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Research Paper

Surfactant-polymer Nanoparticles: A Novel Platform for Sustained and Enhanced Cellular Delivery of Water-soluble Molecules

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Purpose. Nanoparticles, drug carriers in the sub-micron size range, can enhance the therapeutic efficacy of encapsulated drug by increasing and sustaining the delivery of the drug inside the cell. However, the use of nanoparticles for small molecular weight, water-soluble drugs has been limited by poor drug encapsulation efficiency and rapid release of the encapsulated drug. Here we report enhanced cellular delivery of water-soluble molecules using novel Aerosol OT™ (AOT)-alginate nanoparticles recently developed in our laboratory.

Materials and Methods. AOT-alginate nanoparticles were formulated using emulsion-crosslinking technology. Rhodamine and doxorubicin were used as model water-soluble molecules. Kinetics and mechanism of nanoparticle-mediated cellular drug delivery and therapeutic efficacy of nanoparticle-encapsulated doxorubicin were evaluated in two model breast cancer cell lines.

Results. AOT-alginate nanoparticles demonstrated sustained release of doxorubicin over a 15-day period *in vitro*. Cell culture studies indicated that nanoparticles enhanced the cellular delivery of rhodamine by about two–tenfold compared to drug in solution. Nanoparticle uptake into cells was dose-, time- and energy-dependent. Treatment with nanoparticles resulted in significantly higher cellular retention of drug than treatment with drug in solution. Cytotoxicity studies demonstrated that doxorubicin in nanoparticles resulted in significantly higher and more sustained cytotoxicity than drug in solution.

Conclusions. AOT-alginate nanoparticles significantly enhance the cellular delivery of basic, water-soluble drugs. This translates into enhanced therapeutic efficacy for drugs like doxorubicin that have intracellular site of action. Based on these results, AOT-alginate nanoparticles appear to be suitable carriers for enhanced and sustained cellular delivery of basic, water-soluble drugs.

KEY WORDS: cellular drug delivery; cytotoxicity; endocytosis; exocytosis; sustained release.

INTRODUCTION

Many clinically important small molecular weight drugs including anticancer agents (1,2), corticosteroids (3), and immunomodulators (4) have site of action inside the cell. There are a number of biological barriers to drugs reaching their intracellular site of action (5,6). Simple diffusion into the cell across the cell membrane is feasible for only low molecular weight lipophilic drugs. Most drug molecules, however, are weak acids or bases, containing at least one site that can reversibly disassociate or associate a proton to form a negatively charged anion or a positively charged cation at physiologic pH (7). Because the cell membrane is lipophilic and limits the diffusion of compounds that are ionized or polar, availability of many drugs at their intracellular site of action is limited (8). For drug molecules that get into the cell, cellular concentrations are maintained only as long as the concentration (or activity) gradient is maintained outside the cells. Once the concentration gradient is removed, drugs diffuse back out of the cell rapidly (9,10). As a result, a single-dose treatment with most drugs results in only a transient therapeutic effect (9).

Previous studies have shown that nanoparticles can significantly increase and sustain cellular levels of the encapsulated drug (9,11). This results in enhanced therapeutic efficacy of nanoparticle-encapsulated drug. However, the use of nanoparticles for cellular delivery of small molecular weight, water-soluble drugs has been limited by poor drug encapsulation efficiency and rapid release of the encapsulated drug (12–15).

We have recently fabricated novel surfactant-polymer nanoparticles for efficient encapsulation and sustained release of water-soluble drugs (16). These nanoparticles are formulated using dioctyl sodium sulfosuccinate (Aerosol OT™; AOT) and sodium alginate. AOT is an anionic surfactant that is approved as oral, topical and intramuscu-

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NON-STANDARD NOTATIONS: AOT, aerosol OT™; PLGA, poly(D,L-lactide-*co*-glycolide); PBS, phosphate buffered saline; SA, sodium azide; DG, deoxy glucose.

lar excipient (US Food and Drug Administration's Inactive Ingredients Database). Sodium alginate is a naturally occurring polysaccharide polymer that has been extensively investigated for drug delivery and tissue engineering applications (17,18). We have shown that AOT-alginate nanoparticles can sustain the release of water-soluble drugs such as doxorubicin and verapamil over a period of 4 weeks (16).

The objective of the present study was to investigate the suitability of AOT-alginate nanoparticles as carriers for cellular delivery of water-soluble molecules. Using rhodamine and doxorubicin as model water-soluble molecules, we have investigated the kinetics and mechanism of nanoparticle-mediated cellular drug delivery.

MATERIALS AND METHODS

Materials

Rhodamine 123, sodium alginate, polyvinyl alcohol and calcium chloride were purchased from Sigma-Aldrich (St. Louis, MO). Aerosol OT, methanol and methylene chloride were purchased from Fisher Scientific (Chicago, IL).

Methods

Nanoparticle Formulation

Nanoparticles were formulated by emulsification-cross-linking technology developed in our laboratory (16). Sodium alginate solution in water (1.0% w/v; 1 ml) was emulsified into AOT solution in methylene chloride (5% w/v; 1 ml) by vortexing (Genie™, Fisher Scientific) for 1 min over ice bath. The primary emulsion was further emulsified into 15 ml of aqueous PVA solution (2% w/v) by sonication for 1 min over ice bath to form a secondary water-in-oil-in-water emulsion. The emulsion was stirred using a magnetic stirrer, and 5 ml of aqueous calcium chloride solution (60% w/v) was added gradually to the above emulsion. The emulsion was stirred further at room temperature for ~18 h to evaporate methylene chloride. For preparing drug-loaded nanoparticles, drug (5 mg) was dissolved in the aqueous alginate solution, which was then processed as above. Nanoparticles formed were recovered by ultracentrifugation (Beckman, Palo Alta, CA) at 145,000×g, washed two times with distilled water to remove excess PVA and unentrapped drug, resuspended in water, and lyophilized.

Determination of Drug Loading

Drug loading in nanoparticles was determined by extracting 5 mg of nanoparticles with 5 ml of methanol for 30 min and analyzing the methanol extract for drug content. Doxorubicin concentration was determined by fluorescence spectroscopy (excitation/emission wavelengths of 485/528 nm; FLX 8000, Bio-Tek® Instruments, Winooski, VT). Rhodamine concentration was determined by HPLC (see below). Drug loading was defined as the amount of drug encapsulated in 100 mg of nanoparticles.

Determination of Particle Size and Zeta Potential

Particle size and zeta potential were determined using dynamic light scattering. Brookhaven 90Plus zeta potential equipment fitted with particle sizing software (Brookhaven Instruments, Holtsville, NY) was used. About 1 mg of nanoparticles was dispersed in 1 ml of distilled water by sonication, and was subjected to both particle size and zeta potential analysis.

In Vitro Release Studies

Drug release from doxorubicin containing nanoparticles was determined in phosphate buffer saline (PBS, 0.15 M, pH 7.4) or in cell culture medium (RPMI medium supplemented with 10% FBS). A previously used experimental setup was used (19,20). Nanoparticle suspension (1 mg/0.5 ml) was placed in dialysis chamber (MWCO 10,000 Da, Pierce), and the dialysis chamber was immersed in 10 ml of the release medium in a 15-ml centrifuge tube. The centrifuge tube containing dialysis chamber was placed in an incubator shaker (Brunswick Scientific, C24 incubator shaker, NJ) set at 100 rpm and 37°C. At predetermined time intervals, 0.5 ml of the release medium was removed from the tube and was replaced with fresh release medium. Studies with free drug in solution demonstrate that the dialysis membrane used does not retain the drug. Doxorubicin concentration in the release medium was determined by HPLC. Our previous studies (16) indicate that doxorubicin undergoes first-order degradation under the release conditions. Degradation rate constants were determined, and were used to correct the *in vitro* release of doxorubicin for degradation.

HPLC Determination of Doxorubicin and Rhodamine

A Beckman Coulter HPLC system with System Gold® 125 solvent module and System Gold® 508 autoinjector connected to Linear Fluor LC 305 fluorescence detector (Altech) set at 505/550 nm wavelengths for doxorubicin and 490/526 nm for rhodamine was used. A Beckman® C-18 (Ultrasphere) column (Octadecyl silane 4.6×250 mm) was used. For doxorubicin, acetonitrile: water (adjusted to pH 3 with glacial acetic acid) (70:30) was used as mobile phase at a flow rate of 1 ml/min. Retention time of doxorubicin was 7 min. For rhodamine, acetonitrile: sodium acetate (adjusted to pH 4 with glacial acetic acid):tetrabutyl ammonium bromide (50:20:30) was used as mobile phase at a flow rate of 1 ml/min. Retention time of rhodamine was ~3.2 min.

Cell Culture

Human breast cancer cells (MDA-Kb2 and MCF-7) were used as model cell lines. MDA-Kb2 cells were cultured in Leibovitz's medium supplemented with 10% FBS at 37°C. MCF-7 cells were grown in RPMI medium supplemented with 10% FBS at 37°C and 5% CO_2. Both cell lines are sensitive to doxorubicin-induced cytotoxicity.

Cellular Accumulation of Nanoparticles

Nanoparticles containing rhodamine were used for the study. Doxorubicin-loaded nanoparticles were not used to avoid the complication of doxorubicin-induced cytotoxicity while determining uptake. All the studies were performed at 37°C, unless specified differently. MDA-kb2 or MCF-7 cells were seeded in a 24-well plate at a density of 50,000 cells/well and allowed to attach overnight. Cells were then treated with nanoparticle suspension or equivalent dose of rhodamine solution in complete growth medium. To determine the effect of dose of nanoparticles on uptake, cells were treated with various doses (12.5–200 μg/ml) of nanoparticles for 2 h. To determine the effect of time of treatment, cells were treated with constant dose (100 μg/ml) of nanoparticles for varying periods of time (30–120 min). At the end of the treatment period, the cell monolayer was washed three times with cold PBS. Cells were then lysed using 100 μl of 1X cell culture lysis reagent (Promega). The protein content of the cell lysate was determined using the Pierce protein assay reagents (Rockford, IL). Cell lysates were then analyzed for rhodamine content. To study the effect of metabolic inhibition on nanoparticle uptake, cells were preincubated with growth medium containing 0.1% w/v sodium azide and 50 mM deoxyglucose for 1 h, and then incubated with nanoparticle suspension (100 μg/ml) containing 0.1% w/v sodium azide and 50 mM deoxyglucose for 2 h. To study the effect of temperature on cellular uptake of nanoparticles, cells were preincubated at 4°C for 1 h and then treated with the nanoparticle suspension (100 μg/ml) at 4°C for 2 h.

Exocytosis of Rhodamine

A previously reported exocytosis assay was used (21). In brief, cells were incubated with nanoparticles (100 μg/ml) or equivalent dose of rhodamine in solution for 2 h in growth medium, followed by washing with PBS twice. The intracellular rhodamine concentration at the end of the 2-h incubation period was taken as the zero time point value. Cells were then incubated with fresh medium. At different time intervals, medium was removed; cells were washed twice with PBS and lysed as described above. Rhodamine concentration in cell lysate was determined as described below. Data was expressed as percent of rhodamine that was retained at different time intervals relative to the zero time point value.

Quantification of Rhodamine in Cell Lysates

Cell lysates were mixed with 300 μl of methanol and incubated at 37°C for 6 h at 100 rpm. The samples were centrifuged at 14,000 rpm for 10 min at 4°C. Rhodamine-associated fluorescence in the supernatants was determined using HPLC as described earlier. A standard plot was constructed for rhodamine in cell lysate reagent under identical extraction conditions. Data was expressed as rhodamine accumulation normalized to total cell protein.

In Vitro Cytotoxicity with Doxorubicin-loaded Nanoparticles

MCF-7 cells were plated in 96-well plates at 5,000 cells/well/0.1 ml medium. On Day 0, cells were treated with either 50 or 75 μg/ml doxorubicin in solution or encapsulated in nanoparticles. Untreated cells and blank nanoparticle-treated cells were used as controls for solution-treated and nanoparticles-treated cells, respectively. On Day 2, cells were washed to remove the treatments and fresh medium was added. Medium was changed every other day with no fresh dose of the treatments added. Cytotoxicity was determined at different time points using a commercially available cytotoxicity assay (CellTiter 96 AQueous, Promega). Data was expressed as cytotoxicity of treatments as a percent of respective controls.

Table I. AOT-alginate Nanoparticles Loaded with Rhodamine or Doxorubicin

Drug	z-Average Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)	Drug Loading (mg/100 mg)
Rhodamine	515	0.284	−14.6±2.1	4.6±0.2
Doxorubicin	689	0.286	−13.4±1.0	3.8±0.1

RESULTS

Nanoparticle Characterization

Nanoparticles were initially characterized for particle size, polydispersity, zeta potential, and drug loading. As can be seen from Table I, both rhodamine-loaded nanoparticles and doxorubicin-loaded nanoparticles had sub-micron particle size (500 –700 nm) and similar polydispersity indices (~0.28). The zeta potential of nanoparticles was around −13– 14 mV. Both rhodamine and doxorubicin could be efficiently encapsulated in nanoparticles (4.6% w/w drug loading for rhodamine and 3.8% w/w for doxorubicin). Nanoparticles were stable to lyophilization and in various buffers and cell culture medium. Nanoparticles did not aggregate in the presence of serum (data not shown).

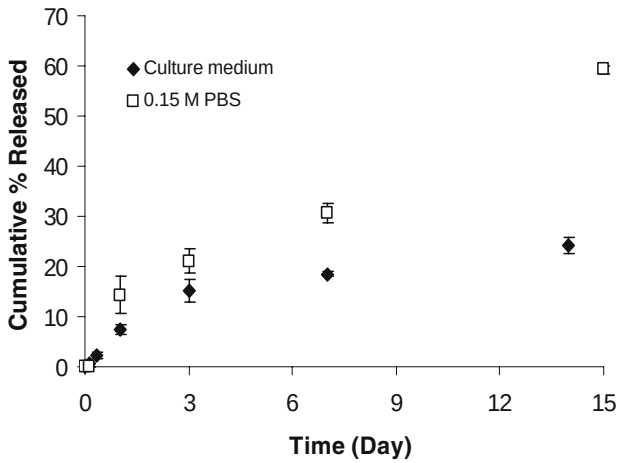

Fig. 1. *In vitro* release of doxorubicin from nanoparticles. Data as mean ± SD (n=3).

In Vitro Drug Release

In vitro release studies in phosphate buffered saline demonstrated an initial burst release phase, with about 18% of the encapsulated drug released at the end of 24 h. Following this, the drug release was near zero-order (~60% of encapsulated drug released) over the 15 days of the study (Fig. 1). In this time period, nanoparticles released doxorubicin at the rate of 2.3 μg/day/mg nanoparticles. *In vitro* release of doxorubicin in cell culture medium was slower (average release rate of 1.3 μg/day/mg) than in PBS.

Kinetics and Mechanism of Nanoparticle Uptake

To determine the efficacy of cellular drug delivery with AOT-alginate nanoparticles, we compared cellular accumulation of rhodamine following treatment with rhodamine in solution and in nanoparticles. As can be seen from Fig. 2, treatment with rhodamine in nanoparticles resulted in a significantly ($P<0.05$) higher accumulation of rhodamine than treatment with rhodamine in solution in both MDA-Kb2 and MCF-7 cells. The enhancement in rhodamine accumulation with nanoparticles was dose and cell line-dependent. Treatment with rhodamine in nanoparticles resulted in a 7.5- to 10-fold higher accumulation of rhodamine in MDA-Kb2 cells, whereas a two–threefold higher accumulation was observed in MCF-7 cells. We further studied the kinetics of cellular rhodamine accumulation with nanoparticles. Rhodamine accumulation in cells with nanoparticles was both dose- and time-dependent (Fig. 3). Rhodamine accumulation increased proportionately with dose at lower doses (up to 50 μg/ml dose), but was disproportionate at higher doses (100–200 μg/ml). Also, nanoparticle uptake into the cells increased with time of incubation, reaching a steady state at about 90 min. In order to determine the mechanism of nanoparticle uptake into cells, we evaluated the energy dependence of nanoparticle uptake in cells. Reducing the cellular ATP production by incubating cells with metabolic inhibitors sodium azide and deoxyglucose resulted in ~50% reduction in cellular uptake of nanoparticles (Fig. 4). Decreasing active processes in cells by incubating cells at 4°C had a similar effect on nanoparticle uptake into cells (Fig. 4). Energy dependence of nanoparticle uptake, along with dose- and time-dependence, suggests that cells internalize AOT-alginate nanoparticles through an endocytic process.

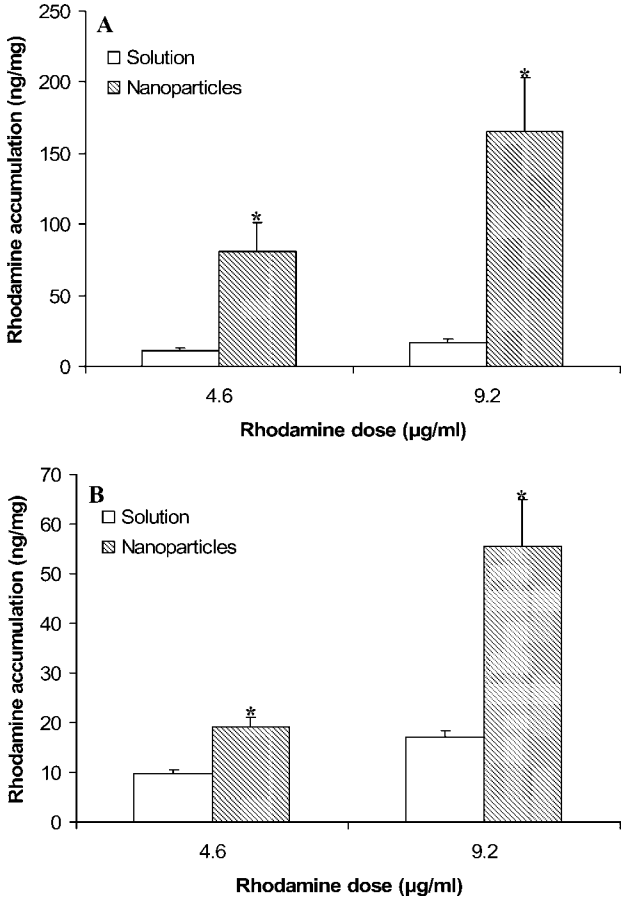

Fig. 2. Cellular accumulation of rhodamine in (A) MDA-Kb2 and (B) MCF-7 cells. Data as mean ± SD (n=4). *$P<0.05$, t test.

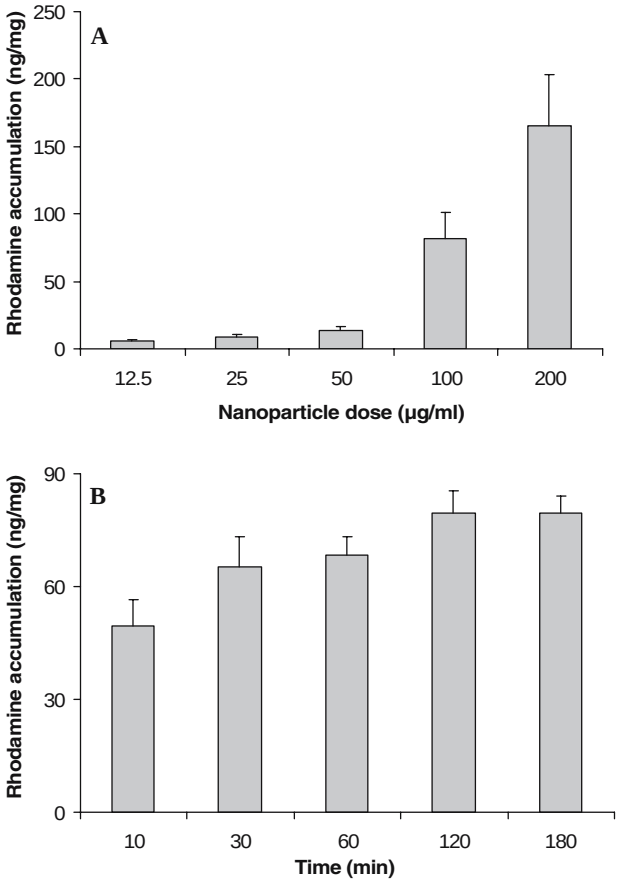

Fig. 3. Kinetics of nanoparticle uptake into MDA-Kb2 cells. (A) Cells were incubated with various doses of nanoparticles containing rhodamine for 2 h. (B) Cells were incubated with 100 μg/ml of nanoparticles for different time intervals. Data as mean ± SD (n=4).

Exocytosis and Retention of Rhodamine

As indicated in Fig. 3B, continuous incubation of cells with nanoparticles resulted in an increase in drug accumulation, followed by steady state cellular levels. However, when

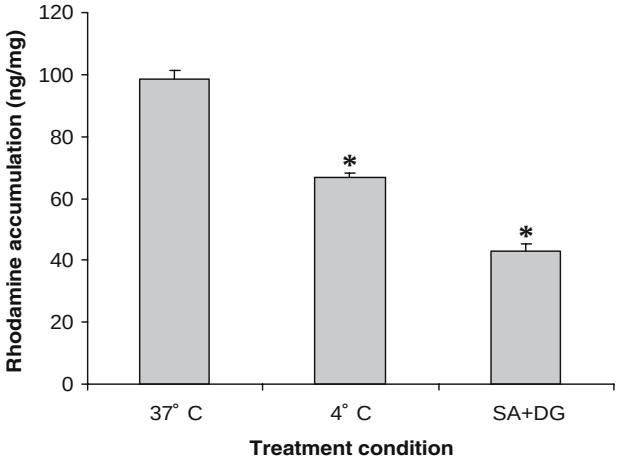

Fig. 4. Mechanism of nanoparticle uptake into cells. Data as mean ± SD (n=4). *P< 0.05 compared to uptake at 37°C and in the absence of inhibitors, t test.

nanoparticle treatment was removed following initial incubation with cells, intracellular levels began to decline. Previous studies have shown that this decline is due to exocytosis of the delivery system from cells (21,22). As can be seen from Fig. 5, exocytosis of AOT-alginate nanoparticles was relatively rapid immediately after the treatment was removed; about 50% of the internalized particles exited in 10 min. Cellular levels appeared to increase beyond 10 min. Cellular retention of the drug following treatment with drug in solution was significantly less than that following treatment with drug in nanoparticles. At the end of 120 min, there was almost a twofold difference in the fraction of internalized drug retained within the cells between the two treatments. Also, the drop in cellular drug levels following treatment with drug in solution was biphasic; a initial rapid drop immediately following the removal of the treatment, followed by a much slower rate of decrease beyond 10 min.

Cytotoxicity of Doxorubicin-loaded Nanoparticles

In order to determine the therapeutic efficacy of nanoparticle-encapsulated drug, we evaluated the cytotoxicity of nanoparticle-encapsulated doxorubicin *in vitro*. Doxorubicin in nanoparticles demonstrated significantly higher cytotoxicity than doxorubicin in solution (Fig. 6). This enhancement in cytotoxicity with nanoparticles was dose-responsive and was sustained for the 10 days of our study. There was no significant difference in the viability of untreated cells and cells treated with blank nanoparticles, indicating that at the concentration tested, blank nanoparticles were not toxic to cells.

DISCUSSION

Nanoparticle-mediated cellular drug delivery is governed by the dynamics of cellular uptake and retention of nanoparticles (21,22) and the rate of drug release from nanoparticles (9). Previous studies demonstrate that uptake and retention of drug carriers like nanoparticles are affected by cellular processes such as endocytosis and exocytosis (21). These cellular processes are, in turn, influenced by nanoparticle properties such as particle size and zeta potential (23–25).

AOT-alginate nanoparticles investigated in this study were developed specifically for sustained release of water-soluble drugs like doxorubicin (16). Alginate polymers have been widely used in biomedical applications as they are biodegradable and biocompatible, but suffer from limitations of rapid drug release in physiologic salt concentration (26,27). In the presence of sodium ions, insoluble calcium alginate gets converted into soluble sodium alginate, resulting in rapid disintegration of the delivery system and drug release (26). We rationalized that introduction of stronger acid groups in alginate nanoparticles will result in stronger cross-linking and drug-matrix interaction, resulting in enhanced drug encapsulation and sustained release of the encapsulated drug. Based on this rationale, we developed nanoparticles composed of alginate and anionic surfactant AOT. AOT has a sulfonic group (pKa < 1) in its polar sulfosuccinate head group with a large and branching hydrocarbon tail group. AOT forms reverse micelles in non-polar solvents and because AOT is a double chain amphiphile, it is expected to form a bilayered structure in multiple emulsion (28). Based on the multiple emulsion-crosslinking process used, AOT-alginate nanoparticles are expected to have a calcium-crosslinked core composed of alginate and AOT head groups, surrounded by a hydrophobic matrix composed of AOT tails, with the drug of interest encapsulated in the core (Fig. 7). Following encapsulation of weakly basic drugs, nanoparticles have a net negative charge, which stabilizes nanoparticles in buffer and in medium containing serum. This is an advantage over other nanoparticle delivery systems such as polycyanoacrylate nanoparticles that become cationic following encapsulation of weakly basic drugs like doxorubicin (29).

In vitro release studies show that nanoparticles sustained doxorubicin release over a 15-day period. Drug release was

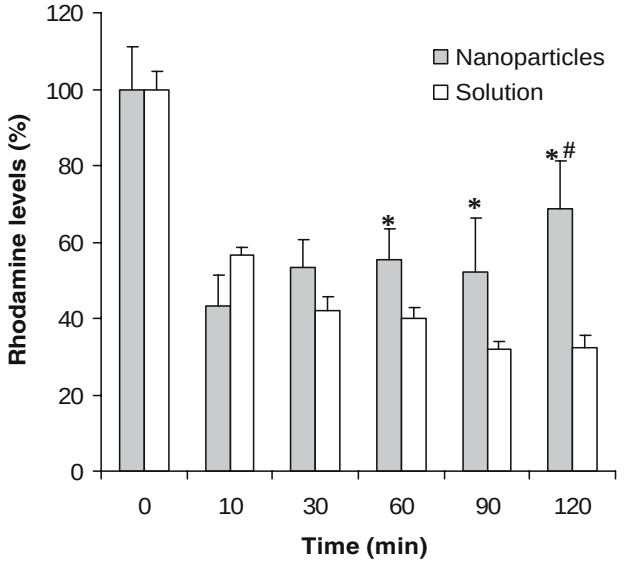

Fig. 5. Cellular retention of rhodamine. Data represented as a percent of rhodamine levels (± SD; n=4) at the end of 2-h incubation. *P< 0.05 compared to drug solution; #P< 0.05 compared to rhodamine level at 10 min; t test.

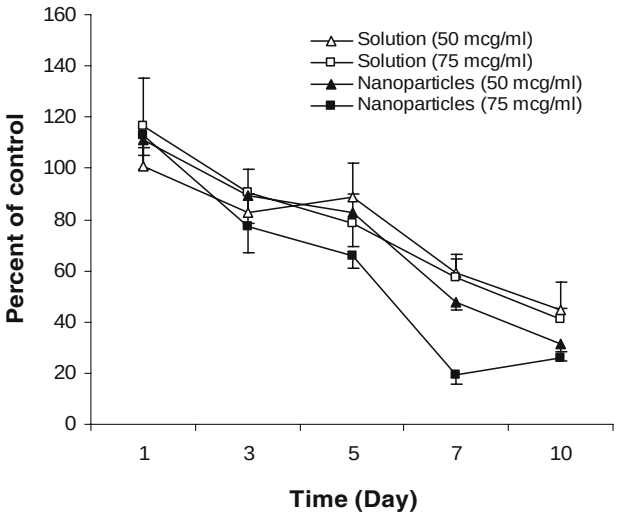

Fig. 6. Enhanced cytotoxicity with doxorubicin nanoparticles in MCF-7 cells. Cytotoxicity presented as a percent of respective controls (n=6).

slower when cell culture medium was used as the release medium. We have shown in our previous study that slow exchange of calcium in nanoparticles for sodium in the release medium is an important determinant of rate of drug release from AOT-alginate nanoparticles (16). Presence of divalent metal salts (Ca^{2+}, Mg^{2+}) in cell culture medium could have decreased the rate of sodium-calcium exchange, resulting in slower drug release in cell culture medium.

Nanoparticles resulted in significantly higher cellular drug accumulation than drug in solution. Weak bases such as rhodamine and doxorubicin are positively charged at physiologic pH (7). For example, doxorubicin, which has a pKa of ~8.2 (30), is about 86% ionized at pH 7.4. Because the cell membrane is lipophilic and limits the diffusion of compounds that are ionized (8), availability of doxorubicin at its intracellular site of action can be limited. Higher drug accumulation with nanoparticles than with solution suggests that processes other than simple diffusion are involved in nanoparticle-mediated cellular drug delivery. Previous studies have shown that nanoparticles formulated using polymers such poly(D,L-lactide-*co*-glycolide) (PLGA) are taken up

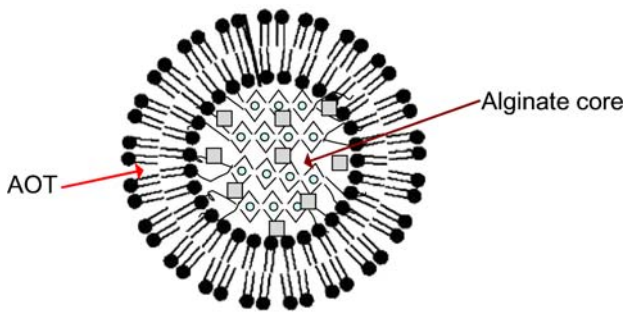

Fig. 7. Proposed structure of AOT-alginate nanoparticles. *Inner core* consists of alginate crosslinked with calcium. This is surrounded by one or more bilayers of AOT. *Gray squares* represent drug molecules. Figure not drawn to scale.

into cells through active process such as endocytosis (31). Energy dependence of nanoparticle uptake into cells (Fig. 4) suggests that cellular uptake of AOT-alginate nanoparticles involves endocytosis (32). This is further confirmed by the achievement of steady state in cellular drug accumulation with prolonged incubation time. Because endocytosis is an active process and is limited by the number of endocytic vesicles originating from the cell membrane, drug accumulation involving endocytosis eventually reaches steady state (21).

Retention studies suggest that a fraction of internalized rhodamine comes out of the cell following the removal of treatment from the external medium. Differences in the kinetics of drug loss from the cells following treatment with drug in solution and drug in nanoparticles suggest that different processes may be involved in drug loss from cells for the two treatments. Simple diffusion out of the cell could be responsible for drug loss following treatment with drug solution, whereas exocytosis may be involved in the case of drug in nanoparticles (21). Exocytosis has been observed for other delivery systems including liposomes (33) and PLGA nanoparticles (21). Exocytosis is a process by which cells release cellular signals and expel waste into the external environment (34,35). The current model for endocytosis and exocytosis suggests the existence of three different cellular compartments in the endocytosis/exocytosis pathway (36). Cells internalize external materials through early endocytic vesicles (early endosomes), which are then trafficked to sorting endosomes. Sorting endosomes sort the incoming materials. Depending on the signals present in the incoming molecules, they are recycled back to the outside of the cell through recycling endosomes, diverted to other cellular organelles such as endoplasmic reticulum, or forwarded to lysosomes for degradation. An increase in cellular levels of rhodamine was observed beyond the first 10 min of the exocytosis study, and the cellular retention of rhodamine at 120 min was significantly higher ($P < 0.05$) than at 10 min. It may be postulated that if rhodamine is exocytosed as nanoparticles, it may also be recaptured by the cells afterwards from the media as nanoparticles, leading to the observed increase in intracellular rhodamine concentration noted beyond 10 min.

Enhanced accumulation and sustained cellular retention of the drug following treatment with nanoparticles suggests that nanoparticles could enhance the efficacy of drugs whose site of action is intracellular. We used doxorubicin as a model drug to study therapeutic efficacy, because doxorubicin causes cytotoxicity by intercalation with DNA in the nucleus. As predicted from enhanced cellular drug accumulation with nanoparticles, doxorubicin in nanoparticles was significantly more cytotoxic than doxorubicin in solution. For example, cytotoxicity of nanoparticle-encapsulated doxorubicin was about three and two-fold higher on day 7 and day 10, respectively, than that with drug in solution. Enhanced cellular uptake and sustained release of nanoparticle-encapsulated doxorubicin within the cells could be responsible for the sustained enhancement of cytotoxicity observed with nanoparticle-encapsulated doxorubicin. A number of previous studies have reported enhancement of doxorubicin-induced cytotoxicity in cancer cell lines. For example, Serpe *et al.* (37) compared *in vitro* cytotoxicity of doxorubicin-

loaded solid lipid nanoparticles with that of doxorubicin in pegylated liposomes and free doxorubicin. Doxorubicin-loaded nanoparticles inhibited cell growth more strongly than either free or liposomal doxorubicin in a variety of cancer cell lines. In a different study (38), the same group demonstrated that solid lipid nanoparticles enhanced the cytotoxicity of doxorubicin in human colorectal cancer cell line HT-29. The IC_{50} of free doxorubicin and nanoparticle-encapsulated doxorubicin were, respectively, 126.75 ± 0.72 and 81.87 ± 4.11 nM at 72 h exposure. Miglietta *et al.* (39) demonstrated that solid-lipid nanoparticles formulated with hexadecylphosphate as doxorubicin counter ion decreased the IC_{50} of doxorubicin from 10 to 1 ng/ml in MCF-7 cells. Wong *et al.* (40) reported enhanced cytotoxicity of doxorubicin loaded in a novel polymer-lipid hybrid nanoparticle system. It was found that nanoparticles resulted in over 8-fold increase in cell kill when compared to doxorubicin solution treatment in drug-resistant human breast cancer cells but cytotoxicity of nanoparticle-encapsulated drug was similar to that of drug in solution in drug-sensitive wild type. It has to be noted that in the above studies, nanoparticle formulations were evaluated for cytotoxicity over a short duration (typically $\leq$ 72 h after incubation). In our studies, we have demonstrated sustained (over 10 days) cytotoxicity of nanoparticle-encapsulated doxorubicin.

CONCLUSION

Our studies show that AOT-alginate nanoparticles significantly enhance and sustain the cellular delivery of basic, water-soluble drugs. This translates into enhanced therapeutic efficacy for drugs like doxorubicin that have intracellular site of action. Based on these results, we conclude that AOT-alginate nanoparticles are suitable carriers for enhanced and sustained cellular delivery of basic, water-soluble drugs.

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