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Research Paper

Intracellular Delivery of Saquinavir in Biodegradable Polymeric Nanoparticles for HIV/AIDS

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Purpose. This study aims at developing poly(ethylene oxide)-modified poly(epsilon-caprolactone) (PEO-PCL) nanoparticulate system as an intracellular delivery vehicle for saquinavir, an anti-HIV protease inhibitor.
Materials and Methods. Saquinavir-loaded PEO-PCL nanoparticles were prepared by a solvent displacement process. The formed nanoparticles were characterized for size, surface charge, and surface presence of PEO chains. Cellular uptake and distribution of the nanoparticle was examined in THP-1 human monocyte/macrophage (Mo/Mac) cell line. Intracellular saquinavir concentrations were measured as a function of dose and duration of incubation.
Results. The PEO-PCL nanoparticles had a smooth surface and spherical shape and showed a relatively uniform size distribution with a mean particle diameter of approximately 200 nm. The surface presence of PEO chains was confirmed by an increase in the –C–O–(ether) signature of the C1s spectra in electron spectroscopy for chemical analysis. Rapid cellular uptake of rhodamine-123 encapsulated PEO-PCL nanoparticles was observed in THP-1 cells. Intracellular saquinavir concentrations when administered in the nanoparticle formulation were significantly higher than from aqueous solution.
Conclusions. This study shows that PEO-PCL nanoparticles provide a versatile platform for encapsulation of saquinavir and subsequent intracellular delivery in Mo/Mac cells.

KEY WORDS: intracellular delivery; nanoparticles; poly(ethylene oxide)-modified poly(epsilon-caprolactone); saquivanir; THP-1 monocytes/macrophages.

INTRODUCTION

Therapeutic use of anti-HIV protease inhibitors suffers from problems of poor solubility, low and variable oral bioavailability with limited penetration into lymphatic and central nervous systems (1–3). The high cost of therapy has added to the cause of significant crisis in the management of HIV/AIDS patients, especially in developing nations. A major barrier to the current therapy is the development of resistance due to the persistence of HIV in the sanctuary sites where the virus thrives (4). A paradigm shift in the last couple of decades has led to utilization of targeted delivery mechanism for various therapeutic agents in order to increase efficacy and reduce toxicity (5). Anti-HIV therapy may explore novel drug delivery strategies as a new dimension along with multiple drug therapy (the highly active antiretroviral therapy, HAART).

One of the recent trends has been the use of nanocarrier delivery technology, where the payload is trapped within a carrier system of 1 to 1,000 nm in diameter (6,7). Nanocarriers, due to their small size and target specific localization properties, are being actively investigated for preferential drug delivery to various disease sites in the body. Higher concentrations and increased residence time of the drugs can be achieved at the sanctuary sites and thus help to reduce the viral load significantly (8). Regardless of the inherent properties of the drug candidates, the pharmacokinetics and distribution pattern will be dictated by the properties of the nanocarrier system (9). Different types of nanoparticle systems can be fabricated for enhancing oral absorption as well as transport across the blood–brain barrier by either passive diffusion or by carrier-mediated endocytosis (10). For instance, Boudad *et al.* (11) have prepared hydroxypropyl-beta-cyclodextrin inclusion complex of saquinavir and encapsulated in poly(alkylcyanoacrylate) nanoparticles. With an aim to increase the intracellular concentration of saquinavir, we have developed a stable formulation using poly(ethylene oxide)-modified poly(epsilon-caprolactone) (PEO-PCL) nanoparticulate system and characterized it. PCL is a synthetic pre-formed biodegradable polymer. It possesses unique properties such as excellent biocompatibility, high hydrophobicity, and neutral biodegradation end products, which do not disturb the pH balance of the degradation medium. It is a crystalline polymer and degrades very slowly *in vitro* in the absence of enzymes and *in vivo* as well (12). PCL has a melting range of 59–64°C, depending on its crystalline state. The crystallinity of PCL varies with its

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molecular weight and plays an important role in determining both permeability and biodegradability because of the fact that bulk crystalline phase is inaccessible to water and other permeating agents (13). An increase in crystallinity reduces the permeability by reducing the solubility of the drug and increasing the tortuosity of the diffusional pathway. The biodegradation rate is also significantly reduced by the decrease in accessibility of the ester bond (14). High permeability to many drugs and a lack of toxicity has made PCL and its derivatives well suited for colloidal drug delivery (14,15).

Surface modification of PCL nanoparticles with PEO chains prevents aggregation and allows for efficient systemic delivery especially after intravenous administration. Pluronics® (also known as poloxamers) are non-ionic ABA-type triblock copolymers with the general formula $HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_aH$, consisting of two PEO chains and a center block made of poly(propylene oxide) (PPO). They are commercially available in different grades and vary from liquids, semisolids, to solids based on the ratio of PEO to PPO and the molecular weight. Pluronics have been used as emulsifying agents, solubilizing agents, surfactants, and wetting agents. As the "*a*" and "*b*" block numbers change, hydrophilic–lipophilic balance changes, thereby changing the physical chemical properties of the triblock copolymer, changing its applicability (15). Pluronic® F-108/NF (poloxamer 338) has a bulky central block as well as long side arms ($a = 122$; $b = 56$).

Saquinavir, the first HIV-protease inhibitor to be marketed as Invirase® for the treatment of HIV, is a peptide derivative that inhibits HIV-1 and HIV-2 protease-mediated cleavage of the gag and pol polyproteins of the HIV genome, thus preventing the post-translational processing required for virus maturation and spread (16). The oral bioavailability of a single dose of saquinavir is only 4% (16–18). Its low bioavailability is mainly attributed to its metabolism by cytochrome *P*450 3A4 and to the membrane transporters namely the P-glycoprotein (P-gp) (4,19). After performing the loading and release studies, intracellular uptake studies were carried out in monocyte/macrophage (Mo/Mac) cells. It has been known that the HIV infects peripheral mononuclear phagocytic cells and the central nervous system in the early stages (19). The phagocytic cells (Mo/Mac) also act as a shuttle for the viruses to go to other sites in the body and thrive there (2). Some of the phagocytic cells also express P-gp efflux pump, which is known to present a therapeutic limitation by preventing the penetration and retention of anti-HIV protease inhibitors.

Bender *et al*. (20) have showed that at a concentration of 100 nM, free saquinavir was completely inactive in chronically HIV-infected Mo/Mac cells, but when bound to poly(-hexylcyanoacrylate) nanoparticles, caused a 35% decrease in virus production. We hypothesize that the Mo/Mac would endocytose the nanoparticulate formulation and provide optimal concentrations in Mo/Mac cells as well as transport the drug to CNS and other sites, which are a potential reservoirs for the HIV. The drug would be released at these sites and help in targeted and effective eradication of the viral load. The preliminary studies shown here revealed that PEO-PCL nanoparticulate system is an efficient delivery vehicle for the release of anti-HIV protease inhibitor and its intracellular delivery to the Mo/Mac cells.

MATERIALS AND METHODS

Materials

Saquinavir base, a protease inhibitor with molecular weight of 670.9 Da, was purchased from Aapin Chemicals, (London, UK). PCL of molecular weight of 14,800 Da., as confirmed by gel electrophoresis, was purchased from Polysciences, (Warrington, PA). National Formulary grade Pluronic® F-108 was kindly supplied by the Performance Chemical Division of BASF Inc., (Parsippany, NJ). Rhodamine-123 was obtained from Molecular Probes (Eugene, OR). Lipase (90 U/g) was purchased from ICN Biochemicals, (Aurora, OH). THP-1 human monocyte/macrophage cell line was purchased from American Type Culture Collection (ATCC, Manassas, VA). Deionized distilled water (NanoPureII, Barnstead, and Dubuque, IA) was used for the preparation of aqueous solutions. All other reagents were purchased from Fischer Scientific.

Preparation of PEO-PCL Nanoparticles

The PEO-PCL nanoparticles were prepared by a solvent displacement method similar to the one used by Chawla *et al*. (14). Briefly, 850 mg of PCL and 150 mg of Pluronic® F-108NF were dissolved in 30-ml acetone by mild heating. The polymer solution was added drop wise into 200 ml of deionized distilled water under continuous stirring over a magnetic stirrer. Stirring was continued overnight to evaporate the organic solvent. The resulting suspension of nanoparticles was centrifuged at 10,000 × *g* for 20 min. The supernatant was discarded and pellet washed twice with deionized distilled water and freeze dried. Saquinavir (5% w/w)-loaded nanoparticles were prepared by a similar procedure as described above, except a known quantity of saquinavir was dissolved in acetone and added to the PCL and Pluronic® F108 solution before addition into aqueous medium thereby encapsulating the drug with the solid polymeric matrix formed.

Characterization of the Nanoparticles

Particle Size Analysis

A sample of nanoparticles suspension prepared was immediately analyzed for particle size. The suspension was diluted suitably in deionized distilled water, sonicated for 1 min and the particle size was determined for unloaded and saquinavir-loaded nanoparticles with a 90Plus ZetaPALS particle sizer (Brookhaven Instruments, Holtsville, NY).

Measurements of the Surface Charge

A suitably diluted aqueous suspension of nanoparticles was mounted in a ZePALS zeta potential analyzer (Brookhaven Instruments, Holtsville, NY) and the mean zeta potential was measured for unloaded and saquinavir-loaded nanoparticles, suspended in deionized distilled water.

Scanning Electron Microscopy (SEM)

Surface morphology of freeze dried sample was observed. A sample of freeze-dried nanoparticles was diluted with deionized distilled water and mounted on an aluminum sample mount and sputter-coated with a gold-palladium alloy to minimize surface charging. SEM was performed using Hitachi S-4800 environmental scanning electron microscope (Hitachi Instruments, San Jose, CA) at an accelerating voltage of 3 kV.

Electron Spectroscopy for Chemical Analysis (ESCA) Studies

Surface modification of the nanoparticles with Pluronic® F108 allows for physical association of the PCL with poly(propylene oxide) block, while leaving the two flanking PEO chains extending out into the surrounding medium. The surface presence of PEO chains was confirmed with ESCA. ESCA was performed on the control and PEO-modified nanoparticle samples at the National ESCA and Surface Analysis for Biomedical Applications Center (NESAC/BIO), University of Washington in Seattle, WA. The spectra of freeze-dried samples were recorded on the spectrophotometer. Standard Scofield photoemission cross-sections were used to determine the surface elemental composition. Identification of chemical functional groups was obtained from the high-resolution peak analysis of the carbon 1s (C1s) envelopes.

Saquinavir Incorporation and *In Vitro* Release Studies

Saquinavir Loading Studies

High performance liquid chromatography (HPLC) analysis of saquinavir was carried out with Waters 2695 Separations Module using the slightly modified method as described by Janoly *et al*. (22). Stationary phase was a Zorbax SB-C18 column (4.6 × 150 mm, 5 μm) and the mobile phase consisted of acetonitrile, tetrahydrofuran, and 0.1-M dihydrogen phosphate buffer (pH 4.0) in a 32:10:58 volume ratio. The sample injection volume was 60 μl and flow-rate of mobile phase was 1.0 ml/min. Detection of saquinavir was performed at 238 nm using a UV detector. For the loading studies, a predetermined volume of saquinavir solution was added to PCL-Pluronic® F108 solution in acetone. Nanoparticles were prepared as described above and freeze dried. A known amount of nanoparticles was re-dissolved in acetone and subsequent dilutions were made with methanol. The amount of drug loaded per milligram of nanoparticles (i.e., loading capacity) and percent drug loading (i.e., loading efficiency) were determined from a calibration curve of the drug in methanol.

In Vitro Saquinavir Release Studies

The *in vitro* release of saquinavir was carried out in phosphate-buffered saline (PBS, pH 7.4). Since the drug is insoluble in this medium, 0.5% (w/v) of Tween® 80 was added to enhance the solubility. Tween® 80 also prevents adsorption of the drug to the container surfaces. The drug release from the formulations was performed by adding 15.0 mg of saquinavir nanoparticles in 15.0 ml of the release medium and kept in a rocker maintained at 37°C. At various time intervals, the tubes were centrifuged at 10,000 × *g* for 10 min. Carefully, 2 ml of supernatant was withdrawn and replaced by 2.0 ml of fresh medium in order to maintain sink conditions. The supernatant was filtered through a 0.45-μm filter and the concentration of the drug was determined by HPLC assay. Release studies were performed in absence and presence of the enzyme *Pseudomonas* lipase, which is known to enhance the degradation of the polymer (14). Cumulative amount and percent saquinavir released from the nanoparticles was calculated from a calibration curve of the drug in Tween®80-containing PBS.

Uptake and Intracellular Distribution of Nanoparticles in Mo/Mac Cells

Cell Culture

THP-1 human monocyte/macrophage, a myelomonocytic cell line derived from the blood of a 1-year-old boy with acute monocytic leukemia (23) were maintained in RPMI® 1640 growth medium and incubated at 37°C under 5% CO_2 atmosphere. They were sub-cultured four times during the experimental time period. Cell viability was periodically examined using the Trypan blue exclusion assay for the entire duration of the study.

Cell Uptake and Distribution Studies

Rhodamine123, a hydrophobic fluorescent dye was encapsulated in PEO-PCL nanoparticles at 0.4% (w/w) concentration. The dye-containing nanoparticles were prepared exactly similar to those described above and characterized for particle size and charge. The nanoparticles were re-suspended in serum-free media to a 1 mg per mL concentration. Twenty microliter of this suspension was added to approximately 20,000 THP-1 cells per well in a six-well microplate. Cells were incubated for 1 h and directly viewed with a Nikon TE-2000U scanning fluorescence confocal microscope (Melville, NY) at original magnification of 60X and Adobe Photoshop and Image-J software were used for digitization and processing.

Intracellular Saquinavir Concentration Measurements

Preparation of [^{3}H]-Saquinavir Loaded PEO-PCL Nanoparticles

Tritiated [^{3}H]-saquinavir loaded nanoparticles were prepared by incorporating 4.6 μCi of labeled saquinavir (Moravek Biochemicals (Brea, CA) to 20.0 mg of cold saquinavir and following the procedure as described above. The nanoparticles obtained in aqueous suspension were used for further experiments.

Evaluation of Intracellular Drug Concentrations as a Function of Dose

Tritiated [^{3}H]-saquinavir-loaded PEO-PCL nanoparticles in suspension were incubated with the THP-1 cells suspended in

Table IParticle Size and Surface Charge of the Control and Saquinavir-Containing Poly(Ethylene Oxide)-Modified Poly(Epsilon-Caprolactone) Nanoparticle Formulations[a]

Nanoparticle formulation	Particle size (nm)	Surface charge (mV)
Blank PEO-PCL	198.9±1.6[b]	−23.5±1.7
Saquinavir-Loaded PEO-PCL	271.0±1.6	−26.2±1.8

[a] Control and saquinavir-loaded poly(ethylene oxide)-modified poly(epsilon-caprolactone) (PEO-PCL) were formulated using the solvent displacement method.
[b] Mean ± S.E. (n=5).

serum-free cell culture media, at different doses of saquinavir ranging from 10 to 250 nM. After drug incubation, the media was changed back to RPMI® 1640. Blank nanoparticles and ^{3}H-saquinavir aqueous solution at the above mentioned concentrations served as controls. After a pre-determined time interval of 6 h, the suspension of cells were subjected to centrifugation and cell pellet obtained was washed with sterile PBS to ensure removal of extracellular drug, re-suspended in PBS, lysed with Triton X-100, and the intracellular drug concentration determined by liquid scintillation counting. All the cell lysate were collected in pre-labeled scintillation vials. To each milliliter of the cell lysate, 10 ml of the ScintiSafe Econo® (scintillation cocktail) was added and the samples were allowed to quench for 4 h in the dark before measuring with a liquid scintillation analyzer. The protein content in the cell pellet was determined using the NanoOrange® Protein Quantitation Kit obtained from Molecular Probes (Eugene, Oregon). Saquinavir concentrations in the cell were calculated as nanomolar (nM) of drug per milligram (mg) of protein.

Evaluation of Intracellular Drug Concentrations as a Function of Time

Following a similar protocol as above, intracellular drug concentrations as a function of time were evaluated by incubating cells with a 50-nM dose of ^{3}H-saquinavir labeled nanoparticles and ^{3}H-saquinavir solution. After several pre-determined time intervals (0.5 to 12 h), the cells were centrifuged, washed, lysed and assayed for drug as described in Section Evaluation of Intracellular Drug Concentrations as a Function of Dose above.

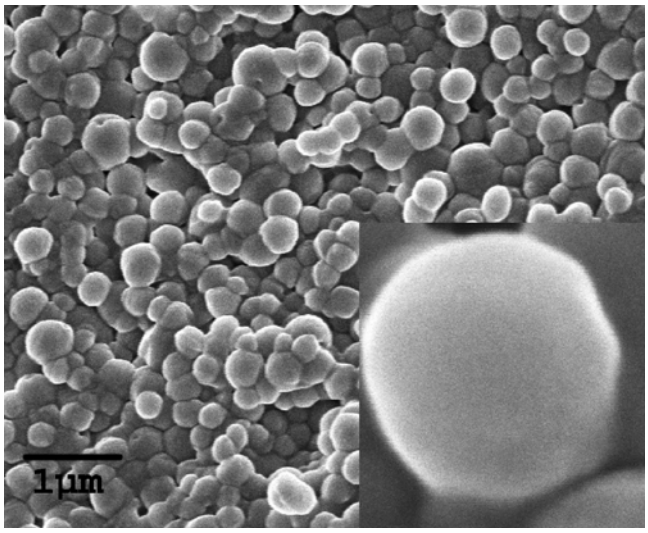

Fig. 1. Scanning electron micrograph of poly(ethylene oxide)-modified poly(epsilon-caprolactone) (PEO-PCL) nanoparticles. The *inset* shows one nanoparticle at higher magnification. The *scale bar* represents a distance of 1 μm.

Table II. High Resolution C_{1s} Peak Analysis of ESCA on the Surface of Poly(Ethylene Oxide)-Modified Poly(Epsilon-Caprolactone) Nanoparticles[a]

Nanoparticle system	Relative C_{1s} peak area (%)		
	–C–H–	–C–O–	–C=O–
PCL nanoparticles (in the absence of PF-108)	67.5	18.7	13.8
PCL-PF-108 with 5% (w/w) PF-108, as is	65.6	21.4	13.0
PCL-PF-108 with 5% (w/w) PF-108, washed once	66.6	20.1	13.4
PCL-PF-108 with 5% (w/w) PF-108, washed twice	67.5	19.7	11.7
PCL-PF-108 with 5% (w/w) PF-108, washed thrice	67.1	20.1	12.8
PCL-PF-108 with 15% (w/w) PF-108, as is	34.0	60.3	5.75
PCL-PF-108 with 15% (w/w) PF-108, washed once	63.8	22.0	14.3
PCL-PF-108 with 15% (w/w) PF-108, washed twice	65.8	20.3	14.0
PCL-PF-108 with 15% (w/w) PF-108, washed thrice	63.9	22.0	14.2

[a] Poly(ethylene oxide) (PEO)-modified poly(epsilon-caprolactone) (PCL) were prepared by blending PCL with Pluronic® F-108 (PF-108) triblock copolymer. ESCA was performed at the NESAC/BIO, University of Washington, Seattle, WA.

RESULTS AND DISCUSSION

Nanoparticulate Systems for Intracellular Drug Delivery

Previous studies from our lab have shown that long circulating PEO-modified polymeric nanoparticles are efficient carriers for intracellular delivery of drugs and genes (15). For instance, PEO-PCL nanoparticles were found to efficiently encapsulate hydrophobic drugs, such as tamoxifen and paclitaxel, and deliver in tumor cells and *in vivo* (15,24). PCL was selected as a hydrophobic slow-eroding matrix that can provide sustained intracellular drug delivery. In addition, unlike other biodegradable polymers, such as poly(D,L-lactide-co-glycolide), the by-products of PCL degradation are not acidic and do not cause any toxicity *in vivo*.

Preparation and Characterization of PEO-PCL Nanoparticles

The PEO-PCL nanoparticles were formed instantaneously by solvent displacement method. The nanoparticles were characterized for size uniformity, surface charge, morphology and presence of surface PEO side arms. The PEO-PCL particles were in the size range of 189.8 to 208.3 nm with a mean diameter of 198.9 nm. Drug loaded nano-

particles had a slightly greater diameter with a mean of 271.0 nm and a size range of 267.7 to 275.3 nm (Table I). SEM image of nanoparticles, as shown in Fig. 1, illustrates a distinct spherical shape with smooth surface and confirms the diameter of the nanoparticles. The suspension of nanoparticles without any drug had almost the same negative surface charge of −23.5 mV as that of the loaded nanoparticles (−26.2 mV) indicating that saquinavir was encapsulated into the nanoparticles rather than being adsorbed on to the surface.

Table II summarizes the results obtained from ESCA, showing high resolution –C–H–(hydrocarbon), –C–O–(ether) and –C=O (carbonyl) peaks of the nanoparticle samples with and without Pluronic® F-108NF. The surface presence of PEO chains was confirmed by an increase in the –C–O–(ether) signature of the spectra, which is indicative

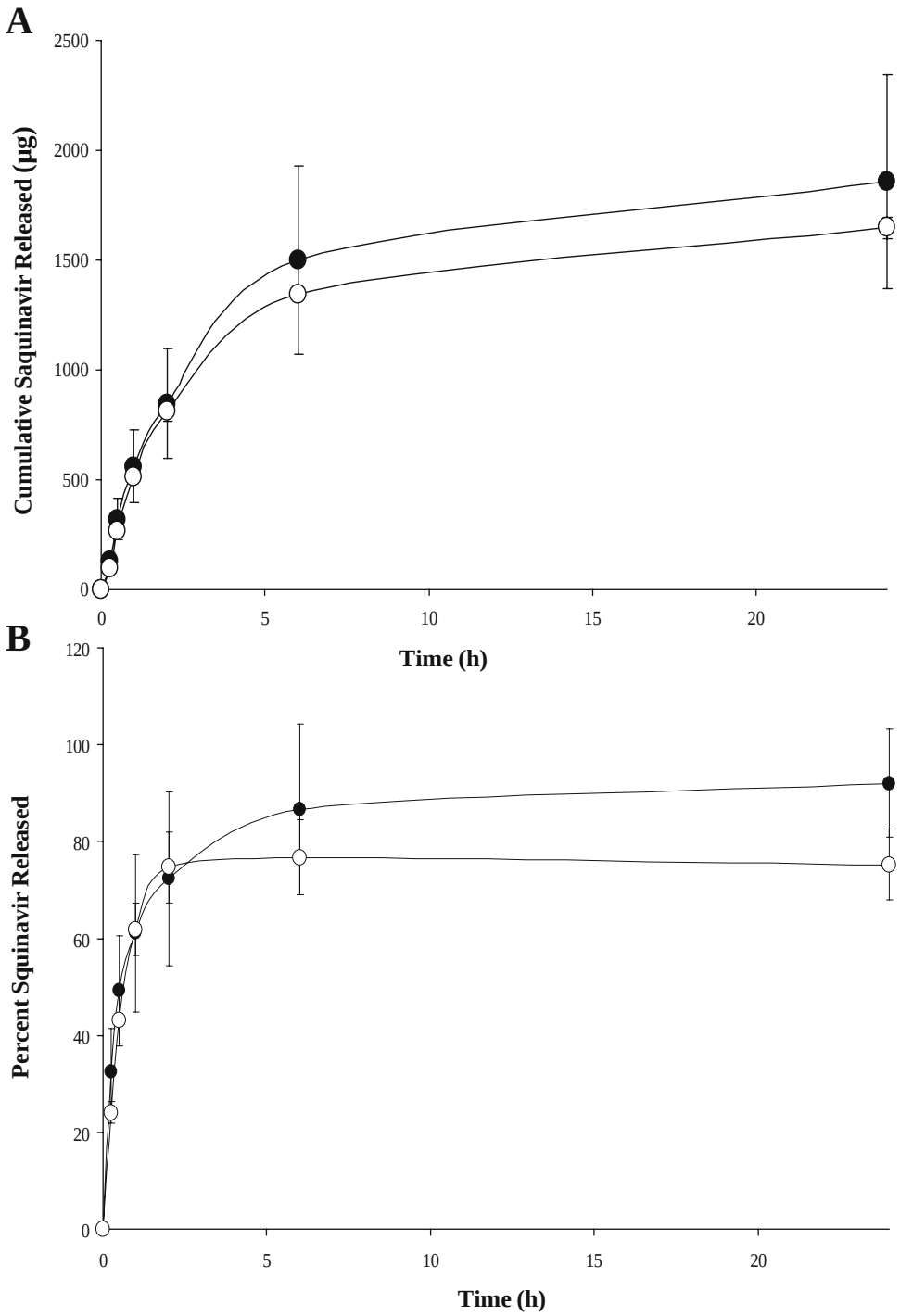

Fig. 2. *In vitro* release profiles of saquinavir from poly(ethylene oxide)-modified poly(epsilon-caprolactone) (PEO-PCL) nanoparticles in the absence: *filled circles* (●) and presence of *Pseudomonas* lipase: *empty circles* (○). The results show cumulative amount (A) and percent (B) released at 37°C in Tween® 80-containing phosphate buffered saline (pH 7.4).

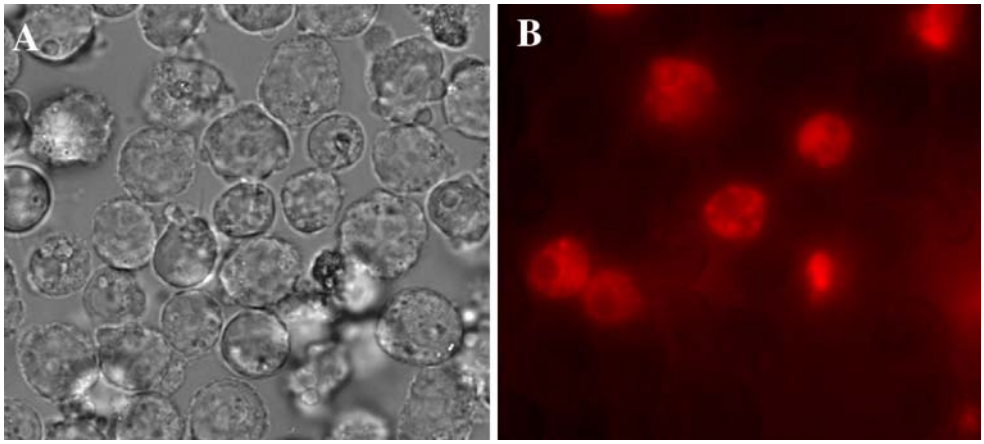

Fig. 3. Uptake of rhodamine123-containing poly(ethylene oxide)-modified poly(epsilon-caprolactone) (PEO-PCL) nanoparticles by the THP-1 monocyte/macrophage cells. Cells were incubated with nanoparticles for 1 h at 37°C. Brightfield (A) and corresponding fluorescent (B) images were acquired at ×40 original magnification.

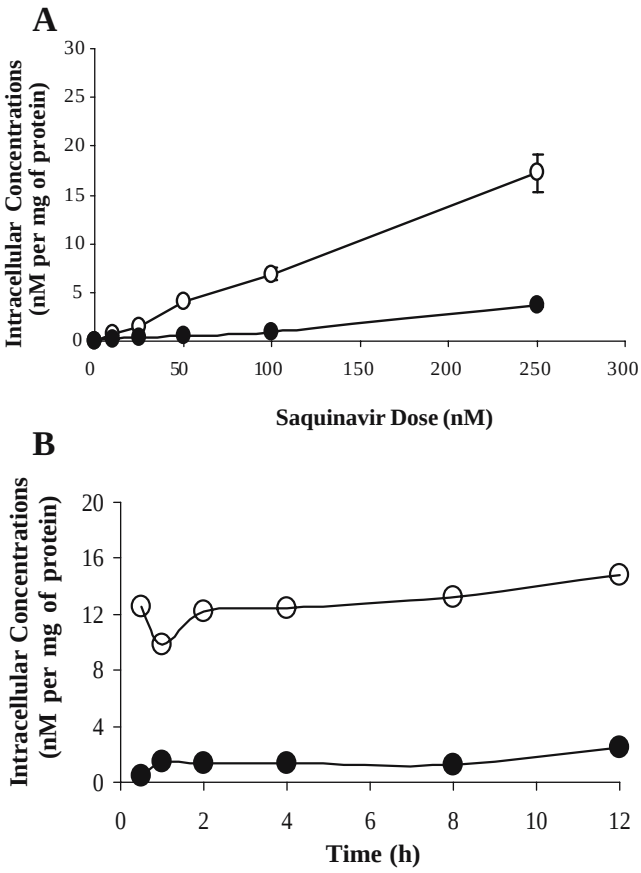

Fig. 4. Intracellular concentrations of saquinavir as a function of dose administered (A) and time of incubation (B) to THP-1 monocyte/macrophage cells. Tritiated [^{3}H]-saquinavir was administered in aqueous solution: *filled circles* (●) and in poly(ethylene oxide)-modified poly(epsilon-capolactone) (PEO-PCL) nanoparticles: *empty circles* (○) at different doses and incubated for different duration. In order to evaluate the effect of incubation time, saquinavir concentration was held constant at 50 nM.

of the presence of ethylene oxide residues. Repeated washings with water showed gradual stripping of the hydrophilic coating resulting in exposure of underlying PCL substrate. The ESCA results show that when Pluronic F-108 copolymer was added as a blend, there was greater stability of the PEO chains on the surface even after repeated washing.

Saquinavir Incorporation and *In Vitro* Release Studies

The loading capacity of saquinavir in the PEO-PCL nanoparticles was found to be 28 μg/mg and the efficiency was calculated to be 60%. The *in vitro* release studies of saquinavir were examined in the absence and presence of lipase. Our previous studies have shown that lipase enhances the degradation of PCL matrix (13). Lipase was added to the release medium, therefore, in order to appreciate the differences in the release profiles based on diffusion alone in the absence of lipase and diffusion/degradation when lipase was present. From the release profile of saquinavir, as shown in Fig. 2A and B, an initial burst release of about 60% was observed within the first hour of the study in PBS containing Tween® 80 at 37°C with and without lipase. Almost a maximum amount of drug was release at the end of 24. It has been established that PCL and its copolymers have a high permeability to low molecular weight drugs (13,14). There is not much difference in the release profile of the entrapped drug with or without lipase, which shows that the predominant mechanism by which the drug is released is by diffusion and not by polymer degradation. The initial burst release may be attributed to the smaller size nanoparticles or to the low molecular weight of the PCL polymer employed (25).

Uptake and Distribution of PEO-PCL Nanoparticles in Macrophages

THP-1 Mo/Mac cell line was used to examine the uptake of rhodamine-123 encapsulated PEO-PCL nanoparticles by

the cells. From a previous study (23), we have observed that the rhodamine-123 encapsulated nanoparticles do not allow for any fluorescent dye to leach out for up to 6 h. To confirm the cell viability during incubation with nanoparticles, Trypan blue exclusion experiment was carried out and the results confirmed that there was no cytotoxicity induced by PEO-PCL nanoparticles. After 2 h of incubation, the bright-field images (Fig. 3A) show the intact cells and the corresponding fluorescent images (Fig. 3B) show the uptake of the rhodamine-123 labeled nanoparticles by these cells. The qualitative images show that a significantly high percentage of the administered dose of nanoparticles was internalized in THP-1 cells.

Intracellular Saquinavir Concentrations

To further quantitate the uptake and examine the intracellular delivery potential of saquinavir with PEO-PCL nanoparticle formulation in THP-1 cells, we carried out the incubation of ^{3}H-saquinavir-loaded nanoparticles as a suspension and ^{3}H-saquinavir aqueous solution at different concentrations and for various time intervals. The protein content of the cell populations used for the study was determined and the results of intracellular concentration of saquinavir were calculated and plotted as nanomolar (nM) of saquinavir in the cells per mg of protein as a function of saquinavir dose administered (Fig. 4A). It is clear that the intracellular saquinavir concentration in the cells was significantly higher when administered in PEO-PCL nanoparticle formulation than when administered as an aqueous solution. This trend was observed at each dose administered. For instance, at a 50 nM dose, an intracellular concentration of 4 nM of saquinavir/mg of protein was obtained for the nanoparticle formulation as compared to only 0.54 nM/mg with the aqueous solution formulation. A similar trend was observed when the cells were treated with a 50 nM dose of saquinavir for different time intervals (Fig. 4B). At 4 h, an intracellular concentration of 12.4 nM/mg of protein was obtained with the nanoparticle formulation, whereas only a 1.4 nM/mg protein concentration was achieved with the aqueous solution formulation.

CONCLUSIONS

Our study shows that an efficient nanoparticle formulation can be developed for saquinavir to ensure better encapsulation and release of the drug. A significant uptake of nanoparticles was also observed in the THP-1 cells of the monocyte/macrophage origin, which are known to shuttle the infected virions. The intracellular concentrations of saquinavir when administered in the nanoparticle formulations was significantly higher than from an aqueous solution. This strategy can potentially serve as a useful targeted drug delivery system for eradicating the viral sanctuaries in patients infected with HIV-1/AIDS.

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