EXPERT OPINION

Nanoparticles as delivery carriers for anticancer prodrugs

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Introduction: Prodrugs are inactive compounds which are metabolized in the body to produce parent active agents. It has been shown that prodrugs hold some advantages over conventional drugs, such as increased solubility, improved permeability and bioavailability, reduced adverse effects and prolonged half-lives. Optimization of the vehicles used is very important in order to employ the advantages of prodrugs. Nanocarriers are currently being widely used as prodrug vehicles because of their ability to enhance storage stability, modulate prodrug release and tumor-targeted delivery and protect against enzymatic attack. This combined approach of prodrugs and nanoparticles has a particular attraction for developing anticancer therapies.
Areas covered: This paper discusses liposomes, polymeric nanoparticles and lipid nanoparticles, which are all carriers commonly used for prodrug encapsulation. Macromolecular prodrugs can spontaneously form self-assembled nanoparticles with no intervention of other additives. This review also describes recent developments in prodrug delivery using nanoparticulate strategies. Pharmacokinetic, pharmacodynamic and cytotoxicity evaluations of anticancer prodrugs are systematically elucidated in this review.
Expert opinion: More profiles involved in animal and clinical studies will encourage the future applicability of prodrug nanocarrier therapy. The possible toxicity associated with nanoparticles is a concern for development of prodrug delivery.

Keywords: cancer, drug delivery, nanoparticles, prodrugs

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1. Introduction

Prodrugs are inactive compounds which can be either chemically or enzymatically degraded in a controlled or predictable manner to the parent active drug inside the body. The term 'prodrug' was first introduced by Adrien Albert [1] in 1958, who described compounds that undergo biotransformation prior to eliciting biological activity. Prodrugs generally should be either inactive or much less potent than the parent drug [2,3]. The prodrug structure generally contains a covalent link between the drug and a moiety or promoiety. The inactive prodrug should ideally release the active agent at the action site either by specific enzymes or by non-enzymatic methods [4]. Prodrugs provide a rationale for achieving target physicochemical, pharmacokinetic and pharmacological characteristics. They can be designed to overcome different barriers for drug delivery, including poor chemical stability, insufficient solubility, rapid metabolism, low blood–brain barrier (BBB) transport and toxicity [5]. The prodrug strategy is usually used for increasing the lipophilicity of a drug, thus enhancing passive membrane permeation [6]. A prodrug technique can overcome drawbacks associated with hydrophilic drugs such as high toxicity and low bioavailability. Currently, approximately 10% of drugs approved worldwide are classified as prodrugs [7]. In addition to prodrugs themselves, optimization of carriers or vehicles is also important when using

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Article highlights.

- Nanotechnology has provided new insights into enhancing possibilities of prodrug strategies for *in vivo* and clinical applications.
- This review describes recent developments in prodrug delivery using nanoparticulate strategies.
- The combined strategy of prodrugs and nanoparticles has a particular attraction for developing anticancer therapies.
- In addition to intravenous administration, oral and transdermal routes are pathways for prodrug permeation from nanocarriers.
- More results in animal and clinical studies will encourage future application of prodrug nanoparticle therapy.

This box summarizes key points contained in the article.

prodrugs in clinical situations. An optimized formulation may help a prodrug reach its target site and minimize adverse effects. In recent years, nanoparticles have extensively been used as carriers for prodrug delivery. The poor solubility of some prodrugs can be resolved by encapsulating into nanoparticles. Combinations of prodrugs and nanoparticles for cancer therapy, anti-inflammation and anti-viral treatment were investigated to achieve successful outcomes of drug therapy. This review shows current advances in combined strategy of prodrugs and nanoparticles for treating cancers. The various types of nanoparticles for prodrug loading and possible delivery routes are also described below.

2. Types of nanoparticles

One aspect in the emerging field of medical therapy is drug delivery via nanoparticles. The use of nanoparticles allows for enhanced therapeutic efficacy with reduced risks of adverse reactions. Advantages of nanocarriers for drugs include greater solubility, improved stability, longer exposure durations, selective delivery to action sites and decreased permeation resistance [8,9]. Different nanoparticulate systems, including liposomes, polymeric nanoparticles, lipid nanoparticles and self-assembled nanoparticles, were developed to deliver drugs and prodrugs. Liposomes were the first nanocarriers used for prodrug encapsulation. Liposomes are microscopic vesicles consisting of membrane-like phospholipid bilayers surrounding water (**Figure 1**) [10]. The purpose of using liposomes as delivery systems is to enhance the therapeutic index of a drug since incorporation alters the pharmacokinetics and biodistribution. The liposomal surface is sometimes conjugated with a monoclonal antibody, for example, immunoliposomes can achieve site-specific drug targeting [11]. Seven liposomal drug formulations have so far received regulatory approval nowadays [12].

A drug or prodrug can either be covalently bound to a polymer backbone or physically incorporated into a polymer matrix (**Figure 1**). The drug/prodrug solubility, stability, circulation life and side effects can be improved using polymeric nanoparticles [13]. Another polymer-related nanosystem is called amphiphilic block copolymer micelles. Copolymer micelles are self-assembled carriers for drug delivery, and possess characteristics of biocompatibility, a greater solubilization capacity and tumor targeting [14]. Copolymers such as poloxamers, polystyrene-*block*-poly(acrylic acid) (PS-*b*-PAA) and polyethylene glycol-*block*-poly(ε-caprolactone) (PEG-*b*-PCL) are often used to construct nanoparticles. Lipid nanocarriers, such as solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) and lipid emulsions (LEs), appear suitable as drug-carrier systems due to their very low cytotoxicity relative to polymeric nanoparticles [15]. The predominant difference among SLNs, NLCs and LEs is the composition of the inner core (**Figure 1**). SLNs are particles made from crystalline solid lipids, whereas NLCs are composed of a solid lipid matrix with a certain content of a liquid lipid and are a more-advanced generation of SLNs. LEs are nanocarriers with neat liquid oil in the inner phase [16]. Lipid nanocarriers were introduced as parenteral drug carriers and offer sustained release and tumor targeting [17]. Self-assembled delivery systems are defined as self-assembled amphiphilic prodrugs [18]. Amphiphilic prodrugs are capable of forming nanoparticles in water in the absence of surfactants or emulsifiers (**Figure 1**). Ideal self-assembled nanoparticles should possess characteristics of stable self-assembly and feasible degradation of prodrugs [19]. Some macromolecular prodrugs allow the preparation of nanoparticles, which can control drug release based on the feature of linkages between macromolecules and the drugs [20].

3. Tumor delivery

Drug delivery to specific targets has become an important issue for promoting the selectivity of drugs to diseased sites and reducing toxicity against normal cells. Combined prodrugs and nanoparticles can induce drug release in target cells [21]. The active ingredient is transformed from the inactive prodrug to reveal cell-killing functions. By modulating the size, surface charge, materials and antibody conjugation, specific targeting efficiencies of carriers can be attained. Nanoparticles have advantages of incorporating lipophilic or hydrophilic compounds for variable routes of administration.

Cancer is the most frequent cause of death in the world. Besides discoveries of novel therapeutic molecules, the clinical use of conventional anticancer drugs is still hampered by nonspecific biodistribution and the difficulty of delivering sufficient concentrations to tumors. The introduction of prodrugs has influenced cancer therapy. Prodrug design for cancer treatment is based on molecular targeting that is responsible for cell transformation [22,23]. Owing to the high proliferation of tumor cells, in addition to a bioreductive effect, levels of specific enzymes can be increased in these cells and targeted tumor delivery can be achieved [4]. On the other hand, nanotechnology strategies can be exploited as means to administer

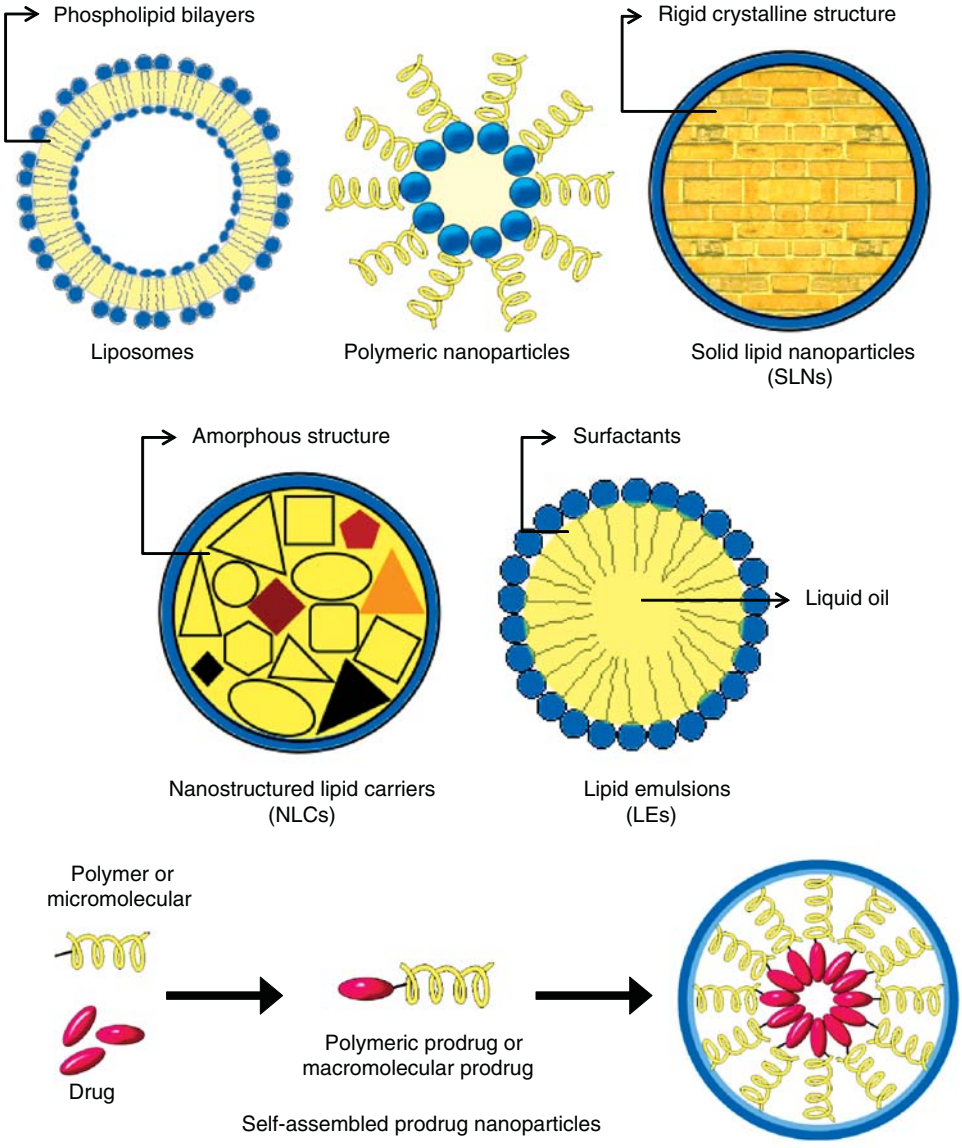


Figure 1. The proposed structures of nanoparticles, including liposomes, polymeric nanoparticles, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), lipid emulsions (LEs) and self-assembled prodrug nanoparticles.

drugs in a controlled manner, deliver drugs to tumors and overcome resistance of drug diffusion [22]. Predominant types of nanocarriers for anticancer drugs are liposomes. PEGylated liposomal doxorubicin (DaunoXome®, Doxil® and Caelyx®) and paclitaxel albumin-bound nanoparticles (Abraxane®) are two formulations approved by the US Food and Drug Administration for treating Kaposi's sarcoma in humans [8]. Liposomal doxorubicin without PEG (Myocet®) and daunorubicin are approved in Europe for treating breast cancer and Kaposi's sarcoma. There are > 100 nanocarriers for anticancer agents that are in preclinical development and clinical trials.

The brain is a delicate organ, is isolated from the general circulation and is characterized by the presence of relatively impermeable endothelial cells with tight junctions, enzymatic activity and the presence of active efflux transporter mechanisms [24]. Pharmacological treatments of central nervous system (CNS) diseases such as brain tumors are often confined by the inability of potent drugs to pass the BBB [25]. One possibility for non-invasively delivering drugs to the brain is using nanocarriers. These nanocarriers not only mask the BBB's limiting characteristics against therapeutic agents, but also protect drugs from chemical/enzymatic degradation. One example of prodrug nanoparticles targeting the brain is the application of SLNs. SLNs carrying the prodrug, cholesteryl butyrate, were tested for treating cerebral gliomas [26]. SLNs provide advantages of opening tight junctions and increasing brain cell uptake, resulting in the possibility of specific brain targeting.

Table 1. The summary of nanoparticles loaded with prodrugs for anticancer treatments.

Cancer type	Model drug	Nanoparticle type	Ref.
Breast cancer	Paclitaxel	Block copolymer micelles	[28]
	Paclitaxel	Adenovirus nanoparticles	[29]
	Paclitaxel	Self-assembled micelles	[31-33]
	Gemcitabine	Polymeric nanospheres	[34]
	Capecitabine	Self-assembled micelles	[35]
	Capecitabine	SLNs	[36]
Colon cancer	5-FU	Calcium phosphate nanoparticles	[37]
	5-FU	Immunoliposomes	[39]
	5-FU	PLGA nanoparticles	[40]
	Gemcitabine	Liposomes	[41]
	Gemcitabine	Self-assembled nanoparticles	[42,44]
	Gemcitabine	Magnetic nanoparticles	[45]
	Paclitaxel	Lipid nanoparticles	[46]
	Tubulysins	Self-assembled nanoparticles	[47]
Leukemia	6-Mercaptopurine	Liposomes	[49]
	Ezatiostat	Liposomes	[51]
	Gemcitabine	Self-assembled nanoparticles	[52]
	Gemcitabine	Magnetite nanocrystals	[53]
	Nucleosides	Self-assembled nanoparticles	[55,56]
	Paclitaxel	Polymeric nanoparticles	[57]
	NO	Block copolymer nanoparticles	[59]
Lung cancer	Ara-C	Liposomes	[62]
	Ara-C	Immunoliposomes	[63]
	Mitomycin C	Liposomes	[64]
	Mitomycin C	PEGylated liposomes	[65]
	Cisplatin	Gold nanoparticles	[66]
Prostate cancer	Cisplatin	PLGA nanoparticles	[69,70]
	Cisplatin	Self-assembled nanoparticles	[71]
	K-182	Cationic nanoparticles	[73]
Liver cancer	Daunorubicin	ApoE liposomes	[74]
	5-FU	SLNs	[76]
Glioblastoma	Paclitaxel	ApoB-100 nanoparticles	[78]
Ovary cancer	Curcumin	Self-assembled nanoparticles	[80]
Gastric cancer	5-FU	Calcium phosphate nanoparticles	[81]
Non-melanoma skin cancer	ALA	Nanoemulsions	[83]

ALA: 5-aminolevulinic acid; ApoE: Apolipoprotein E; 5-FU: 5-fluorouracil; NO: Nitric oxide; PLGA: Poly(lactide-*co*-glycolide); SLN: Solid lipid nanoparticle.

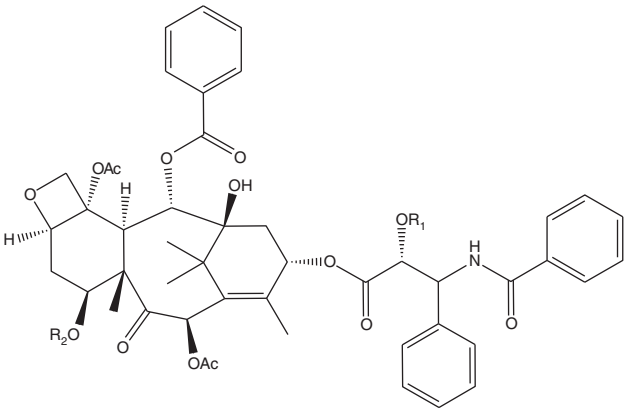
4. Prodrug-loaded nanoparticles for anticancer therapy

Most drug therapies administered via prodrug-loaded nanoparticles are involved in tumor targeting. Cancer therapies are mainly discussed in this section. The profiles of these prodrugs for nanoparticulate loading in the treatment of cancers are summarized in **Table 1**.

4.1 Breast cancer

Breast tumors are an important target for prodrug-loaded nanoparticles. Both paclitaxel and gemcitabine have shown promise as effective antitumor agents against breast cancer. Many paclitaxel prodrugs are synthesized for incorporation into nanoparticles to avoid the toxicity of Cremophor®, a commercial product containing paclitaxel. Moreover, the half-life of paclitaxel is only 0.3 h [27], emerging the development of prodrugs to prolong the pharmacological duration. Paclitaxel is conjugated at the 2′ and 7′ hydroxyl positions to fatty acids via anhydride chemistry (**Figure 2**, compounds 1 – 5c). The lipophilic prodrugs can be loaded into PEG-*b*-PCL to form block copolymer micelles [28]. The nanoparticles have a diameter of < 50 nm. The micelles can release the prodrugs over several days ($t_{1/2}$ > 3 days). The 7′ derivative with the hexanoate acyl chain retains cytotoxicity against breast cancer cells similar to the parent drug. Prodrug-loaded micelles increased the mean residence time from 1.1 to 4.1 h compared with Taxol®. Paclitaxel is conjugated to folate-modified adenovirus nanoparticles using succinic anhydride and Fmoc-Glu(OtBu)-OH linkers to form prodrugs [29]. Adenovirus nanoparticles in MDA-MB-231 breast tumor-bearing mice showed enhanced targeting and residence time in tumors. It is helpful for paclitaxel since this drug lacks selective cytotoxicity between cancer and normal cells [30]. Uptake of the prodrugs into breast cancer cells also increased.

Recently, paclitaxel was conjugated with macromolecules to prepare self-assembled micelles. Heparin or hyaluronic



	R_1	R_2
1	H	H
2	Si(*tert*-butyl)	H
3	Si(*tert*-butyl)	$CO(CH_2)_{14}CH_3$
4a	H	$CO(CH_2)_4CH_3$
4b	H	$CO(CH_2)_{10}CH_3$
4c	H	$CO(CH_2)_{14}CH_3$
5a	$CO(CH_2)_4CH_3$	H
5b	$CO(CH_2)_{10}CH_3$	H
5c	$CO(CH_2)_{14}CH_3$	H

Figure 2. Chemical structures of paclitaxel and its prodrugs conjugated by ester binding.

acid is conjugated to paclitaxel via a single amino acid spacer, either phenylalanine, valine or leucine [31,32]. The prepared prodrugs can self-assemble to form nanoparticles with a narrow size distribution of 140 ~ 180 nm. The prodrugs exhibit better *in vitro* MCF-7 cell inhibition than free paclitaxel (IC_{50} 0.058 vs 0.19 μg/ml). For example, respective IC_{50} values of free paclitaxel and the prodrug conjugated to hyaluronic acid via leucine were 0.80 and 0.25 nM [32]. The heparin–paclitaxel conjugate via leucine revealed similar tumor growth inhibition to that of paclitaxel with no sign of body-weight loss [31], indicating safer administration. Li *et al.* [33] grafted paclitaxel onto the surface of hydrophilic hyperbranched poly(ether ester) to form self-assembled micelles. Hydrolysis of this conjugate in serum results in cumulative release of the parent drug. Results showed that these micelles display potent growth inhibition against MCF-7 cells. An *in vivo* examination suggested that nude mice tolerated a dose of 45 mg/kg prodrug treatment, which is much greater than that of free paclitaxel (15 mg/kg).

Gemcitabine has a very short half-life when administered intravenously. The prodrug/nanomedicine strategy is useful for overcoming this drawback. Lipophilic derivatives of gemcitabine were synthesized in which acyl chains were coupled to a 4-amino moiety (**Figure 3**) [34]. Although the prodrugs exhibited greater cytotoxicity against cancer cells, the chemical modification of gemcitabine had led to strong diminish of water solubility. This may constitute a drawback for intravenous injection. Protection of these prodrugs was attempted by loading them into poly[aminopoly(ethylene glycol)cyanoacrylate-*co*-hexadecyl cyanoacrylate] nanospheres with an average diameter of ~ 150 nm. The IC_{50} against MCF-7 was 29 μM for free gemcitabine, which was reduced to 12.3 μM by prodrug modification (4-(*N*)-stearoylgemcitabine) and nanosphere incorporation.

5-Fluorouracil (5-FU) is a highly toxic anticancer drug. Due to its hydrophilic nature, it possesses a low absorption efficiency and bioavailability. Capecitabine was developed to overcome this limitation. It is an inactive precursor of 5-FU. Capecitabine's structure was further altered to prepare the amphiphilic prodrugs, 5′-deoxy-5-fluoro-N^4-(phytanyloxycarbonyl) cytidine and 5′-deoxy-5-fluoro-N^4-(palmityloxycarbonyl) cytidine [35,36]. These prodrugs possess a lamellar crystalline structure, which can be dispersed in lipid nanoparticles and thus has the advantages of selective, localized activation of the prodrugs combined with sustained release [19]. The amphiphilic prodrugs form self-assembled nanoparticles were loaded into SLNs for *in vivo* cytotoxicity assays. Breast-tumor volumes in mice treated with self-assembled nanoparticles or SLNs were significantly smaller than those treated with free capecitabine. The inhibition of tumor size displayed a trend of increasing efficiency with an increasing dose.

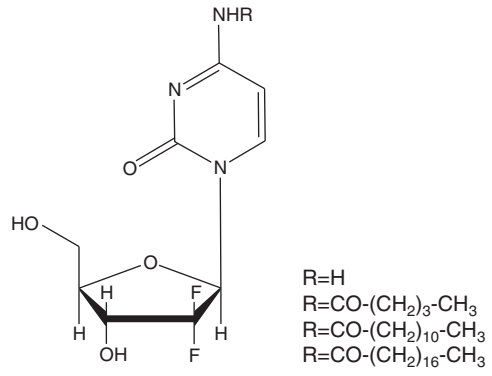


Figure 3. Chemical structures of gemcitabine and its prodrugs.

4.2 Colon cancer

Colon cancer is one of the most frequently encountered malignancies in the world. The yearly incidence is estimated to be one million [37]. New approaches need to be exploited to increase the low survival rate of patients with colon cancer. 5-Fluorouridine is an anticancer drug for treating colon carcinogenesis. It is not in clinical use because of the strong side effects such as leucopenia, thrombopenia and gastrointestinal toxicity [38]. 5′-Palmitoyl-5-fluorouridine as the prodrug of 5-fluorouridine was synthesized and incorporated into immunoliposomes composed of egg phosphatidylcholine and the thiolated antibody, AR-3 [39]. In a mouse model, immunoliposomes containing the prodrug exhibited better activity than the controls, since on day 27 postgraft, only 5% of residual tumors were present. At the same time, a 20% of residual tumor was shown for the group treated with conventional liposomes (without antibody). Another 5-FU prodrug with 1-alkylcarbonyloxymethyl moieties was loaded into poly(lactide-*co*-glycolide) (PLGA) nanoparticles [40]. PLGA can form spherical particles of ~ 200 nm in diameter. Controlled and sustained prodrug release was achieved, with prodrugs possessing longer acyl chains showing lower release. The results indicated a possibility of a more-prolonged half-life in plasma. It is not necessary to load prodrugs into nanoparticles to produce antitumor activity. They can be administered separately. Zhang *et al.* [37] used a suicide gene encapsulated by calcium phosphate nanoparticles to treat colon cancer, followed by 5-FU prodrug treatment. The prodrug used in that study was 5-fluorocysteine. The *in vivo* study showed that nanoparticle delivery together with free 5-fluorocysteine treatment resulted in a significant xenograft colon carcinoma growth delay compared with simple prodrug application.

Gemcitabine prodrugs with an acyl chain in a 4-amino group were also used to treat colon cancer [41]. Liposomes provide 98% encapsulation efficiency of 4-(*N*)-stearoylgemcitabine. Respective cytotoxicity levels against HT-29 cells by the free prodrug and liposomal prodrug were two- and sevenfold higher than that of the free parent drug. The half-life of free gemcitabine is 33 min, which was increased to 184 min by prodrug loading in liposomes. Recently, Couvreur *et al.* [42] introduced the concept of 'squalenoylation' to improve the efficacy of gemcitabine and reduce rapid deamination into inactive uracil derivatives. Squalenoyl gemcitabine can form nanoparticles by self-assembly. Structural study has underlined that the self-assembled micelles strongly interacted with phospholipids, enhancing the entrance into cancer cells by passive diffusion [43]. This conjugate of nanoparticles is considerably more toxic to HT-29 cells than the parent drug above 10 μM [44]. The cell uptake of this prodrug leads to the formation of non-lamellar structures and membrane permeation, resulting in potent cytotoxicity. Magnetic nanoparticles are used to encapsulate squalenoyl gemcitabine for definite tumor targeting [45]. Magnetic nanoparticles are predominantly prepared by $FeCl_3$ and the Pluronic F-68 surfactant. Electrophoretic measurements suggested that the magnetite core was coated by the gemcitabine prodrug. Pluronic F-68 revealed an important role in completely stabilizing nanoparticles. The heterogeneous structure of nanoparticles confers active targeting to solid tumors.

Ansell *et al.* [46] used succinate or a diglycolate cross-linker to synthesize paclitaxel prodrugs with various lipid anchors (**Figure 4**, compounds 6 – 14). The prodrugs were then formulated in lipid nanoparticles. The nanoparticles showed an elimination half-life of 24 h *in vivo*. In HT-29 tumor xenograft mice, inhibition of the tumor size by diglycolate prodrug nanoparticles increased as the anchor lipophilicity increased, whereas succinate prodrugs demonstrated no evidence of efficacy.

Tubulysins belong to naturally occurring tetrapeptides with antiproliferative effects against colon tumors. However, they are highly toxic to animal models. Tubulysins also reveal low solubility to water. A thiol derivative of tubulysin was covalently attached to β-cyclodextrin to form a macromolecular prodrug [47]. The macromolecules were assembled into stable nanoparticles. It is reported that macromolecular nanocarriers can overcome multidrug resistance by intracellular delivery through endocytosis rather than diffusion, a process that is less susceptible to surface pump-mediated multidrug resistance [48]. The IC_{50} of the nanoparticles was 5 versus 1 nM for free tubulysin in the HT-29 cell line. The maximum tolerated dose of nanoparticles was 6 versus 0.05 mg/kg for the parent peptide in nude mice. Potent antitumor activity was also observed for self-assembled cyclodextrin particles.

4.3 Leukemia

Leukemia is a type of cancer of the blood or bone marrow characterized by an abnormal increase in immature white blood cells. 6-Mercaptopurine is an antineoplastic agent for treating leukemia. It is a powerful anticancer drug with severe side effects and short biological half-life. It is covalently coupled with glyceryl monostearate via a succinic anhydride

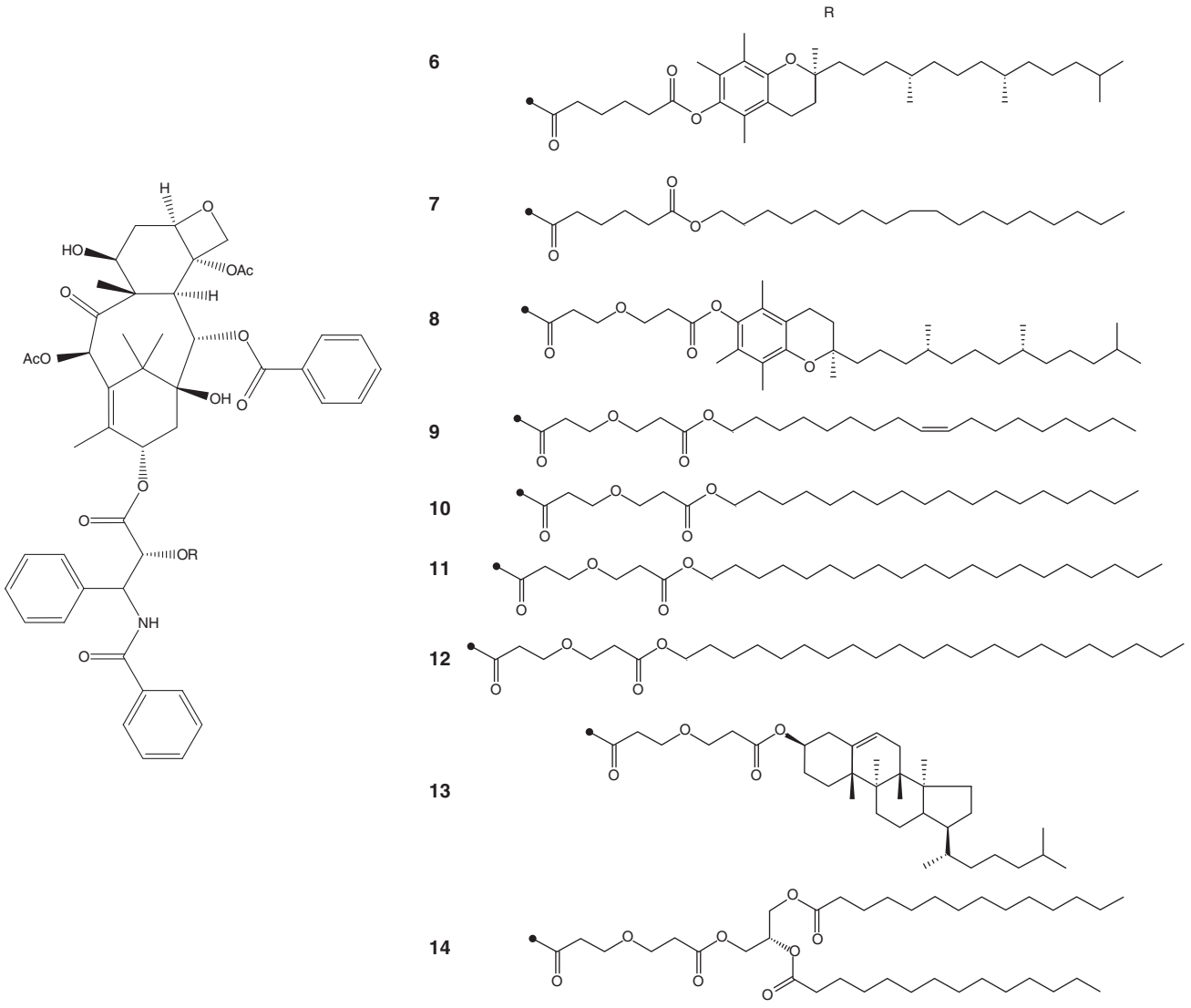


Figure 4. Chemical structures of paclitaxel and its prodrugs conjugated by succinate or diglycolate.

spacer to form a prodrug for prolonged activity. For intravenous administration, the lipophilic prodrug was difficult to be dissolved in injection water. Liposomes were provided as vehicles for 6-mercaptopurine prodrug. Entrapment of the prodrug in liposomes was 92%, which is much higher compared with that of 6-mercaptopurine (2%) [49]. Improved cytotoxicity of L1210 leukemia cells was detected with the liposomal prodrug compared with free 6-mercaptopurine. The pharmacokinetics revealed respective increases in the half-life from 61 to 120 and 296 min for conventional liposomes and sphingomyelin-containing liposomes.

Ezatiostat is a glutathione *S*-transferase PI-I inhibitor, which generates active metabolites for healing myelodysplastic syndrome. This syndrome is a heterogeneous group of clonal hematopoietic stem-cell disorders which may transform to acute myeloid leukemia [50]. The elimination half-life of ezatiostat is only 0.2 h. Phase I and IIa clinical trials were conducted to investigate ezatiostat liposomes for injection [51]. Patients received five doses (50 ~ 600 mg/m^2). The plasma concentration of the active metabolites, including TLK235, TLK236 and TLK117, proportionally increased with the prodrug dose. The unacceptable toxicity rate with liposomal carriers was low (< 19%). Improvements in bone marrow maturation and cellularity were observed.

Squalenoyl gemcitabine is used to treat leukemia, in addition to colon cancer. Self-assembled squalenoyl gemcitabine nanoparticles accumulated within cellular membranes, especially in those of the endoplasmic reticulum [52]. *In vitro* cytotoxicity assays showed that the nanoparticles were more efficient than free gemcitabine in a transporter-deficient human resistant leukemia model. Magnetite nanocrystals were added to self-assembled nanoparticles of squalenoyl gemcitabine for cancer application [53]. In recent years, much attention has been paid to magnetic nanoparticles for

drug delivery, taking advantage of the unique ability of magnetic particles to be guided by an applied magnetic field [54]. The targeted delivery to tumor site can be achieved. When injected into L1210 subcutaneous tumors in mice, a similar response was detected between magnetic nanoparticles and free gemcitabine. However, the administered dose of nanoparticles was 20-fold lower than that of the parent drug (5 vs 100 mg/kg). This demonstrates better therapy and tolerability of the nanoparticulate prodrug.

The concept of squalenoylation can be extended to nucleosides. Nucleosides show widespread anticancer and antiviral therapeutics. Amphiphilic squalenoylated nucleosides spontaneously form nanoparticles [55,56]. A major concern with nucleosides is their poor storage stability. Nanoparticles can be stored for up to 4 months after freeze-drying, with no loss of stability. Cytotoxicity against P388 murine leukemia cell lines was preserved after rehydration of the freeze-dried products.

A recent investigation [57] prepared poly(β-amino ester) and poly(ε-caprolactone) nanoparticles for paclitaxel oleate. A diameter of 70 nm was obtained for these nanoparticles. Good physical stability was achieved when they were stored at 4°C. Leukemia cell uptake and prodrug release were quicker when administered in poly(β-amino ester) than poly (ε-caprolactone). Poly(β-amino ester) provided an IC_{50} of 128 nM, which was much less than that of the formulation with poly(ε-caprolactone) (2.5 μM). This indicates the importance of selecting optimal materials for superior anticancer activity.

Nitric oxide (NO) is a mediator of diverse physiological processes, and shows antiproliferative activity against leukemia [58]. NO prodrugs such as diazeniumdiolates were synthesized for efficient application because of the poor bioavailability of NO. Prodrugs were formulated in polystyrene-*block*-poly (ethylene glycol) (PS-*b*-PEG) or polylactide-*block*-poly (ethylene glycol) (PLA-*b*-PEG) copolymer nanoparticles with sizes of 220 ~ 450 nm [59]. The PEG-based nanoparticles showed longer circulation time and reduced liver uptake compared with the nanoparticles without PEG due to its steric hindrance on particulate shell [60]. The copolymers slow down NO prodrug activation by glutathione, thus prolonging its half-life. The time taken for 50% decomposition can be extended from 4.5 to 40 min by nanoparticulate incorporation. Identical IC_{50} values against U937 leukemia cells for the free prodrug and nanoparticle-loaded prodrug were obtained.

4.4 Lung cancer

1-β-D-Arabinofuranosylcytosine (Ara-C) is an important chemotherapeutic agent in the treatment of leukemia and lung cancer. After intravenous injection, Ara-C is rapidly converted to inactive metabolite by cytidine deaminase, which is widely distributed in both normal and neoplastic tissues [61]. As a consequence, a frequent bolus or continuous infusion is needed. A lipophilic prodrug of Ara-C, namely N^4-[*N*-(cholesteryl-oxycarbonyl)glycyl]-Ara-C, was synthesized and formulated by liposomes for injection [62]. The vesicle size was about 110 nm with almost complete prodrug encapsulation. Intravenously injected liposomes inhibited A549 lung adenocarcinomas implanted in mice more efficiently than did Ara-C in an aqueous solution. Mori *et al.* [63] further entrapped the Ara-C prodrug in immunoliposomes. Monosialoganglioside and PEG conjugated to phosphatidylethanolamine were used to produce long-circulation immunoliposomes. Successful targeting to lung tumors in an animal model was observed. Adriamycin and 5-FU prodrugs were also encapsulated in immunoliposomes in that study. Both prodrugs exhibited similar tumor targeting efficiencies to that of the Ara-C prodrug.

Another anticancer drug for treating lung cancer is mitomycin C. Its clinical efficacy is only modest because of problematic toxicity to bone marrow [64]. Long-circulation liposomes can produce sustained mitomycin C release to avoid the burst effect of this drug. A lipophilic prodrug of mitomycin C (2,3-(distearoyloxy)propane-1-dithio-4′-benzyloxycarbonyl-mitomycin C) was designed to be incorporated with liposomes composed of PEG [65]. Nearly 100% entrapment of the prodrug was observed. After an intravenous injection in rats, the liposomal prodrug resulted in slower clearance of 2.3 ml/h/kg compared with 2831 ml/h/kg for free mitomycin C. The liposomes were threefold less toxic than the free parent drug. Prodrug-loaded liposomes were significantly more active than doxorubicin in PEGylated liposomes in an M109R lung tumor model. It has the potential for future therapy against lung cancer, since M109 is a multidrug-resistant phenotype.

Polyvalent oligonucleotide gold (DNA-Au) nanoparticles with a cisplatin prodrug are another nanocarrier that reveals lung-carcinoma inhibition [66]. DNA-Au nanoparticles have appealing properties for drug application, including high cell uptake, no demonstrated toxicity to normal cells and resistance to enzymatic degradation [67]. A cisplatin prodrug (*c,c,t*-[$Pt(NH_3)_2Cl_2(OH)(O_2CCH_2CH_2CO_2H)$]) was conjugated with the surface of DNA-Au nanoparticles via amide linkages. Cytotoxicity profiles of the A549 cell line showed an IC_{50} of 0.9 μM for the nanoparticulate prodrug, whereas that of free cisplatin was 11 μM. This indicates a superior tumor killing ability by the nanoparticle-prodrug conjugate.

4.5 Prostate cancer

Prostate cancer is the most common carcinoma and the second leading cause of death after lung cancer in the USA [68]. The prodrug *c,c,t*-[$Pt(NH_3)_2Cl_2(OH)(O_2CCH_2CH_2CO_2H)$ incorporated in nanoparticles is also employed to treat prostate cancer [69,70]. In that case, PLGA-PEG was the polymer material for the nanoparticles. A prostate-specific membrane antigen (PSMA) targeting an aptamer on the nanoparticulate surface promotes LNCaP prostate cancer cell uptake by endocytosis. The maximum tolerated dose following an intravenous injection of PLGA-PEG nanoparticles was 40 mg/kg for rats [70]. A pharmacokinetic study revealed prolonged

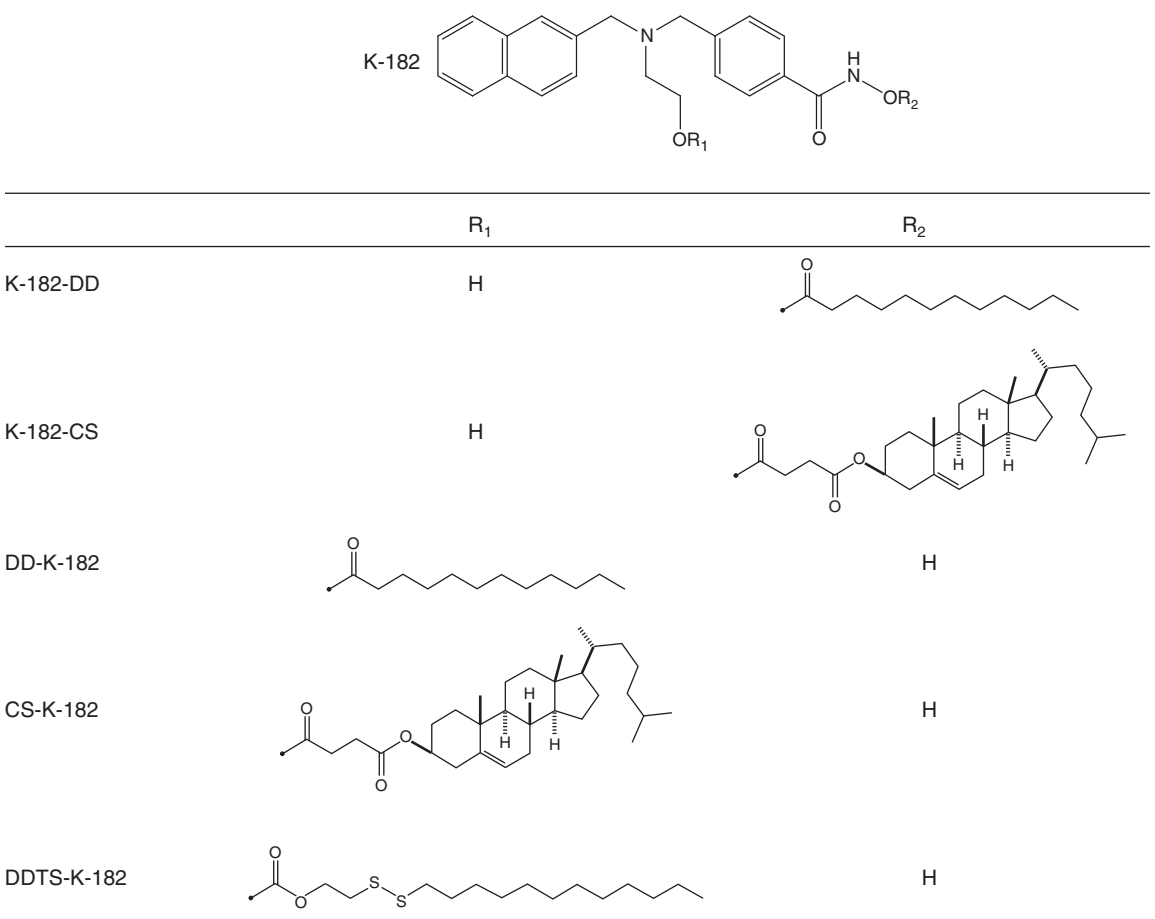


Figure 5. Chemical structure of K-182 and its prodrugs.

persistence of the prodrug in the circulation. Platinum accumulation in the kidneys, the major organ of cisplatin toxicity, also decreased. Equivalent antitumor activity against LNCaP xenografts at one-third of the dose of free cisplatin was shown for prodrug nanoparticles. The cisplatin prodrug can form self-assembled nanoparticles by conjugating the prodrug to PLA with pendant hydroxyl groups [71]. Self-assembly is further assisted by PLGA-PEG and docetaxel for synergistic activity against prostate cancer. The nanoparticulate surface is derivatized with the A10 aptamer, which binds to PSMA. The nanosystems undergo sustained release of both drugs over 72 h. The cytotoxicity indicates a superiority of the dual-drug carriers over the nanoparticles with single drug or non-A10 formulations.

The histone deacetylase inhibitor, K-182, can increase the transfection efficiency due to the improved gene transport in cytoplasm by hyperacetylation of microtubules [72]. K-182 has two hydroxyl groups that can link with functional groups via ester bonds (**Figure 5**). Ishii *et al.* [73] synthesized five kinds of lipid-linked K-182 by ester binding or a disulfide carbonate linker. Nanoparticles with cationic surface charges encapsulated the prodrugs to a satisfactory level. The nanocomplexes containing K-182 prodrugs with *n*-dodecanoic acid exhibited two- ~ fourfold greater gene expression compared with the control. The experimental results indicated that free K-182 was released from nanoparticles in PC-3 prostate cancer cells after endocytosis.

4.6 Liver cancer

Liver carcinoma expresses an elevated level of low-density lipoprotein (LDL) receptors. Apolipoprotein E (ApoE)-exposed liposomes are recognized by LDL receptors. Versluis *et al.* [74] incorporated a lipophilic prodrug of daunorubicin into ApoE liposomes. Daunorubicin is a very potent anticancer drug, but its cardiotoxicity is dose limiting [75]. Prodrug strategy and association with liposomes is expected to reduce the disposition of the drug in the heart. The prodrug was a conjugate of daunorubicin and a litochol-oleate anchor with a tetrapeptide spacer. Compared with free daunorubicin, the circulation half-life of the liposomal prodrug was substantially prolonged. Rats with upregulated LDL receptors showed that the prodrug carrier increased liver uptake by fivefold compared with the free control.

In fact, liver targeting can simply be achieved by SLNs with no surface modifications. The lipophilic characteristics of lipid matrix inside SLNs are feasible to efficiently load

lipophilic prodrugs. N_1-Stearyl-5-FU loaded into SLNs showed a particle size of 240 nm [76]. The distribution of 5-FU from prodrug SLNs in the mice liver doubled compared with that from a 5-FU injection, suggesting significant targeting to liver tissues.

4.7 Other cancers

In addition to liver tumors, LDL receptors are upregulated in glioblastoma multiforme tumors. They are an aggressive malignancy that accounts for approximately 40% of brain tumors [77]. A paclitaxel oleate-containing LDL nanoparticle system was constructed by combining a synthetic peptide with a lipid-binding motif and apolipoprotein B-100 for LDL receptor targeting [78]. LDL is a 22 ~ 27 nm particle composed of a core of lipids, primarily cholesteryl esters with small amounts of triglyceride, which can be used for incorporation of lipophilic compounds [79]. Glioblastoma multiforme cells were killed with a short prodrug incubation and exhibited saturation at 6 h. The LDL receptor inhibitor, suramin, improved cell survival, indicating the nanoparticle delivery via an LDL receptor.

Curcumin revealed cytotoxicity toward various cancer cell lines. However, the extremely low aqueous solubility led to low bioavailability and *in vivo* antitumor activity. The conjugation of curcumin with two short oligo(ethylene glycol) chains forms a surfactant-like prodrug [80]. This prodrug is labile to esterase and intracellular glutathione. This curcumin prodrug forms stable micelles in water. The intravenously injected prodrug significantly reduced tumor volumes in athymic mice containing xenograft SKOV-3 ovary tumors. A preliminary toxicity assay provided evidence of the safety of the nanoparticles.

Liu *et al.* [81] tried to treat gastric cancer by applying nanoparticles containing the suicide gene and the following 5-FU prodrug, 5-fluorocytosine. Calcium phosphate nanoparticles with a cytomegalovirus (CMV) enhancer and carcinoembryonic antigen promoter were fused to the suicide gene. An intratumoral injection of nanocomplexes followed by intraperitoneal administration of 5-fluorocytosine produced marked regression of gastric cancer xenografts.

Topical administration of 5-aminolevulinic acid (ALA) as a precursor of the photosensitizer, protoporphyrin IX, is used in photodynamic therapy for curing non-melanoma skin cancers [82]. This approach was used to enhance cellular uptake by the more lipophilic methyl ALA. Both oil-in-water and water-in-oil nanoemulsions were prepared for ALA and methyl ALA to increase skin absorption [83]. The loading of the prodrugs into the nanoemulsions resulted in slow release. The nanocarriers increased ALA permeation via skin to 180 nmol/cm^2/h, which was 2.6-fold that of the aqueous solution. The nanoemulsions promoted movement of the prodrug to deeper skin layers. This would be beneficial for treating a nidus in the subepidermal level. The mechanisms of the enhanced skin permeation by nanoemulsions are close contact to skin surface, lipid exchange between oil particles and stratum corneum lipids, as well as skin structure disruption by emulsifiers.

5. Conclusions

The selection of vehicles is important for prodrug delivery to exert maximum activity and minimum adverse effects. Some novel nanocarriers were studied to load prodrugs for anticancer therapy. This review summarizes recent advances in prodrug delivery by nanocarriers. In addition to intravenous administration, oral and transdermal routes are possible pathways for prodrug permeation from nanocarriers. Liposomes and nanoparticles within the nanosize range can be applied to achieve high efficacy, especially for tumor targeting. Paclitaxel and gemcitabine are anticancer drugs mostly used to develop prodrugs and subsequent encapsulation by nanoparticles. Drawbacks of clinically used vehicles can be resolved by using prodrug nanoparticles. Breast and colon cancers are predominant targets for these prodrugs. The strategy of combining prodrugs and nanotechnology is also employed for anti-inflammation, antivirus and analgesia. It is expected that the utility of prodrug nanoparticles in basic research and the clinic will be more extensive in the future, because of urgent needs to discover new anticancer therapies.

6. Expert opinion

Many investigations have examined prodrug design for fewer side effects, longer half-life and higher bioavailability compared with parent drugs. Although a lot of prodrugs have been investigated for clinical practice, however, some prodrugs are failed to be approved because of the infeasible vehicles for carrying them. Nanotechnology has provided new insights into enhancing possibilities of prodrug strategies for *in vivo* and clinical applications. Nowadays, nanoparticles are important devices/materials that make them attractive for clinical and commercial applications. As the need to develop new medicines is pressing, nanotechnology has been utilized for different medical uses such as diagnostics and drug delivery. The prodrug and nanoparticle strategy for drug discovery comprises versatile techniques applied to a wide range of drug therapies, especially anticancer chemotherapy. In order to determine the efficacy of the combined approach, *in vitro* cytotoxicity and *in vivo* pharmacokinetics are useful tools to examine whether the design purpose is achieved. Although *in vivo* pharmacological evaluations such as tumor size measurement and biomarker determination are important as well, investigations of pharmacodynamic assessments for animals and human are few. Also, clinical trials investigating nanocarriers for prodrugs are limited. It is suggested that more results in animal and clinical studies will encourage future application of prodrug nanoparticle therapy. Moreover, it should be cautious to load the prodrugs into nanoparticles which may exhibit some disadvantages. Nanoparticles may slow down the release rate of prodrugs to an extremely low level, resulting in the delayed activity of parent drugs. Sometimes the nanoparticles carry the prodrugs to the undesired sites such as reticuloendothelial system (RES).

The possible toxicity of nanoparticles is another concern for the development of prodrug delivery. Nanomaterials are thought to reveal more serious adverse effects on organisms than materials with larger sizes due to their tiny sizes and corresponding higher surface areas. Until now, there is still limited information regarding the health concerns of materials at the nano-level. There are more than 600 commercial products containing nanoparticles nowadays. The total commerce involved in nanoparticles will soon reach US $1 trillion [84]. Nevertheless, increasing debate has raised concerns about toxicity derived from nanoparticulate use. An ascendant chorus by government agencies, academia, industry and environmentalists is calling for urgent studies on nanoparticulate toxicity [85]. For example, a large amount of macromolecular prodrugs is necessary to form self-assembled nanoparticles. Macromolecules should be administered with caution for toxicity reasons. Most investigations involved in combined prodrugs and nanoparticles are for antitumor aims. Intravenous injections are the main route for administration of nanocarriers incorporated with prodrugs. The effort to develop alternative routes and treat other diseases with prodrug nanocarriers should be continued to extend their applications. Permeation via the gastrointestinal tract and BBB may be a future trend. No nanoparticles for brain delivery are performed for clinical trials until now. The combination of two therapeutically active agents to form a single prodrug, which is called a co-drug, is another consideration for future development. It may also face the challenge for selecting optimal vehicles. The same as prodrugs, nanocarriers provide an opportunity to formulate the co-drugs. Although some advantages of prodrug nanocarriers were demonstrated, the mechanisms for the enhanced efficacy are not fully understood. Hence, further elucidation of the mechanisms should be explored.

Declaration of interest

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