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Review

A review of polysaccharide cytotoxic drug conjugates for cancer therapy

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ABSTRACT

The most considerable challenges facing effective cancer therapy are systemic toxicity of cytotoxic drugs, their lack of tumor localizing and an even distribution throughout the whole body. Besides, short half-lives and undesirable pharmacokinetics are among the other drawbacks that inhibit effective cancer chemotherapy. Conjugation of low molecular weight drugs to polysaccharides has been used as a way to address these problems. This review will focus on polysaccharide drug conjugates and will provide an overview on various conjugation studies which have been accomplished for these carriers with cytotoxic drugs. Although a wide variety of anticancer agents including some toxins have been the subject of conjugation techniques, in this review, low molecular weight cytotoxic drugs conjugated covalently to the carriers are considered.

Contents

Abbreviations: 5-FU, fluorouracil; ADH, adipic acid dihydrazide; ATRA, all-trans retinoic acid; CM, carboxymethyl; C_p, plasma concentration; CPT, camptothecin; DOX, doxorubicin; DS, degree of substitution; DTX, docetaxel; EPR, enhanced permeation and retention; HA, hyaluronic acid; i.v., intravenous; MW, molecular weight; LMWC, low molecular weight chitosan; MMC, mitomycin C; MTX, methotrexate; MW, molecular weight; PEG, polyethylene glycol; pHPMA, poly(2-hydroxypropyl methacrylate); PTX, Paclitaxel.

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1. Introduction

The growing global prevalence of cancer has made cancer therapy one of the most investigated aspects in the recent years. The most considerable challenges facing effective cancer therapy are systemic toxicity of cytotoxic drugs, their lack of tumor localizing and an even distribution throughout the whole body including tumor tissues. Besides, anticancer drugs short half-lives in blood circulation and their undesirable pharmacokinetic behavior are among other drawbacks which are present in the way of cancer chemotherapy. As a matter of fact, conjugation of low molecular weight drugs to macromolecular carriers has been used as a way to address this problem which generally enhances distribution of drug molecule in the body. Natural and synthetic polymers including polysaccharides, proteins, antibodies and poly amino acids are accumulated in tumor tissue due to enhanced permeation and retention (EPR) effect. The introduction of this concept is one of the most notable step forwards leading to more general tumor targeting (Fang, Nakamura, & Maeda, 2011). The conjugation of low MW cytotoxic drugs to appropriate macromolecular vectors is therefore considered to be a promising approach for improving efficacy of cytotoxic drugs on tumor cells along with fewer side effects on normal tissues (Kratz & Beyer, 1998).

These macromolecular conjugated prodrugs have several advantages over their low MW precursors. The main advantages are (Bildstein, Dubernet, & Couvreur, 2011; Khandare & Minko, 2006; Pasut & Veronese, 2007):

(1) An increase in water solubility of low soluble or insoluble drugs (such as paclitaxel (PTX) and camptothecin (CPT)) and an enhancement in biodistribution and therapeutic efficacy (Dosio, Stella, Arpicco, & Cattel, 2011; Park et al., 2010);
(2) Drug accumulation in tumor tissues by EPR effect and a reduction in systemic side effects (Chau, Dang, Tan, & Langer, 2006; Chau, Padera, Dang, & Langer, 2006; Wang et al., 2011);
(3) Improvement in drug pharmacokinetics and a more prolonged plasma half-life (Luo, Wang, Miao, He, & Tang, 2012);
(4) The potential of developing a multifunctional drug delivery system, consisting of several active therapeutic or imaging molecules (Chandna, Khandare, Ber, Rodriguez-Rodriguez, & Minko, 2010; Majoros, Myc, Thomas, Mehta, & Baker, 2006);
(5) Protection of drugs against deactivation during blood circulation, transport to targeted organs or tissues and intracellular trafficking (like CPT and its derivatives) (Singer et al., 2001);

Various macromolecules including proteins, antibodies, polysaccharides, lectins and synthetic polymers, have been conjugated to cytotoxic drugs. Among them polysaccharide-based systems have gained increasing attention due to their cost-effectiveness, abundance in nature, remarkable physicochemical and biological characteristics and simplicity of chemical reactions required for specific modifications. Many polysaccharides contain several reactive functional groups (including amino, hydroxyl and carboxyl moieties), which may readily be utilized as an active site for drug conjugation either directly or via linkers (Liu, Jiao, Wang, Zhou, & Zhang, 2008; Sonia & Sharma, 2011). Polysaccharide based biodegradable matrices are especially attractive because of their natural digestion and degradation in human body. However, natural polymers do not always provide safe outcomes. Some modifications such as the attachment of special linkers or reactive groups may lead to the production of non-biodegradable and toxic products. Therefore the selection of appropriate spacers is a critical step in designing efficient drug conjugates (Duncan, 2006; Danhauser-Riedl et al., 1993).

Polysaccharides have a variety of advantages in comparison with synthetic polymers. Despite the desirable properties of biocompatible and biodegradable synthetic polymers, their higher cost may be considered as one of their main draw backs Furthermore, unlike polysaccharides, inadequate drug loading due to limited loading capacity of some of synthetic polymers (such as PEG) presents a serious problem in preparation of drug–polymer conjugates, especially for less potent drugs (Khandare & Minko, 2006). As a result despite outstanding properties and various applications of PEG in nanomedicine only one PEG–cytotoxic drug conjugate, PEG-camptothecin conjugate (Prothecan), has been able to reach clinical trials (Bildstein et al., 2011). It should be mentioned that some synthetic polymers with multifunctional groups such as poly(2-hydroxypropyl methacrylate) (pHMPA) and polyglutamic acid have been used successfully in the field of polymer–drug conjugates and have reached higher phases of clinical trials (Bildstein et al., 2011; Lammers, 2010). Synthetic polymeric prodrugs including polyethylene glycol (PEG) drug conjugates have been widely reviewed previously (Duncan, 2006; Khandare & Minko, 2006; Li & Wallace, 2008; Pasut & Veronese, 2007; Pasut & Veronese, 2009). This review will focus on polysaccharide drug conjugates and will provide an overview of various conjugation studies which have been accomplished for these carriers with cytotoxic drugs.

2. Drug-conjugate design

Different factors can determine the success of a drug conjugate which should be considered during the design of these prodrugs. Selection of macromolecular carriers, desired target (intracellular, lymphatic system, etc.), type of conjugation (direct or indirect), linker chemistry and the MW have been shown as the key parameters (Bildstein et al., 2011; Homma et al., 2010; Khandare & Minko, 2006; Pasut & Veronese, 2007). An excellent example for a rational drug-conjugate design in the literature is the history of CRLX101 development (formerly known as IT-101). This unique design was achieved by focusing on biocompatibility, favorable particle size and zeta potential and other features of drug conjugates (Davis, 2009; Svenson, Wolfgang, Hwang, Ryan, & Eliasof, 2011; Young, Schluep, Hwang, & Eliasof, 2011).

2.1. Macromolecule selection

Many polysaccharides have been evaluated as drug carriers in the conjugated form, but in contrast to the success of synthetic polymers (Boddy et al., 2005; Homsi et al., 2007; Nemunaitis et al., 2005; Schoemaker et al., 2002; Seymour et al., 2002; Vasey et al., 1999), only few polysaccharides have entered to clinical trials due to their toxicity and efficacy profiles. Hyaluronic acid (HA), dextran and cyclodextrin derivatives are among the successful examples (Bassi et al., 2011; Davis, 2009; Veltkamp et al., 2008). One of the most important barriers against further progress of most conjugates is inherent polymer-related toxicity and/or immunogenicity (Duncan, 2006; íhová, 1996). Furthermore conjugation may alter the structure of safe and biocompatible carriers in a way that the conjugated form may not possess the desired properties of the free carrier anymore. Oxidized dextran is an example for this case which has shown some degrees of toxicity in Phase 1 clinical trial (Danhauser-Riedl et al., 1993). In addition, inadequate drug loading due to limited loading capacity of some polymers (such as PEG) presents a serious problem especially for less potent drugs (Khandare & Minko, 2006) which is not the case for most of the polysaccharides.

2.2. Biodegradability

One of the key characteristics of polymers when used as a part of drug delivery systems is their biodegradation and their fate in body (Kean & Thanou, 2010). An ideal carrier is the one which can be

degraded during favorable time interval. The biodegradation time should be predictable in order to design an acceptable drug delivery system (Ratajska & Boryniec, 1998). If a hydrophilic polymer has a low molecular weight it may undergo renal clearance but for ones with higher molecular weights the necessity of biodegradability increases. Enzymatic or chemical biodegradation produces low molecular weight fractions which may undergo renal clearance more easily (Kean & Thanou, 2010). One of the best known polysaccharide molecules present in nature are cellulose fibers which lack the required biodegradability in human body and have not succeeded as a carrier (Ratajska & Boryniec, 1998).

On the other hand chitosan is mostly degraded by lysozyme and the degree of degradation depends on polymers molecular weight, degree of deacetylation – which its increase will enhance the degradation rate – and degree of n substitution. Some specific modifications such as covalent crosslinking and thiolation have been shown to alter degradation profiles (Kean & Thanou, 2010; Ratajska & Boryniec, 1998).

Hyaluronic acid is also considered to be a biodegradable polymer which is mostly affected by enzymatic degradation and is cleaved into its monosaccharide with hyaluronidase playing the role of the main cleaving enzyme. The esterification of carboxylic groups on hyaluronic acid structure will reduce the enzymatic biodegradation of this carrier (Zhong et al., 1994).

2.3. Molecular weight

Another feature in drug-conjugate design is the molecular weight of the polymer. Although a wide range of MWs is available for every polymer, some physiological and pharmaceutical factors may limit the choices. The main aspect of MW of the polymers in their systemic administration is to ensure eventual renal elimination of these polymers. Apart from polysaccharides as generally biodegradable structures, non-biodegradable polymers (like PEG and pHPMA) are considered to be more critical in this case and should be chosen when their MWs is close to the renal threshold of about 30–60 kDa (Haag & Kratz, 2006) (a hydrodynamic radius of approximately 45 Å; Pasut & Veronese, 2007), which represents the albumin excretion limit. On the other hand, they should have an acceptable MW to permit their enhanced permeation and retention in solid tumors which have been reported to be >50 kDa for hydrophilic polymers (Noguchi et al., 1998). In fact the pharmacokinetic behavior of different polysaccharide carriers is commonly determined by their molecular weight and charge. As a general rule, polysaccharides with larger molecular weights will have less clearance and will stay longer in plasma. This will eventually result in their accumulation in tumor tissues and is considered one of the main reasons behind the conjugation practice. This longer plasma half-life is due to enhanced permeation and retention of macromolecules in tumors is usually called passive targeting and is a result of tumor's poor lymphatic drainage and its higher penetration (Mehvar, 2003).

Other factors such as route of administration, site of action and type of polymer may also be important in choosing the appropriate MW (Bassi et al., 2011; Coradini, Pellizzaro, Miglierini, Daidone, & Perbellini, 1999; Rosato et al., 2006). The role of MW in cytotoxicity of many polysaccharide–drug conjugates is inconsistent and depends on type of the polysaccharide, the range of MW and the desired application (Nevozhay, Budzynska, Jagiello, et al., 2006; Nevozhay, Budzynska, Kanska, et al., 2006). To sum up, even though the most successful polymer prodrugs of anticancer agents which are currently under clinical investigation display MWs between 25 and 50 kDa (Haag & Kratz, 2006), the significance of MW of conjugates remains unclear due to limited clinical data for drug–polymer conjugates.

2.4. Linker chemistry

In macromolecular prodrug design, linkers or spacer arms have been mainly used for two purposes. Firstly, they were used where the anticancer drug and/or the polymer have not suitable functional groups for conjugation reaction or to achieve a specific type of bond (amide, ester, etc.). In these cases, linkers have short and simple chemical structure (Hou et al., 2011; Luo et al., 2012). In other cases, the linker has an active and functional role and controls the drug release by stimuli-responsive mechanisms (Chau, Tan, & Langer, 2004; Homma et al., 2010; Sugahara et al., 2008). Hence different strategies with a variety of linkers have been used for the delivery of anticancer drugs to achieve an optimal entrapment efficiency (Haag & Kratz, 2006), specific tumor targeting (Noguchi et al., 1998; Zheng et al., 2011) and higher cellular uptake (Rosato et al., 2006).

Another way for classifying different linkers and conjugation strategies is by defining direct or indirect methods of conjugation. In direct method, the drug molecule is directly attached to the carrier without the interference of any spacer groups. Various reagents including carbodiimides and active esters are used for this purpose (Garnett, 2001). Glutaraldehyde can be utilized for direct coupling as well (Warnecke, Fichtner, Sass, & Kratz, 2007) which create labile disulphide bonds that can be selectively cleaved in the reductive environment of tumor cells (Ojima et al., 2002; Yeh et al., 2006). On the other hand indirect methods exploit various spacers which are attached to drug molecule and present a new derivative which is further applied for drug polymer conjugation. Peptides, succinyl, and hydrazone spacers are among the molecules which have been used for this reason (Warnecke et al., 2007). Peptidyl spacers are constructed of different amino acids and have been demonstrated plasma stability but are rapidly cleaved by intracellular proteases and subsequently release the drug inside the tumor cells (Garnett, 2001; Warnecke et al., 2007; Dubowchik et al., 2002; Chau, Dang, et al., 2006; Chau, Padera, et al., 2006). Another group of linkers are the so called pH labile linkers including N-cis-aconityl, hydrazone and succinic spacers which are considered to be stable in plasma and normal tissues with an approximate pH of 7.2 but are readily cleaved in mildly acidic environment of the target tumor cells (Garnett, 2001; Luo, Ziebell, & Prestwich, 2000). Series of linkers has been used in polysaccharide–cytotoxic drug conjugates are summarized in Table 1.

3. Polysaccharide drug conjugates

The concept of polymeric macromolecule–drug conjugates was first proposed by Ringsdorf for delivery of hydrophobic small drug molecules to their sites of action (Ringsdorf, 1975). These drug delivery systems are basically composed of a water-soluble polymer (e.g. polysaccharides) that is directly or indirectly conjugated to a drug by a chemical bond.

Many natural and synthetic polymers are attractive polymeric drug carriers and have been used for the development of prodrugs from several therapeutic agents. Polysaccharides are considered an important group of polymers which have been widely used in drug delivery systems. Their pharmacokinetics are greatly influenced by their electric charge, MW, extent of chemical modifications, polydispersity and branching (Mehvar, 2003). In Fig. 1 the chemical structure of polysaccharides used in anti-cancer drug conjugation are shown. Although various water-soluble synthetic polymers have been exploited for the conjugation of hydrophobic drugs; naturally occurring polymers such as hyaluronic acid with intrinsic cell specific binding capacity have also remarkable potential as target-specific drug carriers (Auzenne et al., 2007). Table 1 summarizes various types of these macromolecular carriers and their application in drug-conjugation platform.

Table 1
Polysaccharide cytotoxic drug conjugates.

No.	Polymer	Drug	Linker	Type of bond (name and cleavability)	Cell culture studies (name of cell lines)	In vivo studies (cell lines, route of admin for the conjugate)	Approximate average MW of the conjugate (kDa)	References
1	Hyaluronic acid	DOX	Succinate/adipic dihydrazide	Amide with drug, amide with polymer	Selective uptake by CD44 targeting (HBL-100, SKOV-3, HCT-116)	No	11.2	Luo, Bernshaw, Lu, Kopecek, and Prestwich (2002)
2	Hyaluronic acid	DOX	Adipic dihydrazide	Imine (hydrazone) with drug, amide with polymer	Slightly lower toxicities than free DOX (MDA-MB-468LN, MDA-MB-231, MCF-7)	Higher anticancer efficacy relative to the conventional i.v. DOX therapy (MDA-MB-468LN, s.c.)	Not mentioned	Cai et al. (2010)
3	Hyaluronic acid	PTX	Succinate/adipic dihydrazide	Ester with drug, amide with polymer	Selective uptake, targeted but lower toxicities than free PTX (HBL-100, SK-OV-3, HCT-116, NIH3-T-3 and NMP-1, SKOV-3ip)	Antitumor activity in human ovarian carcinoma Xenografts (NMP-1 and SKOV-3ip, i.p.)	11.2, 40	Auzenne et al. (2007), Luo and Prestwich (1999) and Luo et al. (2000)
4	Hyaluronic acid	PTX	4-Hydroxybutanoic acid derived	Ester with drug, ester with polymer	Much stronger inhibitory effect than conventional PTX (RT-4 and RT-112/84)	PTX did not result more effective than the conjugate (RT-112/84, i.p.)[a]	200	Bassi et al. (2011), Leonelli et al. (2005) and Rosato et al. (2006)
5	Hyaluronic acid	PTX	Direct conjugation	Ester	More cytotoxicity for CD44+ cells, but reduced cytotoxicity for NIH-3T3 (HCT-116, MCF-7, NIH-3T3)	No	64	Lee, Lee, Lee, et al. (2008) and Lee, Lee, and Park (2008)
6	Hyaluronic acid	Sodium butyrate	Direct conjugation	Ester	The MW difference did not influence the biological activity of the compounds via CD44 (MCF-7)	No	85, 166	Coradini et al. (1999)
7	Hyaluronic acid	Curcumin	Direct conjugation	Ester	Improvement in cytotoxicity due to the water solubility and cell internalization ability of the conjugate (L929)	No	560	Manju and Sreenivasan (2011)
8	Hyaluronic acid	MTX	Peptide-linkers (egPhePhe-Alkyl)	Amide with drug, amide with polymer	Not for cancer chemotherapy purposes	Not applicable (arthritis model)	Between 330 and 2180	Homma et al. (2009) and Homma et al. (2010)
9	Dextran, carboxymethyl	7-Ethyl-10-aminopropyloxy-CPT (T-2513)	Gly-Gly-Gly	Amide with drug, amide with polymer	The conjugate (T-0128 or Delimotecan) was approximately 1000-fold less potent than T-2513 (Panel of human cancer cell lines includes WiDr, SK-BR-3, HeLaS3)	Efficacy of T-0128 was 10-fold superior to that of T-2513 against Walker-256 carcinoma, significant antitumor activity against the panel of human tumor xenografts (i.v.)[a]	130	Harada, Imai, et al. (2000), Harada, Sakakibara, et al. (2000), Harada et al. (2001), Okuno et al. (2000) and Veltkamp et al. (2008)
10	Dextran, carboxymethyl polyalcohol	Exatecan (DX-8951, CPT analog)	Gly-Gly-Phe-Gly	Amide with drug, amide with polymer	Not available	A single-dose of the conjugate (DE-310) exhibited similar or greater antitumor activity than multiple administrations of DX-8951f (various human tumor xenografts and murine solid tumors)[a]	360	Inoue et al. (2003), Ochi et al. (2005) and Soepenberg et al. (2005)
11	Dextran, carboxymethyl	PTX	Gly-Gly-Phe-Gly	Ester with drug, amide with polymer	The conjugate (AZ10992) was inactive in vitro (cell lines are not mentioned)	Significant antitumor activity (colon26, MX-1, LX-1, HT-29, i.v.)	150	Sugahara et al. (2007, 2008)

Table 1 (*Continued*)

No.	Polymer	Drug	Linker	Type of bond (name and cleavability)	Cell culture studies (name of cell lines)	In vivo studies (cell lines, route of admin for the conjugate)	Approximate average MW of the conjugate (kDa)	References
12	Dextran, oxidized	DOX	Glycine	Not available	Not available	The conjugate (AD-70) showed higher activity, higher plasma concentration, lower acute toxicity and accumulation in the heart in rats (Walker256, i.v.)[a]	70	Danhauser-Riedl et al. (1993) and Munechika et al. (1994, 1989)
13	Dextran, oxidized	Cytarabine (Ara-c)	N4-(4-carboxybutyryl)-ethylene diamine	Amide	Not available	Improved the life span of leukemic mice (L1210, i.p.)	2000	Onishi et al. (1991)
14	Dextran, oxidized	MTX	Diaminohexane or 5-amino-1-pentanol	Amide amide or amide ester (respectively)	Cytotoxicity of MTX-ester-dextran and MTX-amide-dextran against H80 was equivalent to unmodified MTX (H80 brain tumors)	Modest but significant increases in survival after intracranial polymeric delivery of MTX or MTX-amide-dextran (rats with intracranial 9L gliosarcoma, inserted pellets)	70	Dang, Colvin, Brem, and Saltzman (1994)
15	Dextran	MTX	Direct conjugation	Ester	Four to 10-fold lower antiproliferative effects compared to free MTX (A549, SW707, P388)	Greater toxicity in comparison with the parent drug, but no superiority in terms of antileukemic effect. (P388 mouse leukemia model, i.p.)	10, 40, 70, 110, 500	Nevozhay, Budzynska, Jagiello, et al. (2006) and Nevozhay, Budzynska, Kanska, et al. (2006)
16	Dextran, carboxymethyl	MTX (Peptidyl MTX released)	Jeffamine-Pro-Val-Gly-Leu-Ile-Gly	Amide with drug, amide with polymer	Two times lower potency compared to free MTX	No significant difference in drug accumulation at the tumor site between the MMP-sensitive and the MMP-insensitive conjugates which shows that the tumor targeting via EPR. (HT-1080 bearing mice, overexpresses MMP, i.p.)	70	Chau, Dang, et al. (2006), Chau, Padera, et al. (2006) and Chau et al. (2004)
17	Chitosan, N-succinyl	MMC	Direct conjugation	Amide	Not mentioned	Good antitumor activities against various tumors (P388 and L1210 i.p., M5076 i.v. and i.p., MH134 i.p.)	300	Kato et al. (2004)
18	Chitosan, N-succinyl	MMC	Glutaryl	Amide with drug, amide with polymer	Not mentioned	Same efficacy and less toxicity of the conjugate than the glycol-chitosan counterpart *see below and* significant periority over the counteraprt in i.v. administration (P388 i.p., Sarcoma 180 i.p. and i.v.)	300	Kato et al. (2004), Sato, Onishi, Kitano, Machida, and Nagai (1996) and Sato, Onishi, Takahara, Machida, and Nagai, 1996)
19	Chitosan, glycol	MMC	Glutaryl	Amide with drug, amide with polymer	Not mentioned	Same efficacy but more toxicity than the succinyl-chitosan counterpart (P388 i.p., Sarcoma 180 i.p. and i.v.)	160	Kato et al. (2004), Sato, Onishi, Kitano, et al. (1996) and Sato, Onishi, Takahara, et al. (1996)

20	Chitosan	DOX	Succinate	Amide with drug, amide with polymer	Reduced cytotoxicity compared to of free drug (SKOV-3, MCF-7)	Not mentioned	116	Yousefpour, Atyabi, Farahani, Sakhtianchi, et al. (2011) and Yousefpour, Atyabi, Vasheghani-Farahani, et al. (2011)
21	Chitosan, glycol	DOX	cis-Aconityl	Amide with drug, amide with polymer	Not mentioned	DOX-loaded GC-DOX nanoaggregates exhibit lower systemic toxicity but comparable anti-tumor activity via EPR (B16F10 II45, i.v.)	250	Hyung Park et al. (2006) and Son et al. (2003)
22	Chitosan, low MW	PTX	Succinate	Ester with drug, amide with polymer	Comparable IC_{50} values to that of the parent PTX (NCIH358, SK-OV-3, MDA-MB-231)	Conjugate significant inhibition of tumor growth/42% of bioavailability after oral administration (B16F10, p.o. admin of the conjugate vs. i.v Taxol)	<10	Lee, Lee, Lee, et al. (2008) and Lee, Lee, and Park (2008)
23	Chitosan, low MW	DTX	Succinate	Ester with drug, amide with polymer	Only slight activity loss by two or three fold was observed with the conjugates (NCI-H358, U87MG))	Comparable antitumor efficacy and higher bioavailability (p.o.) than i.v. DTX at the same dose along with prolonged blood circulation time blood and less sub-acute toxicity ((NCI-H358 and U87MG)	~6	Lee, Kim, et al. (2009) and Lee, Ahn, et al. (2009)
24	Chitosan, stearic acid grafted	DOX	cis-Aconityl	Amide with drug, amide with polymer	DOX–Chitosan–Stearic acid micelles could reverse the drug resistant by the power of about 4–10 (QGY, MCF-7 and MCF-7/Adr)	Suppressed the tumor growth and reduced the toxicity better than commercial DOX HCl injection (QGY, i.v.)	18	Hu, Liu, Du, and Yuan (2009)
25	Heparin, low MW	ATRA	Ethylenediamine	Amide two sides	PTX-loaded heparin–ATRA conjugate nanoparticles exhibited higher cytotoxic activity than PTX solution/no significant effects on hemolysis (HepG2)	Longer systemic circulation time relative to that of PTX plus ATRA solution	4.5 approximately	Hou et al. (2011)
26	Heparin, low MW	PTX	Carbonate, ethylenediamine	Amide with polymer, ester with drug	Higher toxicity of heparin PTX conjugate nanoparticles (KB)	No	5 approximately	Park et al. (2010)
27	Heparin, succinylated	PTX	Single aminoacid spacer (Val, Leu, or Phe)	Ester	Better cell inhibition than free PTX (MCF-7)	Similar ovarian tumor growth inhibition as PTX and induces no obvious body weight loss for leucine prodrugs	Not mentioned	Wang, Xin, Liu, and Xiang (2009) and Wang, Xin, Liu, Zhu, et al. (2009)
28	Heparin, O-acetylated	PTX	Direct conjugation or aminoacid spacer (Val, Leu, or Phe)	Ester	The anticoagulant activity is reduced. Treated cells are arrested in the G2/M phase of cell cycle. Conjugates show better solubility and faster hydrolysis rate (MCF-7)	No	12	Wang, Xin, Liu, and Xiang (2009) and Wang, Xin, Liu, Zhu, et al. (2009)

Table 1 (*Continued*)

No.	Polymer	Drug	Linker	Type of bond (name and cleavability)	Cell culture studies (name of cell lines)	In vivo studies (cell lines, route of admin for the conjugate)	Approximate average MW of the conjugate (kDa)	References
29	beta-Cyclodextrin, polyethylene glycol copolymer	CPT	Glycine	Amide with polymer, ester with drug	The conjugate (CRLX101, IT-101) has comparable toxicity to CPT against various cancer cell line (PC3, HT29, LS174T, A2780)	Good tolerability and antitumor activity against a wide range of tumors (LS174Tand HT29, H1299, TC71-luc Ewing's sarcoma, MDA-MB-231, Panc-1, andHT29, A2780 ovarian, H69 SCLC, i.v.)[a]	~70	Cheng, Khin, Jensen, Liu, and Davis (2003), Davis (2009) and Schluep et al. (2006)
30	Alginate, ethylenediamine modified	Daunomycin	cis-Aconityl	Amide with drug, amide with polymer	High MW conjugate displayed dose dependent cytotoxicity but nearly 700-fold less toxic than the free drug (B16F10)	Low MW conjugate showed significant delay in tumor growth and reduced toxicity compared with free Dau (mice with B16 s.c. tumors, i.p.)	250, 61	Al-Shamkhani and Duncan (1995)
31	Pullulan	DOX	Maleimide	Amide with drug, ester with polymer	The non-targeted conjugate had a slightly higher cytotoxicity than the free DOX, while folate targeted conjugates were much more toxic (A2780)	No	133	Zhang et al. (2011)
32	Hydroxyethyl Starch	5-FU acetate	Direct conjugation	Ester with polymer	Conjugates were degraded to 5-FU acetate in plasma with halftime life of 20–24 h	Conjugates showed sustained release of 5-FU acetate after i.v. injection	130	Luo et al. (2012)

[a] The clinical trials are reported for this conjugate.

3.1. Hyaluronic acid

Hyaluronic acid is an anionic biopolymer composed of alternating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine with β (1→4) interglycosidic linkages. It has been widely used for drug delivery applications due to its various biological functions, desirable physicochemical properties, biocompatibility, biodegradability and non-immunogenicity. As a cancer diagnostic tool, elevation of HA has been considered to be a reliable disease progression marker in some types of tumors including bladder cancer. For therapeutic purposes, although high molecular weight HA has been exploited in the clinic because of its physical properties as a lubricant in the ophthalmic surgeries and knee disorders, low MW hyaluronic acid and its oligomers play a noticeable role as a bioactive agent in cancer biology which infers the importance of MW of bioactive polysaccharide selection in development of anti-cancer drug delivery systems. In this case, it has been shown that HA oligomers of 8–50 disaccharides, but not high MW HA, stimulate angiogenesis (Aghcheli et al., 2012; Choi, Saravanakumar, Park, & Park, 2012; Lokeshwar et al., 2000).

Perhaps the most remarkable advantage of HA is its potential for active targeting without any additional targeting ligands. Hyaluronic acid has a strong affinity for cell-specific surface markers such as CD44 and RHAMM and has attracted a lot of interest (Auzenne et al., 2007; Lee, Lee, Lee, et al., 2008; Lee, Lee, & Park, 2008). CD44 is a cell surface protein which acts as a specific membrane receptor for HA, is overexpressed in many cancer cells and cancer stem cells, and has been correlated with invasive properties of the above cells and metastatic processes (Dalerba, Cho, & Clarke, 2007; Rudzki & Jothy, 1997; Schatzlein, 2006). As a consequence, HA has greater uptake by breast cancer cells than normal tissues (Götte & Yip, 2006). Therefore through conjugation of cytotoxic drugs, the mechanism of drug uptake is changed from non-specific uptake to receptor mediated endocytosis by these HA-based systems. Due to the growing evidence of the expression of CD44 on cancer stem cells (Dalerba et al., 2007), the potential for selective targeting of chemotherapeutic agents to CD44 have received a lot of interest (Schatzlein, 2006). In addition, it has been shown that endogenous HA is required for high p-glycoprotein expression which is the primary contributor to drug resistance. It has been shown that physical relation of CD44 and p-glycoproteins inside the cell may lead to overexpression of one while the other is affected and it has been proposed that disturbing constitutive HA–CD44 complex via a competitive antagonist can sensitize cancer cells to the cytotoxic drug by reversing multiple drug resistance via p-gp (Misra, Ghatak, & Toole, 2005). Thus hyaluronic acid–drug conjugate can be considered such an antagonist to enhance susceptibility of drug-resistant cells to carried anti-cancer drug molecule.

The hydroxyl and carboxylic groups on the hyaluronate backbone provides appropriate sites for drug conjugation. Direct conjugation of drugs to HA is inefficient due to steric hindrance of the backbone and low reactivity of the carboxylate groups. In indirect conjugation, HA may be extensively derivatized with adipic acid dihydrazide (ADH). This method provides reactive functional groups (NH_2–NH_2) which allow the attachment of other molecules to the HA backbone (Luo & Prestwich, 1999). In addition, using derivatives such as HA-ADH allows for the control of degree of substitution (DS) of carboxylic acid, which is responsible for active targeting properties of HA. It has been found that a DS amount higher than 25 mol% may decrease the ability of HA to target CD44 (Oh et al., 2010). In this case, an appropriate cytotoxicity by HA–drug conjugates is obtained when a balance between minimal HA modification and maximal drug loading is achieved. It has been shown that high loading of PTX masked the CD44 recognition elements of HA, caused aggregation of the conjugate and thus limited the toxicity of the conjugates compared to free PTX (Auzenne et al.,

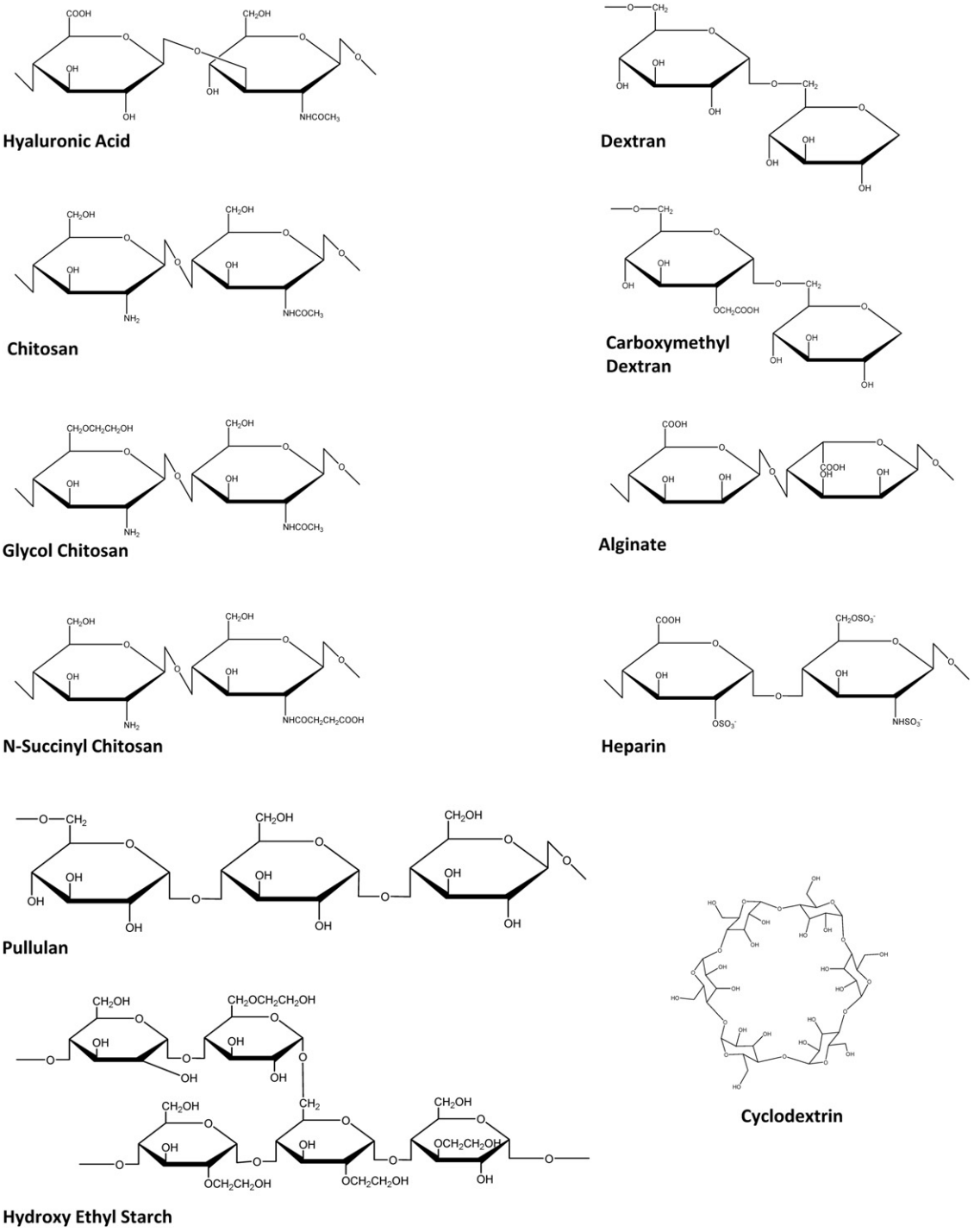

Fig. 1. Chemical structure of polysaccharides.

2007; Luo & Prestwich, 1999; Luo et al., 2000). A new approach to address limited drug substitution in HA-drug conjugates was proposed by Norbedo et al. (2009). 6-Amino-6-deoxyhyaluronan was synthesized as an intermediate to conjugate with CPT. Since C-6 hydroxyl groups of HA are not involved in CD44 recognition, higher degrees of substitution may not significantly affect the targeting properties of the resulted conjugates.

As HA has poor solubility in most organic solvents and moreover the majority of anti-cancer drugs are hydrophobic entities, the hydrophilicity of HA restricts its conjugation reaction with cytotoxic drugs. To address this problem various solubilization methods have been utilized to obtain a homogeneous mixture of HA and hydrophobic reactants in a common solvent. To make it soluble in polar organic solvents, or a mixture of water and polar solvents (e.g. DMSO/H_2O or DMF/H_2O) (Auzenne et al., 2007; Luo & Prestwich, 1999), nano-complexation by dimethoxy polyethylene glycol (Lee, Kim, Lee, & Jon, 2009; Lee, Lee, Lee, et al., 2008; Lee, Lee, & Park, 2008) and ion pair complex with long aliphatic chain cationic salts (Zhang & James, 2005a, 2005b) have been used.

Conclusively variety of HA–drug conjugates has been prepared by above methods which are subjected to in vitro cytotoxicity evaluation. These studies resulted in partially controversial reports on

Table 2
Polysaccharide drug conjugates entered clinical trials.

No.	The conjugate	Route of administration	Efficacy	Toxicity	References
1	Hyaluronic acid–Paclitaxel	Intravesical Instillation	Complete response in 9 of 16 patients with bladder carcinoma refractory to BCG	Minimal toxicity and no systemic absorption	Bassi et al. (2011)
2	Carboxymethyl Dextran–Camptothecin Derivative	i.v. infusion	One partial response and stable disease in 14 patients of 27 patients	Dose-limiting toxicities include thrombocytopenia, neutropenia, and reversible hepatotoxicity	Soepenberg et al. (2005)
3	Carboxymethyl Dextran-Camptothecin Derivative (called Delimotecan)	i.v. infusion	Two partial responses in patients with anal cancer and head and neck cancer	Leucocytopenia, neutropenia, skin rash, fatigue, and diarrhea	Veltkamp et al. (2008)
4	Oxidized Dextran-Doxorubicin	i.v. infusion	No treatment response, one of 13 patients with stable disease for 4 months	Toxicity attributed to uptake of dextran by liver reticuloendothelial cells, reversible thrombocytopenia and hepatotoxicity	Danhauser-Riedl et al. (1993)
5	beta-Cyclodextrin, PEG copolymer-Camptothecin	i.v. infusion	Of 12 patients, 6 with stable disease and 3 with survival of more than 10 months and no progression	No new toxicities because of nanoparticles	Davis (2009)

different cell lines. It has been suggested that improvement in cytotoxicity of HA–drug conjugates compared to free drug is due to better water solubility and cell internalization ability of the conjugates (Manju & Sreenivasan, 2011; Lee, Lee, Lee, et al., 2008; Lee, Lee, & Park, 2008; Leonelli et al., 2005; Rosato et al., 2006) while the reported lower efficacy – even on CD44+ cells – could be related to less available free cytotoxic molecules in conjugated form (Cai et al., 2010; Luo & Prestwich, 1999; Luo et al., 2000). Although different approaches have been assessed to develop a suitable anticancer–drug conjugate (Table 2), only a high molecular weight HA–PTX conjugate form, introduced by an Italian group, has reached clinical trials. It has been shown that the conjugate has minimal toxicity and no systemic absorption after intravesical instillation for refractory bladder carcinoma (Bassi et al., 2011). In addition to its targeting potential, the future application of hyaluronic acid in anti-cancer drug conjugation seems to be focused on the improvement of biodistribution and pharmacokinetics of the conjugated drug after systemic administration.

3.2. Dextran

Dextran is another widely used macromolecule in anticancer drug conjugation strategies containing monosaccharides of simple sugar glucose (Fig. 2). This poly-glucose biopolymer was initially approved as a plasma expander and its desirable physicochemical characteristics along with its low cost and a history of clinical use make it an attractive carrier for drug delivery systems. Although due to polyanionic structure of dextran sulfate, it has been used in a complex form with positively-charged cytotoxic drugs (Nakashima et al., 1999; Tan, Friedhuber, Dunstan, Choong, & Dass, 2010; Yousefpour, Atyabi, Farahani, Sakhtianchi, & Dinarvand, 2011; Yousefpour, Atyabi, Vasheghani-Farahani, Movahedi, & Dinarvand, 2011), there are plenty of primary and secondary hydroxyl groups present on dextran backbone which provide potential functional sites for drug conjugation through direct or indirect methods.

Various anticancer drugs, such as doxorubicin (DOX), CPT, mitomycin C (MMC) and methotrexate (MTX) have been conjugated to dextrans to form cytotoxic–drug conjugates. At least three derivatives of dextran have been used in drug delivery including carboxymethyl dextran (Chau et al., 2004; Harada et al., 2001; Okuno, Harada, & Yano, 2000), oxidized dextran (Danhauser-Riedl et al., 1993; Ueda, Kikukawa, Kanoh, Yamanouchi, & Yokoyama, 1989) and amino-dextran (Shih et al., 1991). These conjugates generally had a prolonged effect, and reduced toxicity and immunogenicity in many in vitro and in vivo studies. Carboxymethyl (CM) dextran contains a sufficient number of carboxyl moieties which provide sufficient conjugation sites for polymer drug conjugation and results in water-soluble CM dextran–drug conjugates.

Peptidyl spacers have been mostly exploited for indirect conjugation of dextrans (Fig. 2). These linkers are substrates of lysosomal cysteine proteinases including cathepsin B. The peptidyl linkers have a profound role in determining the conjugates success in murine xenograft models and in clinical studies (Harada, Imai, Okuno, & Suzuki, 2000; Harada, Sakakibara, Yano, Suzuki, & Okuno, 2000; Ochi, Shiose, Kuga, & Kumazawa, 2005; Veltkamp et al., 2008).

Although most of the studies with dextrans have not progressed past the preclinical phase, oxidized dextran–doxorubicin conjugates (AD-70) have entered Phase 1 clinical trials. Unfortunately this modification resulted in a non-biodegradable polymer which showed toxicity, attributed to uptake of dextran by liver reticuloendothelial cells in human (Danhauser-Riedl et al., 1993). Another dextran–drug conjugate (DE-310) which used CM dextran as the polymer displayed dose-limiting toxicities including thrombocytopenia, neutropenia, and reversible hepatotoxicity in Phase 1 clinical trials (Kumazawa & Ochi, 2004; Masubuchi, 2004; Soepenberg et al., 2005). However, of 27 patients, one patient with metastatic adenocarcinoma of unknown primary achieved complete remission, 1 patient proved by a partial remission, and 14 patients showed disease stabilization (Soepenberg et al., 2005). Another carboxymethyl dextran-CPT derivative conjugate called Delimotecan has shown partial responses in patients with anal cancer and head and neck cancer (Veltkamp et al., 2008).

3.3. Chitosan

Chitosan, the N-deacetylated derivative of chitin, has drawn an increasing interest as a macromolecular carrier due to its desirable properties including biocompatibility and biodegradability (Hyung Park et al., 2006; Kim et al., 2001; Park, Cho, Chung, Kwon, & Jeong, 2003). The reactive amino moieties which are present in the backbone of chitosan make it possible to chemically conjugate various biological molecules to this polymer. Moreover, like hyaluronic acid, chitosan was found to play a remarkable role in cancer biology, and may be exploited to inhibit tumor angiogenesis (Harish Prashanth & Tharanathan, 2005).

In recent years, chitosan–anticancer drug conjugates have also been investigated extensively (Table 1). Considering the chemical

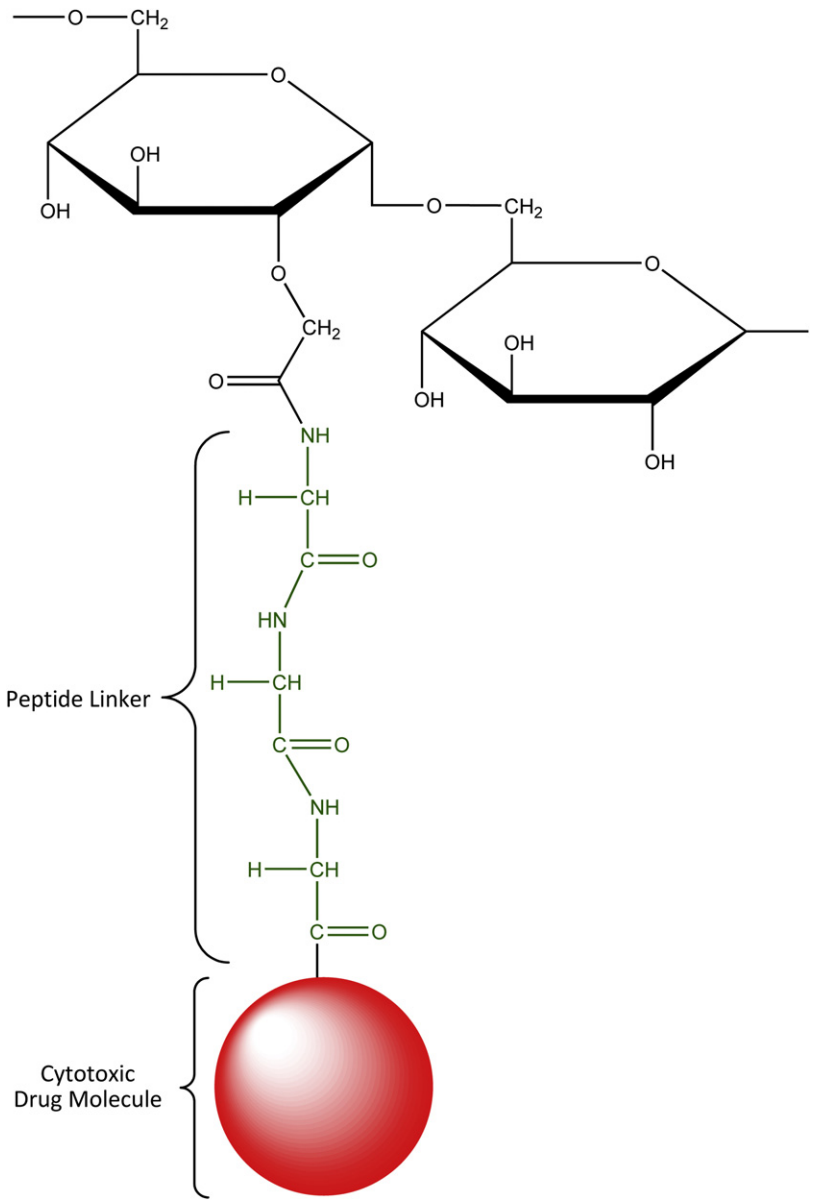

Fig. 2. Peptidyl linkers in dextrans–drug conjugates.

structure of chitosan, several derivatives such as glycol chitosan (Hyung Park et al., 2006), N-succinyl chitosan (Kato, Onishi, & Machida, 2004) and carboxymethyl chitosan (Zheng et al., 2011) in addition to non-modified chitosan have been used. However, one of the major limitations for the application of chitosan is its insolubility at pH 7.4. Although this property has been addressed by fabrication of nanoparticulate systems (Anitha et al., 2011; Atyabi, Moghaddam, Dinarvand, Zohuriaan-Mehr, & Ponchel, 2008; Quiñones et al., 2012; Yousefpour, Atyabi, Farahani, Sakhtianchi, et al., 2011; Yousefpour, Atyabi, Vasheghani-Farahani, et al., 2011), many efforts have been made to develop physiologically-soluble chitosan derivatives (Avadi et al., 2004; Sadeghi et al., 2008) which may find application in systemic cancer chemotherapy (Akhlaghi, Saremi, Ostad, Dinarvand, & Atyabi, 2010; Hyung Park et al., 2006; Park et al., 2003; Saremi et al., 2011).

Due to the established mucoadhesive nature of chitosan and its derivatives, and their use in oral drug delivery systems (Atyabi, Majzoob, Dorkoosh, Sayyah, & Ponchel, 2007; Atyabi, Talaie, & Dinarvand, 2009; Avadi et al., 2010; Moghaddam, Atyabi, & Dinarvand, 2009; Sadeghi et al., 2008; Talaei et al., 2011), chitosan and its derivatives have been widely used for oral administration of poorly soluble cytotoxic drugs (e.g. PTX and docetaxel (DTX)) (Lee, Kim, et al., 2009; Lee, Ahn, & Park, 2009; Lee, Lee, Lee, et al., 2008; Lee, Lee, & Park, 2008). For this purpose, low molecular weight chitosan (LMWC) is used, which is more soluble than high MW chitosan (Fattahi, Golozar, Varshosaz, Sadeghi, & Fathi, 2012; Lee, Lee, Lee, et al., 2008; Lee, Lee, & Park, 2008; Lee et al., 2001). These conjugates led to comparable in vitro and in vivo antitumor efficacy and higher bioavailability (p.o.) than i.v. conventional drug at the same dose along with prolonged blood circulation time and less sub-acute toxicity (Lee, Kim, et al., 2009; Lee, Ahn, et al., 2009; Lee, Lee, Lee, et al., 2008; Lee, Lee, & Park, 2008). One of the most significant advantages of conjugated LMWC is its ability to bypass the P-gp mediated barrier (efflux pump) in the gastrointestinal tract and avoid cytochrome P450-dependent metabolism in the intestine and liver (Lee, Lee, Lee, et al., 2008; Lee, Lee, & Park, 2008). It has also been demonstrated by radiolabeled conjugates that LMWC-PTX was probably absorbed from the ileum mainly and reached the systemic blood circulation in its intact form (Park et al., 2008).

3.4. Heparin

Heparin has a long history of clinical application as anticoagulant and even investigation around its usage as a carrier to improve efficacy of cytotoxic agents (Cremers et al., 1990). It has been shown that heparin itself, inhibits cancer cell adhesion, deactivates heparanase, activates the attack by NK cells in the immune system and interferes with the activity of growth factors such as bFGF and VEGF while through this prevents tumor angiogenesis and metastasis. In this case low molecular weight heparin has been more effective than unfractionated heparin (Lee, Kim, & Byun, 2007; Li et al., 2012; Park et al., 2006). As a consequence, heparin has been widely used as a part of anti-cancer drug delivery systems (Lee et al., 2007; Li et al., 2012; Park et al., 2006).

Heparin has recently received an increasing amount of attention as a drug carrier in macromolecule-anticancer drug conjugate models (Hou et al., 2011; Park et al., 2010; Wang, Xin, Liu, & Xiang, 2009; Wang, Xin, Liu, Zhu, & Xiang, 2009). Although, as a drug carrier the anticoagulant activity will be a concern, this property of heparin is decreased following drug conjugation, and consequently reduces the risk of hemorrhagic complications in clinical applications. In studies applied PTX as cytotoxic drug molecule, it has been shown that the anticoagulant activity is reduced and there was no significant effects on hemolysis, while different heparin–PTX conjugates exhibited more cytotoxicity that free PTX (Park et al., 2010; Wang, Xin, Liu, & Xiang, 2009; Wang, Xin, Liu, Zhu, et al., 2009). Conjugates are provided better solubility for PTX (Wang, Xin, Liu, & Xiang, 2009; Wang, Xin, Liu, Zhu, et al., 2009) and displayed longer systemic circulation time relative to that of free drug solutions of PTX and ATRA (Hou et al., 2011). These special features of heparin make this carrier of interest, along with other promising polysaccharide carriers such as hyaluronic acid and chitosan.

3.5. Cyclodextrin-based polymers

Although there are limited reports on the use of cyclodextrin polymer, it may be one of the most successful polysaccharide among drug conjugates in terms of design and optimization studies to reach CRLX101 (Davis, 2009; Svenson et al., 2011; Young et al., 2011). CRLX101 (formerly IT-101) is a polymeric nanoparticle formulation of 30–40 nm particles formed of CPT covalently conjugated to a linear, β-cyclodextrin-polyethylene glycol copolymer (Svenson et al., 2011). Phase 1 clinical trials with CRLX101 were performed for the treatment of advanced solid tumors, regarding to its cytotoxic efficacy and appropriate toxicity profile in numerous animal studies. IT-101 showed a half-life of about 40 h in human. According to Phase I clinical trials results, Phase II trial has initiated to investigate the efficacy in ovarian cancer (Davis, 2009; Oliver, Yen, Synold, Schluep, & Davis, 2008).

3.6. Other polysaccharides

Among natural and semi-synthetic polysaccharides, hyaluronic acid, chitosan and dextran have been the pioneers in bioconjugation studies. The therapeutic properties of anticancer drugs can be combined with the biological properties of these polymers. A vast number of other polysaccharides including alginate (polyhydroxyl–polycarboxylic anionic backbone) (Al-Shamkhani & Duncan, 1995), pullulan (non-ionic, polyhydroxylic backbone) (Zhang et al., 2011) and their derivatives have been used for the preparation of drug conjugates but they did not present any significant superiority over other players in this field.

4. Conclusion

As reviewed above, a great deal of research has been dedicated to design, synthesis and characterization of polysaccharide anticancer drug conjugates. These prodrugs have been accepted for their improved adverse reaction profiles, targeting properties and enhanced therapeutic efficacy using biodegradable and biocompatible polysaccharides. In rational drug-conjugate design various factors such as biocompatibility with regard to the host response and toxicity of polysaccharides or their derivatives, molecular weight of the final conjugates, type of conjugation in terms of direct or indirect linkage, desired physiological target and favorable particle size and zeta potential (in the case of self-assembled systems) are considered as the main features in successful pharmaceutical development.

It is noteworthy that although generally macromolecular drug conjugates show lower or comparable in vitro cytotoxicity in comparison with the corresponding free drug, their superiority is the significant performance in enzymatic physiological condition where in vivo distribution will affect efficacy and safety of the treatment. Therefore using a more reliable in vitro cell culture model to simulate their fate in relation to tumor, may accelerate screening studies to find the best choice for use in vivo.

However, regarding the fact that some conjugates have been successfully applied in clinic, polysaccharide drug conjugation seems to be an interesting approach and presents a new era in clinical cancer chemotherapy.

Conflict of interest

Authors would like to declare no conflicts of interest.

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