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Recommended Citation
Tao, Zhimin; Toms, Bonnie; Goodisman, Jerry; and Asefa, Tewodros, "Mesoporous Silica Microparticles Enhance the Cytotoxicity of Anticancer Platinum Drugs" (2010). Chemistry Faculty Scholarship. Paper 38.
http://surface.syr.edu/che/38
Mesoporous Silica Microparticles Enhance the Cytotoxicity of Anticancer Platinum Drugs

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The Pt-based anticancer compounds, including cisplatin, carboplatin, oxaliplatin, and nedaplatin, exert their antitumor activity by binding to DNA and promoting cell death.1–7 In the presence of high chloride concentrations (e.g., human plasma), cisplatin (cis-[Pt(NH3)2Cl2]) exists mostly in its neutral form. However, in solutions with lower chloride concentration, the drug goes through a multistep hydrolysis, producing the charged species, cis-[Pt(NH3)(H2O)Cl]2+, and their deprotonated products, cis-[Pt(NH3)(OH)Cl], cis-[Pt(NH3)2(OH)(H2O)]+, and cis-[Pt(NH3)2(OH)2].1–3 All of these species may interact with nitrogen atoms of the bases of nuclear DNA, forming monooxy- and dihydroxyadducts, that is, monohydroxy or dihydroxy cisplatin complexes that can further bind to nitrogen atoms of DNA by losing their hydroxyl groups; the resulting “Pt lesions” impair DNA functions and induce cell death most likely through apoptotic pathways.1–3 Structurally, transplatin (trans-[Pt(NH3)2Cl2]) differs from its cis-isomer in the arrangement of the ligands around Pt in these square planar compounds. This alteration makes transplatin much less lethal than cisplatin, possibly because of the restricted access to DNA due to steric hindrance.4,5 Our previous studies revealed that cisplatin impairs cellular respiration in a dose-dependent and time-dependent manner by inducing intracellular caspase activity, whereas its trans-isomer exhibits no observable inhibition of mitochondrial oxygen consumption.6,7

Mesoporous silica microparticles (MSMs) are a class of nanostructured materials with nanometer pores that are currently thought to have potential applications as drug delivery vehicles because of their large surface area, high pore volume, and tunable nanoscale pores.10–12 Previously, we investigated the adsorption and release of drug molecules including isomeric platinum drugs by mesoporous materials.10,11 The two most common types of mesoporous materials, SBA-15 and MCM-41, exhibited different adsorption and release profiles for both cis- or trans-platin.11 Furthermore, the two materials showed dissimilar capacity in hosting the cisplatin and transplatin isomers.11 The difference in drug encapsulation and release shown by the materials for the two isomers of the platinum drug can be expected to induce different therapeutic effects. We also have recently showed the effect of MCM-41 and SBA-15 on mitochondrial O2 consumption (respiration) of HL-60 (myeloid) cells, Jurkat (lymphoid) cells, and isolated mitochondria.10 While SBA-15 showed a concentration- and time-dependent inhibition of cellular respiration at 25–500 μg/mL, MCM-41

ABSTRACT  We report on the endocytosis and the time-dependent enhanced cytotoxicity of anticancer platinum drugs when the drugs are combined with (or loaded into) one of the two most common types of mesoporous silica materials, MCM-41 or SBA-15. The anticancer drug cisplatin and its isomer transplatin, when loaded on MCM-41 and SBA-15 microparticles, were less cytotoxic to leukemia cells than the drugs alone after 12 h exposure. However, the drug-loaded microparticles exhibited unprecedented enhanced cytotoxicity to the cancerous cells after 24 h of exposure. This cytotoxicity of the drug-loaded microparticles was even higher than that of the pure drugs in solutions, suggesting that mesoporous silica microparticles loaded with cisplatin or transplatin enabled a localized intracellular release of the platinum compounds and possibly also facilitated the drug’s hydrolysis, enhancing the desired cytotoxic effect.

KEYWORDS: mesoporous materials · nanomaterials · adsorption capacity · endocytosis · cell viability
exhibited no noticeable effect on the cell respiration rate. Both materials, however, inhibited the respiration rates of isolated mitochondria and submitochondrial particles with no harm to their cellular glutathione. In another study, we also demonstrated that a high dosage of organic-functionalized mesoporous materials could result in cancerous cell death.21

Herein we report on an unprecedented higher cytotoxicity of platinum drug/mesoporous microparticles combination on cancer cells, more than the sum of the cytotoxicity of the drug and that of the microparticles. Platinum drug-loaded MSMs were prepared using either MCM-41 or SBA-15. The synthesized particles were calcined to remove the templates, followed by a series of characterizations, including transmission electron microscopy (TEM), nitrogen physisorption, and elemental analysis. Cell uptakes of both SBA and MCM particles were next examined and demonstrated by TEM images. As a possible consequence of endocytosis, the cytotoxicity of drug-loaded particles to Jurkat (leukemia) cells was assessed. The drug-loaded microparticles showed an enhanced cytotoxicity to cancer cells after 24 h incubation, compared to either the particles or the drug alone. The cytotoxic effect by the drug and microparticles was higher than the simple sum of cytotoxicities of the drug and the particles. Interestingly, even the less potent transplatin became very cytotoxic when delivered by MSMs. This is the first report that illustrates the enhancement of cytotoxicity of cisplatin or transplatin by using mesoporous microparticles as drug delivery systems.

RESULTS AND DISCUSSION

MSMs, whose organic templates were removed by calcination, were first synthesized as described in the Methods section. They were then characterized by TEM (as shown in Supporting Information Figure S1). TEM images show that the calcined MCM-41 materials contain rather regular particles of ~500–900 nm with a spherical or oval shape, while the calcined SBA-15 materials possess irregularly shaped and micrometer-sized particles of various diameters. The materials were further characterized by nitrogen physisorption measurements (Table S1 and Figure S2), which gave type IV isotherms with steep capillary condensation steps for both materials, confirming the presence of highly ordered mesoporous structures with high surface areas. The surface areas were 876 and 1216 m²/g for MCM-41 and SBA-15 samples, respectively. Furthermore, both samples possessed a high pore volume of quite similar value of ~0.9 cm³/g. However, as expected, the pore diameter of MCM-41 was about two times smaller than that of SBA-15, with values of 59.3 and 28.7 Å, respectively. Elemental analyses of calcined MSMs (Table S2) corroborate the successful removal of surfactants, eliminating the possibility of further induced cytotoxicity by the templating reagents.

The internalization of MSMs was next investigated and demonstrated by TEM images (Figure 1 and Supporting Information Figure S3). To examine the cellular uptake of these particles, 0.5 × 10⁶ Jurkat cells/mL were incubated with 200 μg/mL of SBA-15 or MCM-41 at 37 °C for 1 h, kept gently stirring. At the end of incubation, 1.5-mL cell suspensions were collected, processed, and visualized by TEM (see Methods for details). As shown in Figure 1, it is obvious that both mesoporous SBA-15 and MCM-41 microparticles can be efficiently ingested by cells, although the uptake pathways appear to be different. This agrees with the report on endocytosis of MSMs that described the internalization of particles within a short time of exposure in human lung cancer cells (A549). However, it is worthwhile to note that the ingestion of MCM-41 particles by Jurkat cells re-
sulted in a pseudopodium folded by cell membrane, indicating a possible phagocytosis, whereas cell uptake of SBA-15 suggested a receptor-mediated endocytotic process, typified by a formation of intracellular endosomes as indicated in Figure 1A (upper right and lower left panels). The difference in the uptake mechanism for these two mesoporous microparticles could be mainly a result of their different shapes rather than their distinctive mesopore sizes (the pore size of SBA-15 is twice as large as that of MCM-41).

Given the possibility that the cellular uptake of microparticles themselves could lead to severe cytotoxicity, Jurkat cells were incubated with and without 200 μg/mL of SBA-15 or MCM-41 at 37 °C for 24 h (Figure 2). As a negative control, 20 μM doxorubicin was added separately into cell suspension. As a potent antitumor agent, doxorubicin executes cell death by inducing caspase activation and impairing mitochondrial respiration. It is also worth mentioning that the delivery of doxorubicin aided by nanomaterials has recently been reported by a few research groups.24,25 However, the possible enhancement of drug cytotoxicity by the drug / nanomaterials combination compared to the drug or the materials alone as we had observed here was not reported to be the case for doxorubicin in these previous recent reports.24,25 Although a doxorubicin/MSM combination was not studied here, doxorubicin was used as a control to verify the cell viability tests. Cell viability was found to be 100 ± 12.4% for untreated cells, 1.6 ± 0.7% for 20 μM doxorubicin-treated cells (p < 0.001), 71.6 ± 7.2% for 200 μg/mL of SBA microparticle-treated cells (p < 0.01), and 82.0 ± 7.5% for 200 μg/mL of MCM microparticle-treated cells (p < 0.03) (Figure 2A). Hence, at a dosage of 200 μg/mL, the cytotoxicities of mesoporous MCM-41 and SBA-15 microparticles on Jurkat cells are small, making both materials good candidates for drug carriers. Note that this cytotoxicity could not result from the physical wrapping or surrounding of the cell by microparticles because cytotoxicity in adhesive cell lines was unchanged when the particles were layered first in the well.26,27

Cisplatin and transplatin were next loaded on either SBA-15 or MCM-41, and the cytotoxicities of the drug-loaded microparticles were then analyzed. First, a 5 mg/mL SBA-15 or MCM-41 suspension was soaked in 1 mM cisplatin or transplatin solution in PBS for 48 h, with continued stirring. Afterward, 4 μL of the drug–microparticle mixture was added to a 100-μL Jurkat cell solution (equivalently, ∼8000 cells were exposed to 200 μg/mL microparticles plus 40 μM platinum drugs). In parallel, cells were incubated with 40 μM cisplatin or transplatin previously aged in PBS for 48 h (i.e., hydrolysis of drugs is complete). Cell viability was checked in each condition at 12 and 24 h incubation. Results are shown in Figure 2B.

At 12 h (solid bars), cell viability was 100 ± 3.2% for untreated cells, 15.1 ± 2.9% for cisplatin-treated cells (p < 0.001), 83.8 ± 5.9% for cisplatin + SBA-treated cells (p < 0.001), 78.5 ± 6.7% for cisplatin + MCM-treated cells (p < 0.03), 67.7 ± 13.9% for transplatin-treated cells (p < 0.01), 106.2 ± 9.5% for transplatin + SBA-treated cells (p < 0.01), and 105.6 ± 13.3% for transplatin + MCM-treated cells (p < 0.01). At a concentration of 40 μM, cisplatin exhibited a significant toxicity to Jurkat cells in 12 h incubation, inactivating 84.9% of the cells. Transplatin was much less toxic: only 32.3% of cells were killed. Furthermore, packaging drug in MSMs decreased the drug cytotoxicity in the first 12 h. In the presence of cisplatin, either SBA-15 or MCM-41 protected cells from the treatment for the first 12 h. Forty micromolar transplatin had also very little effect on cancer cells in 12 h, and loading transplatin on MSM microparticles made the effect even smaller. Thus, both MSMs protected cells from chemotherapy for the first 12 h. This is most probably because the diffusion of the drug-loaded microparticles into the cell occurred much
slower than the diffusion of the naked drug. Furthermore, there is the possibility of the slow intracellular release of the MSM-adsorbed drugs. Consequently, the cytotoxic effects of these drug-loaded microparticles were postponed. Interestingly, however, after a total 24 h incubation (open bars), these drug-loaded microparticles showed a significantly enhanced cytotoxicity, more so than the drug or the particles alone. Cell viability after 24 h was found to be 100 ± 18.8% for untreated cells and 6.9 ± 13.3% for cisplatin-treated cells (p < 0.001). The combination of cisplatin with either SBA-15 or MCM-41 caused complete cell death, as no absorbance corresponding to living cells was read. The calculated viability for cisplatin + SBA at 24 h is −12.3 ± 20.1% and for cisplatin + MCM −6.8 ± 11.8%, both essentially zero (see Figure 2B). Thus, the microparticles enhance cell killing by cisplatin. For transplatin-treated cells, cell viability became 45.9 ± 13.6% (p < 0.01). Transplatin had an inhibitory effect on cell growth, although less acute and lethal than its cis-isomer. However, only 2.6 ± 26.7% transplatin + SBA-treated cells (p < 0.02) and 12.7 ± 10.1% transplatin + MCM-treated cells (p < 0.01) survived the 1 day treatment. The large errors reflect the variable degree of inhibition of particles on cell survival. Since we indicated that MSM itself had a limited cytotoxicity as we showed above or reported previously, it was apparent that the MSM greatly augmented also the cytotoxicity of transplatin, as it did for cisplatin. This is possibly because the materials may have assisted transplatin in reaching an intracellular site or even increased its hydrolysis, leading to more toxicity. We recently found out that mesoporous materials could accelerate the oxidation of epinephrine (a hormone molecule), perhaps due to the formation of hydroxyl or oxygen radicals in their mesoporous channels. Therefore, the possible generation or presence of free radicals in MSMs in aqueous solutions could potentially also catalyze the hydrolytic reactions of platinum drugs, which in turn lead to their enhanced pharmaceutical effects. It is worth noting again that the hydrolysis reactions of cis- and trans-platinum drugs involve the substitution of chloride(s) by hydroxyl groups.1-3

CONCLUSIONS

In this study, we reported on the cytotoxicity and the associated pharmaceutical effect of drug/MSMs combination compared to the drug or MSMs alone. The drug-loaded materials exhibited much improved antitumor activities, more than either the drug or the material alone, and this enhanced anticancer activity appears to be much more than the simple sum of the cytotoxicities of the particles and the drug. This is the first time that an enhanced anticancer property on malignant cells by combining platinum compounds with mesoporous silicates has been demonstrated. It is also of great importance that, with a much lessened potency when compared to its renowned cis-isomer, even transplatin exhibited a surprisingly high cytotoxicity when it is combined and delivered with these nanostructured materials. Transplatin has been traditionally known to be inactive and has, therefore, been not so useful for cancer treatment, but when loaded on mesoporous silica particles, it can be made to have an unprecedented high anticancer property, almost to the same magnitude as cisplatin. Also, the particles probably protect transplatin from hydrolysis (its hydrolysis rate is larger than that of cisplatin because of the trans effect) and subsequent reactions with extracellular species; when it is released inside the cell, it can readily react with DNA and other intracellular species and cause cell death.34 This work shows that drugs and even less potent drug isomers can become effective and valuable pharmaceuticals by proper combination with micro- or nano-sized materials. It appears that the drug-loaded mesoporous silica SBA-15 and MCM-41 microparticles, after entering cells, release the encapsulated platinum drugs and increase their potency, possibly by improving localized drug delivery or targeting as well as facilitating their drug hydrolysis.

METHODS

Materials and Reagents. Cetyltrimethylammonium bromide (CTAB), tetraethoxysilicate (TEOS), and poly(ethylene oxide)-block-poly(tertbutyl oxide)-block-poly(ethylene oxide) (Pluronic P123 or P1213, MW = 5800) were obtained from Sigma-Aldrich. Cisplatin (cis) and transplatin (yellow powder, MW = 300.05) (Trans) were obtained from Sigma-Aldrich and dissolved in PBS before experiments. Phosphate-buffered saline (1× PBS, without Mg2+ and Ca2+, pH = 7.4) was purchased from Mediatech (Herdon, VA). Doxorubicin HCl (3.45 mM) was purchased from Bedford Laboratories (Bedford, OH).

Synthesis of SBA-15 and MCM-41. Mesosstructured MCM-41 and SBA-15-type materials were synthesized by previously reported procedures with minor modification.29-33 For the MCM-41 synthesis, 11.0 mmol CTAB was mixed with 960 mL of distilled water (dH2O) and 14 mL of 2.0 M NaOH solution and stirred at 80 °C for 30 min. Then 101.2 mmol TEOS was slowly dripped into the reactant solution, which was stirred for another 2 h at 80 °C. It was filtered when still hot, and the solid was washed with copious amounts of dH2O and ethanol. The resulting precipitate was dried in an 80 °C oven overnight, which gave the MCM-41 “as synthesized”. To remove the surfactant template from the material, as-synthesized MCM was calcined at 550 °C for 5 h (heating rate = 1 °C min⁻¹). The resultant CTAB-free mesoporous silica MCM-41 was then collected.

To synthesize SBA-15-type mesoporous silica material, 1.4 mmol (8 g) Pluronic P123 was dissolved in a mixture of 60 mL of distilled water and 240 mL of 2 M HCl that was prewarmed at 40 °C and stirred for 4 h until no longer chunky. Then 81.7 mmol of TEOS was added into the solution while stirring at 40 °C for 20 h. The mixture was then moved to an 80 °C oven to age for 24 h under static conditions. After that, 20 mL of ethanol was added and mixed well. The solution was filtered, and the cake was washed with distilled water 4–5 times (20 mL each time). The precipitate was then left to dry overnight, producing as just synthesized SBA-15 material, which was further calcined at 600
**REFERENCES AND NOTES**


