In Vitro Investigation of Ionic Polysaccharide Microspheres for Simultaneous Delivery of Chemosensitizer and Antineoplastic Agent to Multidrug-Resistant Cells†

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Abstract □ Insufficient intratumoral concentration of therapeutic agents and multidrug resistance are major factors responsible for failure of treatment of solid tumors. Simultaneous delivery of chemosensitizing and antineoplastic agents by microspheres could lead to enhanced chemotherapy of multidrug-resistant (MDR) tumors. Ionic polysaccharide microspheres derived from dextran were used to load chemosensitizers (e.g., verapamil) and anticancer drugs such as vinblastine. High drug loading was achieved for both a single agent and dual agents. The equilibrium drug loading was dependent on the ratio of the microspheres (MS) to the drug, as well as the relative affinity of the agents to the MS in the case of dual agents. The drug release from drug–MS involved hydration and swelling of the MS in addition to ion exchange. The effectiveness of MS-delivered chemosensitizers in the reversal of drug resistance was evaluated by measuring the uptake of [^{3}H]vinblastine by MDR cells (CH^{R}C5). The concomitant delivery of verapamil with vinblastine by the MS led to a 6–7-fold increase in the uptake of vinblastine, a level similar to the uptake obtained with free drug solutions. The results suggest that the antineoplastic and chemosensitizing agents were released effectively from the MS and the bioactivity of the chemosensitizer was preserved during the process.

Introduction

The efficacy of cancer chemotherapy may be limited by the drug toxicity, drug concentration achievable in the tumor, and the development of multidrug resistance (MDR). The maximum systemic drug concentration is set by the drug toxicity to the normal tissues (e.g., leukopenia of vinblastine, and cardiotoxicity and immunosuppressive activity of doxorubicin). In addition, it is difficult to obtain an effective therapeutic drug level in solid tumors because of higher intratumoral pressure and poor blood supply.[1] Therefore, targeted drug delivery has been extensively investigated to increase the drug exposure of the tumor relative to that of normal tissues. Of all the targeting approaches, the most direct one is the intratumoral or intra-arterial injection of drug solutions to the tumor site.[2−4] This technique, however, is often associated with significant systemic exposure due to rapid egress of the drug from the tumor mass. To maintain the intratumoral drug concentration and reduce the systemic exposure, slow-release formulations, especially in the form of microspheres (MS), have been utilized in the place of free drug solutions.[5−14]

To date, slow-release MS have been tested in regional cancer chemotherapy in over a thousand patients worldwide[6−9] as a potential, effective treatment of solid tumors in the liver, kidney, breast, lung, head, and neck. Pharmacokinetic and pharmacodynamic studies in animals and humans have shown enhanced drug exposure of tumors and diminished systemic toxicity as compared with organ perfusion with free drug solutions.[2−14] Moreover, there is little indication of increased local toxicity in most studies.[5,7,8] Despite these positive results, a considerable percentage of chemotherapy failure is still observed in animal tumor models and in clinical trials, which may be partly attributed to MDR to chemotherapy.[8,15−19]

It has been demonstrated that chronic exposure of cancer cells to sublethal concentrations of chemotherapeutic agents can lead to the outgrowth of the MDR phenotype.[15−19] MDR, characterized by diminished cellular drug accumulation, usually derives from an increased rate of drug efflux by specific membrane proteins [e.g., P-glycoprotein (P-gp)[16−19]]. Integration of MDR-reversing agents, such as verapamil and cyclosporins, in conventional chemotherapy has been used for treatment of MDR tumors.[18−22] However, there has been limited success in clinical trials, especially in the treatment of solid tumors, which is believed to be a consequence of insufficient drug concentration in the tumors.[23,24] We hypothesized that simultaneously delivery of both chemosensitizing and antineoplastic agents to the tumor sites by MS could increase local drug concentration and thus enhance the therapeutic efficacy while reducing the systemic side effects.

Among the numerous methods for incorporating drugs into MS, loading of ionic drugs into ion-exchange MS is of particular interest because of their high drug-loading capacity and ease of the loading process.[25−28] Furthermore, biocompatible and biodegradable materials, such as polysaccharides and albumin, can be used to prepare the MS.[5,25,29] The biodegradable MS drug carriers are more suitable for in vivo application than nonbiodegradable polystyrene MS, although the latter have also led to positive therapeutic results.[26−28]

Our group has initiated the development of microspherical delivery systems for enhanced therapy of MDR tumors via regional, simultaneous delivery of chemosensitizing and antineoplastic agents.[30,31] The purpose of this work was to undertake in vitro characterization and evaluation of MS for simultaneous delivery of the dual agents to MDR cells. Ionic polysaccharide MS derived from cross-linked dextran were chosen as the drug carriers because of their biocompatibility and biodegradability.[29,32−34] More importantly, the original material, dextran, has been applied in vivo as a blood expander for years. Although the cross-linked dextran MS have not been approved officially, they have been used clinically in cancer therapy[32,33] and wound treatment[34] without observed adverse effects. To achieve a sustained release of the therapeutic agents and retention of the MS in the tumor or the arteries leading to the tumor

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while maintaining the ease of injection, MS with diameters ranging from 40 to 125 μm were selected. As our previous work suggested,[35,36] the therapeutic agents may interfere with each other or redistribute among the MS after having been released into a confined compartment in the tumor, thus altering their bioavailability. To elucidate the possible mutual effect of the dual agents on the release kinetics, the competitive loading and release of the dual agents were investigated. Moreover, because of the concern about loss of biological activity of the agents during the process of loading and release, in vitro efficacy of the agents in the reversal of MDR was evaluated using an MDR model cell line with high expression of P-gp.

Experimental Section

Characterization of the Microspheres. The morphology of the MS before and after drug loading was examined by microscopy. The diameters of the MS with or without a loaded drug were measured by a Wild M420 stereomicroscope equipped with a Wild MMS 235 digital optical accessory and an automated camera.[35] The swelling kinetics and swelling ratio were determined based on the diameter change measured by microscopy.

Drug Loading into the Microspheres. Cross-linked dextran MS containing sulfonic groups (Sephadex SP C-25, Pharmacia) were washed several times with deionized water prior to use. Vinblastine sulfate and verapamil hydrochloride (Sigma Chemical Company) were used in this study because the former is a P-gp substrate and broadly used anticancer drug and the latter is a well-known effective chemosensitizer for P-gp-mediated MDR. In a typical loading process, 0.05 g of the dry, ionic MS were added to 10 mL of 0.5% verapamil aqueous solution. After incubation at room temperature for predetermined time intervals, the MS were separated by centrifugation, and the drug concentration in the supernatant was analyzed by ultraviolet−visible (UV−VIS) spectrophotometry (Hewlett-Packard 8452A) at wavelengths of 270 nm for vinblastine and 278 nm for verapamil. When the drug absorption reached equilibrium (after ~30 h), the MS were harvested by centrifugation and washed extensively with deionized water. The amount of drug loaded was calculated from the difference between the initial drug concentration and the final one after incubation with the ion-exchange MS. Unbound drug in the washout was also determined by spectrophotometry for the calibration of the drug loading. The MS were then lyophilized. In the case of loading of dual agents (i.e., vinblastine and verapamil), the same procedures were applied except that the drug assay was carried out by high performance liquid chromatography (HPLC; see next section).

Analysis of Drug Mixture by HPLC. Because of the interference of UV absorbance of the two drugs, for the studies of competitive drug loading and dual-agent release, the concentration of vinblastine and verapamil in the solution was analyzed using a Waters HPLC system including a spectrophotometer (Model 481), an HPLC pump (Waters 501), and a System Interface Module. The mobile phase consisted of phosphate buffer (ionic strength, 0.1 M; pH 7.0), tetrahydrofuran, and methanol, with a volume ratio 0.43:0.41:0.16. Vinblastine and verapamil were separated in a reverse-phase column (Norva-pak C-18, Waters) by the mobile phase at a flow rate of 0.6 mL/min. The drug concentration in the solution was determined based on the standard curves by a UV detector at a wavelength of 270 nm.

In Vitro Drug Release. Release rate of vinblastine and verapamil from single-agent-loaded and dual-agent-loaded MS was determined at 37 °C with the addition of 1.4 mg of dry MS in 10 mL of pH 7.4 phosphate buffer with ionic strength of 0.05 M. At predetermined time intervals, the suspension was centrifuged and the supernatant was analyzed by spectrophotometry or by HPLC. The release kinetics of both agents from dual-agent-loaded MS was also studied at 37 °C in an Earle's Balanced Salt Solution (EBSS), a pH 7.4 buffer solution commonly used in studies of drug uptake by cultured cells. The solution consists of $CaCl_2$ (1.8 mM), KCl (5.3 mM), $MgS0_4$ (0.8 mM), NaCl (138 mM), Na_2HP0_4 (1.0 mM), D-glucose (5.5 mM), *N*-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; 20 mM), and Trizma base to bring the pH to 7.4. In this case, the concentration of the individual drugs in the dual-agent solution was monitored by spectrophotometry (for verapamil) and liquid scintillation counting (for [^{3}H]vinblastine).

Tissue Cell Culture and Drug Accumulation Studies. Parent (AUXB1) and MDR (CH^RC5) Chinese hamster ovary (CHO) cells were initially grown in Dr. V. Ling's laboratory.[37,38] The resistant cell line, CH^RC5, is chosen because it expresses high levels of P-gp and has been shown to be resistant to a variety of structurally unrelated drugs, such as vinblastine and doxorubicin. The cells were grown in plastic culture flasks containing alpha minimal essential medium (α-MEM), 10% fetal bovine serum, and 0.5% penicillin-streptomycin at 37 °C, under an atmosphere of 95% air and 5% CO_2. Subculture of the cells was undertaken by trypsinization with 0.05% trypsin-EDTA when a confluent monolayer was formed.

Drug accumulation tests were carried out using monolayer cells grown on 24-well plates.[39] Initially, the accumulation of vinblastine over time by the parent and resistant cells was determined in the absence (control) or presence of chemosensitizers. Cyclosporin A (20 μM, a gift from Sandoz Canada) and verapamil hydrochloride (50 μM) were used as the chemosensitizers to verify the MDR characteristic of the resistant cells. Drug uptake was initiated in the presence or absence of a chemosensitizer by the addition of 0.5 mL of an EBSS containing 21 nM [^{3}H]vinblastine sulfate (11.7 Ci/mmol, Moravek Biochemicals) with 1/3 ^{3}H-labeled and 2/3 cold drug. At various time intervals (e.g., 0.5, 1, and 2 h), drug accumulation by the monolayer cells was stopped by aspirating the medium and washing the cells twice with an excess of ice-cold 0.16 N NaCl. The cells were then lysed with 1 mL of 1 N NaOH for 30 min and then transferred to scintillation vials containing 0.5 mL of 2 N HCl. Radioactivity was measured by a standard liquid scintillation technique using a Beckman Scintillation Counter and the standard Beckman scintillation fluid cocktail "Ready Safe". The protein concentration of the cells was determined by a colorimetric method using bovine serum albumin as a standard.[40]

The effect of blank MS and MS-immobilized agent(s) on the cellular uptake of vinblastine was determined using the same method as already described. Typically, in the place of a free drug solution, 0.5 mL of EBSS containing 0.2 wt % MS were introduced to the monolayer cells followed by the addition of 0.5 mL EBSS containing 21 nM vinblastine. After the medium was aspirated, the MS were removed by washing with an excess of ice-cold 0.16 N NaCl.

Cell Viability Tests. Cell viability in the presence of blank MS was tested by the standard trypan blue method. This procedure monitors the integrity of the plasma membrane. An aliquot (100 μL) of CH^RC5 cells incubated with the blank MS at various times was rapidly added to an equal volume of 0.8% trypan blue solution in isotonic saline and examined by optical microscopy. The percentage of nonviable cells was evaluated from the percentage of cells taking up the stain.

Statistical Analysis. For the drug uptake studies, each experiment was performed using at least two different sets of cultured cells. Within each experiment, the experimental data points were determined in quadruplicate. The results are expressed as means ± SD from data obtained from at least two separate experiments. Two-way analysis of variance (ANOVA) was applied to compare the results from different experiments. A value of $p < 0.05$ is considered statistically significant.

Results and Discussion

Characterization of the Microspheres. *Morphology of the Microspheres.* Figure 1 shows the microscopic photographs of the unloaded (Figure 1a) and verapamil (VER)-loaded (Figure 1b) dry MS with the same magnification. As illustrated, the process of drug loading did not rupture the MS. Instead, the surface of the MS became smoother and the size of the MS became larger, indicating an increase in the volume of the MS due to the drug loading.

Swelling of the MS in Various Media. Equilibrium swelling and swelling rate are two important parameters associated with the release mechanism and kinetics of a solute from hydrogels. The MS used in this study are essentially polyelectrolyte hydrogels. Therefore, their swelling in various media, such as deionized water, 0.05 M pH

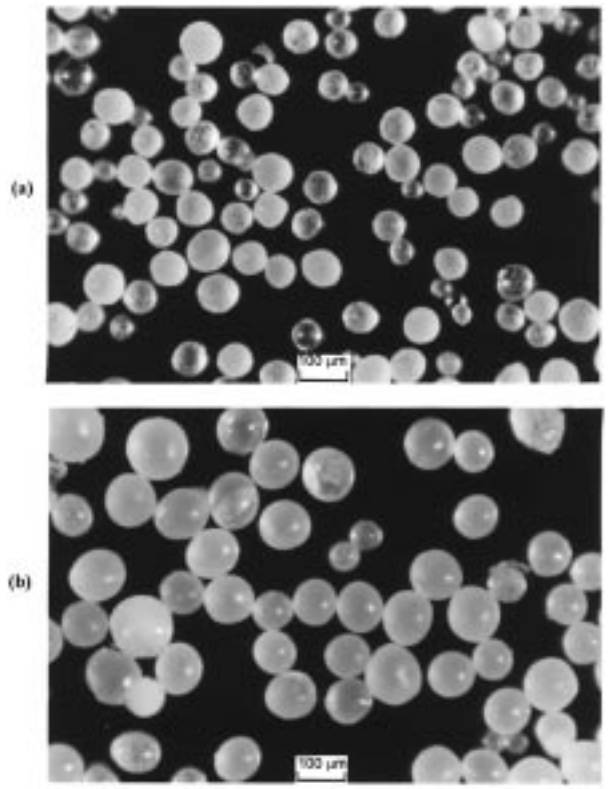

Figure 1—Microscopic photographs of (a) unloaded and (b) verapamil-loaded MS in dry state.

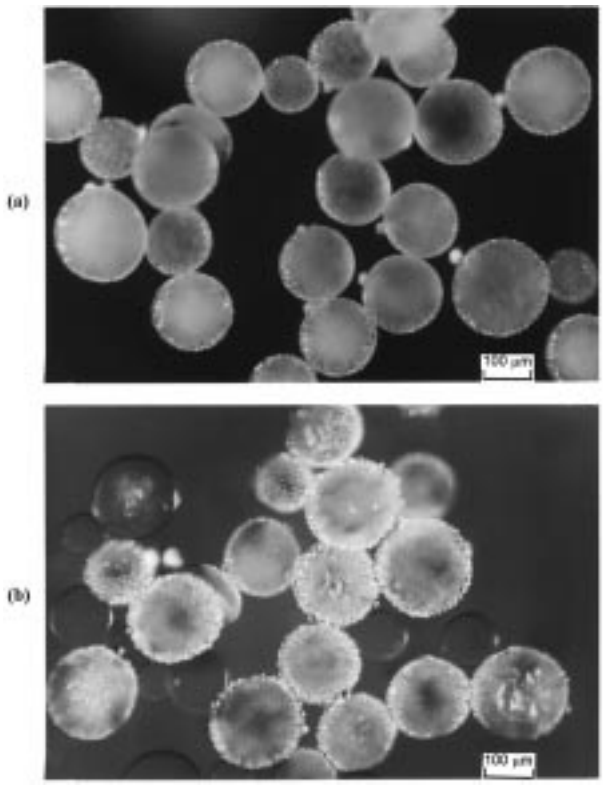

Figure 2—Microscopic photographs of verapamil-loaded MS in 0.05 M pH 7.4 buffer solution at room temperature for (a) 5 min and (b) 20 min.

7.4 phosphate buffer (Fisher Scientific), and 0.9% NaCl, was investigated microscopically. The MS without loaded drug swelled instantaneously like "pop corns" in all three media. Their swelling ratio, H, defined as the volume of the MS at the swollen state divided by the volume at the dry state, was 12−13. In contrast, the drug-loaded MS exhibited slower swelling and a much smaller swelling ratio, especially in deionized water. The VER-loaded MS reached the equilibrium swelling ($H \approx 3$) in deionized water in about 30 min, with a clear swelling or solvent penetration front, like the observation with hydrophobic gels.[41] In the isotonic saline and the buffer solution, the maximal size ($H \approx 6$) was observed within 3−10 min, followed by a size reduction. The peak size may be an indication of an initial swelling-dominated stage and thereafter solute depletion, which is typical in hydrophobic hydrogel loaded with high drug content.[42] The diminished swelling ratio and rate for drug-loaded MS suggest an increased hydrophobicity of the MS due to the bound drug molecules (i.e., VER) that are more hydrophobic than Na^+ present in the unloaded MS. In pH 7.4 buffer solution, the swelling or penetration front disappeared, probably as a result of quick ion exchange of the drug with the counterions (i.e., K^+ or Na^+), which converts the hydrophobic drug−MS complex into hydrophilic MS.

Microscopic photographs of the VER-loaded MS in the buffer solution for 5 and 20 min are shown in Figures 2a and 2b, respectively. It was observed that oily droplets accumulated on the surface of the MS at a later time. The droplets disappeared as more buffer solution was added and more vigorous shaking was applied. This result may be ascribed to small volume of the solution and insufficient mixing during the examination of microscopy. It implies that the rate of removal of the released drug from the MS surface is lower than the drug release from the MS, resulting in the drug accumulation on the surface. This accumulation may be the case in the body cavities, such as a solid tumor, where circulation of the body fluid is poor.[35,36]

Interestingly, the oily droplets were absent when 0.9% NaCl was used as a releasing medium. Moreover, the boundary of undissolved drug that was observed in the pH 7.4 buffer solution disappeared in the saline. These results agree with the observations of the dependence of the drug moving front on the drug solubility,[41] suggesting that the solubility of verapamil salts may vary with its salt form. In a NaCl solution, the released verapamil salt is in a chloride form, whereas in the phosphate buffer, it is in a phosphate form. The latter may exhibit lower solubility than the former. Based on this observation, it may be concluded that in the present system, ion exchange is a major mechanism of the drug release from the MS, but drug solubility and drug diffusion away from the surface of the MS into the bulk fluid may play important roles in the release kinetics.

Determinants of Drug Loading. To obtain optimal drug loading, several factors influencing the amount of drug bound to the MS were investigated, including incubation time, ratio of MS to drug (M/D), drug affinity to the MS, and initial drug concentration.

Effect of Incubation Time. Curve A in Figure 3 depicts the fraction of remaining verapamil in the solution as a function of time for the MS incubated in a 0.025-mg/mL verapamil solution. A rapid decrease in the remaining drug is seen in the initial 10 h, followed by a slower change in the subsequent 10 h. A plateau in the curve after 20 h indicates an equilibrium state. A similar trend was also observed for vinblastine. Therefore, incubation was carried out for 30 h for all the drug loading to ensure completion of the process. The fraction of the drug loaded into the MS, as shown by curve B in Figure 3, follows typical first-order sorption kinetics, suggesting that the drug loading is essentially a diffusion-controlled process like drug release.[43]

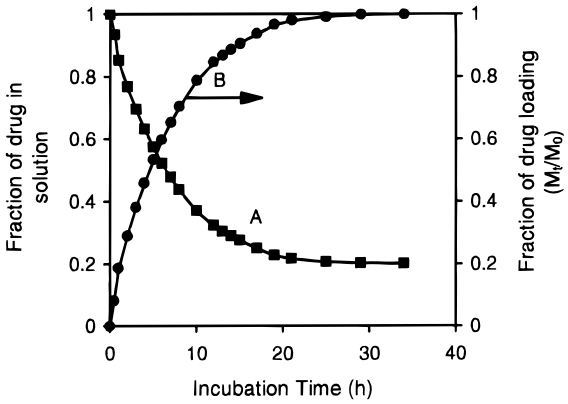

Figure 3—Dynamics of verapamil absorption into the MS through ion exchange. The initial concentration of verapamil is 0.025 mg/mL; curve A, Fraction of drug remains in the solution; curve B, fraction of drug loaded into the MS calculated using the amount of drug loaded at time *t* divided by the equilibrium amount of drug loaded. Experiments in Figures 3–6 were performed as described in the Experimental Section (SD < 10%).

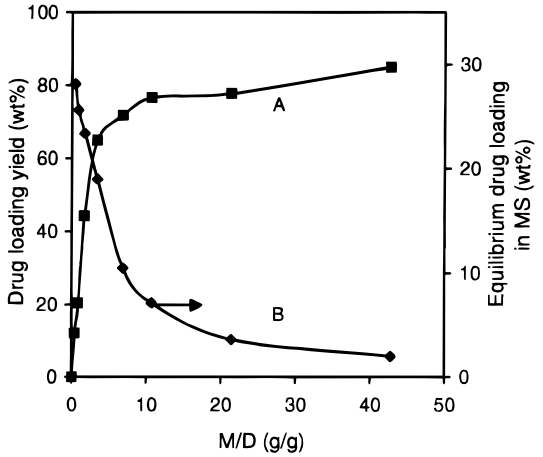

Figure 4—Effect of the MS/drug ratio (M/D) on the equilibrium level of verapamil loaded and the yield of the loading. The initial verapamil concentration is 0.05 mg/mL.

Although swelling of the dry MS is observed, the swelling in an aqueous medium is very quick compared with the drug absorption, as discussed previously.

Effect of Ratio of Microspheres to Drug. The amount of MS relative to drug is an important factor influencing the equilibrium drug content and the yield of drug loading. However, there has been little work in this area, though various conditions have been used by different researchers.[25−28] Using verapamil as a model drug, the effect of the M/D ratio was investigated. The yield of drug loading and the equilibrium level of verapamil loaded are plotted against the M/D ratio in Figure 4. As the M/D ratio increases, the yield of drug loading increases while the equilibrium level of drug loaded decreases (Curve A). This result indicates that to raise the equilibrium drug content in the MS, one has to sacrifice the loading efficiency. Therefore, a compromise approach is to control the M/D ratio between 1 and 3 and thus the drug content can reach ~30% and the yield of drug loading is in the range 40−60%.

Effect of Drug Concentration. The effect of drug concentration on the equilibrium drug content was investigated for initial verapamil concentrations ranging from 10 to 85 mg/mL at an M/D ratio of 1. In this wide range of verapamil concentrations, the equilibrium loading is only slightly reduced from 24 to 22%. This observation is not abnormal. At a fixed M/D ratio, the amount of drug bound to MS is determined by the competition between the drug and the counterions (e.g., Na^+). Addition of water to the mixture causes an equal dilution of both cations, but the equilibrium constant remains unchanged. Consequently, the amount of drug bound to the MS undergoes little change.

Relative Affinity of Vinblastine and Verapamil. The relative affinity of vinblastine (VIN) and verapamil (VER) was determined by the sorption method. The competitive sorption of VIN and VER was carried out using known amounts of MS (e.g., M/D ratio = 1.5) in the solution of dual agents with various VIN/VER ratios. The separation and assay of the dual agents were performed with HPLC. The dual agents were effectively separated with a retention time of 5.95 min for VIN and 6.82 min for VER.

When the MS are added to a solution containing a cationic drug, the drug competes with Na^+ to bind to the MS as illustrated by the following formula

$$\mathrm{Re{-}SO_3^-\ Na^+ + DRUG^+ \rightleftharpoons Re{-}SO_3^-\ DRUG^+ + Na^+}$$

The equilibrium of ion exchange determines the maximum of the drug loaded into the MS, which can be correlated with selective coefficients that are expressed by the following equations[44]

$$K_{VIN} = \frac{[VIN]_m[Na]_s}{[VIN]_s[Na]_m} \quad (1)$$

$$K_{VER} = \frac{[VER]_m[Na]_s}{[VER]_s[Na]_m} \quad (2)$$

where K_{VIN} and K_{VER} are the selectivity coefficients for the drug and the competing ions, Na^+; and the subscripts m and s denote the concentration in the microspheres and in the solution, respectively. In the process of absorption of dual agents, the Na^+ concentration in the solution and the MS should be the same in eqs 1 and 2. Therefore, the relative selectivity coefficient of VIN and VER, K_R, can then be obtained from eq 3

$$K_R = \frac{K_{VIN}}{K_{VER}} = \frac{[VIN]_m/[VER]_m}{[VIN]_s/[VER]_s} \quad (3)$$

By plotting the [VIN]/[VER] ratio in the MS against that in the solution at the equilibrium as illustrated by Figure 5, the value of K_R was estimated from the slope of the straight line to be 1.25. The K_R value greater than unity reflects slightly higher affinity of VIN to the MS than that of VER.

Kinetics of Drug Release. Figure 6a shows the release profiles of verapamil and vinblastine from single-agent-loaded MS in 0.05 M pH 7.4 buffer at 37 °C. It appears that verapamil is released completely within 2 h and vinblastine by 3 h. Similarly, Figure 6b illustrates a higher release rate of verapamil than vinblastine from the dual-agent-loaded MS. In EBBS, the same trend was also observed. The lower release rate of vinblastine may be a reflection of its higher affinity to the MS in addition to its larger molecular size. When the MS loaded with the drugs without drying were added to the release medium, the release rate was increased significantly. This result suggests that hydration of the MS or the polymer relaxation may play some role in the kinetics of drug release. Microscopic studies revealed a short period time of swelling of the MS, as discussed previously. The completion of the swelling was within 10 min which was relatively short compared with other hydrogel beads[41,42] because of their much smaller diameter and higher hydrophilicity. Never-

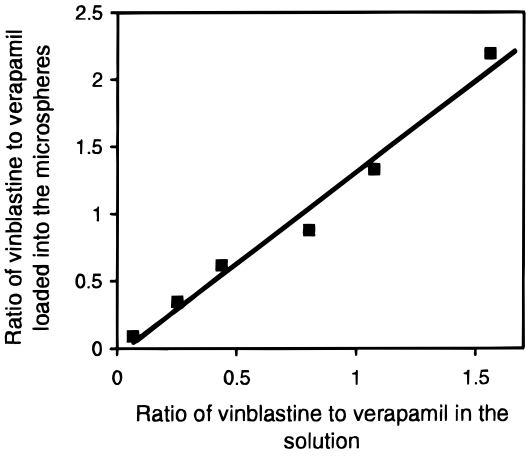

Figure 5—A plot of [VIN]/[VER] ratio in the MS against that in the solution at the equilibrium (M/D = 1.5, the total drug loading is ~20 wt %). The relative selective coefficient of vinblastine is evaluated from the slope of the straight line (K_R = 1.25).

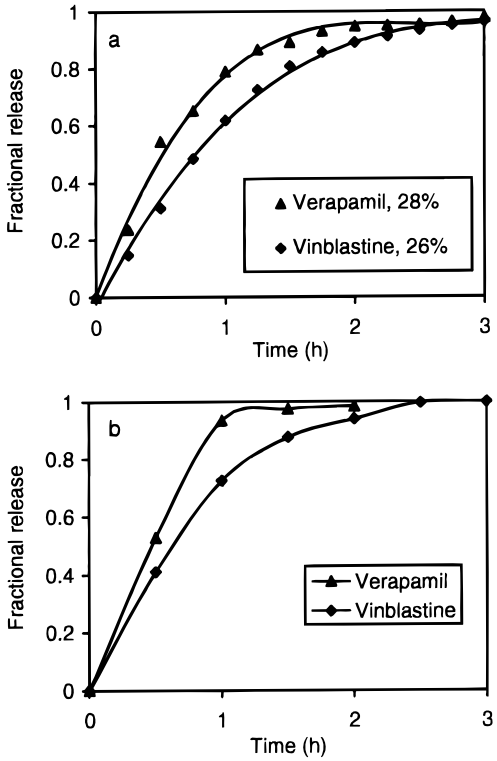

Figure 6—Fractional release of vinblastine and verapamil in 0.05 M pH 7.4 buffer at 37 °C as a function of time from (a) single-agent-loaded MS (drug loading for verapamil is 28 wt % and for vinblastine is 26 wt %); and (b) dual-agent-loaded MS (the total drug loading is ~20 wt %).

theless, this time span is about 10% of the total release time of the drug (i.e., VER). Therefore, the hydration of the MS is not negligible in the present system.

In Vitro Evaluation of the MS Delivery System. *Drug Resistance of CH^{R}C5 Monolayer Cells.* The effectiveness of CH^{R}C5 monolayer cells as a MDR cell model was investigated by measuring drug uptake by the cells in the absence (control) and presence of a chemosensitizer, either verapamil (50 μM) or cyclosporin A (20 μM). Figure 7a shows that in the presence of verapamil or cyclosporin A, the cellular uptake of vinblastine by CH^{R}C5 cells significantly ($p < 0.01$) increases 7 and 9 times, respectively. In contrast, only up to 3-fold ($p < 0.01$) increase in drug uptake by nonresistant cells, AUXB1, is observed in the

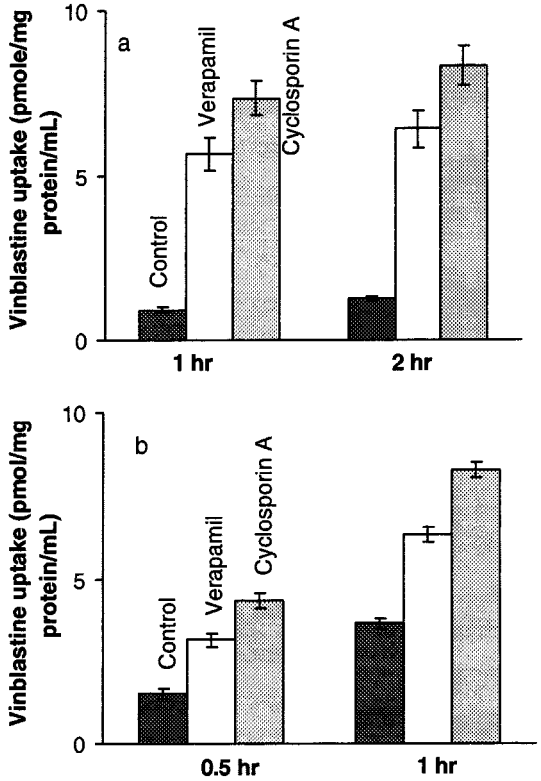

Figure 7—Vinblastine uptake by (a) MDR (CH^{R}C5) monolayer cells and (b) parent cells (AuxB1) after incubation for 1 h and 2 h. The bars represent control (i.e., without a chemosensitizer), with addition of verapamil (50 μM), and with addition of cyclosporin A (20 μM). The concentration of vinblastine used in the uptake is 21 nM with 1/3 radiolabeled and 2/3 cold drug. Results are expressed as mean ± SD from at least two different experiments. A statistically significant difference between the control and the uptake in the presence of chemosensitizers was found ($p < 0.01$) for Figure 7a and 7b.

presence of chemosensitizers (Figure 7b). These results confirm that CH^{R}C5 cell line grown as a monolayer is an effective model for the in vitro evaluation of chemosensitization.

Effect of Blank Microspheres on Cell Viability and Cellular Drug Uptake. Cell viability in the presence of blank MS was comparable to the control (data not shown), suggesting that the blank MS did not cause cell toxicity. As shown in Figure 8a, in the presence of the blank MS and absence of chemosensitizers, the cellular drug uptake (bar 2) does not statistically differ from the control (bar 1; $p > 0.05$). In the presence of chemosensitizers (bar 3: 50 μM verapamil, and bar 4: 20 μM cyclosporin A), the blank MS do not have any significant effect on the drug uptake as evidenced by the equivalent efficacy of MDR reversal in the absence (Figure 7a, bars 2 & 3) and presence of the MS (Figure 8a, bars 3 & 4). As presented in Section 2, the relative equilibrium loading of vinblastine to verapamil is 1.25; that is, the amount of vinblastine loaded in the MS can reach 1.25 times of that of verapamil if both are of the same concentration in the solution. However, the concentration of vinblastine used in the uptake studies is 21 nM, which is about or less than the one-thousandth of the concentration of the chemosensitizers (20 and 50 μM). Therefore, the competitive binding of vinblastine with verapamil to the MS is expected to be negligible.

Effectiveness of the Chemosensitizer Delivered by the Microspheres. Two different approaches of delivering chemosensitizers and anticancer drugs to MDR cells were evaluated: (1) verapamil-loaded MS plus free vinblastine (bar 2 in Figure 8b), and (2) vinblastine- and-verapamil-

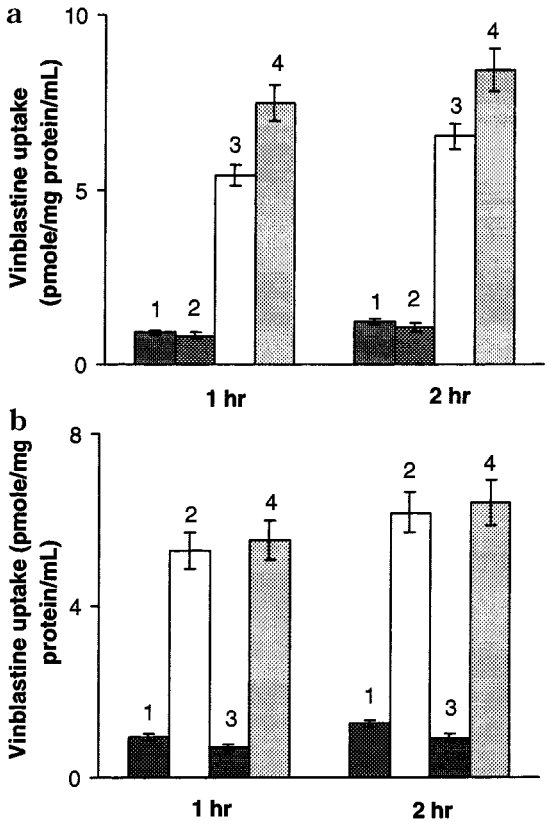

Figure 8—(a) Effect of blank MS on vinblastine uptake by MDR (CH^RC5) monolayer cells at 1 and 2 h. The bars represent (1) control; (2) blank MS; (3) free verapamil plus the blank MS; and (4) free cyclosporin A plus the blank MS. The concentration of vinblastine used is the same as in Figure 7. Results are expressed as mean ± SD from at least two different experiments. A statistically significant difference between the control and the uptake in the presence of chemosensitizers/blank MS was found for bar 3 and bar 4 ($p < 0.01$). (b) Effect of different microspherical formulations on vinblastine uptake by MDR (CH^RC5) monolayer cells. The bars represent (1) control; (2) verapamil-loaded MS plus free vinblastine (21 nM); (3) vinblastine-loaded MS; and (4) vinblastine- and-verapamil-loaded MS. Results are expressed as mean ± SD from at least two different experiments. A statistically significant difference between the control and the uptake in the presence of chemosensitizer-loaded MS was found for bar 2 and bar 4 ($p < 0.01$).

loaded MS (bar 4 in Figure 8b). For comparison, a control (bar 1) and vinblastine-loaded MS (bar 3) are also presented in the figure. In the absence of verapamil, the drug uptake in the presence of vinblastine-loaded MS (bar 3) is almost the same ($p > 0.05$) as the control. In contrast, the verapamil-loaded MS (Figure 8b, bar 2) exhibit a comparable efficacy in the reversal of MDR as free verapamil (Figure 7a, bar 2), suggesting that the bioactivity of the chemosensitizer has been preserved. Moreover, the MS loaded with both verapamil and vinblastine display a significant ($p < 0.01$) enhancement of drug uptake up to 7-fold, a level achieved with free drug solutions.

The diameter of the MS ranges from 40 to 125 μm in dry form (even larger in wet form), much bigger than the size of CHO cells, so it is unlikely that the increase in drug uptake is due to the phagocytosis of the particles by the cells. On the other hand, the blank MS have no effect on drug uptake. Therefore, the enhancement of the drug uptake is likely a reflection of the effect of the chemosensitizer released from the MS. These results demonstrate that the bioactivity of the MS-loaded verapamil in reversing MDR remains unchanged in both formulations of single-agent and dual-agent loading. The results also suggest that both antineoplastic and chemosensitizing agents are released from the MS effectively.

Conclusion

The ionic polysaccharide MS possess a fairly good loading capacity for ionic chemosensitizer and anticancer drug. The loading of a single agent or dual agents could readily reach 28 wt %. The amount of drug loaded was influenced by the M/D ratio and drug affinity to the MS. Release rate of the loaded drugs was likely controlled by ion exchange; that is, the counterions diffuse in and the ionic drugs diffuse out of the MS. However, hydration of the MS, solubility of the drug, as well as the rate of drug leaving the surface of the MS each might play an important role in release kinetics. The delivery system appeared effective at releasing both antineoplastic and chemosensitizing agents in vitro. More importantly, similar enhancement of anticancer drug uptake was achieved with the MS-delivered chemosensitizer as that with the free drug, suggesting that the biological activity of the chemosensitizer was preserved. To further evaluate the therapeutic efficacy of the new formulation, in vivo investigation is presently being undertaken using an animal tumor model.

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