For the cellular concentration of paclitaxel, an octadecylsilane column (Gemini C18, 4.6 mm × 250 mm, 5 µm; Phenomenex, Torrance, CA) was eluted with the mobile phase (acetonitrile:distilled water = 40:60, v/v) and the column was maintained at 40 °C. The flow rate was 1.0 mL min⁻¹ and the detection wavelength was 229 nm. For plasma and tumor concentrations of paclitaxel, 2 mL of ethyl acetate was added to each sample. After vigorous vortexing, each sample was centrifuged at 13,000 g for 10 min and the supernatant was evaporated under nitrogen gas. The residue was reconstituted with methanol/1% acetic acid (1:1), and then a 50 µL aliquot was injected into the high performance liquid chromatography (HPLC) system. An octadecylsilane column (Gemini C18, 4.6 mm × 250 mm, 5 µm; Phenomenex, Torrance, CA) was eluted with the mobile phase (acetonitrile/distilled water = 37:63, v/v) and the column was maintained at 40 °C. The flow rate was 1.0 mL min⁻¹ and the detection wavelength was 229 nm. The calibration curve from the standard samples was linear over the concentration range of 20–500 ng/mL. The detection limit of paclitaxel was 20 ng/mL.

1.4. Preparation and characterization of paclitaxel (PTX), DiR or rhodamine-loaded MSNs

PTX-MSNs were prepared by the following method: PTX (2.5 mg in 0.5 mL of chloroform) was added to MSNs in ethanol (7.5 mg) with a 1:3 drug/carrier ratio. The volume of ethanol was 1.5 mL. After mixing and incubation for 12 hours, the samples were centrifuged at 14,000 g for 10 minutes and the MSNs were washed three times with deionized water, and then dried in a vacuum chamber. DiR or rhodamine-loaded MSNs were similarly prepared as described above, using DiR or rhodamine instead of PTX. In order to ensure that the dye was strongly associated with MSNs, the release of DiR from MSNs was studied by incubating DiR loaded MSNs in PBS containing 0.1% Tween for 24 hours. The morphology and size distribution of drug-free MSNs were observed using transmission electron microscopy (TEM) after negative staining. To determine the amount of PTX in MSNs, PTX was extracted from MSNs using ethanol with sonication and then centrifuged at 14,000 g for 10 minutes. The process was repeated three times to ensure the complete extraction. PTX in ethanol was quantified using HPLC. The drug encapsulation efficiency (EE) and loading capacity (LC) were calculated according to the following formulas:

$$EE\% = \frac{\text{Amount of PTX in MSNs}}{\text{Total amount of PTX}} \times 100$$
$$LC\% = \frac{\text{Amount of PTX in MSNs}}{\text{Weight of nanoparticles}} \times 100$$

1.5. In vitro drug release study

An in vitro drug release study was carried out using PBS (PH = 7.4) containing 0.1% (w/v) Tween 80 as the release medium.²¹ PTX-MSNs, containing about 3 µg/mL of paclitaxel were suspended in release medium at 37 °C in an orbital shaker. At specified time points, samples were centrifuged and the resulting supernatant was analyzed for the concentration of free drug (i.e., not bound to MSNs). The concentration of PTX remaining in the 50 mL tubes at various time points was measured by HPLC. The mean values of triplicate samples were reported. Initial burst was defined as the dose fraction released during the first 24 hours. The duration of the release study was 7 days.

1.6. Effect of MSNs on the cellular accumulation of paclitaxel

The human pancreatic carcinoma cell line (MIA PaCa-2), obtained from American Type Culture Collection, was maintained in DMEM supplemented with fetal calf serum (10%, Sigma, MO), penicillin (1%), and streptomycin (1%). Cells were seeded into 12-well plates at a density of 4×10^4 cells/cm². Two days post-seeding, cells were incubated with drug solution and drug loaded MSNs (5 µM paclitaxel). After 2 hours of incubation, the drug solution and MSNs with drug were removed, and the cells were washed three times with ice-cold phosphate-buffered saline. After lysis, the cells were harvested and sonicated for 5 minutes. Acetonitrile was added to the cell lysate, vortexed vigorously for 10 minutes and centrifuged for 5 minutes at 13,000 g. The supernatant was collected and the paclitaxel concentration of each sample was determined by HPLC. The amount of protein in each sample was determined using the BCA protein assay kit following the manufacturer's instructions (Sigma Chemical Co., St. Louis, MO).

1.7. Cellular uptake of rhodamine-loaded MSNs

The cellular uptake study was conducted using rhodamine dye as a fluorescent marker. Rhodamine-loaded MSNs [3:1 (w/w), MSNs/rhodamine] were prepared as described above. MIA PaCa-2 cells were seeded into 6-well plates at a density of 4×10^4 cells in 1 mL complete DMEM and cultured for 48 hours, followed by removal of culture medium and addition of rhodamine-loaded MSNs at a rhodamine concentration of 4 $\mu\text{g/mL}$. The cells were incubated at 37 °C with 5% CO₂ for 2 hours. Subsequently, the cells were washed with DPBS three times and fixed with 4% paraformaldehyde for 30 minutes at room temperature. Finally, the slides were rinsed with DPBS three times, mounted with cover slips, and observed using confocal laser-scanning microscopy (Nikon A1R, Japan) with excitation/emission maxima of 560/580 nm.

1.8. In vivo studies using human tumor xenographic model

Male athymic nude mice, 6–8 weeks old, were purchased from Charles River. All animals were housed under pathogen-free conditions according to AAALAC guidelines. All animal related experiments were performed in full compliance with institutional guidelines and approved by the Animal Use and Care Administrative Advisory Committee at the University of Connecticut. Humane use and care of vertebrate animals were ensured in the research. For the mouse xenograft model established from cultured cells, 1×10^7 cell of MIA PaCa-2 were collected in cold PBS (0.3 mL) and injected into the peritoneal cavity of the nude mice. Mice were then randomly divided into 3 groups ($n = 4$) after 4 weeks of tumor growth.

1.8.1. In vivo imaging and tissue distribution—The in vivo biodistribution and passive tumor targeting efficiency of MSNs were investigated using a near infrared fluorescence dye, DiR. In order to ensure the labeling stability of the DiR loaded MSNs, the in vitro release of DiR from MSNs was studied using the same method as the paclitaxel release study described above. Three nude mice bearing IP MIA PaCa-2 xenografts were used in this study. Two hundred microliters of DiR-loaded MSNs (0.1 mg/mL of DiR) were injected intraperitoneally into each mouse. At predetermined times, the three mice were scanned using the IVIS Lumina Series III Pre-clinical In Vivo Imaging system (Perkin Elmer, USA) with excitation of 745 nm. Mice were continuously anesthetized by isoflurane inhalation during the imaging process. After imaging, the mice were euthanized by CO₂ overdose. The tumor tissues were removed, frozen and cryo-sectioned, followed by preparation of 20 μm sections of the tumors. Finally, the depth of penetration of fluorescent nanoparticles in tumor sections was examined using confocal laser-scanning microscopy (Nikon A1R, Japan).

1.8.2. Pharmacokinetics of paclitaxel in nude mice with intraperitoneal tumor

—A peritoneal and systemic absorption PK model was established in peritoneal MIA-PaCa-2 tumor bearing nude mice. The mice were administered an intraperitoneal or intravenous dose of the Cremophor EL formulation (10 mg/kg paclitaxel). IP administration was achieved through an angiocatheter (18-gauge, 1.3 mm; Becton-Dickinson; Sandy, UT) inserted into the peritoneal cavity. Intravenous administration was done by tail vein injection. The paclitaxel solution for IP injection was prepared by dissolving paclitaxel in a Cremophor EL and ethanol mixture (1:1, v/v) and diluted with saline. A suspension of PTX-

MSNs was prepared using sterile physiological saline and dosed with equivalent paclitaxel dose of 10 mg/kg. At predetermined times, mice were anesthetized, and ascites samples (50 μ L) were collected at 0, 0.083, 1, 5, 12, 24, 48, 72, 96, 120, 144, 168 hours after administration of the formulations using a syringe and needle. Blood samples (50 μ L) were collected at 0, 0.25, 0.5, 1, 1.5, 2, 4, 8, 12, 24 hours following submandibular or tail prick bleeding. Ascites and blood samples were centrifuged and the obtained supernatant was stored at -70°C until analyzed.

1.8.3. Pharmacokinetic data analysis—Non-compartmental pharmacokinetic analysis was performed using the WinNonlin[®] version 6.3 (Pharsight Corporation, Mountain View, CA, USA). For peritoneal absorption, the area under the ascites concentration–time curve (AUC) and the area under moment curve (AUMC) for paclitaxel from 0 to 168 hours were calculated using the trapezoidal rule. The apparent peritoneal absorption rate constant was calculated from the initial slope of the log-linear plot of the percent of dose–time curve. For systemic absorption, the area under the plasma concentration–time curve (AUC) and the area under moment curve (AUMC) for paclitaxel from 0 to 24 hours were calculated using the trapezoid rule. The mean peritoneal and plasma residence time (MRT) after intraperitoneal administration was calculated as AUMC plasma/AUC plasma. Peritoneal cavity residence time was calculated as ratios of AUC in the peritoneal cavity from 0 to 172 hours to AUC in plasma from 0 to 24 hours. The systemic bioavailability of an IP dose of paclitaxel with formulation was calculated using the pharmacokinetics of an intravenous dose of the Cremophor EL formulation as the reference.

1.8.4. Drug concentrations in tumors in vivo—Tumor-bearing mice were given IP injections of paclitaxel with or without MSNs (10 mg/kg paclitaxel-equivalents). After 7 days of treatment, the tumor nodules were excised. Paclitaxel was extracted from homogenized tumor samples with ethyl acetate using cephalomannine as the internal standard, and the paclitaxel concentration was analyzed using HPLC.

1.9. Statistical analysis

All mean values were presented with their standard deviation (mean $\pm$ S.D.). Statistical analysis was conducted using a one-way ANOVA followed by a posteriori testing with Dunnett correction. A P value less than 0.05 was considered statistically significant.

2. Results

2.1. Synthesis and characterization of mesoporous silica nanoparticles (MSNs)

Transmission electron microscopy analysis showed that the synthesized MSNs were spherical in shape and approximately 100 nm in diameter with pores in hexagonal arrays (Figure 1). The pore size of the synthesized MSNs is 2.3 ± 0.3 nm, with narrow distribution. These MSNs tend to aggregate and settle in aqueous due to high density of the silica material (~ 2 g/cm³) and the hydrophobic surface. After sonication, the particles can be homogeneously suspended. The measured particle size using dynamic light scattering was consistent with the TEM result and the zeta potential was -37 ± 10 mv. The encapsulation efficiency was 25.6%, and drug loading was determined to be 17.8%.

2.2. Release kinetics of PTX-loaded MSNs

The release of PTX from the MSNs was studied using PBS (pH = 7.4) containing 0.1% Tween 80 (w/v) as the release medium. The concentration of MSN-associated paclitaxel was 3 µg/mL. As shown in Figure 2, PTX formulated in MSNs exhibited significantly extended release compared to PTX alone. Paclitaxel was released gradually from MSNs, with a much lower but steady release rate after 48 hours and reached 82% of drug release in about 7 days (172 hours).

2.3. Cellular uptake study

The cellular uptake of paclitaxel-loaded MSNs in human pancreatic carcinoma MIA PaCa-2 cells was investigated. A 3.5-fold increase in cellular accumulation of paclitaxel was achieved using paclitaxel-loaded MSNs compared with free paclitaxel after a 2 hour incubation (Figure 3). Fluorescence was observed both on the cell membrane and inside the cells, which was confirmed by confocal orthogonal views of the z-stack images and a representative image of this MSN and cell interaction can be observed in Figure 4. A spotted distribution was observed both on the cell surface and inside of the cells, suggesting that rhodamine is associated with MSNs and that the particles accumulate on the cell membrane or in the vesicles after being internalized through endocytosis. Escape of the delivered cargos from endosomes into cytoplasm is important as this is where the drug target is located. Although more studies are needed to understand the intracellular trafficking and the underlying mechanisms, the present results suggest that MSNs have the ability to mediate the intracellular delivery of formulated drugs.

2.4. Biodistribution of MSNs via DiR optical imaging

The biodistribution and tumor targeting efficiency of DiR-MSNs were evaluated in a mouse xenograft model of human pancreatic carcinoma cell lines (MIA PaCa-2), using a fluorescence dye, DiR. It was confirmed that no release of the dye from MSNs was detected after incubating DiR loaded MSNs in PBS containing 0.1% tween 80 for 24 hours. MSNs containing DiR were intraperitoneally injected into three mice bearing MIA PaCa-2 tumors. The three mice were then optically imaged over time using the IVIS Lumina Series III Pre-clinical In Vivo Imaging System. Figure 5 shows the images of a tumor-bearing mouse at 0.08, 2, 6, 12, and 24 hours following IP injection of DiR-MSNs. A noticeable signal in the peritoneal cavity was observed post injection, which indicates the dynamic distribution of the MSNs; the signal intensified around the tumor tissue between 2 and 24 hours and remained clearly visible. Interestingly, ex-vivo biodistribution 24 hours after injection showed a statistically significant higher accumulation of DiR-MSNs in tumor tissues in comparison to other peritoneal tissues. A weak fluorescence signal was observed in liver, spleen and intestines, while no fluorescence was detected in heart, lung and kidney regions.

2.5. Pharmacokinetics of paclitaxel in nude mice with intraperitoneal tumor

Figure 6, A illustrates the kinetic model of free paclitaxel and MSNs loaded with paclitaxel in IP tumor therapy. This involves drug and vehicle disposition in the peritoneal cavity and systemic circulation of the free drug after IP administration. Based on our design, the kinetic model suggests that only the free drug is absorbed into the systemic circulation. Larger

particles cannot be transported across the peritoneum or through the lymphatics, and drug absorption into the systemic circulation occurs only after it is released from the particles. Similarly, a multi-scale kinetic model described the drug disposition in peritoneal tumors during IP treatments.

Figure 6, *B* shows the kinetics of paclitaxel in the peritoneal fluid, and Table 1 summarizes the results. The amount of free drug was measured in the peritoneal cavity through collection of ascites fluid. The MSNs significantly altered the peritoneal exposure of paclitaxel compared to the control group (paclitaxel alone). The peritoneal mean residence time (MRT) and AUC of paclitaxel increased by 3.2 and 1.7 fold, respectively. Consequently, the $C_{\max}$ of paclitaxel in MSNs decreased significantly by 3.4 fold compare to paclitaxel in the Cremophor formulation. There was a marked decrease in peritoneal clearance of paclitaxel, implying a sustained release of paclitaxel in the presence of the MSN formulation (Table 1). Pretreatment with MSNs significantly enhanced the peritoneal exposure of paclitaxel.

Figure 6, *C* shows the plasma paclitaxel concentration time-profiles resulting from IP administration of the PTX with and without MSNs. Administration of the PTX-MSNs by IP injection significantly altered the systemic exposure (AUC) of PTX compared to the control group given PTX alone (in Cremophor, $P < 0.05$). The PTX-MSN formulation showed a 4.2 fold decrease in $C_{\max}$ and 2.8 fold decrease in AUC over 24 hours. Furthermore, this formulation increased the MRT of PTX in plasma by 1.7 fold which is shown in Table 1. Consequently, the bioavailability values of PTX decreased significantly ($P < 0.05$) with the MSNs formulation implying that the PTX was released slower into the systemic circulation from the MSNs formulation compared to free PTX. The ability of PTX to remain in the peritoneal cavity for extended periods of time was increased by 4.7 fold when in the MSN formulation.

2.6. Drug concentrations in tumors in vivo

To examine the ability of MSNs to deliver anticancer drugs to suppress tumor growth in mice, PTX-MSNs were administered to human pancreatic cancer bearing nude mice by i.p. The amount of paclitaxel was determined in tumor tissues 168 hours after intraperitoneal administration of free paclitaxel in Cremophor suspension and paclitaxel loaded MSNs (10 mg/kg). Compared with the free paclitaxel, MSNs increased paclitaxel tumor accumulation 6.5 fold (Figure 7).

3. Discussion

The present study was designed to demonstrate the positive impact of MSN on the disposition of paclitaxel in IP chemotherapy. According to our prior report, the MSNs accumulated preferentially in tumors with limited exposure to other tissues after IP delivery of radioactive MSNs.¹¹ We report here that paclitaxel carried by MSNs exhibited a sustained release profile from MSNs compared to paclitaxel only. The relatively slow and sustained release from PTX-loaded MSNs may be ascribed to the strong interaction between the MSNs and PTX and limited diffusion of PTX from the inner pores/channels of the MSNs. Hence, sustained-release formulations may reduce the frequency of treatment. 5-

Fluorouracil-loaded poly(lactide-co-glycolide; PLG) microspheres, which released drug over a period of 3 weeks, yielded significantly higher drug concentrations in peritoneal tissues compared with systemic tissues after intraperitoneal administration.^{22,23}

The disposition of MSN-loaded drug administered by IP injection consists of multiple kinetic processes: drug release from nanoparticles, nanoparticle accumulation in tumors facilitating the drug delivery to cancer cells, absorption of the drug from the peritoneal cavity to the systemic circulation and elimination through hepatic metabolism. Based on the previous reports on drug clearance from the peritoneal cavity, the transport across the peritoneum is a major pathway for compounds with MW less than 20 kD.²⁴ Some drugs may bind to cells and debris in ascites fluid in the peritoneal cavity.²⁵ The clearance of particulates from the peritoneal cavity is dependent on size and primarily takes place through lymphatic duct drainage. Small particles (<50 nm) can pass through lymph nodes while large particles (>700 nm) are mostly trapped in lymph nodes.^{26–28} Since MSNs are in the 50–700 nm size range, they most likely remain in the peritoneal cavity while free drug is absorbed through the peritoneum and passes through the lymphatic ducts into the systemic circulation.

Drug release rate from the carriers appeared to be slow in the peritoneal cavity. Released drug will quickly enter into the systemic circulation, as illustrated in the plasma drug profile of IP administered paclitaxel (Figure 6, C). However, MSN-based paclitaxel exhibited much lower plasma drug concentration and longer retention time both in the circulation and in the peritoneal cavity, implying the slow paclitaxel clearance in the peritoneal fluid from the paclitaxel-MSN formulation compared to free paclitaxel.

The tumor specific accumulation of MSNs facilitates effective delivery of paclitaxel to tumor cells. In the in vivo distribution of MSNs study, a noticeable signal in tumor was observed in mice 24 hours after injection demonstrating a statistically significant higher accumulation of DiR-MSNs in tumor tissues in comparison to other peritoneal tissues. In the cellular uptake study, paclitaxel-loaded MSNs were taken up by tumor cells via endocytosis, with a 3.5-fold increase in cellular accumulation of paclitaxel compared to free paclitaxel. Interestingly, no release of the dye DiR was observed in 24 hours while almost 60% of paclitaxel had been released within the same time period in the in vitro release study. After i.p. administration, it is expected that any of the released dye would be quickly cleared or show more signal in kidney/liver during the clearance process. However, the tumor is the only tissue illuminated in Figure 5 indicating that the observed signal is from MSN-associated dye. Paclitaxel and DiR may have different affinities for MSNs and so they exhibited different release profiles. Overall, DiR-MSNs retained the dye in the nanoparticles; thus, the fluorescent signal is indicative of MSNs, while for paclitaxel-MSNs, the release of drug was gradual. This was demonstrated by the pharmacokinetic profile of paclitaxel after the administration of paclitaxel-MSNs. The difference between i.p. paclitaxel-MSNs and i.p. paclitaxel alone is that paclitaxel-MSNs accumulated in tumors while releasing the drug gradually which eventually increased drug content in peritoneal tumors by 6.5 fold (Figure 7) and reduced the systemic exposure of the drug by 3 fold.

The results of the drug distribution study provided several findings that may be applied to improving IP therapy. They further exhibit the effects of the physicochemical properties of MSNs on drug absorption as well as their advantage in peritoneal tumor targeting. The affinity between the drug and MSNs affects the drug release rate. For this IP therapy, drug delivery to peritoneal tumors can be attributed to two sources: (1) redistribution of the absorbed drug from the peritoneal cavity via the systemic circulation; this is expected to be a minor source due to the relatively low drug concentration in blood, and (2) drug diffusion through the ascites fluid to the tumor tissue and drug carried by MSNs accumulating in tumor tissues. Although more studies are needed to understand the mechanism of tumor specific accumulation, the present result suggests that MSNs have the ability to effectively enhance the tumor delivery of formulated drugs.

Most current reports on MSNs focus on i.v. delivery. This work addressed the current challenge in limited drug delivery to peritoneal metastases by using intraperitoneal delivery of tumor-specific MSNs carrying paclitaxel. The pharmacokinetic profile of paclitaxel was greatly improved due to the longer retention of paclitaxel nanoparticles in the peritoneal cavity, predominant tumor accumulation of paclitaxel nanoparticles and the sustained release of paclitaxel from the nanoparticles. This system reduced the drug exposure to healthy tissues, largely increased the tumor drug concentration and may have profound significance for the development of future nanomedicines.

Acknowledgments

The work is supported by National Cancer Institute (NCI)R03CA184394 and the Research Scholar Grant, RSG-15-011-01-CDD from the American Cancer Society.

References

1. Sha H, Zou Z, Xin K, Bian X, Cai X, Lu W, et al. Tumor-penetrating peptide fused EGFR single-domain antibody enhances cancer drug penetration into 3D multicellular spheroids and facilitates effective gastric cancer therapy. *J Control Release*. 2015; 200:188–200. [PubMed: 25553823]
2. Markman M, Rowinsky E, Hakes T, Reichman B, Jones W, Lewis JL Jr, et al. Phase I trial of intraperitoneal taxol: a Gynecologic Oncology Group study. *J Clin Oncol*. 1992; 10:1485–91. [PubMed: 1355523]
3. Fu Q, Lee BJ, Ha I, Kang KW, Woo ER, Han HK. Effect of maceligan on the systemic exposure of paclitaxel: in vitro and in vivo evaluation. *Pharm Sci*. 2010; 41:226–31.
4. Alberts DS, Liu PY, Hannigan EV, O'Toole R, Williams SD, Young JA, et al. Intraperitoneal cisplatin plus intravenous cyclophosphamide versus intravenous cisplatin plus intravenous cyclophosphamide for stage III ovarian cancer. *Med*. 1996; 335:1950–5.
5. Armstrong DK, Bundy B, Wenzel L, Huang HQ, Beargen R, Lele S, et al. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. *Med*. 2006; 354:34–43.
6. Gadducci A, Carnino F, Chiara S, Brunetti I, Tanganelli L, Romanini A, et al. Intraperitoneal versus intravenous cisplatin in combination with intravenous cyclophosphamide and epidoxorubicin in optimally cytoreduced advanced epithelial ovarian cancer: a randomized trial of the Gruppo Oncologico Nord-Ovest. *Gynecol Oncol*. 2000; 76:157–62. [PubMed: 10637064]
7. Walker JL, Armstrong DK, Huang HQ, Fowler J, Webster K, Burger RA, et al. Intraperitoneal catheter outcomes in a phase III trial of intravenous versus intraperitoneal chemotherapy in optimal stage III ovarian and primary peritoneal cancer: a Gynecologic Oncology Group Study. *Gynecol Oncol*. 2006; 100:27–32. [PubMed: 16368440]

8. Szebeni J, Alving CR, Muggia FM. Complement activation by Cremophor EL as a possible contributor to hypersensitivity to paclitaxel: an in vitro study. *J Natl Cancer Inst.* 1998; 90(4):300–6. [PubMed: 9486816]
9. Williamson SK, Johnson GA, Maulhardt HA, Moore KM, McMeekin DS, Schulz TK, et al. A phase I study of intraperitoneal nanoparticulate paclitaxel (Nanotax[®]) in patients with peritoneal malignancies. *Cancer Chemother Pharmacol.* 2015; 75(5):1075–87. [PubMed: 25898813]
10. Yea, Hongye; Tanenbaum Laura, M.; Jeong, Nac Young; Mantzavinoua, Aikaterini; Fulcic, Giulia; del Carmend Marcela, G., et al. Sustained, low-dose intraperitoneal cisplatin improves treatment outcome in ovarian cancer mouse models. *J Control Release.* 2015; 220:358–67. [PubMed: 26548976]
11. Di Pasqua AJ, Yuan H, Chung Y, Kim JK, Huckle JE, Li C, et al. Neutron-activatable holmium-containing mesoporous silica nanoparticles as a potential radionuclide therapeutic agent for ovarian cancer. *J Nucl Med.* 2013; 54:111–6. [PubMed: 23100452]
12. He Q, Shi J. Mesoporous silica nanoparticle based nano drug delivery systems: synthesis, controlled drug release and delivery, pharmacokinetics and biocompatibility. *J Mater Chem.* 2011; 21:5845–55. <http://dx.doi.org/10.1039/C0JM03851B>.
13. Argyo H, Weiss V, Bräuchle C, Bein T. Multifunctional mesoporous silica nanoparticles as a universal platform for drug delivery. *Chem Mater.* 2014; 26:435–51.
14. Song N, Yang YW. Molecular and supramolecular switches on mesoporous silica nanoparticles. *Chem Soc Rev.* 2015; 44:3474–504. [PubMed: 25904466]
15. Aznar E, Oroval M, Pascual L, Murguía JR, Martínez-Mañez R. Sancenón gated materials for on-command release of guest molecules. *Chem Rev.* 2016; 116:561–718. <http://dx.doi.org/10.1021/acs.chemrev.5b00456>. [PubMed: 26730615]
16. Ang CY, Tan SY, Zhao Y. Recent advances in biocompatible nanocarriers for delivery of chemotherapeutic cargoes towards cancer therapy. *Org Biomol Chem.* 2014; 12:4776–806. [PubMed: 24737243]
17. Fu C, Liu T, Li L, Liu H, Chen D, Tang F. The absorption, distribution, excretion and toxicity of mesoporous silica nanoparticles in mice following different exposure routes. *Biomaterials.* 2013; 34(10):2565–75. [PubMed: 23332175]
18. Lu J, Liong M, Li Z, Zink JJ, Tamanoi F. Biocompatibility, biodistribution, and drug-delivery efficiency of mesoporous silica nanoparticles for cancer therapy in animals. *Small.* 2010; 6:1794–805. [PubMed: 20623530]
19. Jia L, Shen J, Li Z, Zhang D, Zhang Q, Liu G, et al. In vitro and in vivo evaluation of paclitaxel-loaded mesoporous silica nanoparticles with three pore sizes. *Pharm.* 2013; 10:4451–2.
20. Jia L, Shen J, Li Z, Zhang D, Zhang Q, Duan C, et al. Successfully tailoring the pore size of mesoporous silica nanoparticles: exploitation of delivery systems for poorly water-soluble drugs. *Pharm.* 2012; 439:81–91.
21. Yang T, Cui FD, Choi MK, Cho JW, Chung SJ, Shim CK, et al. Enhanced solubility and stability of PEGylated liposomal paclitaxel: in vitro and in vivo evaluation. *Pharm.* 2007; 338:317–26.
22. Hagiwara A, Takahashi T, Sawai K, Sakakura C, Tsujimoto H, Imanishi T, et al. Pharmacological effects of 5-fluorouracil microspheres on peritoneal carcinomatosis in animals. *Cancer.* 1996; 74:1392–996.
23. Hagiwara A, Sakakura C, Tsujimoto H, Imanishi T, Ohgaki M, Yamasaki J, et al. Selective delivery of 5-fluorouracil (5-FU) to i.p. tissues using 5-FU microspheres in rats. *Anticancer Drugs.* 1997; 8:182–8. [PubMed: 9073314]
24. Lu Z, Wang J, Wientjes MG, Au JL. Intraperitoneal therapy for peritoneal cancer. *Future Oncol.* 2010; 6:1625–41. [PubMed: 21062160]
25. Jessie, L-S Au; Guo, Peng; Gao, Y.; Lu, Z.; Wientjes, MG.; Tsai, M., et al. Multiscale tumor spatiokinetic model for intraperitoneal therapy. *AAPS J.* 2014; 16:424–39. [PubMed: 24570339]
26. Flessner MF, Fenstermacher JD, Blasberg RG, Dedrick RL. Peritoneal absorption of macromolecules studied by quantitative autoradiography. *Physiol.* 1985; 248:H26–32.
27. Shih WJ, Coupal JJ, Chia HL. Communication between peritoneal cavity and mediastinal lymph nodes demonstrated by Tc-99 m albumin nanocolloid intraperitoneal injection. *Proc Natl Sci Counc Repub China B.* 1993; 17:103–5. [PubMed: 8290652]

28. Hirano K, Hunt CA. Lymphatic transport of liposome-encapsulated agents: effects of liposome size following intraperitoneal administration. *J Pharm Sci.* 1985; 74:915–21. [PubMed: 4067845]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.
FT-TEM images of MSNs.

Figure 2. Release of paclitaxel from MSNs at 37 °C. Paclitaxel release was measured in PBS (pH 7.4) with 0.1% Tween 80 (mean $\pm$ S.D., $n = 3$).

Figure 3. Cellular accumulation of paclitaxel with or without MSNs in MIA-paca-2 cells (mean $\pm$ S.D., $n = 3$). $P < 0.01$, compared with the control given paclitaxel alone.

Figure 4. Cellular accumulation of MSNs loaded with rhodamine in MIA PaCa-2 cells after a 2 hour-incubation. Confocal image with z sections every 0.5 μm .

Figure 5.
Peritoneal distribution of MSNs with imaging at 0.08, 2, 6, 12, 24 hours post i.p. administration. Tumors are circled.

Figure 6.

(A) Kinetic model for drug disposition during IP therapy. (B) Paclitaxel concentration–time profiles in peritoneal ascites. Paclitaxel or paclitaxel loaded MSNs were administered by intraperitoneal injections at 10 mg/kg. (C) Paclitaxel plasma concentration profiles of paclitaxel and paclitaxel loaded MSNs administered by intraperitoneal injections as well as paclitaxel administered by intravenous injections at 10 mg/kg.

Figure 7. Tumor accumulation of paclitaxel with or without MSNs in mice after i.p. administration (mean $\pm$ S.D., $n = 3$). $P < 0.01$, compared with the control (paclitaxel alone).

Pharmacokinetic parameters of paclitaxel after an IP or intravenous administration of paclitaxel (10 mg/kg) to nude mice in the presence and the absence of MSNs (mean $\pm$ S.D., $n = 3$).

Table 1

	Peritoneal cavity				Plasma			PTA ^a	
	AUC _(0-172 hr) (μ g hour/mL)	T _{max} (hours)	C _{max} (μ g/mL)	MRT ^b (hours)	AUC _(0-24 hr) (μ g hour/mL)	T _{max} (hours)	C _{max} (μ g/mL)	MRT ^b (hours)	F (%)
PTX (Control)	324.53 $\pm$ 33.32	0	28.09 $\pm$ 0.18	17.83	8 $\pm$ 1.32	1	1.38 $\pm$ 0.24	4.98	32
PTX + MSN	547.97 $\pm$ 26.9 [*]	8	8.24 $\pm$ 0.69 [*]	56.86 [*]	2.88 $\pm$ 0.54 [*]	2	0.33 $\pm$ 0.07 [*]	8.38 [*]	11.6 [*]

^{*} $P < 0.05$ compared to control group given PTX alone.

^a Peritoneal targeting advantage = AUC_{peritoneal}/AUC_{plasma} $\times$ 100.

^b The mean peritoneal or plasma residence time.