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Polyarginine Nanocapsules as a Potential Oral Peptide Delivery Carrier

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ABSTRACT

We have previously reported the development of novel nanocapsules made of polyarginine (PArg) specifically designed for the delivery of small anticancer drugs into cells. Our goal, in this work, has been to investigate the potential of these nanocarriers for oral delivery of peptide anticancer drugs. To reach this objective, we chose the antitumoral peptide, elisidepsin, and evaluated the characteristics of the PArg nanocapsules in terms of drug loading capacity, stability in simulated intestinal fluids, and ability to interact with the intestinal epithelium both *in vitro* (Caco-2 model cell line) and *in vivo*. Our results suggest that elisidepsin can be effectively loaded into the nanocapsules by adjusting the formulation parameters, using a solvent displacement technique. The resulting nanocapsules were stable upon incubation in simulated intestinal fluids and had the ability to reduce, in a transient manner, the transepithelial electrical resistance of the Caco-2 cell monolayer. Confocal images also revealed that PArg nanocapsules were internalized by the monolayer without evident signs of cytotoxicity. Finally, the *in vivo* fluorescent imaging study illustrates the retention of the nanocapsules in the gastrointestinal tract upon oral administration. Overall, the results underline the potential interest of PArg nanocapsules as carriers for the oral administration of peptide drugs.

Introduction

Cancer is a leading cause of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million cancer-related deaths. In the next 2 decades, the number of new cases per year is expected to rise by about 70%.[1] Not surprisingly, over the last years, the discovery and delivery of new cancer drugs has been a research priority. As a result, many new drugs have become available. Several of these new drugs are complex macromolecules, including peptides, proteins, and monoclonal antibodies, which are gaining increasing relevance in oncology.[2] Likewise, progress in the drug delivery field has led to a reduction in the toxicity and in the multidrug resistance problems associated with several anticancer drugs.[3] Despite these advances, the optimum delivery approach, especially for complex macromolecules is still unclear. In fact, these macromolecules are known to be highly unstable in biological media and have great difficulties for overcoming cell and epithelial barriers. As a consequence, these molecules cannot be administered orally and have significant difficulties to reach their targets.[3,4]

Over the last decades, a substantial amount of research has focused on the development of drug delivery strategies that would make possible the oral administration of peptides.[5,6] To this end, nanotechnology-based delivery strategies have aimed at protecting the drug from degradation, promoting its interaction with the intestinal mucosa, prolonging its intestinal residence time, and reversibly increasing the permeability of the mucosal epithelium in order to enhance drug absorption.[5-7]

Within this context, our group has shown that polymer nanocapsules, vesicular systems in which an oily core is surrounded by a polymeric shell, are useful carriers that increase the oral bioavailability of drugs. In particular, we have shown that chitosan nanocapsules are able to adhere to a mucus-producing model epithelium (Caco-2-HT29 co-culture) and facilitate the transport of

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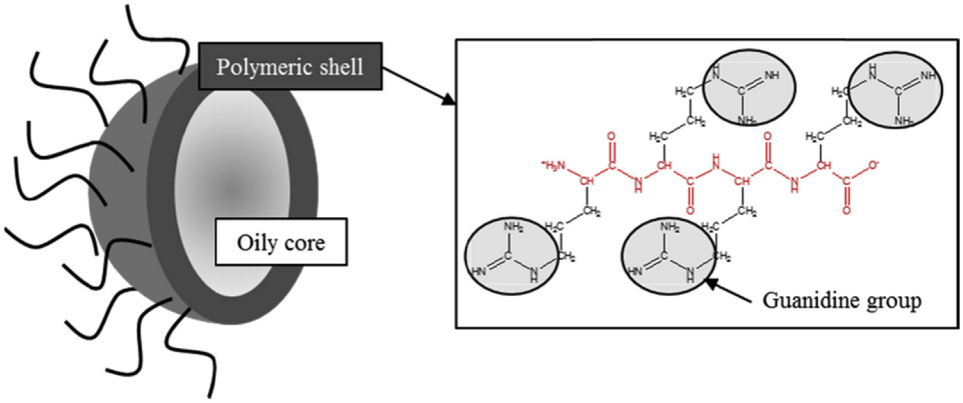

Figure 1. Illustration of the structure of PArg nanocapsules.

an associated peptide, that is, salmon calcitonin, into the cells.[8] More recently, our efforts have also been focused on the identification of positively charged polymers with potential interest for transmucosal drug delivery. Among them, we have identified poly-L-arginine (PArg), a biodegradable polyaminoacid belonging to the cell-penetrating peptide (CPP) family, as a promising biomaterial for the design of such delivery systems.[9]

The cell-penetrating properties of arginine-rich polyaminoacids have been attributed to the presence of guanidine functional groups, which are known to specifically interact with cell surface domains before its internalization (Fig. 1).[10-14] In fact, the use of arginine-based CPPs for the intracellular delivery of peptide anticancer drugs has already been described.[13] In addition, it has been indicated that PArg interacts with the tight junction proteins, occludin and ZO-1, thereby favoring the paracellular transport of hydrophilic drugs.[15,16] These properties are considered the basis for the reported ability of PArg to improve the transport of peptides across the Caco-2 intestinal epithelium model,[16] as well as across the nasal and ocular mucosae.[17,18]

Recently, we developed a new type of nanocapsules with a PArg shell and showed their ability to deliver the anticancer drug docetaxel intracellularly.[19] As a follow-up, the main goal of the present work was to evaluate their potential for the encapsulation of the anticancer peptide drug elisidepsin, their stability, and their ability to cross the epithelial barriers. Elisidepsin (PM02734, Irvalec®; PharmaMar S.A., Madrid, Spain) is a synthetic marine-derived cyclic peptide of the Kahalalide F family, which showed a potent and broad antitumor activity in colon and prostate cancer cell lines. The drug is currently in phase II of a clinical trial.[20,21]

Materials and Methods

Materials

PArg (molecular weight [Mr], 5000-15,000) and Poloxamer 188 (Pluronic® F68) were purchased from Sigma-Aldrich (Madrid, Spain). Miglyol® 812 was purchased from Sasol (Johannesburg, South Africa). Epikuron® 170 was donated by Cargill (Madrid, Spain). DiD (1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate) and fluorescein-DHPE (N-(fluorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt) were supplied by Invitrogen (Waltham, MA). All solvents employed were of analytical grade and supplied by Merck. Elisidepsin (Mr, 1591.90) was provided by PharmaMar S.A. The peptide is very slightly soluble in water (0.78 mg/mL) whereas sparingly soluble in ethanol (27.1 mg/mL).

Preparation of PArg Nanocapsules

PArg nanocapsules were obtained by a modification of the solvent displacement technique.[19] The general method to obtain PArg nanocapsules is as follows: 0.125 mL of Miglyol® 812 and 30 mg of Epikuron® 170 were dissolved in 0.5 mL of ethanol and 9 mL of acetone. This organic phase was poured over an aqueous phase (20 mL) containing the surfactant poloxamer 188 (0.25% w/v) and PArg (0.05% w/v). Finally, the organic solvents were removed under vacuum to a final volume of 10 mL. Nanoemulsions were also prepared and used as controls to test the need for the polymeric coating. They were obtained by the same technique described above, with the exception that no PArg was added to the external water phase.

Physicochemical Characterization of PArg Nanocapsules

Particle size and polydispersity index were determined by photon correlation spectroscopy (PCS) after dilution with bidistilled water. Analyses were carried out at 25°C with an angle detection of 173°. The zeta potential values were calculated from the mean electrophoretic mobility values, as determined by laser Doppler anemometry (LDA). For LDA measurements, samples were diluted with KCl 1 mM and placed in an electrophoretic cell. PCS and LDA analyses were performed in triplicate using a NanoZS® (Malvern Instruments, Malvern, UK). The morphological analysis of the nanocapsules was performed by transmission electron microscopy (TEM; CM12 Philips, Amsterdam, The Netherlands). For TEM imaging, samples were stained with 2% w/v phosphotungstic acid solution and placed on a copper grid with Formvar® films for analysis.

Elisidepsin Encapsulation Into PArg Nanocapsules

Elisidepsin-loaded nanocapsules were prepared by dissolving 2.5 mg or 10 mg of the active compound in the ethanol solution before adding the other components of the organic phase. Then, the method described above was followed. PArg nanocapsules with the highest drug concentration (elisidepsin concentration 1.6 mg/mL) were obtained in a similar manner, but solvents were evaporated under vacuum to a final volume of 6.5 mL.

Elisidepsin encapsulation efficiency of PArg nanocapsules was determined indirectly by calculating the difference between the total amount of the active compound in the formulation and the free drug measured in the aqueous phase. The total amount of drug was estimated by dissolving an aliquot of PArg nanocapsule