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REVIEW ARTICLE

Application of cyclodextrins in cancer treatment

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Abstract Cancer is one of the major fatal diseases. Chemotherapy is a typical treatment method that uses a combination of drugs to either destroy cancer cells or slow down the growth of cancer cells. However, most of the cytotoxic chemotherapeutic drugs are water insoluble resulting in formulation difficulty. One promising strategy is to use cyclodextrins (CDs) which have been widely employed to enhance the solubility, bioavailability, stability and safety of drug molecules by forming non covalent inclusion complexes. The objective of this review is to explain the use of CDs in the different approaches for cancer treatment. Of specific interest is that CDs are shown to have anticancer activity both in vitro and in vivo. The use of CDs as anticancer agent and the possible mechanism to inhibit cancer cell growth are discussed. CDs/anti-neoplastic-drug complexes with improved solubility, increased stability and enhanced anticancer activity are described and possible future applications are discussed. Use and their advantages of CDs in the different drug delivery systems like liposomes, conjugates, nanoparticles and siRNA carriers for cancer treatment are detailed in this review. These CDs modified delivery system for anticancer drugs are shown to provide improved encapsulation, prolonged release, increased therapeutic efficacy and reduced toxicity. Furthermore, there is a prerequisite to exploit the utility of CDs and CDs-based carriers using in vivo tumor models for pharmacokinetics, pharmacodynamics and toxicity studies to validate their safety and efficacy. In future, we believe that CDs and CDs-based delivery system for anticancer drugs have great potential to serve as an alternative for cancer treatment.

Keywords Cyclodextrin · Anti-cancer, · Inclusion complex · Delivery · Solubilization

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Introduction

Cancer is the leading cause of death worldwide, accounting yearly for 8 million deaths. Chemotherapy is a major therapeutic approach for the treatment of localized and metastasized cancers. Most of the chemotherapeutics are administered intravenously to ensure complete bioavailability and accurate dosing. One of the main challenges in current chemotherapeutic treatment is that the majority of anticancer drugs have poor aqueous solubility, produce adverse effects in healthy tissue, and thus impose major limitations on both clinical efficacy and therapeutic safety of cancer treatment [1, 2]. Any drug to be absorbed must be present in the form of solution at the site of absorption. Solubility is one of the important parameters to achieve desired concentration of drug in systemic circulation for achieving required pharmacological response [3]. To help circumvent problems associated with solubility, most cancer drugs are formulated with co-solubilizers. However, these solubilize agents often also introduce severe side effects, thereby restricting effective treatments and patient quality of life. A promising approach to addressing problems in anticancer drug solubility is their complexation with cyclodextrins (CDs) to form inclusion complexes [4, 5].

CDs comprise a family of cyclic oligosaccharides produced by enzymatic degradation of starch. They are crystalline, homogeneous, and nonhygroscopic substances. CDs derive their system of nomenclature from the number of glucose residues in their structure, such that the glucose hexamer is referred to as α-CD, the heptamer as β-CD and the octomer as γ-CD [6] (Fig. 1). Because of the large number of hydroxyl groups on CDs, they are water-soluble. The water solublities of α-CD, β-CD and γ-CD are approximately 14.5 g/100 ml, 1.85 mg/100 ml and 23.2 g/100 ml (25 °C), respectively [7]. The lower solublity of β-CD compared with α-CD and γ-CD is due to the formation of an internal hydrogen-bond between the C-2-OH group of one glucopyranoside unit and C-3-OH group of the adjacent glucopyranose unit. In the β-CD molecule, a complete secondary belt is formed by these hydrogen-bonds, therefore, the β-CD is a rather rigid structure. However, the hydrogen-bond belt is incomplete in the α-CD molecule. Only four hydrogen-bonds rather than six can be established because one glucopyranose unit is in a distorted position. The γ-CD is a non-coplanar, more flexible structure; therefore, it is more soluble than α-CD and β-CD [8]. CDs are widely used as complexing agents for lipophilic and amphiphilic substances and functional excipients that have gained widespread attention because of their ability to solubilize, and in some instances stabilize poorly water-soluble drug candidates enabling both oral and parenteral formulation.

CDs are presently manufactured globally on an industrial scale. The raw material for CD (α-CD, β-CD and γ-CD) production is the readily available carbohydrate polymer starch. β-CD is the most commonly employed CDs in the pharmaceutical formulations. The reason for this lies in the ease of its production and subsequent low price. More than 10,000 tons of β-CD are produced annually with an average bulk price of approximately 5 USD per kg [9]. However, the low aqueous solubility and nephrotoxicity limited the use of β-CD especially in parenteral drug delivery [10]. A universal solution to this problem was found in the substitution of multiple β-CD hydroxyls on both rims of the molecule. Many CD derivatives have been synthesized. These derivatives usually are produced by aminations, esterifications, or etherifications of the primary and secondary hydroxyl

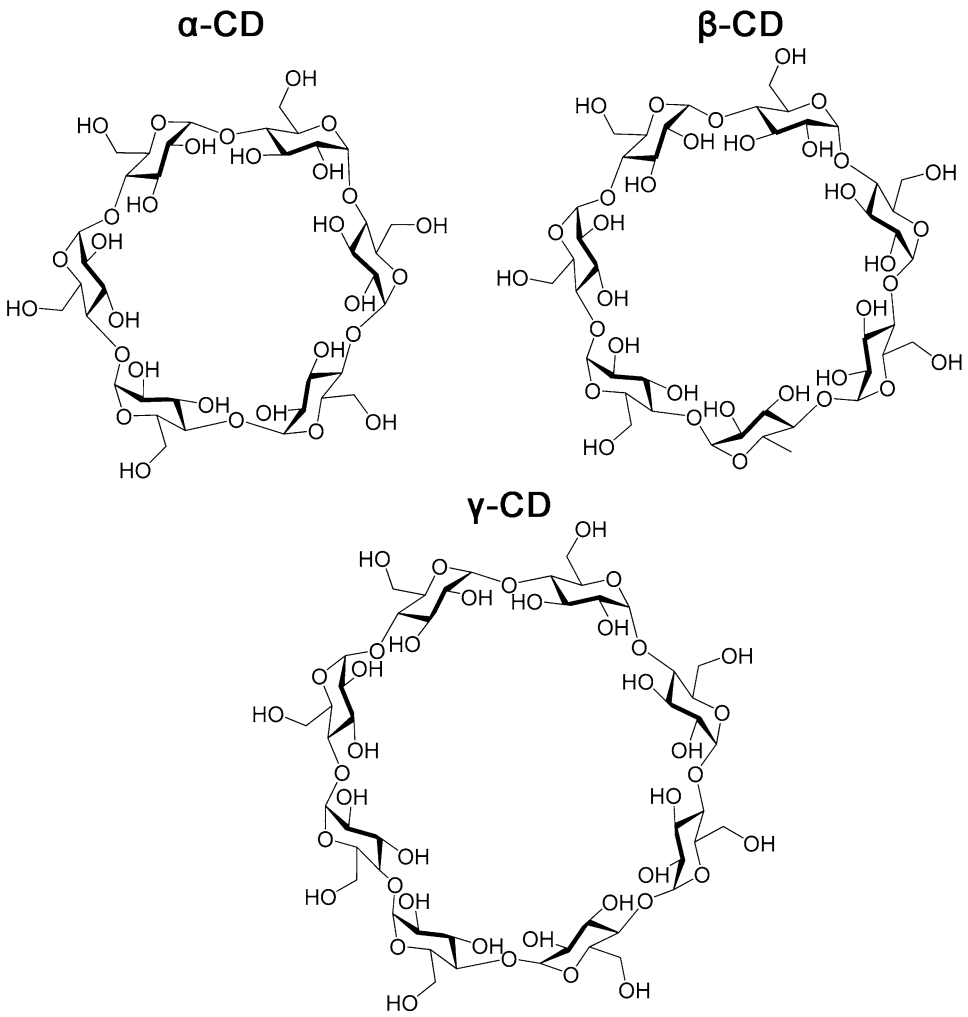

Fig. 1 Chemical structure of α-CD, β-CD and γ-CD

groups. These chemical modifications caused breakage of intramolecular hydrogen bond construction resulted in a notably improved aqueous solubility. Moreover, some derivatives, such as 2-hydroxypropyl-CD (HP-β-CD) and sulfobutylether-CD (SBE-β-CD), possess improved toxicological profiles in comparison to their parent CDs. Because of these advantages, substituted CDs cover more than 1/3 of all CD-containing medicines.

Due to the chair formation of the glucopyranose units, CD molecules are shaped like cones with secondary hydroxy groups extending from the wider edge and the primary groups from the narrow edge (Fig. 2a). The large number of hydroxyl groups on the outer surface make them water soluble [11]. The cavity is lined by the hydrogen atoms and the glycosidic oxygen bridges. Therefore, the interior of the cone is relatively apolar and creates a hydrophobic microenvironment. The hydrophilic cavity exteriors and hydrophobic interiors of CDs are responsible for their aqueous solubility and ability to encapsulate lipophilic moieties within their cavities. The incorporation of 'guest' molecules in CD (host molecule) inclusion complexes in aqueous media has been the basis for most pharmaceutical applications [6] (Fig. 2b). The formation of inclusion complex is possible with entire drug molecule or only a portion of it. One, two, or three CD molecules contain one or more entrapped "guest" molecules. Most frequently the host: guest ratio is 1:1. The ability of CDs to form inclusion complexes is the function of steric as well as thermodynamic factors [7, 12]. If the guest is the wrong size, it will not fit properly into the CD cavity. Because of cavity size, α-CD can typically complex low molecular weight molecules or compounds with aliphatic side chains, whereas β-CD is appropriate for aromatics and heterocycles and γ-CD can accommodate larger molecules such macrocycles and steroids [13]. No covalent bonds are broken or formed during the complex formation, and drug molecules in the complex are in rapid equilibrium with free molecules in the solution [13, 14]. The driving forces for the complex formation include release of cavity-bound enthalpy-rich water molecules, electrostatic interactions, van der Waals' interactions, hydrophobic interactions, hydrogen bonding, release of conformational strain and charge-transfer interactions. In bulk aqueous solution, the repulsive interactions between the hydrophobic guest and the aqueous environment are very strong, the lipophilic cavity of CD molecules provides a microenvironment into which appropriately sized non-polar moieties can enter to form inclusion complexes. Enthalpy-rich molecules are displaced by more hydrophobic guest molecules present in the aqueous solution to attain an apolar–apolar association. A reduction of the repulsive interactions between the lipophilic guest and the aqueous environment and decrease of CD ring strain result in a more stable lower energy state [15, 16].

Inclusion in CDs exerts a profound effect on the physiochemical properties of guest molecules. These effects are as follows: solubility enhancement of poorly soluble molecules, bioavailability improvement, stabilisation of labile guests against degradative effects of oxidation, radiation and heat, reduction of irritation, prevention of incompatibility of drugs or inactive ingredients, taste modification by masking off flavors, unpleasant odors and controlled release of drugs

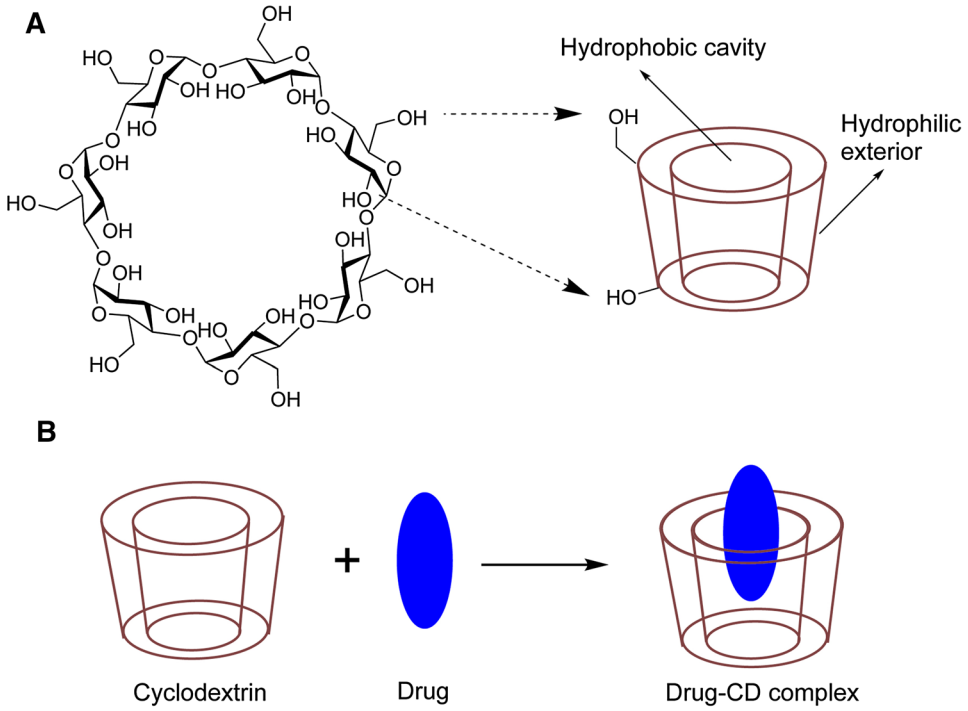

Fig. 2 **a** The architecture of CD and hydrophilic/hydrophobic regions of the CD molecules; **b** Schematic illustration of the association of CD and drug to form drug-CD complex

and flavors [17–22]. Therefore, CDs are widely used in the pharmaceutical, agrochemical, food and cosmetic industries [23].

Although CDs have many advantages, they must be biocompatible and safe when considered for use in pharmaceutical formulations. The toxicities of CDs are dependent on their route of administration. Many studies have shown that orally administered CDs of pharmaceutical interest are practically nontoxic due to their lack of absorption from the gastrointestinal tract [24, 25]. Furthermore, a number of safety evaluations have shown that α-CD, HP-β-CD, SBE-β-CD, γ-CD and HP-γ-CD appear to be safe even when administered parenterally. HP-β-CD and SBE-β-CD are available in FDA-approved products for human use and some parenteral injections. For instance, itraconazole parenteral injection (containing HP-β-CD, 40% w/v) has been commercialized in the United States and Europe [26]. Due to their nephrotoxicity, β-CD and methylated β-CD, such as RM-β-CD are not suitable for parenteral administration. Although α-CD can be found in parenteral formulations, its concentrations are very low. RM-β-CD is currently only used in topical or nasal drug formulations at relatively low concentrations [9]. Cyclodextrins can be found listing in several Pharmacopoeias and European Medicines Agency give the suggested thresholds of CDs for safety use (Table 1).

The purpose of this review is to discuss and summarize some of the potential findings and applications of CDs for the treatment of cancer. First, the use of CDs as anticancer agent and the possible mechanism to inhibit cancer cell growth are discussed. Recent achievements in the study of CDs complexation with anticancer drugs are included in the second part. The third part of the review is devoted to various aspects related to the utility of CDs based carriers for effective delivery of anticancer drugs.

Table 1 Suggested thresholds (TH) above which adverse effects may occur [27]

CD/route	α-CD	β-CD	γ-CD	RM-β-CD	HP-β-CD/SBE-β-CD[a]
Oral tox					
PDE, mg/kg/day	120	**10**	**200**	N	**160**
TH adult	120	**10**	**200**	–	**160**
TH neonate	12	**1**	**20**	–	**16**
Nasal					
Safe solution, %	N	1.5	N	**10**	10
TH adult	–	1.5	–	**10**	10
TH neonate	–	0.15	–	**1**	1
Rectal					
Safe amount mg/kg/day or %	N	**5**	N	N	**12%**
TH adult	–	**5**	–	–	**12%**
TH neonate	–	**0.5**	–	–	**1.2%**
Dermal					
Safe amount, %	N	**±0.1**	**±0.1**	N	**±0.1**
TH adult	–	**0.1**	**0.1**	–	**0.1**
TH neonate	–	**0.01**	**0.01**	–	**0.01**
Ocular					
Safe solution, %	<4	**±1**	N	**<5**	**10**
TH adult	1	**1**	–	**1**	**10**
TH neonate	0.1	**0.1**	–	**0.1**	**1**
Parenteral					
PDE, mg/kg/day	**0.2**	N	0.8	N	**300**
TH adult	**0.2**	–	0.8	–	**300**
TH neonate	**0.02**	–	0.08	–	**10**

Bold: used in medical products; N: no data indication for respective route of administration; ±: estimation based on properties

[a]Although the molecular weights of HP-β-CD and SBE-β-CD differ ca. 1.5 times, they can be taken together from a property- and toxicological point of view

CDs act as anticancer agents

Methylated-β-CD

Membrane lipids are known to be associated with cell death. CDs and their hydrophilic derivatives can form inclusion complexes with hydrophobic molecules. A number of studies have demonstrated that CDs can interact with cell membrane constituents such as cholesterol and phospholipids, resulting in the induction of hemolysis of human and rabbit red blood cells [28–30]. Methylated CDs are more hydrophobic and their affinity to cholesterol are stronger than the parent CDs themselves. Therefore, methylation of CDs lead to an increase in hemolytic activity [31]. The hemolytic activity of CDs is reported to be in the order of γ-CD<α-CD<HP-β-CD<β-CD<TM-β-CD (2,3,6-tri-O-methyl-β-CD)<DM-β-CD (2,6-di-O-methyl-β-CD) [25]. It is reported that cholesterol and sphingolipids in cell membrane are contributed to apoptosis via FasL/Fas and Bad, an apoptosis inducible factor of Bcl-2 family [32–34]. The depletion of cholesterol by M-β-CD led to a spontaneous clustering of Fas in the non-raft compartment of the plasma membrane, formation of Fas-FADD (Fas-associated death domain protein), activation of caspase-8, and apoptosis [35]. M-β-CD markedly caused apoptotic cell-death in KB cells, a human oral squamous carcinoma cell line, Ihara cells, a highly pigmented melanoma cell line, and M213 cells, a human cholangiocarcinoma cell line, through cholesterol extraction in cell membranes [36]. On the other hand, DM-β-CD and TM-β-CD could also induce apoptosis in A549 cells, an adenocarcinomic human alveolar basal epithelial cells, through the depletion of cholesterol in cell membranes. Apoptosis induced by DM-β-CD could result from the inhibition of the activation of PI3K-Akt-Bad pathway. α-CD, β-CD and γ-CD provided less cytotoxicity than methylated CDs [37].

In vivo studies demonstrated that the intraperitoneal injections of M-β-CD (800 mg/kg) once a week significantly suppressed tumor growth of MCF-7 cells xenograft in nude mice, compared to those of doxorubicin(2 mg/kg). The tumor volume of the xenografted mice (MCF-7 cell and A2780 cell) treated with M-β-CD was at least twofold reduced compared with the control group [38]. An intratumoral injection of the M-β-CD at a dose of 10, 50, and 100 mg/kg drastically inhibited the tumor growth of mice bearing Colon-26 carcinoma cells [36]. However, an intravenous administration of M-β-CD did not show any significant antitumor activity, probably due to the lack of target specificity against tumor cells and rapid renal clearance from body. Modification of M-β-CD with folic acid (FA) as a tumor targeting ligand could obtain a tumor cell-selectivity. Potent antitumor activity and cellular association of the FA modified M-β-CD (FA-M-β-CD) were higher than those of M-β-CD in KB cells, folate receptor (FR)-positive cells. The FA-M-β-CD drastically inhibited the tumor growth after intratumoral or intravenous injection to FR-positive Colon-26 cells bearing mice. The antitumor activity of FA-M-β-CD was comparable and superior to that of doxorubicin after both intratumoral and intravenous administrations, respectively, at the same dose (30 mg/kg), in the tumor bearing mice. Survival rate studies showed that all of the tumor-bearing mice after intravenous injection of FA-M-β-CD survived for at least 140 days without any relapse, while the mice treated with doxorubicin and M-β-CD died sickness within 70 days [39, 40].

Methylated-β-CDs showed potential therapeutic application in cancer therapy. The possible mechanism of cell death induced by Methylated-β-CDs is apoptosis through cholesterol depletion in cell membranes. It is reported that there is a positive correlation between the hemolytic activity of CDs and their capacity to solubilize cholesterol [37]. Meanwhile, Methylated-β-CDs are shown to have a relatively higher toxicity than the parent β-CD [25]. Thus, toxicological issues should be deeply investigated before Methylated-β-CDs are developed for cancer therapy.

HP-β-CD

HP-β-CD is available in registered oral, buccal, rectal, ophthalmic, and intravenous products [4]. Cholesterol provides signaling platform capable of activating various cellular signaling pathways [41]. Cholesterol accumulation and/or dysregulated cholesterol metabolism is reported in various malignancies [42–44]. HP-β-CD could reduce intracellular cholesterol and result in significant leukemic cell growth inhibition through G_2/M cell-cycle arrest and apoptosis. Single use of HP-β-CD effectively inhibits not only the growth of various leukemia cells, such as acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML), but also imatinib-resistant CML cell growth, including cells harboring the T3151 mutation [which are resistant to first- and second-generation tyrosine kinase inhibitor (TKIs)], and stem cell-like CML cells. Intraperitoneal injection of HP-β-CD treatment inhibited leukemic cell growth and significantly improved survival time in Ba/F3 BCR-ABL^{WT} and BV173-xenografted leukemic mice at the dose of 50 and 150 mM. The dosage required to inhibit tumor growth are relatively high. However, HP-β-CD is well tolerated and showed limited toxicity after intraperitoneal injections for 5 consecutive days every week for 13 weeks at the dose of 150 mM (2086.5 mg/kg). Meanwhile, M-β-CD-injected mice died of diffuse alveolar hemorrhage within 24 h of intraperitoneal administration at the same dose (150 mM) [45]. A study revealed that HP-β-CD is tolerable by the mice at the dose up to 10,000 mg/kg through intraperitoneal injection [46].

Although there are studies in which transport of CDs across the intestinal membrane has been reported, it is widely accepted that CDs are poorly absorbed from the gastrointestinal tracts follow oral administration due to their bulky and hydrophilic nature. Thus, it is suggested that CDs can be used in parenteral formulations. However, CDs are known to induce human erythrocytes to change their biconcave shape to monoconcave and at higher concentrations induce the lysis. The dosage of CDs required for tumor growth inhibition are relatively high. Low doses are required for safely use of CDs in parenteral formulations. HP-β-CD is more promising for parenteral use compared to Methylated-β-CDs. HP-β-CD has generally been found to be safe when administered parenterally in animals and humans. No adverse effects were observed in the human studies [11, 18, 25].

CDs complexation with anticancer drugs

The search for innovative medicines in disease management without compromising on safety and efficacy is a challenge. In the last few decades, many anticancer drug compounds were discovered and developed, most notably the newer chemotherapeutic agents or "chemo-drugs" such as taxanes (e.g.paclitaxel, docetaxel) and platinum-based drugs. In addition, some existing drugs originally indicated for non-cancer diseases have been "repurposed" for cancer treatment. However, many existing chemotherapeutic drugs, repurposed drugs and newly developed small-molecule anticancer compounds have high lipophilicity and low water-solubility. The lack of good aqueous solubility has been frequently identified as a key obstacle of the development and clinical use of these anticancer compounds [47–50]. The FDA and other drug organizations have defined a biopharmaceutical classification system (BCS) in which drugs are divided into four types on the basis of their solubility and permeability characteristics (Table 2). According to BCS, a drug is considered to be poorly water-soluble if its highest dose strength is not soluble in 250 ml or less of aqueous media over the pH range of 1–7.5 [6, 48]. Unfortunately, an estimated 40% of approved drugs and nearly 90% of molecules in the discovery pipeline are poorly water-soluble [51].

Table 2 Biopharmaceutical classification system (BCS) characterization of drugs based on solubility and permeability measures

BCS class	Solubility	Permeability
1	High	High
2	Low	High
3	High	Low
4	Low	Low

Since 1990s, the newer drug pipeline tends to have lower solubility resulting in an increase in poorly water-soluble BCS class 2 (i.e. generally aqueous solubility ≤ 0.1 mg/ml) compounds from ~30 to 50–60% and the corresponding decrease in water-soluble BSC class 1 compounds from ~40 to 10–20% [52]. Anticancer drugs are by nature toxic compounds, so IV infusion is often needed to achieve more predictable pharmacokinetics and reduced gastrointestinal toxicity. However, the aqueous solubility of anticancer drugs is typically in the microgram per milliliter range (e.g. paclitaxel < 0.3 µg/ml) [50]. These poorly soluble anticancer drugs are simply not suitable for this route of administration. Thus, a novel formulation with high solubility and the concomitant safety, efficacy and convenience is currently an unmet medical need for anticancer drugs.

Enhancement of solubility can be achieved by various means, such as solid dispersion, size reduction, formation of water soluble prodrug, use of co-solvents and/or surfactants and encapsulation into nanodelivery systems [53]. Cyclodextrin complexation is one of the most extensively investigated ways to improve drug solubility and bioavailability [51, 54]. Because of the large number of hydroxyl groups on CDs, they are water-soluble. Although the entire CD molecule is water soluble, the interior of the cavity is relatively apolar and creates a hydrophobic micro-environment. In an aqueous solution, the slightly apolar CD cavity is occupied by water molecules that are energetically unflavored (polar-apolar interaction), and therefore can be readily substituted by appropriate "guest molecules", which are less polar than water. The dissolved CD is the "host" molecule, and part of the "driving force" of the complex formation is the substitution of the high-enthalpy water molecules by an appropriate "guest" molecule. A dynamic equilibrium between free CDs, free drug molecules and their formed inclusion complexes is established and the dissociation of the CD/guest complex formed is governed by the thermodynamic equilibrium [6, 31]. The formation of inclusion complex with CDs leads to improvement in physicochemical properties and changing in absorption process of drug. As depicted in Fig. 3, once solid drug is delivered to the gastrointestinal tract as either free drug or complex with CDs, dissolution and permeation across the intestinal membrane must occur in order for the drug to be absorbed. Only a free form of the drug is capable of penetrating lipophilic barriers consisting of either mucosal or stratified cell layers and eventually entering the systemic circulation. Free drug with low solubility will precipitate if its concentration in solution is higher than its equilibrium solubility. CDs may enhance free drug concentration by slowing the precipitation of free drug. The inclusion of lipophilic drugs or certain lipophilic moieties into the CDs creates a hydrophilic shield that notably increases the drug solubility in the physiological fluids, promoting a fast dissolution of solid drug-CD complexes. Such complexes

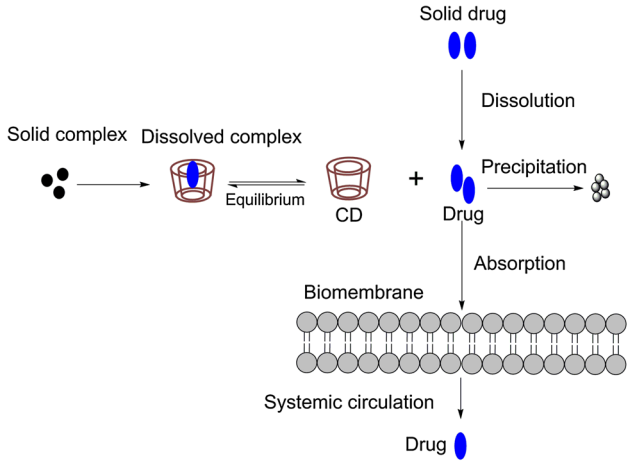

Fig. 3 Diagram of the process of systemic absorption of drug from its CD complex form or free form. Processes are influenced by the dissolution of drug and/or complex, precipitation of drug, complexation/decomplexation of drug and CDs, and absorption of drug

diffuse easier than the free drug through the aqueous layers at the surface of the mucosa, increasing the number of drug molecules available for permeation at the membrane surface. The drug molecules in complex are in rapid equilibrium with free molecules in the solution. It is generally accepted that neither the complex nor free CDs are absorbed to an appreciable extent. As the free drug permeates the membrane, progressive decomplexation occurs in order to replace the absorbed free molecules and maintain the equilibrium [18, 55, 56]. A number of anticancer drugs have been complexed with CDs and their derivatives to improve the solubility and stability, increase the bioavailability and dissolution, reduce the toxicity, and modify the physicochemical characteristics [57]. Table 3 enlists the complexation of various anticancer drugs with CDs and their derivatives.

The solubility of chemotherapeutics can be improved by complexation with CDs and their derivatives. However, the improvement of solubility may vary with drugs. For example, the solubility of camptothecin (CPT) is about 1.3 μg/ml, after complexation with HP-β-CD, the solubility increased to 43.69 μg/ml (about 33-fold) [62]. Meanwhile, complexation with HP-β-CD increased the solubility of barbigerone (an natural anti-cancer agent) from 0.5 μg/ml to 6.83 mg/ml, about ~12,000-fold [71]. In addition, solubility enhancement of antineoplastic agent by complexation may also vary with CDs. For instance, the solubility of CPT increased by approximately six-fold with 1.5% (w/v, near limiting solubility in water) β-CD in 0.02N HCl. In contrast, three- and five-fold solubility increase achieved with 2.5% α-CD and γ-CD, respectively. For modified CDs, DM-β-CD solubilized CPT to the greatest extent. At a 25% w/v concentration of DM-γ-CD, HP-β-CD and DM-β-CD, the solubility of CPT increased by factors of 9, 33 and 170, respectively [62]. The dramatically higher solubilizing effect of DM-β-CD

Table 3 Complexation of anticancer drugs with cyclodextrin and their derivatives

Drug	Treatment	Cyclodextrin	Solubility	Ref
Paclitaxel	Breast and ovarian cancer, Leukemia	HP-β-CD	Enhancement of aqueous solubility	[58]
		M-β-CD	Enhancement of solubility (from ~0.3 μg/ml to 5 mg/ml) and stability	[59]
		Tetraethylenepentaamino-bridged bis(β-CD-cyclodextrin)s	Water solubility is dramatically increased from less than 1 μg/ml to approximately 2.0 mg/ml	[60]
Methotrexate	Breast and skin cancer	β-CD	Increase in solubility and dissolution rate, enhancement of bioavailability and antitumor activity	[61]
camptothecin	Leukemia	DM-β-CD	Improvement of solubility (171 times) and stability, enhancement of cytotoxicity	[62]
		HP-β-CD	Increase in solubility and cytotoxicity	[62, 63]
Doxorubicin	Lymphoma and leukemia	HP-β-CD	Improvement of stability and reduction in photo-degradation	[64]
Cisplatin	Testicular cancer, ovarian cancer, and breast cancer	HP-β-CD	Increase in solubility and dissolution rate, improvement of stability	[65]
5-Fluorouracil	Colon, rectal and breast cancer	α-CD and β-CD	Improvement of solubility and increase in the anticancer activity	[66]
Lapatinib	Breast cancer	SBE-β-CD	Significant improvement in solubility (more than-600 fold)	[67]
curcumin	Neck cancer	HP-β-CD	Improvement of solubility and stability, enhancement of cytotoxicity	[68, 69]
		β-CD	Increase stability to light, pH and heating	[70]

compared to other CDs (α-CD, β-CD, γ-CD, DM-γ-CD and HP-β-CD) can be attributed to the methyl groups, which not only disrupt intramolecular hydrogen bonding, making DM-β-CD molecule highly soluble in aqueous solution, but also enlarge the whole cavity of the molecule by extending the secondary hydroxyl side and narrowing the primary hydroxyl side of the cone [72]. The relatively smaller change in the solubility of CPT by γ-CD and DM-γ-CD is probably due to their larger cavity size, allowing CPT molecule to readily move in and out of the cavity.

Interestingly, for some antineoplastics, an increase in anticancer activity can be achieved by complexation with CDs. For example, complexation of 5-fluorouracil with α-CD and β-CD is able to obtain a significantly ten-fold increase in the anticancer activity [66]. The improvement of 5-fluorouracil activity is probably ascribable to the enhancement of cellular membranes permeability due to the interaction between CDs and cholesterol contained in cell membranes. Albendazole is a benzimidazole derivative with potent anticancer activity in both experimental animals and in a pilot clinical trial. Complexation of albendazole with HP-β-CD increased the antiproliferative efficacy and reduced the number of OVCAR-3 colonies formed [73]. After complexation with DM-β-CD and HP-β-CD, the cytotoxicity of CPT inclusion complexes were almost two-fold more active than free CPT in THP-1 (human myeloid leukemia) cells. Another study reported the 9-nitrocomptothecin/HP-β-CD complex showed significant anti-tumor activity toward Skov-3 (human ovarian carcinoma), MCF-7 (human breast cancer), HeLa (human epithelial cervical cancer) and S180 (mice sarcoma) cell lines, with less than 1/2 IC_{50} values compared to that of free 9-nitrocomptothecin [74]. The increase in cytotoxicity of CPTs complexes can be attributed to the improved stability of free CPTs by complexation with CDs. Because the complexed form of CPT is less prone to hydrolysis and hence serves as a depot for continuous supply of CPT in its active form [62]. The increased anti-cancer activity for CPTs complex probably also ascribed to the enhanced membrane permeability and distribution of hydrophobic molecule into the cell nucleus.

Limited aqueous solubility, degradation in gastrointestinal fluids, short in vivo stability, poor intestinal permeabilities, and strong dose dependent side effects of promising anticancer drug candidates have long ben obstacles in treatment of cancer. The formation of inclusion complex with CDs leads to improvement in physicochemical properties of drug. Many chemotherapeutics have been complexed with CDs to improve/enhance the solubility and stability, increase the bioavailability and dissolution, reduce toxicity and modify the physicochemical characteristics [18, 57]. To date, one of the main obstacles in cancer treatment has been selective delivery of drugs to tumor site, a feat which holds great promise at enhancing drug therapeutic efficacy and lowering off-target toxicities. Most of the cytotoxic chemotherapeutic agents are distributed nonspecifically throughout the body and effect both the normal and tumor cells even complexed with CDs. To deliver chemotherapeutics to the tumor site, chemical modification of CDs with tumor targeting ligands or combination with nanotechnology could be two useful strategies.

CDs application in anti-cancer drug delivery system

Liposomes

Since first being described by English hematologist Alec Bangham and coworkers in 1961, liposomes have been recognized and extensively used as delivery vehicles for pharmaceuticals [75]. Liposomes are self-assembled small and spherical vesicles consisting of an aqueous core which is surrounded by one or more phospholipid bilayers. Because of their amphiphilic nature, liposomes are able to entrap hydrophilic drug in the aqueous phase and hydrophobic drug in the lipid bilayer and retain drugs in route to their destination [76]. Liposomes are usually made of natural, biodegradable, non-toxic and non-immunogenic lipid molecules. They have been used as artificial model membranes and as carriers to deliver active molecules in vivo. Liposomes offer an excellent opportunity to selective drug targeting which is expected to optimize the pharmacokinetic parameters and pharmacological effects, prevent local irritation, and reduce the drug toxicity [77]. However, accommodation of lipophilic compounds in the lipid phase can be problematic, as some drugs can interfere with bilayer formation and stability, thus limiting the range and amount of valuable drugs that can be associated with liposomes. Accommodation of water-insoluble drugs in the lipid bilayer of liposomes is often limited in terms of drug to lipid mass ratio. Entrapping water soluble drug/CDs inclusion complexes in the aqueous phase of liposomes has been proposed in order to avoid such drawbacks [78].

The concept of entrapping CD-drug complexes into liposomes combines the advantages of both CDs (such as increasing the solubility and stability of drugs) and liposomes (such as passive targeting of drugs and prolonged circulating time) into a single system and thus circumvents the problems associated with each system [22]. By forming water-soluble complexes, CDs would allow insoluble drugs to accommodate in the aqueous phase of vesicles (Fig. 4) and thus potentially increase drug-to-lipid mass ratio levels, enlarge the range of insoluble drugs amenable for encapsulation, allow drug targeting and reduce drug toxicity [79]. Problems associated with intravenous administration of CD complexes such as their rapid removal into urine and toxicity

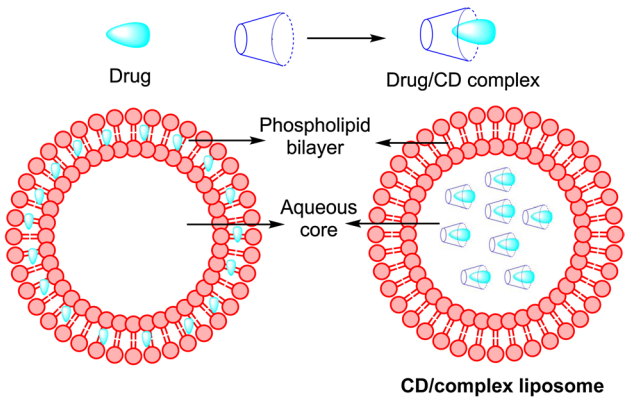

Fig. 4 Schematic illustration of conventional and CD based liposomes

to kidneys, especially after chronic use, can be circumvented by their entrapment in liposomes [80].

CDs has been shown to increase the entrapment of a series of anti-cancer agents. For instance, the encapsulation efficiency for barbigerone (an anticancer agent extracted from *Tephrosia barbigera*) liposome was 12.78%. By forming inclusion complex (Bar/HP-β-CD), more than four-fold increase in encapsulation efficiency could be achieved [81]. Similarly, the encapsulation efficiency of Ro-282653 (a synthetic MMP inhibitor) was improved when formulated as HP-β-CD inclusion complexes entrapped in liposomes [82]. Dhule et al. reported that the entrapment of curcumin increased from 30% in CD-free liposomes to 50% when HP-γ-CD-curcumin-complex was used, the antiproliferative activity of curcumin was increased by HP-γ-CD complexation and encapsulation into liposomes [83]. CDs can modulate in vivo dissociation of the drug/CD complex by forming complexes/liposomes, thereby contributing to improvements in the pharmacokinetic profile, therapeutic efficacy and reduced toxicity of the drugs. Hagiwara et al. reported that γ-CD enhanced the stability of liposomes in fetal calf serum (FCS) through complexation of doxorubicin with γ-CD. The cellular release of doxorubicin from colon-26 cells, a mouse rectal carcinoma cell line after cellular uptake of PEGylated liposomes entrapping the doxorubicin complex with γ-CD was markedly slower than that in PEGylated liposomes entrapping doxorubicin alone [84]. When injected to mice bearing colon-26 tumor cells, the plasma doxorubicin levels after intravenous injection in the γ-CD/complex-in-liposome system were significantly higher than those in the doxorubicin-in-liposome system. The $t_{1/2}$(half-life) of the plasma doxorubicin level in the γ-CD/complex-in-liposome system was 21.5 ± 1.6 h, while 15.4 ± 1.1 h in doxorubicin-in-liposome system. γ-CD/complex-in-liposome system provided superior accumulation of doxorubicin in solid tumors to doxorubicin-in-liposome. The AUC value of doxorubicin in solid tumors up to 72 h in the γ-CD/complex-in-liposome system was higher approximately 2.3 times than that of the doxorubicin-in-liposome system. γ-CD/complex-in-liposome provided a decrease in side effects of doxorubicin and the inhibitory effect on the tumor growth more than those of doxorubicin-in-liposome and doxorubicin solution. Treatment with γ-CD/complex-in-liposome elicited the survival time longer than those of doxorubicin-in-liposome and doxorubicin [85]. Cui et al. developed stable PEGylated liposomal vincristine formulation using sulfobutyl ether cyclodextrin (SBE-β-CD). The SBE-β-CD modified liposome was more able to stabilize entrapped vincristine than normal liposome. The vincristine half-life of SBE-β-CD modified liposome was considerably longer than that of the normal liposome (67.3 vs. 27.6 h). The SBE-β-CD modified liposome formulations exhibited enhanced anti-tumor effects and reduced toxicity [86]. Similarly, nanoencapsulation of LPSF/AC04-HP-β-CD inclusion complexes into liposomes enhanced the drug antiproliferative activity (two fold stranger than that of LPSF/AC04-loaded liposomes) [87].

CDs based drug conjugate

CDs-drug conjugate

CD-drug conjugate was first designed for site-specific delivery of drugs to the colon. Colon targeting is essentially classified as a delayed release with a fairly long lag-time, because the time required to reach the colon after oral administration is expected to be about 8 h in human [88]. When a CD complex is given orally, it will readily dissociate in the gastrointestinal fluid. This indicates that CD complexes are not suitable for colon-specific delivery due to release of the drug before it reaches the colon. However, when a drug conjugated with CD covalently, it can survive passage through the stomach and small intestine and reach the cecum and colon in an intact form, and release drugs after enzymatic actions of microflora existing in these lower intestinal tracts [89]. Udo et al. synthesized 5-Fluorouracil-1-acetic acid-β-CD (5-FUA-β-CD) conjugates through ester or amide bond. 5-FUA-β-CD ester conjugate may survive passing through stomach and small intestine and release 5-FUA preferentially in cecum and large intestinal tracts of rats after fermentation of β-CD to small oligosaccharides. On the other hand, the 5-FUA-β-CD amide conjugate do not release the drug even in the cecum and colon, despite the ring-opening of CDs by bacterial enzymes [90]. When conjugated with CDs, the aqueous solubility and anti-cancer activity were usually higher than the free drug alone. Scutellarin was covalently bound to the primary hydroxyl groups of β-CD. The aqueous solubility of the conjugate was much

higher than that of scutellarin (>40 times increase). The conjugate presents more satisfactory antiproliferative activity than free scutellarin in colon cancer cell line HT-29, SW480, Lovo and HCT116 [91]. Similar results were reported for artesunate-β-CD conjugate with a >26-fold increase in solubility and about two-fold decrease in IC_{50} values in colon cancer cell lines HCT116 and SW480 [92]. A more significant increase in aqueous solubility was obtained for Rhein-β-CDs conjugates. The solubility of Rhein-β-CDs conjugates rose up to ~1 mg/ml from 0.0052 mg/ml (Rhein) and the cytotoxicity of the conjugates against hepatocellular carcinoma (HepG2) were also improved [93]. The improvement in water solubility is attributable to the phase change from a crystalline state of drugs with lower solubility to an amorphous state of the conjugates with higher solubility [91]. However, the mechanisms for the increase in anti-cancer activity of drug-CD conjugates are unconfirmed. Previous studies reported the β-CD cavities of the drug-CD conjugates had the capability of including cholesterol or lecithin of the damaged cell membranes [94, 95]. It has been known that the junction gap in the cancer cell membrane was loose due to the enhanced permeability and retention (EPR) effect, which allowed the conjugates to include cholesterols of the cell membrane. Therefore, the drug molecule was displaced competitively by the cholesterols out of the cavity and was hydrolyzed by peptidases in endosomes from the conjugates to release into the cancer cells. The ability of drug-CD conjugates to release free drug molecule into cancer cells was supported by Jiang's studies [91–93]. To maximize therapeutic effects, folic acid modified drug-CD conjugates has been developed. Folate is a known target ligand in the treatment of malignant cancers that overexpress folate receptor (FR) [96]. Mizusako et al. developed a folate-based CD-doxorubicin conjugate (per-FOL-β-CD-ss-DOX), which has folic acid molecules covalently attached at the end of primary hydroxyl groups of β-CD. The per-FOL-β-CD-ss-DOX exhibited a significant higher cellular uptake and a remarkable greater cytotoxic effects against doxorubicin-resistant cells as compared with the free doxorubicin [97].

CDs-polymer-drug conjugate (CDP-drug conjugate)

Cylcodextrin-based polymers (CDPs) contain alternating repeat units of β-CD and polyethylene glycol (PEG) with two carboxylate groups per repeat unit for drug conjugation. PEG is often used in pharmaceutical applications to increase the solubility, stability and plasma half-life of drugs [98]. The CDPs is highly water soluble and neutrally charged when fully conjugated with drug through various linkers. The resulting CDP-drug conjugate, in the absence of drug, is biocompatible with no observable side effects or immune responses at intravenous dose up to 240 mg/kg in mice [99]. Several antineoplastic drugs such as CPT and tubulysin has been attached to CDP through various linkers. The chemical structure of CPT includes an unstable lactone ring that is highly susceptible to spontaneous and reversible hydrolysis, which yields an inactive, but more water soluble, carboxylate form that predominates at physiologic pH. When covalently conjugated to the CD-PEG polymer, the resulting CDP-CPT conjugate (CRLX101, or IT-101, Fig. 5) successfully stabilizes the labile lactone ring of CPT in its closed, active form and prevents premature inactivation by pH-mediated ring-opening upon systemic administration [100]. The aqueous solubility of CRLX101 increased more than three orders of magnitude compared with CPT [101].

One of the unique features of CDP-drug conjugate is that the CD blocks form inclusion complexes with hydrophobic small-molecule drugs through both intra- and intermolecular interactions. Such interactions between adjacent polymer strands catalyzed the self-assembly of several CD-PEG polymer strands into highly reproducible nanoparticles [100]. These CDP-drug conjugate nanoparticles dramatically change the pharmacokinetics (PK) and biodistributions of free drugs. For instance, pharmacokinetics of CRLX101 had a larger area under the curve, lower volume of distribution, longer terminal half-life, and slower systemic clearance. In animals that were dosed with CRLX101, plasma concentrations of released CPT stayed well below those of conjugated-CPT by a factor of >30-fold at all time points, and the long-terminal half-life was 13–20 h in mice and rats, respectively. This was in contrast to the PK of CPT alone, which showed a high volume of distribution and short terminal half-life of 1.3 h. Tumor concentrations of active CPT released from the conjugate were more than 160-fold higher when administered as a CDP-CPT conjugate rather than as CPT alone. These illustrated that CRLX101 is an effective drug depot, providing a slow, continuous drug supply while remaining in circulation and accumulating in target tissues [102, 103]. The changes in pharmacokinetics is key to achieve the desired improvements in pharmacodynamics (PD) and, ultimately, therapeutic index. CPT essentially has no therapeutic window and its development was abandoned due to excessive toxicity. CRLX101, in contrast, has shown to be highly active in multiple human subcutaneous and disseminated cancer models. One treatment cycle of 3 weekly doses of CRLX101 resulted in significant antitumor activity that was superior to irinotecan or topotecan, two small-molecule analogs of CPT which have been used clinically [100, 104]. Furthermore, CRLX101 significantly prolonged the survival of animals bearing s.c. and disseminated human cancer xenografts when compared with irinotecan at its maximum tolerated dose (MTD) in mice [105].The increase in therapeutic index was even more impressive for tubulysin A, showing a >100-fold increase in MTD (6 mg/kg versus 0.05 mg/kg). The CD-PEG-tubulysin A conjugate showed equal or superior efficacy compared with vinblastine and paclitaxel

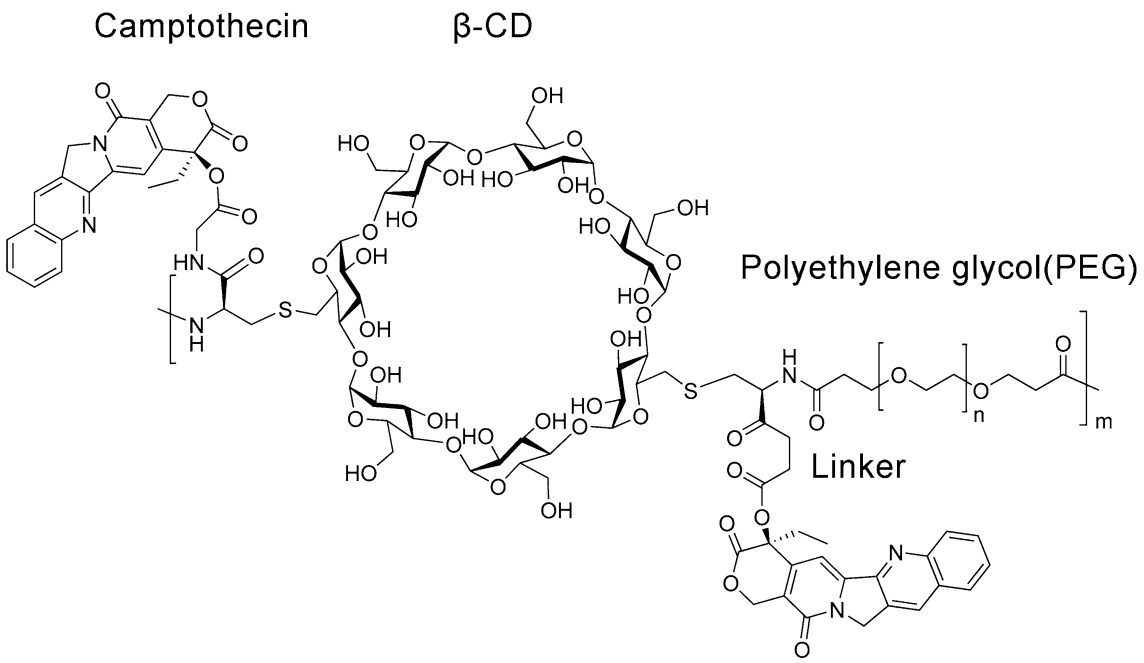

Fig. 5 Chemical structure of CRLX101

reference treatments with minimal observed toxicity. A single treatment cycle of 3 weekly doses of CD-PEG-tubulysin A conjugate showed a potent antitumor effect and significantly prolonged survival compared with tubulysin A alone [106]. These preclinical studies of CDP-drug conjugate have confirmed that these CD based conjugation technology has successfully conferred superior pharmacokinetics, enhanced pharmacodynamics, and increased efficacy. These biological properties have a strong potential to translate therapy into significant impact on clinical outcome. Actually, a phase 1/2a study of CRLX101 has been completed and a randomized phase II study in patients with advanced NSCLC has been initiated [107]. Results from these upcoming studies will be critical for establishing the potential of CRLX101 as a new oncology agent.

CDs based nanoparticles

One of the most attractive areas of research in drug delivery system is the design of nanomedicines consisting of nanosystems that are able to deliver drugs to the right place, at appropriate time. Nanoparticles can be formed from inorganic and polymer materials. Polymeric nanoparticles are more desirable because they can be chemically designed to be biodegradable and biocompatible. Over the past few decades, nanoparticle delivery systems have been developed using polymers like alginate, chitosan, gelatin or synthetic polymers such as poly(lactic acid), poly(lactide-*co*-glycolide) and poly-ε-caprolactone [108]. However, these polymeric nanoparticles also display an important drawback related with their low capability to encapsulate lipophilic drugs. Recently, CDs have been included in polymeric nanoparticle systems to increase the loading capacity of nanoparticles. For example, paclitaxel is an antineoplastic with a proven activity against a number of tumors including lung, neck and advanced ovarian cancers. However, paclitaxel is highly lipophilic and insoluble in water. Amphiphilic CD (6-O-CAPRO-β-CD) was developed as nanodelivery system for paclitaxel. 6-O-CAPRO-β-CD was CD derivative modified on the primary face with 6C aliphatic ester. The major advantage of amphiphilic CDs is their self-assembly properties which are sufficient to form different nanosized carriers spontaneously without the presence of surfactants along with their demonstrated ability of forming inclusion complexes with various drugs in their cavity and within the long aliphatic chains [109, 110]. Nanospheres and nanocapsules could be made directly from the formation of inclusion complex between paclitaxel and 6-O-CAPRO-β-CD by the nanoprecipitation technique. High encapsulation efficiency with a three-fold increase was achieved in the loading capacity of nanoparticles when formed directly from the inclusion complex. Prolonged release rates for paclitaxel up to 12 h for nanospheres and 24 h for nanocapsules were obtained [111]. Agüeros et al. reported that poly(anhydride) nanoparticles were not capable to efficiently incorporate paclitaxel and displayed low drug loading values (less than 1 μg/mg nanoparticles). On the contrary, when paclitaxel was incorporated

in the nanopraticles as a complex with HP-β-CD, the drug loading was found to be close to 170 μg/mg nanoparticles (500-fold higher than in the absence of HP-β-CD) and the permeability of paclitaxel was found to be about 12-times higher than when the drug was formulated as Taxol® [112]. CPT is highly insoluble and instable under physiological conditions. Various nanoparticulate drug delivery system with CPT have been tried and the mean drug content was less than 2%. When nanoparticles were prepared from preformed inclusion complexes of CPT with CDs, the drug loading values were significantly increased when compared with those for PLGA and PCL nanoparticles with CPT alone. The release of CPT extended up to 12 days for CDs complex nanoparticles and 48 h for conventional polymeric nanoparticles. Cytotoxicity studies against MCR-7 breast adenocarcinoma cells revealed that the CD nanoparticles showed higher anticancer efficacy than PLGA and PCL nanoparticles loaded CPT and free CPT [113].

Docetaxel is being used in clinical for the treatment of prostate and breast cancers as well as lung carcinoma but faces difficulties as dosage formulation during intravenous delivery. a nanoparticulate system made of the amphiphilic CD derivative heptakis (2-O-) oligo (ethyleneoxide)-6-hexadecylthio-)-β-CD (SC16OH) was developed for incorporate of docetaxel with almost 100% encapsulation efficiency of the drug. Release studies showed a prolonged release up to 8 weeks for docetaxel loaded nanoparticles as compared with docetaxel commercial formulation Taxotere® (24 h). Superior cell killing and cell damage were observed for docetaxel-loaded SC16OH nanoparticles in comparison with unloaded docetaxel, which can be attributed to the controlled and prolonged release of the drug from the nanoparticles causing cell arrest during mitosis. Hemolytic studies performed on human red blood cells showed that docetaxel-loaded SC16OH nanoparticles were about 10 order of magnitude less toxic than Taxotere® [114]. In another example, mucoadhensive nanoparticles composed of M-β-CD in combination with poly(isobutylcyanoacrylate) and coated with thiolated chitosan were developed to enhance the intestinal permeability of docetaxel. Permeation studies performed on rat intestine using the Ussing chamber model showed that the intestinal permeation of docetaxel loaded into nanoparticles was found to be improved in comparison with ethanol control solution of docetaxel [115].

In another study, chitosan-CD nanospheres with a size less than 300 nm were developed by in situ formation through Michael addition of N-maleated chitosan and per-6-thio-β-CD sodium salt for delivery of anticancer drug, doxorubicin, via inclusion complex formation between doxorubicin and β-CD moiety. The resulting nanospheres exhibited a high encapsulation efficiency for doxorubicin. The released of doxorubicin from the nanospheres could be effectively sustained and the drug loaded nanospheres exhibited efficient inhibition on HeLa cells [116]. The polymer-associated CD (β-CD-epichlorohydrin crosslinked polymer) has shown to be able to load doxorubicin and artemisinin by spontaneously forming nanoparticles in aqueous solution. The polymer-associated CD was able to disrupt the doxorubicin dimer, which is the predominant form of doxorubicin in solution. Spectroscopic and photophysical studies of the complexes evidenced an alcohol-like environment for artemisinin and an improved inherent emission ability for doxorubicin in the nanoparticles [117].

CDs based siRNA delivery system

Gene silencing by RNA interference (RNAi) technology has recently gained widespread attention in pharmaceutical biotechnology in view of its ability to mitigate diseases such as cancer, viral diseases and genetic disorders. RNAi is triggered by siRNA (short interfering RNA), which is a double stranded RNA (ds RNA) consisting of two 21–22 bp complementary strands. siRNAs are one of the promising carriers for delivery of anticancer drugs. However, the delivery of siRNA remains a major challenge, due to the difficulty in its cellular uptake. Naked siRNA has a biological half-life of less than 1 h in human plasma and are rapidly excreted by the kidney without penetrating through the cellular phospholipid membrane [118]. Thus, to increase the life time and improve its therapeutic efficacy, non-viral vectors have been developed. Polymeric nanoparticles have been designed as siRNA carriers to attain the optimal activity, improved stability, increased plasma half-life, tissue or cell specificity and improved cellular penetration. Nevertheless, the positively charged siRNA-encapsulated nanoparticles tend to aggregate and increase their hydrodynamic radius and decrease their ability to penetrate into cells [119]. CDs and PEG may be used to reduce the surface charge and nanoparticle aggregation [120]. So far, CD polymer, CD polyrotaxane and CD/dendrimer conjugate have been developed as gene and siRNA carriers for the treatment of cancer. Currently, CD-modified dendritic polyamines (DexAMs) were developed as a multicomponent delivery vehicle for translocating siRNA and anticancer drugs to achieve a synergistic therapeutic effect. β-CD presented in DexAMs facilitated complexation and intracellular uptake of hydrophobic anticancer drugs-Suberoylanilide hydroxamic acid and Erlotinib, whereas the cationic polyamine backbone allowed for electrostatic interaction with negatively charged siRNA. The DexAMs complexes had minimal cytotoxicity over a wide range of concentrations and efficiently delivered siRNA, thereby silencing the expression of targeted genes. Co-delivery of siRNA-EGFRvIII and SAHA/Erlotinib in glioblastoma cells significantly inhibited cell proliferation and induced apoptosis compared to individual

treatments [121]. In another study, a hepta-guanidino-β-CD associated anisamide-terminated PEG conjugate (G-CD-PEG-AA) was synthesized as a delivery vector for siRNA. The PEGylated chain masked the cationic charge and facilitated the attachment of the targeting group anisamide. The G-CD-PEG-AA siRNA formulations induced prostate cell-specific internalization of siRNA resulting in approximately 80% knockdown of the reporter gene. Intravenous administration of the G-CD-PEG-AA siRNA formulations showed significant tumor inactivation with corresponding reductions in the level of vascular endothelial growth factor (VEGF) mRNA with reduced toxicity when studied in a mouse prostate tumor model [122]. Antibody-targeted CD-polymer nanoparticles (CD.DSPE-PEG-Fab) have also been studied for siRNA delivery in the treatment of acute myeloid leukemia (AML). This system consist of Fab as the targeting ligand for binding to the IL-3 α-chain (IL3Rα, also known as CD123, a cell surface antigen for human AML LSCs), achieved antigen-mediated cellular uptake in KG1 cells (an AML leukemia stem and progenitor cell line). Efficient delivery of bromodomain-containing protein 4 (BRD4) siRNA using the targeted formulation resulted in downregulation of the corresponding mRNA and protein in KG1 cells and in ex vivo primary AML patient derived samples. The resulting silencing of BRD4 induced myeloid differentiation, triggered leukemia apoptosis and reduced blast proliferation. In addition, the targeted formulation achieved synergistic therapeutic effects when combined with the clinically available chemotherapeutic cytarabine (Ara-C). Interestingly, the targeted formulation achieved superior therapeutic effects in relapsed AML samples where IL3Rα is highly expressed compared to newly diagnosed AML where IL3Rα is partially expressed [123].

Within the gene delivery field, CD-based vectors are emerging as promising novel candidates for the safe and effective delivery of siRNA to target sites. The presence of several different variants with a wide range of physicochemical and biological properties is a major asset of these complexes. Among the CD based polymeric carriers for siRNA, CD-containing polycations for the targeted delivery of siRNA developed by Davis group are an excellent choices as a delivery system since these have almost reached the clinical success. One of their products based on a CD, that is CALAA-01, has entered clinical trials for solid tumors [118]. This system consist of a CD-containing polymer, PEG for stability, and human transferrin as the targeting ligand for binding to transferrin receptors, which are often overexpressed on cancer cells. Removal of the targeting ligand or the use of a control siRNA sequence eliminates the antitumor effects [124]. The siRNA in CALLA-01 was designed to inhibit tumor growth via a mechanism to reduce expression of the M2 subunit of ribonucleotide reductase (RRM2), a protein involved in DNA replication whose function is required to complete cell division [125]. This multi-component delivery system provides low toxicity when administered intravenously to patients. No abnormalities in interleukin-12 and INF-α, liver and kidney function tests, complete blood counts, or pathology of major organs are observed from long-term, low-pressure, low-volume tail-vain administration performed on a murine model of metastatic Ewing's sarcoma [124]. Preclinical safety and efficacy testing performed in non-human primates (cynomolgus monkeys) revealed that good tolerability at the 3 and 9 mg/kg dose levels, in the range for antitumor effects had been observed [126]. A twice-weekly dosing regimen of CALAA-01 yielded a significant reduction in tumor burden in mouse subcutaneous tumor models including liver and melanoma, at the dose levels in the range of 2.5–10 mg/kg [100, 127]. In phase I clinical trials, patients with solid cancers refractory to standard-of-care therapies were administered targeted nanoparticles via IV infusion on days 1, 3, 8 and 10 of a 21-day cycle. Successful delivery of targeted nanoparticles was confirmed by the presence of intracellular nanoparticles in tumor biopsies from melanoma patients after treatment. siRNA cleavage occurred specifically at the site predicted for an RNAi mechanism from a patient who received the highest dose of the nanoparticles. Furthermore, reduction of both the specific messenger RNA (RRM2) and the protein levels were confirmed in tumor biopsies from these patients by quantitative reverse transcription-polymerase chain reaction (Qrt-PCR) and by immunohistochemical staining in the patients treated with the highest dosage (30 mg/m^2). No dose limiting toxicities (DLTs) observed in five dose escalations (3, 9, 18, 24 and 30 mg/m^2). Pharmacokinetics indicate relatively fast clearance, consistent with preclinical findings, and some transient elevations of cytokines (IL-6, IL-10, and TNF-α) were seen. More importantly, the first evidence of the RNAi mechanism in humans (for any siRNA) and the first evidence of dose-dependent tumor accumulation of targeted nanoparticles injected systemically in humans (for any nanoparticles) have been observed in this study [128, 129]. These studies demonstrated that siRNA administered systemically to humans may result in specific gene inhibition by an RNAi-mediated mechanism and the potential of CALAA-01 for human cancer treatment.

Future prospects CDs based anti-cancer drug delivery system

Various nanotherapeutic approaches have been developed for delivery of anticancer drugs. Nevertheless, none of the available treatments for cancer is safe, effective, and able to treat the disease completely. Most of these are expensive, unacceptable, and inconvenient for long-term use or associated with significant toxicity. Introducing CDs into

nano-delivery system may render enhanced biocompatibility and drug loading capacity, reduced toxicity and prolonged the existence of the drug in systemic circulation, as partly demonstrated by intensive studies on CD-containing nanocarriers. Recent advances in cyclodextrin chemistry and self-assembly nanotechnology make it possible to design diverse nano-delivery system such as liposomes, general nanoparticles, micelles as well as highly complex superstructures and multifunctional systems simply by combinatorial strategy, taking full advantage of host–guest interactions and other non-covalent forces by utilizing CDs or CD derived materials. However, lack of toxicity and pharmacokinetic studies of these CD-based carriers are still the major challenge. Most of the studies are in the proof-of-concept stage and the information about toxicity is mainly based on in vitro cell models. The majority of them lack systemic biocompatibility evaluation and comprehensive toxicological study. Limited studies concerning the mechanism of elimination of these CDs-based nanocarriers within the body [57, 108, 130, 131]. The establishment of the nonclinical safety and pharmacokinetic data is necessary for clinical trials of new developed pharmaceutical products. Although clinical studies of CRLX101 and CALAA-01 showed that the future of CD-based nanocarriers in anticancer drug and gene delivery is promising, manufacturing approaches that can be easily scaled up with low costs need to be developed before introducing into marketing.

Conclusion

The excellent biocompatibility and unique inclusion capability as well as powerful functionalization capacity of CDs and their derivatives make them especially attractive for applications in cancer treatment. The formation of inclusion complex with non-toxic CDs leads to enhancement of solubility, reduced toxicity and improvement of physicochemical characteristics of anti-cancer drug. CDs-based nanocarriers involved combination of two different approaches in a single delivery system increase the advantage of both CDs and nanoparticles. Interestingly, CDs show promising as anti-cancer agents for various cancers treatment in vitro and in vivo. During the last two decades, the number of the CDs-containing pharmaceutical products approved and marketed has been continuously increasing. However, there is no formulation containing anti-cancer drug with CDs in the market. The successful translation of CDs or CDs-based nanocarriers for anticancer agents to clinical reality remains challenging. There is a prerequisite to conduct systemic biocompatibility evaluation and comprehensive toxicological study for CDs or CDs-based carriers. Furthermore, the route of administration, the type of formulation and the mechanism of elimination in the body also needs to be investigated before these products move out of the laboratory and into the clinics. Although most of the CDs based cancer treatments are only at the early age of investigation, CDs have significant potential as anticancer drug alone or as carriers in advanced dosage forms. The future will see that CDs or CDs based carriers could be employed for cancer therapy with maximal efficacy and minimal toxicity.

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