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Design, synthesis and evaluation of biotin decorated inulin-based polymeric micelles as long-circulating nanocarriers for targeted drug delivery

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Abstract

Here, long-circulating behaviors of Inulin-based nanomicelles are demonstrated for the first time *in vivo*. We show the synthesis and evaluation of biotin (BIO)-decorated polymeric INVITE micelles constituted of substances of natural origin, Inulin (INU) and Vitamin E (VITE), as long-circulating carriers for receptor-mediated targeted drug delivery. The resulting INVITE or INVITE-BIO micelles, nanometrically sized, did not reveal any cytotoxicity after 24 h of incubation with Caco-2 cells. Moreover, *in vitro* studies on Caco-2 cells monolayers indicated that the transport of INVITE-BIO micelles was faster than surface unmodified INVITE micelles. *In vivo* optical imaging studies evidenced that, upon intravenous administration, INVITE-BIO micelles were quantitatively present in the body up to 48 h. Instead, after oral administration, the micelles were not found in the systemic circulation but eliminated with the normal intestinal content. In conclusion, INVITE-BIO micelles may enhance drug accumulation in tumor-cells over-expressing the receptor for biotin through receptor mediated endocytosis.

Key words: Inulin; Vitamin E; Biotin; Cancer; Optical imaging; Micelle

Polymeric micelles are interesting nanostructured platforms characterized by a core-shell structure and obtained by self-assembly of amphiphilic polymers in aqueous solutions. The core is formed by the hydrophobic portion of the polymer, while the hydrophilic part constitutes the shell. Due to their small size (10–100 nm), low toxicity, capacity to solubilize lipophilic drugs in the core, and high drug loading these nanocarriers are effective drug delivery systems.[1] Among the hydrophobic polymers, poly(propylene glycol (PPG), poly(D,L-lactide) polycaprolactone are often employed, while polyethylene glycol (PEG) is frequently used as hydrophilic moiety, and therefore the majority of polymeric micelles described in literature are based on biodegradable and synthetic copolymers.[2]

In the context of our research project aimed at evaluating biodegradable amphiphilic polymers of natural origin and from renewable resources, according to the sentence "learning from Nature, discovering through Nature", we designed nanomicelle systems based on Inulin (INU, a fructan-type oligosaccharide) and Vitamin E (VITE), denoted as INVITE. The INVITE nanomicelles, previously demonstrated effective biomedical and pharmaceutical properties, such as high biocompatibility, suitability for intravenous administration, rapid uptake by the cellular membrane, solubilization and delivery of highly

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Inulin (PubChem CID: 16219508); Vitamin E (PubChem CID: 14985); Vitamin E Succinate (PubChem CID: 20353); Biotin (PubChem CID: 171548); Biotin-NHS (PubChem CID: 6710714); Cy5.5 NHS ester (PubChem CID: 52918950).

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hydrophobic drugs, and favorable pharmacokinetic *in vivo* after intravenous administration.[3–6]

We selected INU as the main component because it is a natural polysaccharide extracted from many plants, hydrophilic, cheap, FDA-approved and routinely used by intravenous injection, *i.e.*, it has an exceptionally-high safety profile. Furthermore, it has been used for pharmaceutical applications in different forms such as hydrogels,[7–11] micelles,[12–14] nanoparticles[15] and iron-supplementing systems.[16] When INU is intravenously administered, it does not bind to plasmatic proteins[17,18] and it is freely filtered by the kidney where it is neither secreted nor reabsorbed and it is not metabolized by the kidney.[19] INU shows a mean molecular radius of 1.5 nm and a molecular weight of approximately 5000 Da.[20] Moreover, notable applications of inulin concern stabilization of proteins, modified drug delivery and targeting and adjuvanting vaccine formulations.[20]

VITE is a vitamin normally found in many foods, especially in olive oil and other fat-derived nutrients.[21] It is one of the most powerful anti-oxidant that nature uses in its cycles, and in the human body is involved in several processes including cancer and oxidative stress.[22,23] VITE is hydrophobic and its use for pharmaceutical applications is widely documented.[24,25]

Concerning the targeting properties of polymeric micelles, their small size allows passive targeting to be achieved by extravasation through the leaky tumor vessels *via* enhanced permeability and retention effect (EPR) effect. However, to increase the intracellular uptake of these drug delivery systems to the target site, the presence of an active targeting moiety on the surface of these nanocarriers would enable and exploit the receptor-mediated active targeting strategy.

The aim of the present work was to evaluate BIO surface modified INVITE nanomicelles as carriers for targeted drug delivery. Among the cellular surface targets potentially useful for receptor mediated targeted drug delivery, BIO, a natural nutrient, is widely employed because of its overexpression in several tumors and for its strong interaction with avidin.[26] Indeed, several aggressive cancer lines such as leukemia (L1210FR), ovarian (OV 2008, ID8), colon (Colo-26), mastocytoma (P815), lung (M109), renal (RENCA, RD0995), and breast (4 T1, JC, MMT06056) cancer cell lines[27,28] overexpress receptors for BIO.[29] It is important to note that BIO cannot be synthesized by mammalian cells, thus, BIO must be obtained from exogenous sources *via* intestinal absorption.[30]

The so called sodium-dependent multivitamin transporter (SMVT) is an important membrane transporter for BIO which is found along the small and large intestines. Several essential nutrients such as BIO, are taken-up by this transporter which have been shown as the responsible for the antitumor activity of BIO functionalized camptothecin on multi-drug resistant human ovarian cancer cell line A2780.[31,32] Interestingly, its SMVT overexpression was found to exceed that of its folate receptor.[33] This is mostly due to the fact that BIO belongs to a particular category of exogenous micronutrients which are required for cellular functions and, particularly, for cell growth.[34] Consequently, the BIO demand in tumors is higher than normal tissues.[35]

In 2006 Park Keun-Hong and coworkers were among the pioneers in preparing nanogels from pullulan and BIO (PU/Bio) as a valuable method to deliver anticancer drugs using specific receptor-mediated targeting between BIO and tumor cells.[36] In the last years, more and more evidence points on the effectiveness in using BIO as a drug targeting molecule.[27,37,38] Moreover, BIO binds to plasmatic protein only in very small amount.[39] In this way, we would not substantially modify the plasmatic behaviors of INU, when modifying the hydrodynamic properties of the polymer.

These premises led us to hypothesize that a drug delivery system composed by INU-based micelles would retain the main behaviors of the parent polymer, since the external shell of the micelle would be chemically composed by the polysaccharide and, eventually, by the non-plasma-protein binder BIO. What would be modified should essentially be the spatial conformation of the polymer especially when BIO is found on the surface of the micelle. Since glomerular filtration is strongly influenced by size and shape of the substances we thought that such a system, based on INU, would "acquire" long-circulation behaviors to be exploited for drug delivery purposes.

Thus, herein we rationally designed and investigated an amphiphilic inulin-vitamin E (INVITE) bioconjugate, surface modified with BIO (INVITE-BIO), as specific carriers with long-circulating and targeting behaviors. In particular, the synthesis and characterization of INVITE-BIO nanomicelles are described in this paper. Moreover, the fate of the targeted micelles was monitored *in vitro* on Caco-2 cells as well as *in vivo* by optical imaging biodistribution studies.

Methods

Materials and cell lines

All reagents were of analytical grade, unless otherwise stated. Anhydrous *N*,*N*-dimethyl formamide 99.8% (DMF), triethylamine ≥99% (TEA), N,N′-dicyclohexyl carbodiimide 99% (DCC), pyrene, d-α-tocopherol succinate semisynthetic 1210 IU/g, inulin from dahlia tubers (INU, approx. 5500 Da), fluorescein 5-isothiocyanate (FITC) and *N*-Hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (Milan, Italy). *N*-hydroxysulfosuccinimide sodium salt ≥98% (NHSS), biotin (BIO) and DMSO-d6 99.96 atom % D were purchased from TCI Europe, Zwijndrecht, Belgium. Cyanine5.5 NHS ester (Cy5.5) was from Lumiprobe, Hallandale Beach, USA. Caco-2 cells (Caco-2 Passage 43) were obtained from the European Collection of Authenticated Cell Cultures Cell Bank (ECACC, Salisbury, UK). All reagents used for cell cultures were purchased from Eurcolone (Milan, Italy). Fetal bovine serum was obtained from Hyclone (GE Healthcare, Milan, Italy).

Six to eight week-old female BALB/c mice derived from and were housed in our specific pathogen free (SPF) animal facility. Procedures involving animals and their care were in conformity with institutional guidelines that comply with national and international laws and policies (D.L. 26/2014 and subsequent implementing circulars), and the experimental protocol (Authorization n. 1050/2015-PR) was approved by the Italian Ministry of Health.