Review Article

Inulin for Cancer Therapy: Present and Perspectives

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ABSTRACT

Inulin is an extremely adaptable polysaccharides consisting of glucopyranose end-capped (β-1,2) fructose repeating units and, as it is, can be classified as an inherently multifunctional polymeric scaffold. It may be further functionalized employing mild conditions to give rise desired biological and physicochemical properties exploitable for targeted anticancer applications (e.g., active targeting toward specific cytotypes, self-assembling behavior, selective cytoxicity and hyperthermia features). In this review, the main chemical features and the inulin derivatives applications in the field of targeted anticancer therapy is reported and discussed

Keywords: Inulin, polysaccharide, glucose, targeted cancer therapy, doxorubicin, folate

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I. INTRODUCTION

The last forty years have witnessed the progress in polymeric materials in the field of healthcare. Special attention has been directed to the development of new nanomedicines for targeted cancer therapy, conceived to be accumulated in the tumor site in order to release their drug payload nearby the site of action thus increasing the bioavailability and, as consequence, the effectiveness of anticancer drugs [1]. Among these, the synthesis of complex functional polymers, namely polymers endowed with reactive groups whose features (i.e., kind of function/s, number, macromolecular architecture, etc.) have been arranged, has attracted great interest for the development of anticancer nanomedicine with selective toxicity toward tumor mass instead of healthy tissues.

The aim of this article is to highlight the huge potential of a biopolymer named inulin as multifunctional vehicle for anticancer drug and to provide an updated state-of-the-art on chemistry and the rational design of innovative drug delivery systems based on this peculiar polysaccharide.

SYNTHETIC FEATURES

Inulin is a carbohydrate highly available in the root of many Arsteracee such as chicory, and its hydrolysis by means of inulinase leads to a mixture of fructose and glucose [2,3]. Recent reliable estimations of the molecular weight of inulin have yielded values which vary from 5200-200, suggesting that its average degree of polymerization (DP_n) varies from 28-3 as function of the natural source.

From a chemical standpoint it consists of glucopyranose end-capped (β-1,2) fructose repeating units carrying a high amount of hydroxyl functional groups regularly arranged in the polymer Chain (**Figure 1**). Apart from the end-chain, inulin exhibits in principle three reactive hydroxyl groups per repeating unit, two secondary and one primary. Notwithstanding the interest in obtaining heterodifunctional polymers in several biomedical fields [4], nobody has yet performed functionalization of this polysaccharide exploiting its end-chain groups so far. On the opposite, many research groups have attempted to realize branched tailor-made inulin derivatives as polymer therapeutics using its side functions [5-13]. The structures of most inulin

derivative described so far in which different side chains were grafted are reported in (**Table 1**).

It seems reasonable to assume that only one alcoholic group per repeating unit may react and, among these, the primary hydroxyl functions should be more nucleophilic than the other ones. Hence, inulin derivatives reported in the literature are essentially random copolymers obtained in most cases activating the primary hydroxyl groups using Bis(4-nitrophenyl) carbonate (BPNPC). Noteworthy, using this approach all conceivable nucleophiles (e.g., mono- and bis- amines, thiols, etc.) are eligible as side chains to confer to the main backbone desired properties. Another strategy encompass the activation of aliphatic carboxylic acid employing either a mixture of N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-(dimethylamino) propyl)-carbodiimide hydrochloride.

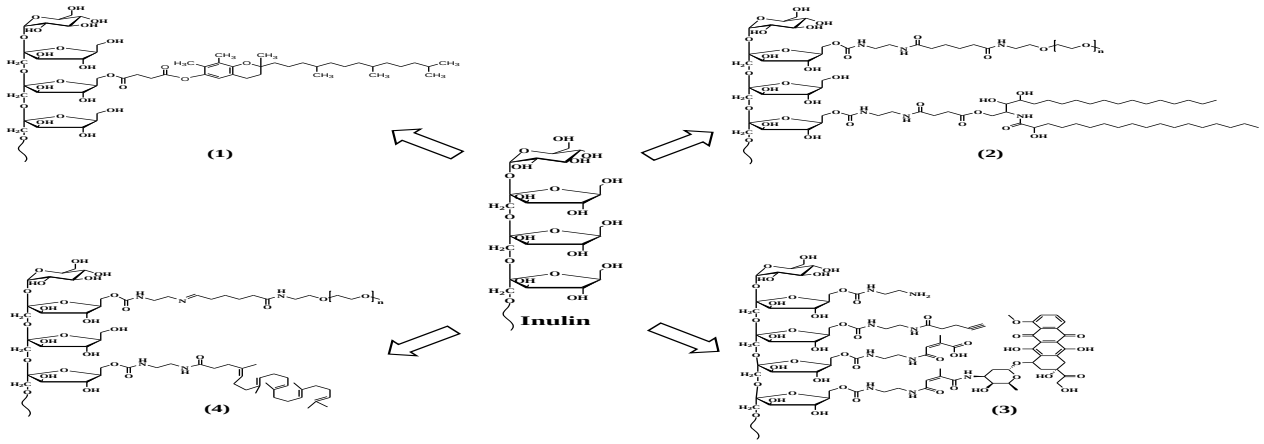
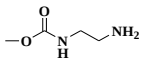
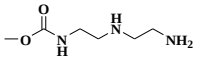
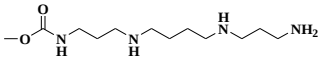
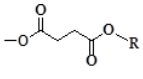
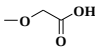
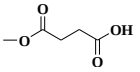
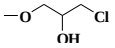
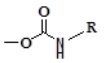


Figure 1: Structure of inulin and its derivatives recently employed in targeted cancer applications.

Table 1: OH derivatization of inulin backbone with purpose tailored groups.

No.	Functionalization	Coupling Agent	References
1		BPNPC[a]	5
2		BPNPC[a]	6
3		BPNPC[a]	7
4		DCC[b]/NHSS[c]	8
5		-	9
6		EDC[d]/NHS[e]	10
7		-	11
8		-	12

9		-	12
10		-	13

Bis(4-nitrophenyl) carbonate; [b]N,N'-dicyclohexylcarbodiimide; [c]N-hydroxysulfosuccinimide; [d]1- ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride; [e]N-hydroxysuccinimide.

PHYSICOCHEMICAL FEATURES

The physicochemical properties of inulin strictly depend on its molecular weight and distribution. The molecular weight-dependent behavior of inulin was clearly showed by several studies in which the inulin conformation in water was related to its DP_n. It seems that inulin with a DP_n smaller than 9 arranges into helical conformations [14], as for inulins sized up to DP_n 9 more rigid architectures are predominant due to steric hindrance [15], thus reflecting in a lower water solubility. In the light of this, owing to the bigger dimension and the assembling abilities, it was realized that high-molecular-weight-based cancer delivery systems were preferred to achieve an effective accumulation into the tumor site by means of the enhanced permeability retention (EPR) effect [16].

The amount of reducing sugar, being formed as consequence of hydrolysis, is a measure of the chemical stability of inulin. Influence of many parameters of the chemical stability was widely reported in the literature [17]. On the whole, inulin results stable under neutral and slightly basic conditions, where the free sugar in solution revealed only 0.1% hydrolysis, whereas it is hydrolyzed under acidic conditions especially during heating [18]. The acid-dependent hydrolysis of inulin may play a crucial rule in its degradation inside lysosomes upon cell internalization and, hence, should be better studied in future works to obtain smart lysosome-triggered delivery systems.

BIOLOGICAL FEATURES

Inulin is a FDA approved polymer for intravenous injection and for the formulation of functional foods. Among the relevant biological effects of inulin, the most interesting is its prebiotic characteristics. In particular, colonic fermentation of inulin leads to low molecular weight carboxylic acids which decrease the cecal pH with benefic effects on the tissue perfusion accompanied with higher colon motility [19]. Besides, inulin represses the growth of pathogens (bacteroids, fusobacteria and clostridia) [20] for promoting the predominance of bifidobacteria in the large intestine [21]. Along this line, several authors have described a rule of inulin in inhibiting colon-rectal pre-neoplastic lesions thus suggesting that inulin may suppress colon tumorigenesis [19,22].

For these reasons it represents a good scaffold, in term of biocompatibility and synergistic anticancer effect, to prudently design novel anticancer delivery systems endowed with an exceptional combination of prebiotic and pharmacological activities.

SELF-ASSEMBLED NANOSYSTEMS

Micelles

Highly hydrophobized inulin derivatives, obtained from the partial substitution of the hydroxyl groups of inulin with functional hydrophobic chains, were considered for anticancer delivery applications. Inulin-D-α-Tocopherol succinate (INVITE) amphiphilic copolymers with different degree of derivatization were synthesized to show theirs aptitude to form nano-sized micelles at low concentration (from 2.4×10^{-3} to 22.3×10^{-3} mM as function of the amount of INVITE chains per macromolecule) able to efficiently delivery curcumin inside tumor cells (**Figure 1**, (1)) [23]. They are biocompatible, non-hemolytic and can load suitable amount of drug (3% w/w) to be delivered under acidic and neutral conditions. The pharmacokinetic profile of INVITE-based micelles, obtained on Balb/C mice e via intravenous tail vein injection at the dose of 50 μg per animal, plainly point out that they provide a sustained release of curcumin assuring an excellent bioavailability up to 6 h from the administration. Besides, uptake studied carried out on human embryonic kidney 293 cells clearly showed that they can also

rapidly enter cells thus releasing their payload inside cells.

In a second investigation [5], an inulin conjugate containing ceramide and PEG_{2000} was prepared and tested. It was suggested that PEGylation would prevent recognition by the reticuloendothelial system and prolong bloodstream circulation, as ceramide in addition to its hydrophobic effect play a crucial role in executing antiproliferative signaling inside cells (**Figures 1** and **2**). More interesting, these amphiphilic copolymer assemblies into micelles once dissolved in a solution of doxorubicin in DMSO and placed in a dialysis tube against water, with a loading capability of 18% on a weight basis. Cytotoxicity evaluation on both cancer (HCT116) and normal (16 HBE) cell lines evidenced a slight preferential cytotoxic activity of DOXO-loaded micelles toward cancer cells perhaps due to the enhanced uptake activity observed on cancer cells.

Micellar Prodrugs

Considerable attention has been recently focused on a inulin-doxorubicin conjugate capable itself of assembling into micelles [24]. An efficient conjugation of doxorubicin (> 18%w/w) with inulin to give an environment-sensitive amphiphile named INU-EDA-P,C-Doxo was accomplished via amidic bond, employing citraconic acid as a pH-sensitive spacer (**Figures 1** and **3**). The authors grafted also pentynoic pendants to provide Π-Π structuring in water thus yielding to organized assembled micelles of about 20 nm at concentration beyond 0.33 mg mL^{-1}. Cytotoxicity studies carried out on a huge array of cancer (HCT 116, MDA-MB 231 and Sk-Hep-1) and normal (16 HBE, HB-2) cell lines point out an evident selective cytotoxic effect on cancer cells. Being this study performed *in vitro* this phenomenon can be ascribed to biochemical mechanisms instead of the EPR effect [16]. Flow cytometry measures carried out following the fluorescence of the FITC-labelled copolymer and the auto-fluorescence of doxorubicin suggest a crucial rule of the cancer culture microenvironment, that owing to its acidic condition provokes the hydrolysis of the conjugate at the membrane interface bringing about independent doxorubicin diffusion throughout the cells. On the contrary, in normal cell cultures (pH 7.4) the unmodified conjugate pass through the cell membrane. It was proposed that while a partially hydrolyzed conjugate losses the self-assembling ability and reverses its net charge inside lysosomes (from negative to positive), efficiently releasing its payload, the unmodified one undergo a contraction of volume inside lysosome (the volume here is 10^5 lower than cytosol or interstitial liquid) implying its assembling into micelles which slowly release the drug being highly organized (**Figure 2**).

There are no doubt that these approach must be confirmed in future works, by definitely represents a novel frontier to cleverly delivery anticancer drugs in order to impart selectivity to advanced therapies

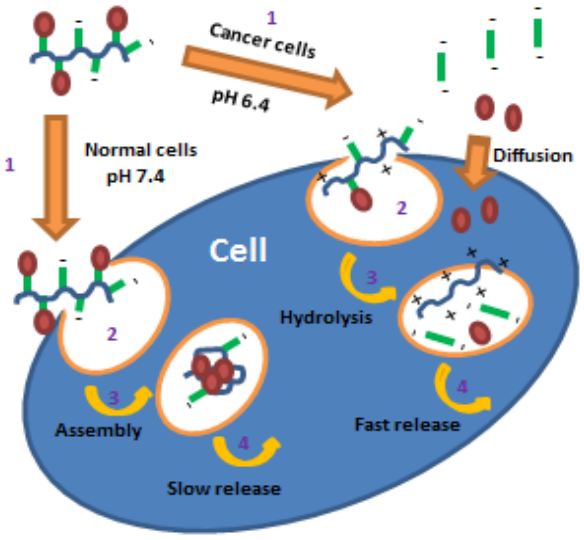


Figure 2: Schematic representation of the mode of action of INU-EDA-P,C-Doxo. pH-sensitive citraconylamide spacer (green), doxorubicin (red), inulin backbone (blue).

Nanostructured carbon-nanosheets

The inulin-doxorubicin conjugate reported above (INU-EDA-P,C-Doxo) is not only an intrinsically self-assembling anticancer prodrug, but is also liable to further functionalization via click-chemistry reactions. The alkyne moieties available in this conjugate were functionalized with a discrete PEG-biotin harm, a targeting agent widely employed to delivery drugs into cancers which overexpress biotin receptors, using the bio-orthogonal Huisgen cycloaddition [25]. This biotinylated widely employed to delivery drugs into cancers which overexpress biotin receptors, using the bio-orthogonal Huisgen cycloaddition [25]. This biotinylated anticancer prodrug was combined with reduced graphene oxide (RGO) nanosheets, capable of acting as an energy-converting device producing heat, in order to obtain a Π-Π-staked nanosystem simultaneously endowed with synergic anticancer effect (i.e., RGO-induced thermal ablation and anticancer chemotherapeutic effect) and high selectivity (**Figure 3**). Apart from the higher efficacy, the EC50 values on MCF-7 cells showed a notwithstanding higher potency for the biotinylated system instead of the non-biotinylated one (EC50 = 23 μM *vs* EC50 > 50 μM). In addition, after the treatment of cancer cells with a 810 nm laser diode cell viability exponentially decreased as consequence of the increased temperature, following a dose-response curve. The laser treatment also blocked cell proliferation of the cells still alive during 24 h of post-incubation avoiding drug resistance mechanisms. This dual effect nanosystem appears very active both as a selective surgical thermal ablation tool and as hyperthermia-triggered drug release and sensitization

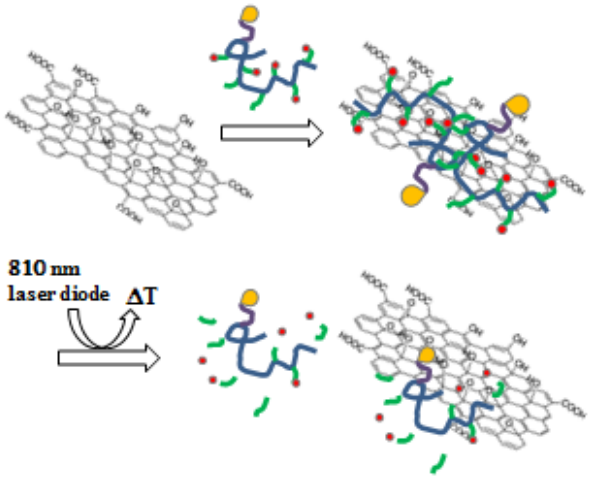


Figure 3: Formation of Π-Π stacked nanosystem between biotin-containing INU-EDA-P,C-Doxo and RGO. Thermal ablation of cells, hyperthermia and the selective drug release is obtained by irradiating the nanosystem with a 810 nm laser diode.

SUPERPARAMAGNETIC NANOPARTICLES (SPIONs)

SPIONs

Superparamgnetic nanoparticles are nanocarriers which has proved capable of targeting anticancer drugs, avoiding the most part of limitations attributable to conventional therapeutic tools. Such systems allow improved tumor bioavailability exploiting a double targeting effect, that is enhanced permeability retention effect (EPR) (passive) and the magnetic field-triggered targeting (active).

An PEGylated inulin derivative containing carboxy-squalenoyl side chains through amidic bond was employed as coating agent of SPIONs to obtain biocompatible stealth doxorubicin delivery systems (**Figure 1**) [26]. These SPIONs were assembled by dialyzing against water a suspension of Fe_3O_4 in a mixture of the copolymer and doxorubicin free base in DMSO. The hydrophobic interactions between the

squlenoyl tails, doxorubicin and the SPIONs surface induced the formation of nanoparticels enriched in doxorubicin (11.6 ± 0.5%). Owing to the small dimension (~ 55 nm, PDI = 0.12) they efficiently enter cells and localized inside cytosol. In HCT 116 cell culture the inulin-coated SPIONs exhibited a significantly higher anticancer activity than the free drug especially under the effect of a magnetic field place at the bottom of the culture (up to three times higher). These results indicated that inulin-based SPIONs are excellent candidates for the treatment of cancers, since they have advantageous therapeutic effects, among which local and amplified drug uptake and increased anticancer activity.

Magnetoplexes

Small interfering RNAs (siRNA) have been received great attention in the last decade for many human disease, comprising cancer. As a rule, they need to be carefully targeted towards the tumor mass, and then inside cancer cells, to efficiently reach the site of action (cytosol) avoiding degradation. Cationic non-viral delivery systems, such as cationic lipids or polyethyleneimine, have been already proposed, but they are relatively toxic and rapidly localize to lung or liver after intravenous administration. Unlike many other polycations inulin-based vectors bearing either prim- or poly-amines as side functions were referred as cytocompatible on different cell lines (16HBE, JHH6, HCT-116) and capable of easily complexing siRNA [6].

More recently highly biocompatible magnetoplexes constituted by inulin-ethylenediamine-coated SPIONs loaded with siRNA were obtained by using the layer-by-layer self-assembly [27] technique [28]. In particular, anionic SPIONs of about 20 nm were covered by an opposite charged layer of inulin-ethylenediamine followed by a last shell of anionic siRNA to get to siRNA-coated inulin/SPIONs nanostructures of 30 nm diameter. This nanosystem stably condensed siRNA at vector/siRNA ratio (R) higher than 10 w/w as a consequence of the net positive charge obtained (Z-potential ~ + 25 mV). The uptake experiments obtained on normal (16HBE) and cancer (JHH6) cell models showed that these magnetoplexes can be internalized with a remarked R-dependent trend (R10 < R20 < R30 = R40), thus reflecting on the higher transfection efficiency observed at the higher R value. Noteworthy, magnetoplexes at R30 (corresponding to an applied dose of siRNA/cell of 63.8 pg) are able to down regulate luciferase expression on normal cells up to about 70%, while in the presence of a magnetic field transfection considerably increased up to 95%. On the contrary, an identical magnetic field applied on the cancer cell culture did not affect luciferase expression. This difference should deserve further investigations.

CONCLUSIONS

Inulin is an FDA approved biopolymer endowed with a combination of relevant properties making it eligible to design a variety of targeted anticancer delivery systems. First of all, it contains primary and secondary hydroxyl groups homogeneously distributed along the main chain which confer it a structural versatility enabling to finely tune its functionalization with a wide array of functional groups such as targeting agents, anticancer drugs, environment-sensitive spacers and long-chain lipophilic moieties. The last but not the least, it intrinsically displays an anticancer prebiotic effect which equips the potential carrier with a synergistic anticancer strategy.

Inulin has been successfully considered for a few numbers of applications in targeted anticancer therapy. Overall, preliminary research data point out that, independently from the EPR effect, it somehow provides selective *in vitro* cytotoxicity toward cancer cells both as drug loaded self-assembled nanocarrier and as self-organized prodrugs. Hence, it might be expected that its impact will sharply increase in the next years.

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