Available online at www.sciencedirect.com
ScienceDirect
Biomaterials 29 (2008) 703–708
Biomaterials
www.elsevier.com/locate/biomaterials

The use of deoxycholic acid to enhance the oral bioavailability of biodegradable nanoparticles

Robert M. Samstein[1], Karlo Perica[1], Fanor Balderrama, Michael Look, Tarek M. Fahmy*

Department of Biomedical Engineering, Yale University, 55 Prospect St., New Haven, CT 06511, USA

Received 26 August 2007; accepted 16 October 2007

Abstract

Oral delivery of nanoparticles encapsulating drugs and proteins remains a challenging route for administration due to the many barriers in the gastrointestinal tract that limit bioavailability. We hypothesized that bile salts could be used to improve the bioavailability of poly(lactide-*co*-glycolide) (PLGA) nanoparticles by protecting them during their transport through the gastrointestinal tract and enhancing their absorption by the intestinal epithelia. A deoxycholic acid emulsion is shown to protect PLGA nanoparticles from degradation in acidic conditions and enhance their permeability across a Caco-2 cell monolayer, an *in vitro* model of human epithelium. Oral administration of loaded PLGA nanoparticles to mice, using a deoxycholic acid emulsion, produced sustained levels of the encapsulant in the blood over 24–48 h with a relative bioavailability of 1.81. Encapsulant concentration was highest in the liver, demonstrating a novel means for targeted delivery to the liver by the oral route.

Keywords: Nanoparticles; Oral delivery; Deoxycholic acid; PLGA

1. Introduction

Oral delivery of peptides and drugs is one of the greatest challenges for drug delivery due to the many obstacles present in the gastrointestinal tract. These obstacles include: (1) the acidity and presence of digestive enzymes in the stomach, which are optimized to degrade many molecules [1,2]; (2) the low absorption of therapeutics from the intestinal lumen due to the tight junctions in the epithelial lining [3,4]; (3) the deactivation or extrusion of many drugs in the epithelial lining [5]; and (4) the exposure of the intestinal lining to toxic levels of the drug resulting in dose-limiting side effects [6]. These barriers significantly decrease the bioavailability of drugs and peptides administered orally while simultaneously limiting the maximum tolerable dosage and thus compel intravenous administration of therapeutics. However, oral delivery remains the most attractive drug delivery route due to its ease and convenience, resulting in improvements in quality of life for patients and reduced administrative costs [7–9].

An objective in designing a drug delivery system for oral administration is to maintain drug levels in the therapeutic range for sustained periods of time. The delivery system must protect the drug at low pH, facilitate absorption in the intestinal tract, bypass unwanted metabolic degradation, and limit intestinal cell exposure. Particulate systems for oral delivery have been attempted to address some of these issues [10]. They can theoretically provide protection from degradation and metabolic deactivation, as well as limit intestinal exposure [11]. Nanoparticles of synthetic poly-esters such as poly(lactic acid), poly(glycolic acid), and their copolymers poly(lactide-*co*-glycolide) (PLGA) are often chosen due to their biocompatibility and versatility in encapsulating a variety of drugs and biologics, as well as the ability to tune the dynamics of drug release by varying monomer ratios and polymer molecular weight [12–14]. Oral delivery of PLGA particles and uptake by intestinal cells has also been well studied [15,16]. However, absorption efficiency of particulates is typically very low

*Corresponding author. P.O. Box 208260, New Haven, CT 06520, USA. Tel.: +1 203 432 1043; fax: +1 203 432 0030.
E-mail address: tarek.fahmy@yale.edu (T.M. Fahmy).
[1]These authors contributed equally to this work.

0142-9612/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2007.10.026

with estimates of only 1% absorbed after oral administration [17]. In addition, PLGA particles are degraded via acid-catalyzed ester hydrolysis and therefore release much of their contents at the low pH of the stomach.

We reasoned that an existing physiological system, the biliary system, could be exploited to address many of the issues associated with oral delivery of therapeutics. Bile acids are produced in the liver and recirculated 10–20 times per day as part of the enterohepatic circulation [18]. After being released into the duodenum, they are passively and selectively reabsorbed in the intestinal lumen and returned to the liver via the portal vascular system. These bile salts are either passively diffused across the membranes of intestinal epithelial cells or are actively taken up at the ileum. The selective reabsorption of bile salts can potentially be used to improve the bioavailability of particulates. Bile salts have also been shown to disrupt tight junctions in the epithelial lining, allowing for paracellular and transcellular transport pathways [19,20]. In addition, bile salts have been shown to aggregate at low pH, offering protection inside the stomach [21]. There is also the potential of targeting drugs to the liver due to the anatomy of the portal circulation [22].

Attempts have been made to take advantage of deoxycholate in drug delivery systems by covalently conjugating drugs to bile salts [23]. For example, heparin conjugated to deoxycholic acid (DCA) was shown to significantly increase heparin absorption in the intestine, particularly in the ileum [24]. Various peptides were conjugated to bile acids and shown to increase absorption [25,26]. Functional insulin conjugates with DCA were also constructed for oral delivery in diabetes but have not been tested orally [26]. However, covalent attachment of DCA to the therapeutic can potentially impair the therapeutic's intended physiological response. Here, we describe a system where PLGA particles are orally delivered in a non-covalently associated emulsion of DCA. We show that this system improved the bioavailability of encapsulated model agent (rhodamine B) in PLGA nanoparticles. The simplicity of preparation combined with the attractive features of biodegradable particle technology positions this formulation as a viable formulation for enhancing oral administration of therapeutic agents.

2. Methods

2.1. Fabrication of PLGA nanoparticles

PLGA particles containing rhodamine B were prepared using a modified double emulsion (W/O/W) process described previously [27]. Briefly, 200 mg of PLGA was dissolved in 4 mL methylene chloride. While vortexing, 1 mg of rhodamine B (10 mg/mL in water) was added dropwise to the methylene chloride–PLGA solution and then sonicated three times for 10 s at an amplitude of 38% (600 W Ultrasonic Processor). The solution was then added drop-wise to a vortexing solution of 8 mL of 5% poly-vinyl alcohol (PVA) and the resulting mixture was sonicated 3X. This mixture was then added drop-wise to 200 mL of 0.3% PVA while stirring and the particles were hardened by evaporation of the organic phase for 3 h under continuous stirring. Particles were collected by centrifugation at 13,000 RPM for 10 min at 4 °C, and washed 3X with deionized water. The resulting particles were then frozen at −80 °C and lyophilized for 48 h.

2.2. Scanning electron microscopy (SEM)

The lyophilized particles were imaged using SEM. The particles were fixed on an aluminum stub using 2-sided carbon tape and then coated with gold in an argon atmosphere using a sputter current of 40 mA (Dynavac Mini Coater, Dynavac, USA). The samples were imaged with a Philips XL30 SEM using a LaB electron gun with an accelerating voltage of 5–10 kV. Particle diameters were determined using the ImageJ particle sizing software from the NIH.

2.3. Controlled release measurement

Release of encapsulated rhodamine was performed in sodium phosphate buffer titrated with hydrochloric acid to pH 2 and 7 at 37 °C. Samples were placed in dialysis tubing (exclusion size 10,000 MW) at 20 mg/mL and dialyzed against 50 mL of buffer. At appropriate time points, 100 μL of buffer was removed and rhodamine concentration was read by fluorescence (Molecular Devices SpectraMax M5; Ex: 540 nm; Em: 620 nm). The fraction of rhodamine B released was calculated by dividing the amount of rhodamine at the indicated time points by the total content of dye in 10 mg of the same stock of particles. Total rhodamine B content was measured by dissolving 10 mg of particles in 1 N dimethyl sulfoxide overnight.

2.4. Permeability in Caco-2 monolayer

Caco-2 cells were seeded at 7×10^4 cells/cm^2 on 0.4 μm pore transwell filters in Dulbecco's modified eagle media containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.1 mM non-essential amino acids. The cells were grown to confluency and allowed to mature for approximately 30 days at 37 °C and 5% CO_2. Cell culture media was changed every 2–3 days. Prior to performing permeability studies, the transepithelial electrical resistance (TEER) was measured using an epithelial voltometer (World Precision Instruments, EVOMX with STX2 electrodes). Confluent cell layers with TEER values greater than $300\,\Omega \times \text{cm}^2$ were used for permeability and cytotoxicity studies. For permeability studies, a solution of 40 mg/mL rhodamine-loaded PLGA nanoparticles was prepared in phenol-free Hank's balanced salt solution (HBSS) containing 25 mM glucose and varying concentrations of DCA. The cells were kept incubated at 37 °C and 5% CO_2. About 400 μL of DCA solution was added to the apical chamber of the transwell filter, and 400 μL of HBSS containing 25 mM glucose was added to the basolateral chamber. Every 30 min, 100 μL of the media in the basolateral chamber was sampled and replaced with 100 μL of fresh HBSS containing 25 mM glucose. Basolateral media samples were obtained for 5 h. The amount of rhodamine in the basolateral sampled media was determined by dissolving it with 100 μL of dimethyl sulfoxide overnight in a 37 °C rotary shaker, and then measuring rhodamine fluorescence (Ex: 540 nm, Em: 620 nm). From these measurements, the cumulative amount of rhodamine transport to the basolateral chamber was determined as a function of time. The rate of cumulative rhodamine transport to the basolateral chamber gave the flux, dQ/dt. The permeability (P) was calculated by dividing the flux by the initial concentration of total rhodamine in the apical chamber (C_0) and the area of the transwell filter (A).

2.5. Cytotoxicity measurements

Cell titer blue assay (Promega®) was used to measure the cytotoxicity of deoxycholate *in vitro*. After the 5 h permeability measurements, cells were washed with buffer, then replaced with 200 μL of varying concentrations of DCA in the apical chamber. About 50 μL of cell titer blue reagent was added to the apical chamber, and allowed to incubate

with cells for an additional 90 min. Following incubation, 100 μL of media from the apical chamber was read in a fluorimeter (Ex: 560 nm, Em: 590 nm). The media was diluted until fluorescence reading was linear with dilution.

2.6. Oral gavage of mice

C3 H mice were obtained from Charles River Laboratories (Wilmington, MA) and maintained under pathogen free conditions and routinely monitored by Yale Animal Resource Center. Mice of 10–12 weeks were fasted overnight and fed 300 μL of solution using a blunted end oral gavage needle containing 25 μg of rhodamine in PLGA in PBS or in an emulsion of DCA.

2.7. Rhodamine extraction and quantification

At indicated time points, mice were sacrificed by CO_2 asphyxiation on dry ice and blood was collected by cardiac puncture. Blood was allowed to clot at 37 °C for 15 min, centrifuged at 3000*g* for 15 min, and serum was collected for analysis. Heart, lung, liver, spleen and kidney were collected and homogenized in water. Rhodamine extraction was performed in a solution of 8:1:1 70% methanol, 10% Triton-X 100, and sample at −20 °C overnight. The samples were then centrifuged at 13,000 RPM and the fluorescence of supernatant was measured (Ex: 540 nm; Em: 620 nm). C_{max} and T_{max} were defined as the maximum concentration measured and the timepoint at which this concentration was observed respectively. Background fluorescent levels were subtracted based on control mice fed PBS. Area under the curve (AUC) values were estimated by the log-linear trapezoidal method applied to the concentration vs. time curve. The relative bioavailability (*F*%) was defined as the AUC of the PLGA with DCA divided by the AUC of PLGA alone.

2.8. Statistical methods

Where appropriate the unpaired, two-tailed student's *t*-test was used to measure significance.

3. Results and discussion

3.1. Preparation and characterization of particles

Biodegradable PLGA nanoparticles containing rhodamine B were fabricated using a commonly used double emulsion process. Particle diameters ranged from 80 to 400 nm with a mean diameter of 212 ± 61 nm (Fig. 1). Nanoparticles were fabricated instead of microparticles since they have been shown to have increased uptake in the intestinal tract by endocytosis, transcellular passage, and lymphatic uptake [15]. The double emulsion method allows for a variety of encapsulants ranging in hydrophobicity including small molecule drugs as well as proteins.

3.2. In vitro release studies

The release of rhodamine B from the PLGA particles was measured at pH 7.4 and found to have a characteristic burst release followed by linear release. The emulsion of DCA had little effect on the release rate of rhodamine at pH 7.4 (Fig. 2a). At pH 2, a large increase in the rate of release was observed, as expected due to acid-catalyzed ester hydrolysis (Fig. 2b). However, with the addition of

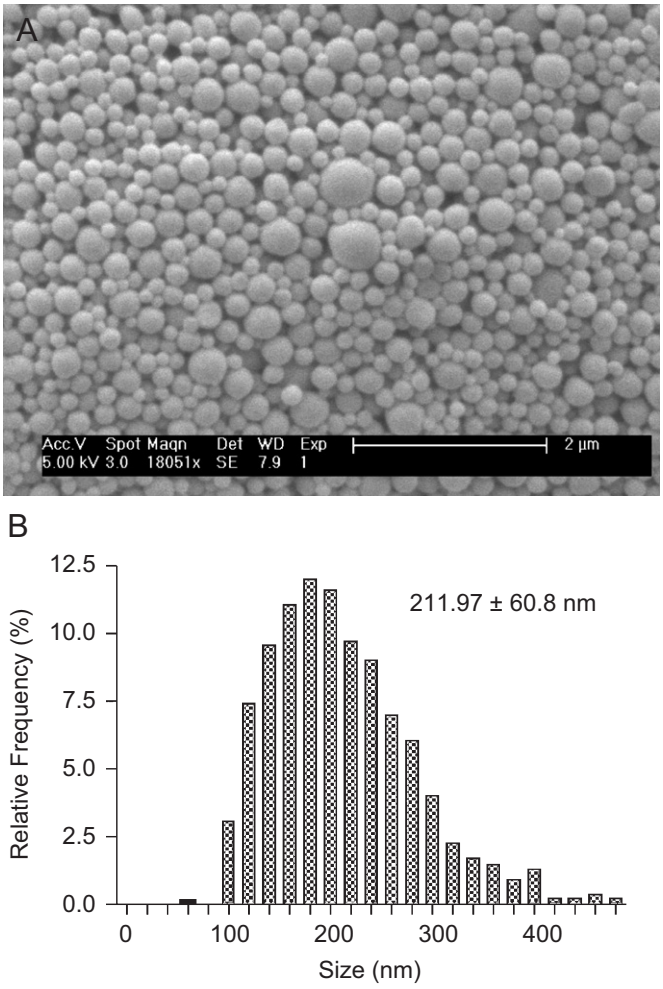

Fig. 1. PLGA nanoparticles were prepared using a modified water–oil–water (W/O/W) emulsion as described under materials and methods. (A) Samples were characterized by scanning electron microscopy. (B) Diameters were quantified using the NIHI ImageJ particle sizing software.

the DCA emulsion visible aggregation was observed (data not shown). This corresponded with a significant difference in the release patterns at pH 2 (Fig. 2c). This release pattern is ideal for oral applications, offering protection in the acidic environment of the stomach but not inhibiting release in the intestine or bloodstream.

3.3. Caco-2 studies of intestinal transport and cytotoxicity

The delivery system was then tested *in vitro* using the Caco-2 cell line, a model for the epithelial lining of the intestine. As seen in Fig. 3a, increased permeability of the monolayer was observed as the concentration of DCA was increased. This has been demonstrated for a wide variety of molecules but never for a nanoparticulate system [28]. Cytotoxicity, using the cell titer blue assay, was also observed at high concentrations (Fig. 3b). An effective but non-toxic range between 1 and 2.5 mg/mL of DCA was demonstrated in these conditions. This suggests that conditions may exist *in vivo* where DCA enhances permeability with limited damage to the epithelial lining.

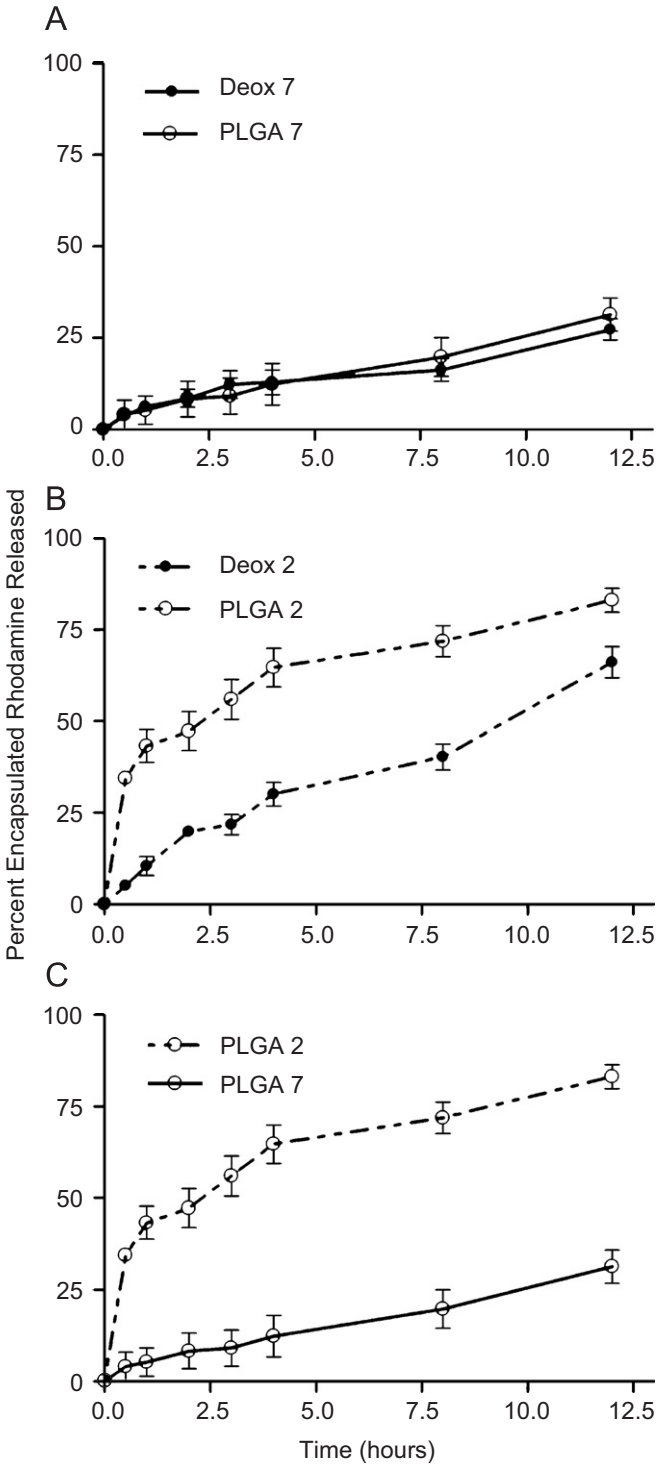

Fig. 2. Release of encapsulated rhodamine was carried out in sodium phosphate buffer titrated with hydrochloric acid to pH 2 and 7.4 at 37 °C as described under Materials and methods. Rhodamine B concentration was measured by fluorescence (Ex: 540; Em: 620). The fraction of dye released was calculated by dividing the amount of rhodamine at the indicated time points by the total content of both model drug in 10 mg of the same stock of particles. (A) At pH 7.4, deoxycholic acid was found to have little effect on the release characteristic of the PLGA particles; (B) as expected, at pH 2 release occurs over several hours; (C) the DCA emulsion protects the particles and slows down release at pH 2.

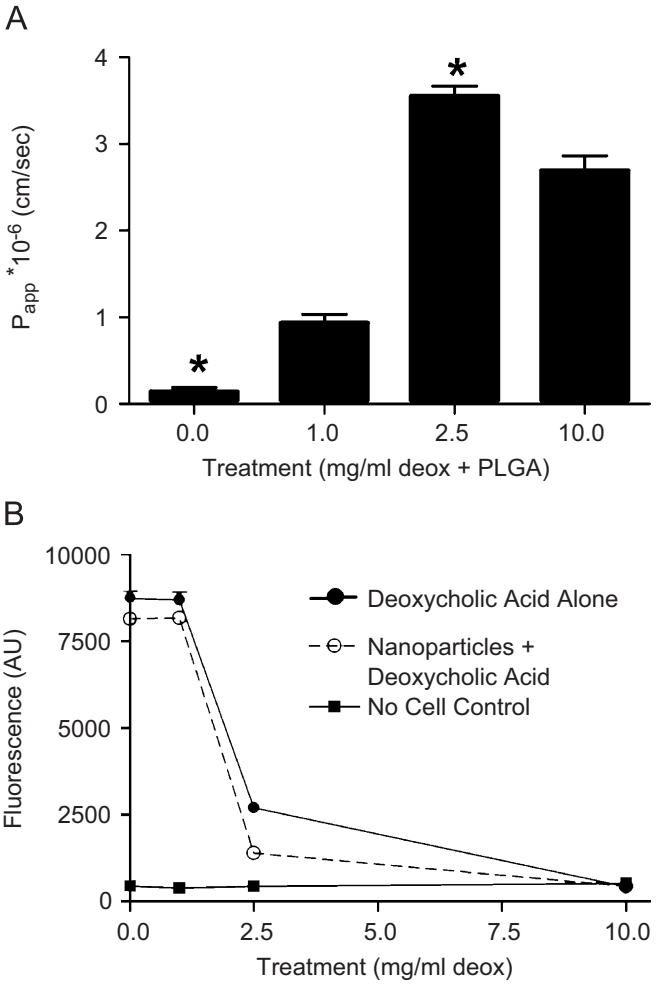

Fig. 3. (A) Enhanced permeability was observed with increasing concentrations of deoxycholic acid on a Caco-2 cell monolayer model. *$p<.05$ compared to 1 mg/mL (B). Cell titer blue assay was used to measure the cytotoxicity of deoxycholate as described under Materials and methods. DCA was found to be toxic at high concentrations but an intermediate range was found *in vitro* with enhanced permeability and limited cytotoxicity.

However, levels of DCA must be maintained in a narrow effective range due to its potential toxicity. DCA has been suggested as a potential tumor promoter and induces apoptosis in cancer cells, suggesting that the chemopreventive ursodeoxycholic acid, which has similar properties to DCA, should potentially be used instead [29–31].

3.4. Pharmacokinetics and the effect of deoxycholic acid

To test whether DCA improves the dynamics of absorption of PLGA, mice were fed PLGA nanoparticles loaded with rhodamine B with and without DCA emulsion, and the concentrations of rhodamine in serum and tissues were measured over time. PLGA particles delivered without deoxycholate resulted in a kinetic profile similar to that observed with free rhodamine (data not shown); peak rhodamine levels were measured 4 h after PLGA administration and levels of the dye were observed up to ~12 h.

Table 1
Pharmacokinetic characteristics after oral administration

	AUC (μg*h/mL)	C_{max} (ng/mL)	T_{max} (h)	F%
PLGA	5.37±.282	14.47	4	—
PLGA + deox	9.74±1.34	6.83	4	1.81

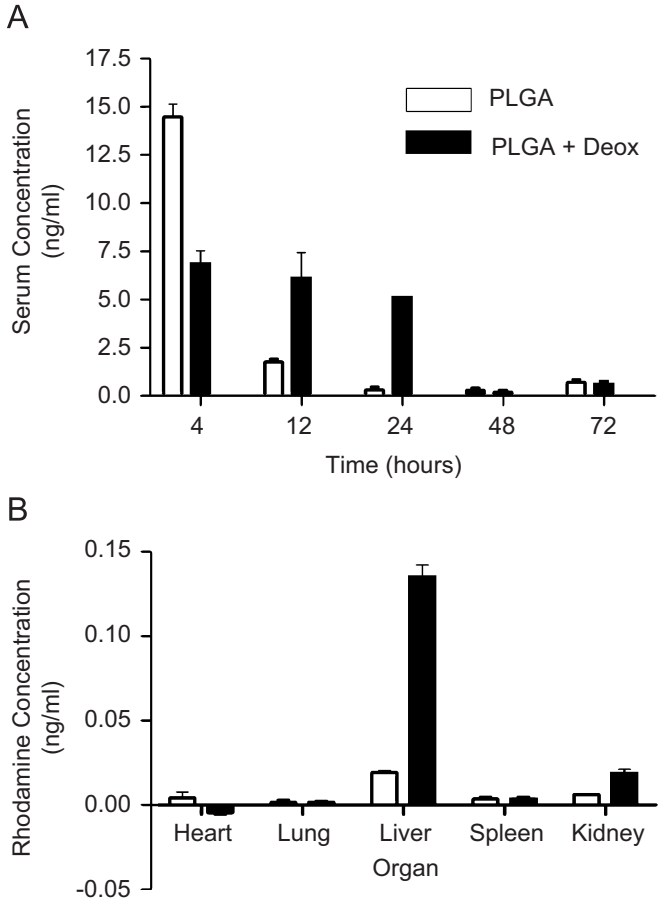

Fig. 4. C3 H mice of 10–12 weeks were fasted overnight and fed 300 μL of solution containing PLGA nanoparticles alone or in an emulsion of deoxycholic acid. At indicated time points, mice were sacrificed and serum and organs were analyzed by fluorescence after extraction as described under Materials and methods. (A) Serum concentrations of rhodamine b at indicated time points. $p<.01$ for all time points. (B) Organ distribution of rhodamine at 24 h demonstrating higher relative levels in the liver.

The PLGA particles in the DCA emulsion produced extended serum levels after oral administration, with peak levels occurring 4–24 h and the duration of delivery extending beyond 24 h. AUC was calculated using the log-linear trapezoidal rule due to the limit on data points [32]. Increased bioavailability was also observed since the particles with DCA yielded an AUC of 9.74±1.34 compared to 5.37±282 without DCA or a relative bioavailability of 1.81 (Table 1, $p<.01$). A significantly higher C_{max} was seen with the free particles, potentially exposing cells to toxic levels as compared to the intermediate levels maintained with the emulsion. Rhodamine concentrations in the liver were significantly higher with DCA potentially due to the hepatic portal reabsorption of deoxycholate (Fig. 4c). Free particles were found to be similar to free rhodamine most likely due to acid-catalyzed degradation of the PLGA in the stomach. DCA/PLGA emulsion showed increased bioavailability with maintenance of rhodamine concentrations at intermediate levels for 24–48 h. Rhodamine B has been shown to be cleared from circulation within hours [33], so the levels observed in the blood can be attributed to additional absorption from the intestine or release from particles. Rhodamine B has also been shown to have several metabolites with different fluorescent emissions, [34] demonstrating that the system delivers significant amounts of unmetabolized dye to the circulation. This increased absorption could be due to several factors. Aggregation of the particles in the stomach could result in delayed transit time, facilitating increased absorption [35]. Disruption of the tight junctions by DCA could allow transport of the nanoparticles across the intestinal lining between the cell [36]. Alternatively, the dye or particles may have been transported through the cells via the active and passive pathways for DCA [37]. In addition, cycling of the dye in the enterohepatic circulation could provide sustained release into the bloodstream [38].

Patient compliance is also a major concern when switching to out-patient oral administration, [39] but the extended levels with a single dose observed with this system can limit the number of required doses and thus increase compliance. In addition, enhanced delivery of rhodamine to the liver was observed, suggesting that the application of deoxycholic delivery systems may be useful for treating liver diseases or for obtaining first-pass metabolism of prodrugs.

4. Conclusion

In this study, we demonstrated the potential of bile salts such as DCA for improving oral delivery. In combination with existing biodegradable systems, DCA can offer protection in the stomach as well as improved bioavailability. The versatility of this system also allows DCA to be combined as an emulsion with many other existing systems for delivery of drugs and biologics to provide further protection and increased bioavailability.

Acknowledgments

We thank Javier Lapiera and Andrew Frederickson for assistance with preliminary experiments, Dr. W. Mark Saltzman, Dr. Seth Feurstein, Dr. Peter Fong, and Jason

Park for helpful comments. We would like to acknowledge Erika Wells and Dr. Michael Caplan for supplying Caco-2 cells, and Dr. Margaret Cartiera for helpful discussions regarding Caco-2 cell culture. This work was supported by a National Science Foundation Grant (NIRT CTS-0609326) to T.M. Fahmy.

References

[1] Hamman JH, Enslin GM, Kotze AF. Oral delivery of peptide drugs: barriers and developments: clinical immunotherapeutics, biopharmaceuticals and gene therapy. Biodrugs 2005;19(3):165–77.
[2] Malik DK, Baboota S, Ahuja A, Hasan S, Ali J. Recent advances in protein and peptide drug delivery systems. Curr Drug Deliv 2007; 4(2):141–51.
[3] Kondoh M, Yagi K. Progress in absorption enhancers based on tight junction. Exp Opin Drug Deliv 2007;4(3):275–86.
[4] Thanou M, Verhoef JC, Junginger HE. Oral drug absorption enhancement by chitosan and its derivatives. Adv Drug Deliv Rev 2001;52(2):117–26.
[5] Breedveld P, Beijnen JH, Schellens JH. Use of *P*-glycoprotein and BCRP inhibitors to improve oral bioavailability and CNS penetration of anticancer drugs. Trends Pharmacol Sci 2006;27(1):17–24.
[6] Blanchette J, Peppas NA. Oral chemotherapeutic delivery: design and cellular response. Ann Biomed Eng 2005;33(2):142–9.
[7] Aisner J. Overview of the changing paradigm in cancer treatment: oral chemotherapy. Am J Health Syst Pharm 2007;64(9 Suppl. 5):S4–7.
[8] Liu G, Franssen E, Fitch MI, Warner E. Patient preferences for oral versus intravenous palliative chemotherapy. J Clin Oncol 1997;15(1): 110–5.
[9] DeMario MD, Ratain MJ. Oral chemotherapy: rationale and future directions. J Clin Oncol 1998;16(7):2557–67.
[10] des Rieux A, Fievez V, Garinot M, Schneider YJ, Preat V. Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. J Control Release 2006;116(1): 1–27.
[11] Dong Y, Feng SS. Poly(D,L-lactide-*co*-glycolide)/montmorillonite nanoparticles for oral delivery of anticancer drugs. Biomaterials 2005;26(30):6068–76.
[12] Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Adv Drug Deliv Rev 2003;55(3): 329–47.
[13] Shive MS, Anderson JM. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Deliv Rev 1997;28(1):5–24.
[14] Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-*co*-glycolide) (PLGA) devices. Biomaterials 2000;21(23):2475–90.
[15] Damge C, Aprahamian M, Marchais H, Benoit JP, Pinget M. Intestinal absorption of PLAGA microspheres in the rat. J Anatom 1996;189(Part 3):491–501.
[16] Sahoo SK, Panyam J, Prabha S, Labhasetwar V. Residual polyvinyl alcohol associated with poly (D,L-lactide-*co*-glycolide) nanoparticles affects their physical properties and cellular uptake. J Control Release 2002;82(1):105–14.
[17] Chen H, Langer R. Oral particulate delivery: status and future trends. Adv Drug Deliv Rev 1998;34(2-3):339–50.
[18] Bahar RJ, Stolz A. Bile acid transport. Gastroenterol Clin North Am 1999;28(1):27–58.
[19] Sakai M, Imai T, Ohtake H, Azuma H, Otagiri M. Effects of absorption enhancers on the transport of model compounds in Caco-2 cell monolayers: assessment by confocal laser scanning microscopy. J Pharm Sci 1997;86(7):779–85.
[20] Lindmark T, Kimura Y, Artursson P. Absorption enhancement through intracellular regulation of tight junction permeability by medium chain fatty acids in Caco-2 cells. J Pharm Exp Ther 1998; 284(1):362–9.
[21] Hofmann AF, Mysels KJ. Bile acid solubility and precipitation in vitro and in vivo: the role of conjugation, pH, and Ca^{2+} ions. J Lipid Res 1992;33(5):617–26.
[22] Kramer W, Wess G, Schubert G, Bickel M, Girbig F, Gutjahr U, et al. Liver-specific drug targeting by coupling to bile acids. J Biol Chem 1992;267(26):18598–604.
[23] Kramer W, Wess G, Enhsen A, Falk E, Hoffmann A, Neckermann G, et al. Modified bile acids as carriers for peptides and drugs. J Control Release 1997;46(1–2):17–30.
[24] Lee YK, Kim SK, Lee DY, Lee S, Kim CY, Shin HC, et al. Efficacy of orally active chemical conjugate of low molecular weight heparin and deoxycholic acid in rats, mice and monkeys. J Control Release : Off J Control Release Soc 2006;111(3):290–8.
[25] Kramer W, Wess G, Neckermann G, Schubert G, Fink J, Girbig F, et al. Intestinal absorption of peptides by coupling to bile acids. J Biol Chem 1994;269(14):10621–7.
[26] Lee S, Kim K, Kumar TS, Lee J, Kim SK, Lee DY, et al. Synthesis and biological properties of insulin-deoxycholic acid chemical conjugates. Bioconjugate Chem 2005;16(3):615–20.
[27] Anthony T, Fong P, Goyal A, Saltzman WM, Moss RL, Breuer C. Development of a parathyroid hormone-controlled release system as a potential surgical treatment for hypoparathyroidism. J Pediatr Surg 2005;40(1):81–5.
[28] Lo YL, Huang JD. Effects of sodium deoxycholate and sodium caprate on the transport of epirubicin in human intestinal epithelial Caco-2 cell layers and everted gut sacs of rats. Biochem Pharm 2000; 59(6):665–72.
[29] Powell AA, LaRue JM, Batta AK, Martinez JD. Bile acid hydrophobicity is correlated with induction of apoptosis and/or growth arrest in HCT116 cells. Biochem J 2001;356(Part 2): 481–6.
[30] Martinez JD, Stratagoules ED, LaRue JM, Powell AA, Gause PR, Craven MT, et al. Different bile acids exhibit distinct biological effects: the tumor promoter deoxycholic acid induces apoptosis and the chemopreventive agent ursodeoxycholic acid inhibits cell proliferation. Nutr Cancer 1998;31(2):111–8.
[31] Pai R, Tarnawski AS, Tran T. Deoxycholic acid activates beta-catenin signaling pathway and increases colon cell cancer growth and invasiveness. Mol Biol Cell 2004;15(5):2156–63.
[32] Toutain PL, Bousquet-Melou A. Bioavailability and its assessment. J Vet Pharm Ther 2004;27(6):455–66.
[33] Elliott GS, Mason RW, Edwards IR. Studies on the pharmacokinetics and mutagenic potential of rhodamine B. J Toxicol Clin Toxicol 1990;28(1):45–59.
[34] Webb JM, Hansen WH. Studies of the metabolism of rhodamine B. Toxicol Appl Pharm 1961;3:86–95.
[35] Davis SS. Formulation strategies for absorption windows. Drug Discov Today 2005;10(4):249–57.
[36] Mauricio AC, Slawik M, Heitzmann D, von Hahn T, Warth R, Bleich M, et al. Deoxycholic acid (DOC) affects the transport properties of distal colon. Pflugers Arch :Eur J Physiol 2000;439(5): 532–40.
[37] Kullak-Ublick GA, Stieger B, Meier PJ. Enterohepatic bile salt transporters in normal physiology and liver disease. Gastroenterology 2004;126(1):322–42.
[38] Roberts MS, Magnusson BM, Burczynski FJ, Weiss M. Enterohepatic circulation: physiological, pharmacokinetic and clinical implications. Clin Pharm 2002;41(10):751–90.
[39] McLeod HL, Evans WE. Oral cancer chemotherapy: the promise and the pitfalls. Clin Cancer Res 1999;5(10):2669–71.