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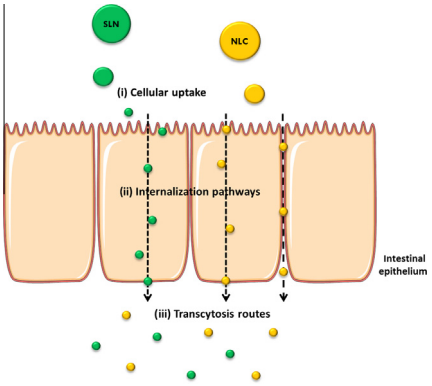
Cellular uptake and transcytosis of lipid-based nanoparticles across the intestinal barrier: Relevance for oral drug delivery

Ana Rute Neves [a], Joana Fontes Queiroz [a], Sofia A. Costa Lima [a], Francisco Figueiredo [b], Rui Fernandes [b], Salette Reis [a,*]

[a] UCIBIO, REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[b] HEMS/IBMC – Institute for Molecular and Cell Biology, Rua do Campo Alegre, 823, 4150-180 Porto, Portugal

GRAPHICAL ABSTRACT



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ABSTRACT

Oral administration is the preferred route for drug delivery and nanosystems represent a promising tool for protection and transport of hardly soluble, chemically unstable and poorly permeable drugs through the intestinal barrier.

In the present work, we have studied lipid nanoparticles cellular uptake, internalization pathways and transcytosis routes through Caco-2 cell monolayers.

Both lipid nanosystems presented similar size (~180 nm) and surface charge (−30 mV). Nanostructured lipid carriers showed a higher cellular uptake and permeability across the barrier, but solid lipid nanoparticles could enter cells faster than the former. The internalization of lipid nanoparticles occurs mainly through a clathrin-mediated endocytosis mechanism, although caveolae-mediated endocytosis is also involved in the uptake.

Both lipid nanoparticles were able to cross the intestinal barrier by a preferential transcellular route. This work contributed to a better knowledge of the developed nanosystems for the oral delivery of a wide spectrum of drugs.

Abbreviations: CLSM, confocal laser scanning microscopy; DAPI, 4′,6-diamidino-2-phenylindole; DLS, dynamic light scattering; DMEM, dulbecco's modified eagle medium; FITC, fluorescein isothiocyanate; MTT, thiazolyl blue tetrazolium bromide; NLCs, nanostructured lipid carriers; PBS, phosphate buffered saline; SLNs, solid lipid nanoparticles; TEER, trans-epithelial electrical resistance; TEM, transmission electron microscopy; WGA, wheat germ agglutinin.

* Corresponding author.
E-mail address: shreis@ff.up.pt (S. Reis).

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1. Introduction

Oral administration is the preferred route for drug delivery, although most drugs have low water solubility, chemical instability, poor intestinal absorption and extensive first-pass metabolism, thereby presenting a low oral bioavailability [1,2]. Nanocarriers represent a promising strategy to overcome these constrains in order to improve the oral delivery of several drugs and bioactive compounds [3].

In the present work, we were interested in developing and validating nanosystems for protection and transport of drugs by the oral route. The ultimate goal would be to protect drugs from degradation in the gastrointestinal tract, increase their oral solubility, enabling an increase in their intestinal permeability and escaping the primary metabolism. Consequently, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) were produced according to an optimized method described before [4]. Lipid nanoparticles are constituted by biocompatible and biodegradable lipids, being ideal for lipophilic and poorly soluble drugs, thereby enhancing their oral absorption [5,6]. The transport of drugs across the intestinal barrier is determinant for oral bioavailability. Hence, Caco-2 cells were used as an intestinal model since after growth they form confluent and differentiated monolayers with microvilli, tight junctions and transport systems [7,8]. The comprehensive study of the mechanisms of internalization and transport through the intestinal epithelium is of the utmost importance when developing drug delivery systems for oral administration. Therefore, the effective cellular uptake, endocytic internalization pathways and transcytosis routes of SLNs and NLCs through the intestinal barrier were accessed by confocal laser scanning microscopy (CLSM), flow cytometry, transmission electron microscopy (TEM), dynamic light scattering (DLS), and the use of endocytic inhibitors and transcellular/paracellular tracers [9,10].

2. Materials and methods

2.1. Materials

Lipid nanoparticles were prepared using cetyl palmitate provided by Gattefossé SAS (Nanterre, France), polysorbate 60 (tween 60) supplied by Merck (Darmstadt, Germany), and miglyol-812 from Acofarma (Madrid, Spain). Caco-2 cell line was purchased from the American Type Culture Collection (ATCC, Wesel, Germany), used between passage number 35 and 55 and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (South America origin), 1% fungizone (amphotericin B, 250 μg mL^{-1}), and 1% Pen Strep (penicillin, streptomycin), all obtained from Gibco (Paisley, UK). Trypsin–EDTA was also purchased from Gibco. Trypan blue, thiazolyl blue tetrazolium bromide (MTT), Phosphate Buffered Saline pH 7.4 (PBS), sucrose, chlorpromazine hydrochloride, filipin, cytochalasin D, ammonium chloride, propranolol, lucifer yellow and fluorescein isothiocyanate (FITC) were obtained from Sigma–Aldrich (St. Louis, USA), while wheat germ agglutinin (WGA) Alexa Fluor 633 conjugate and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Gibco. Vectashield® mounting medium was acquired from Vector Laboratories (Peterborough, UK). Paraformaldehyde was purchased from Merck, glutaraldehyde and Epon resin from Electron Microscopy sciences (Hatfield, USA).

2.2. Preparation and characterization of nanoparticles

SLNs and NLCs were produced according to an optimized method described before in our group of high shear homogenization followed by an ultrasound technique [4]. SLNs were composed by cetyl palmitate (10%) and tween 60 (2%) dispersed in water and NLCs were made by cetyl palmitate (7%), miglyol-812 (3%) and tween 60 (2%) also dispersed in water. Both lipid nanoparticles were labeled with 2 mg of FITC which was added to the lipid phase during the production procedure. A loading percentage of 90% was found for FITC which was satisfactorily high for its use as the fluorescent dye. Moreover, free FTIC was excluded by dialysis against PBS, overnight, in order to not interfere with the NPs quantification during the uptake and permeability assays. Particle size and zeta potential was evaluated by DLS in a Brookhaven Instrument (Holtsville, NY, USA) and bothformulations (SLNs and NLCs) presented a size around 180 nm, polydispersity index of 0.2 and high negative zeta potential of −30 mV [4]. The size below 200 nm, the negative charge and their lipophilic nature suggest these lipid nanoparticles are suitable for oral administration and permeation across the intestinal barrier [11,12].

2.3. Caco-2 cell culture

Caco-2 cells were maintained at 37 °C and 5% CO_2 in DMEM supplemented with 10% fetal bovine serum, 1% fungizone and 1% Pen Strep and were subcultured by treatment with trypsin–EDTA at 80–90% confluency. Cell viability in the presence of FITC-labeled lipid nanoparticles, inhibitors and tracers was assessed by MTT assay, as previously described [13].

2.4. Cellular uptake of nanoparticles

2.4.1. Flow cytometry analysis

Cells were seeded in 24-well plates in a density of 2×10^5 cells per well and incubated 20 h at 37 °C and 5% CO_2. In the next day, cells were incubated with FITC-labeled SLNs or NLCs (2.5 μM) for 0, 0.5, 1, 2, 3 and 4 h. Then, they were washed twice with PBS, detached with 0.25% trypsin–EDTA and analyzed by flow cytometry on a BD Accuri C6 (BD Biosciences, Erembodegem, Belgium), upon 1 min incubation with trypan blue to quench non-internalized nanoparticles. For each sample a minimum of 10,000 events were recorded. The internalization patterns can reflect the delivery efficiency of the studied nanoparticles. Therefore, the kinetics of cellular uptake was analyzed and fitted to the Michaelis–Menten equation:

$$Y = \frac{Y_{max} \cdot t}{k + t}$$

where Y is the uptake level in real time, t is the incubation time, Y_{max} is the maximal uptake level representing the saturated plateau of the kinetic curves, and k is the uptake rate constant, representing the time when the uptake is half of Y_{max}. Hence, a smaller k reflects a quicker cellular uptake.

2.4.2. Confocal laser scanning microscopy

For CLSM imaging, cells were seeded on coverslips inside 24-well plates in a density of 2×10^5 cells per well and incubated 20 h at 37 °C and 5% CO_2. Cells were then incubated with FITC-labeled SLNs or NLCs (2.5 μM) for 4 h. After incubation, cells were washed twice with PBS and fixed with 2% paraformaldehyde for 20 min, at room temperature. After rinsing twice with PBS, coverslips were stained with 5 μg mL^{-1} WGA-Alexa 633 for 10 min at 37 °C. Then, cells were rinsed twice with PBS and nuclei stained with 300 nM DAPI for 5 min, at room temperature. Coverslips were inverted over microscope slides using Vectashield® as mounting medium for fluorescence photobleaching prevention. CLSM images were acquired on a Leica SP5 CLSM (Leica Microsystems, Wetzlar, Germany) and processed using a Leica Application Suite – LAS AF v4.3 software.