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Bioavailability and biodistribution of nanodelivered lutein

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ABSTRACT

The aim of the study was to evaluate the ability of poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NP) to enhance lutein bioavailability. The bioavailability of free lutein and PLGA-NP lutein in rats was assessed by determining plasma pharmacokinetics and deposition in selected tissues. Lutein uptake and secretion was also assessed in Caco-2 cells. Compared to free lutein, PLGA-NP increased the maximal plasma concentration (C_{max}) and area under the time-concentration curve in rats by 54.5- and 77.6-fold, respectively, while promoting tissue accumulation in the mesenteric fat and spleen. In comparison with micellized lutein, PLGA-NP lutein improved the C_{max} in rat plasma by 15.6-fold and in selected tissues by $\geqslant$3.8-fold. In contrast, PLGA-NP lutein had a lower uptake and secretion of lutein in Caco-2 cells by 10.0- and 50.5-fold, respectively, compared to micellized lutein. In conclusion, delivery of lutein with polymeric NP may be an approach to improve the bioavailability of lutein *in vivo*.

1. Introduction

Carotenoids in fruits and vegetables appear to contribute to health promotion through an array of putative bioactions, including antioxidation, anti-inflammation, and modulation of cell signal transduction pathways (Chatterjee, Roy, Janarthan, Das, & Chatterjee, 2012; Stahl & Sies, 2003). Lutein is a fat-soluble xanthophyll present primarily in dark green leafy vegetables. In observational studies, lutein intake and blood status are inversely associated with the risk of age-related macular degeneration, cataracts, cognitive dysfunction, and some forms of cancer (Granado, Olmedilla, & Blanco, 2003; Johnson, 2004). Like other carotenoids, the bioavailability of lutein is relatively low at ~2.0–9.4% (Lienau et al., 2003). Several processes are involved in lutein absorption in the small intestine: incorporation into micelles, transfer into intestinal cells, integration into chylomicrons, and secretion to the lymph system. In addition to its physiological determinants, lutein bioavailability is affected by the food matrix in which it is present: e.g., chopping and sautéing augment the release of lutein from chloroplasts and tissues of vegetables and co-consumption of lutein rich foods with lipids improves the efficiency of micellarization in the small intestine (Yeum & Russell, 2002). Since usual dietary intakes of lutein fall short of those levels associated with many of its benefits, food engineering approaches that increase its bioavailability and distribution to key target tissues could yield important health benefits.

Nanoentrapment can offer a novel approach to enhancing the bioavailability of poorly absorbed nutrients. In a human study, Vishwanathan, Wilson, and Nicolosi (2009) demonstrated that nanoemulsions of lutein, mimicking a synthetic micelle with a greater surface:volume ratio, improved bioavailability by 1.3-fold compared to unmodified lutein. Using an *in vitro* Caco-2 cell model, Yi, Lam, Yokoyama, Cheng, and Zhong (2014) reported that solid lipid nanoentrapment significantly improved cellular uptake of β-carotene. In addition to using lipid-like ingredients as a delivery vehicle, water-soluble polymeric nanoparticles (NP), have emerged as a promising material in the formulation of poorly absorbed nutrients and drugs to improve their bioavailability (He et al., 2013). For example, Arunkumar, Harish Prashanth, and Baskaran (2013) reported that lutein nanoentrapped in chitosan displayed higher bioavailability than unmodified lutein in mice. Poly(lactic-co-glycolic) acid (PLGA) has been used successfully as a biodegradable polymer and is often preferred for NP synthesis because of its well-established safety (Semete et al., 2010) and stability in intestinal environments (Murugeshu, Astete, Leonardi, Morgan, & Sabliov, 2011) as well as its effectiveness as a carrier of hydrophobic compounds (Khalil et al., 2013; Li et al., 2013; Tsai et al., 2011). However, its application with carotenoids has not been reported previously.

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Both *in vitro* and *in vivo* models are common and useful approaches to investigating nutrient bioavailability. *In vitro* models are utilized as simple, inexpensive, and reproducible tools to generate preliminary information on bioavailability in conjunction with factors like digestive stability, micellarization, intestinal transport, and metabolism. However, as several food- and host-related physiological, biochemical, and anatomical factors have an impact on bioavailability *in vivo* that cannot be fully characterized *in vitro*, both approaches should be employed (West & Castenmiller, 1998). Further, available information on the comparison of the bioavailability of polymeric NP using both *in vitro* and *in vivo systems* is limited. We hypothesized that PLGA-NP would increase lutein bioavailability. The objective of the project was to assess the impact of polymeric PLGA nanoentrapment on lutein bioavailability and pharmacokinetics in rats, as well as on the uptake of PLGA-NP entrapped lutein into intestinal cells compared to a physiologically relevant vehicle, the micelle. Further, we explored the effect of added micelles on the absorption of PLGA-NP lutein.

2. Material and methods

2.1. Chemicals and materials

β-Cryptoxanthin (BC, 97%), chlorophyll-a (CA, from *Anacystis nidulans* algae), 1-oleoyl-*rac*-glycerol [monoolein (MO), 99%], 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine (PC, 99%), 1-palmitoyl-*sn*-glycero-3-phosphocholine [lysolecithin, (LC), 99%], polyvinyl alcohol (PVA), sodium glycodeoxycholate (GDC, 97%), sodium taurodeoxycholate hydrate (TDC, 97%), taurocholic acid sodium salt hydrate (TC, 95%), sodium oleate (OA, 99%) and bovine serum albumin (97%) were purchased from Sigma–Aldrich (St. Louis, MO). Advanced Dulbecco's Modified Eagle Medium (DMEM), 200 mM L-glutamine, penicillin–streptomycin (10,000 U/ml) were purchased from Gibco, Life Technologies Inc. (Grand Island, NY). Hyclone phosphate buffer saline (PBS) without Ca^{2+} or Mg^{2+}, Pierce RIPA buffer, and Pierce bicinchoninic acid (BCA) protein assay were purchased from Thermo Fischer Scientific (Rockford, IL). Multi-well tissue culture treated plates, Transwell® permeable supports, cell culture treated flasks, and sterile filters (0.22 μM pores) were purchased from Corning Life Sciences (Tewksbury, MA). Caco-2 cells, fetal bovine serum and trypsin–EDTA (0.25%) were purchased from ATCC (Manassas, VA). All other solvents were of HPLC grade and purchased from Sigma–Aldrich (St. Louis, MO). Purified lutein (70–75%) was a generous gift from Kemin Industries Inc. (Des Moines, IA). Olive oil and refined wheat flour were obtained from Bertolli's and a local supermarket, respectively.

2.2. Preparation of polymeric nanoparticles containing lutein

The polymeric PLGA-NPs containing lutein were synthesized using an emulsion evaporation technique (Astete & Sabliov, 2006). Briefly, 400 mg copolymer PLGA 50:50 with a molecular weight (MW) of 30–60 kDa (Sigma–Aldrich, St. Louis, MO) was dissolved in 8 ml of ethyl acetate and 40 mg lutein was added after polymer dissolution, creating the organic phase. The organic phase was mixed with 60 ml of 2% PVA in water (aqueous phase), microfluidized (Microfluidics Inc., Westwood, MA) at 25,000 psi 4 times in an ice bath. The solvent was evaporated using a Rotovapor Buchi R-124 (Buchi Labortechnik AG, Switzerland) under N_2 gas. Subsequently, the NP suspension was dialyzed with water (replaced every 8 h) for 48 h using a Spectra/Por CE cellulose ester membrane with 100 kDa molecular weight cut off (Spectrum Rancho, Dominquez, CA). Finally, trehalose (Sigma–Aldrich, St. Louis, MO) was added (1:1 w/w theoretical ratio) before freezing the NP suspension, and the sample was freeze-dried for 40 h using a Freezone 2.5 Plus freeze-drier (Labconco, Kansas City, MO).

The morphology of PLGA-NP was examined with transmission electron microscopy (TEM) using a JEOL 100-CX system (JEOL USA Inc., Peabody, MA). Briefly, the sample preparation was performed as followed: 500 μl of NP suspension was mixed with a contrast agent (negative staining, one droplet of 2% uranyl acetate), and a droplet of the mixture was placed on a carbon-coated copper grid of 400 mesh. The excess of the sample was removed with filter paper and the liquid film over the grid was dried at room temperature for 15 min before placing the grid in the microscope.

The PLGA-NP size, polydispersity index (PI), and zeta potential were measured by dynamic light scattering (DLS) using the Malvern Zetasizer Nano ZS (Malvern Instruments Inc., Southborough, MA). For nanoparticle characterization, the resuspended PLGA-NP lutein samples were diluted to 0.5 mg/ml; NPs had a mean size of 124 ± 4 nm with a PI of 0.11 ± 0.09, and zeta potential of −5.3 ± 1.9 mV at pH of 6.5. All samples were measured in triplicate.

The entrapment efficiency of lutein was measured by UV-light spectrophotometry. Briefly, 6 mg PLGA-NP lutein powder was resuspended in 600 μl of water by sonication with further addition of 5.4 ml of acetonitrile. The mixture was vortexed for 4 h and centrifuged at 30,000× *g* for 15 min at 4 °C to obtain a white pellet. The supernatant was collected and the absorbance was measured at 450 nm with a UV–Vis spectrophotometer (Genesys 6, ThermoFisher Scientific, Waltham, MA) to obtain lutein concentration. The samples were measured in triplicate and their concentrations were calculated using a standard constructed with authenticated lutein standard. The PLGA-NP lutein showed an entrapment efficiency of 52 ± 3%.

The stability of PLGA-NP lutein was measured after being suspended in water and PBS over 24 h. Based on our preliminary observations (unpublished), the amount of lutein remaining in PLGA-NP remained the same ⩾24 h.

2.3. Preparation of synthetic micelles containing lutein

Micelles containing lutein were prepared according to Chitchumroonchokchai, Schwartz, and Failla (2004). For the cell culture study, MO, PC, and LC in chloroform (500, 200, and 200 μM, respectively), OA (1500 μM) in methanol, and lutein in ethanol were added to a conical glass tube and dried under N_2 gas at room temperature. Filtered, sterilized (0.22 μM pores) serum-free medium containing 800, 450, and 750 μM GDC, TDC, and TC, respectively, were added to reconstitute the dry residue, and the resulting mixture was sonicated for 30 min at room temperature under red light. For the animal pilot study, the components used to prepare mixed micelles were scaled up 3-fold, and serum-free medium was replaced with PBS.

2.4. Bioavailability of lutein in animal model

Male Fischer 344 rats, body weight (BW) of 238 ± 8.0 g, were obtained from Charles River Laboratories (Wilmington, MA) and housed individually in suspended wire cages at 22 °C, humidity control and with a 12 h light:dark cycle. After they were fed *ad libitum* with a lutein-free AIN-93G semi-purified diet (Teklad, Madison, WI) for 2 wk, 96 rats were randomly assigned to one of two lutein treatment groups, unmodified (free) and PLGA-NP, with 8 rats each tested at 6 different time points. The time points were chosen based on the results of two pilot studies where lutein in plasma was detected 2 h after gastric gavage (results not shown). An additional 8 rats were randomly assigned to serve as negative controls for the flour-oil slurry (30% olive oil + 70% flour slurry