NOVEL CHITOSAN POLYMERIC MICELLES AS A DELIVERY VEHICLE OF HYDROPHOBIC ANTICANCER DRUGS

Andréia Almeida1,2,3, Marco Araújo1,2, Ramon Novoa-Carballal4,5, Fernanda Andrade1,2,6, Marlene Lúcio7,8, Simó Schwartz Jr.6,9, Bruno Sarmento1,2,10

13S – Institute for Research and Innovation in Health, University of Porto, Porto, Portugal
2INEB – Institute of Biomedical Engineering, University of Porto, Porto, Portugal
3ICBAS – Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal
43B’s Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães, Portugal
5ICVS/3B’s PT Government Associate Laboratory, Braga/Guimarães, Portugal
6Molecular Biology and Biochemistry Research Centre for Nanomedicine (CIBBIM-Nanomedicine), Vall d’Hebron Institut de Recerca (VHIR), Universitat Autònoma de Barcelona (UAB), Barcelona, Spain
7CF-UM-UP – Centre of Physics of University of Minho and Porto, Braga, Portugal
8CBMA – Centre of Molecular and Environmental Biology of University of Minho, Braga, Portugal
9Networking Research Centre for Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Instituto de Salud Carlos III, Madrid, Spain
10CESPU, IINFACTS – Institute for Research and Advanced Training in Health Sciences and Technologies, Gandra, Portugal

Background: The synthesis of new chitosan derivatives with amphiphilic properties allows the production of polymeric micelles, which have the ability to encapsulate hydrophobic drugs as camptothecin (CPT). Once encapsulated, the drug is protected from the body fluids, its aqueous solubility is increased as well as its intestinal permeability and therapeutic activity.

Methods: Chitosan was chemically modified with O-methyl-O′-succinylpolyethylene glycol and with oleic acid by a carbodiimide reaction to produce micelles by self-assembly. Characterization of the copolymer included 1H NMR, FTIR, GPC, DSC/TGA and XRD. Size, zeta potential and morphology were determined by DLS and TEM, respectively. The CPT association efficiency was determined by HPLC as well as its in vitro release and lactone ring protection from hydrolysis, both performed in simulated gastrointestinal fluids. Cytotoxic studies were performed against Caco-2 and HT29-MTX intestinal cell lines. CPT intestinal permeability was tested in three different in vitro cell models and biodistribution and pharmacokinetic studies were performed by gavage in male Balb/c nude mice colorectal cancer induced.

Results: The success of the synthesis and the purity of the new copolymer were demonstrated by 1H NMR, FTIR and GPC and, after the grafting, the copolymer showed an increase on its thermal stability and crystallinity. The CMC revealed a good stability of the system after dilution and DLS revealed an average size of 140 nm, a positive superficial charge and CPT association efficiency of 78%. TEM analysis demonstrated a round and smooth shape for both empty and CPT-loaded micelles. The CPT in vitro intestinal release showed a low release in gastric media and a controlled release in intestinal fluids, suggesting a pH-dependent behaviour. Also, these micelles were able to protect CPT from hydrolysis up to 75% of its initial lactone form, exhibiting a good system stability. Regarding the safety profile, copolymer did not present a cytotoxic effect against colorectal cancer cell lines in concentrations equal or bellow 10 mg/mL. More importantly, CPT improved significantly its in vitro intestinal permeability, as compared with free CPT. Moreover, CPT-loaded micelles showed 5% tumor accumulation, a distribution volume of 0.3 L, an elimination half-life of 7.1 h and a clearance rate of 0.02 L/h.

Conclusions: CPT-loaded chitosan micelles proved to be a potential vehicle of hydrophobic anticancer drugs, as CPT.