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Invited review

The effect of dendrimers on the pharmacodynamic and pharmacokinetic behaviors of non-covalently or covalently attached drugs

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Abstract

Dendrimers are a new class of artificial macromolecules with several attractive properties that show promises in several biomedical applications. They can be widely used to increase the cellular uptake, bioavailability and therapeutic efficacy, to optimize the biodistribution and intracellular release profile, and to reduce the systemic toxicity, clearance and degradation rate of non-covalently or covalently attached drugs. Recent studies in this aspect clearly point to the potential advantages of dendrimers for the design of new drug delivery systems. Before final applications of dendrimer-based drug delivery systems in humans, we should not only address the benefits of these systems, but also assess the long-term pharmacodynamic (PD) and pharmacokinetic (PK) behaviors and health risk of them. In this mini-review, we will mainly discuss the influence of dendrimers on the PD and PK behaviors of drugs complexed or conjugated to them.

Keywords: Dendrimer; Pharmacodynamic; Pharmacokinetic; Complex; Conjugate; Drug delivery system

1. Introduction

Drug delivery systems (DDS) have revolutionized the medicine today by significantly improving the therapeutic efficacy and reducing the side effect of clinically established drugs [1–3]. They have caused significant impacts on the clinical treatment of many diseases, especially chronic diseases including cancer, rheumatoid arthritis, HIV infection, drug addiction, epilepsy, heart disease, and diabetes. During the past decades, polymers have been widely used as physical drug carriers or scaffolds of drug complexes, conjugates or prodrugs [2,4]. Among these polymeric DDS, the development of dendrimers as potential drug vehicles or scaffolds now is one of the most active areas of biomedical and pharmaceutical sciences. Dendrimers are a new class of artificial macromolecules with several attractive properties, such as extremely low polydispersity, regular and high degree of branching, multi-valency, nano-sized scale, globular architecture and well-defined molecular weight [5–11]. Compared with traditional linear polymers, dendrimers offer several featured advantages as drug carrier candidates. These advantages include: (1) high density and reactivity of functional groups on the periphery of dendrimers make multifarious bioactive molecules to be easily modified onto the surface [10,12–14]; (2) well-defined globular structure, predictable molecule weight and monodispersity of dendrimers ensure reproductive pharmacokinetics [8,9]; (3) controllable size (generation-dependent) of dendrimers satisfies various biomedical purposes [6,11,15]; (4) high penetration abilities of dendrimers through the cell membrane cause increased cellular uptake level of the drugs complexed or conjugated to them [10,16,17]; (5) the lack of immunogenicity of dendrimers makes them much safer choices than synthesized peptide carriers and natural protein

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carriers [17]; (6) enhanced penetration and retention (EPR) effect of dendrimers offers preferential uptake of the materials by cancer tissues [12,18]; (7) well-established methodologies proposed to construct nanodevices with various functional moieties based on dendrimers provide miscellaneous biomedical applications of these promising materials, such as cancer targeting therapy, magnetic response imaging, photodynamic therapy, neutron capture therapy [11,19–22]; (8) Perfectly programmed release of drugs or other bioactive agents from dendrimers leads to reduced toxicity, increased bioavailability and simplified dosing schedule [3,23–25]. Generally, the size, shape, and surface properties of the polymeric carriers greatly influence the pharmacodynamic (PD) and pharmacokinetic (PK) behaviors of drugs encapsulated in/complexed to/conjugated to the carrier [26]. In the case of dendrimers, the loading ability of drug molecules and other bioactive agents can be altered by varying dendrimer generations, the water solubility, biodistribution, circulation time in blood and therapeutic efficiency of drugs in dendrimer-based formulations can be tuned by varying dendrimer surface components, the release of drugs from dendrimer scaffolds can be controlled by using different degradable linkers between dendrimers and drugs, and the specific accumulation of the dendrimer-based therapeutics can be achieved by further modifying the dendrimers with targeting moieties [26]. These properties together prove dendrimer perfect candidates in the design of new DDS. In this mini-review, we will mainly discuss the influence of dendrimers on the PD and PK behaviors of drugs complexed or conjugated to them.

2. Drugs complexed to dendrimers

Dendrimers were reported to possess non-polar cavities in their interior, which ensures them capable of encapsulating hydrophobic drug molecules [10,27–29]. Also, there are large numbers of positively or negatively charged functional groups on the surface of dendrimers, which make it easy for drug molecules with opposite charges to attach [30–32]. These non-covalent inclusions or complexes offer a variety of promising advantages over the free drug molecules, such as enhanced water solubility and drug stability, programmed release of drugs from the matrixes, and improved PD and PK behaviors [10,28]. A question which should be proposed here is that ''How does dendrimer influence the PD and PK behaviors of drugs associated with dendrimers?''

When generation 3 (G3) amine-terminated poly(amidoamine) (PAMAM) dendrimers were used as drug carriers of sulfonamides [33], a family of widely used anti-bacterial agents, the complexes were definitely more potent than free drugs against *Escherichia coli* (*E. coli*) (the data are shown in Table 1). It is interesting that drugs entrapped in the cavities of PAMAM dendrimers exhibit improved release profiles but without decrease of the anti-bacterial activities. This encapsulation and complexation strategy of hydrophobic drugs to PAMAM dendrimers would be powerful and easy technique generally. The authors presumed that the enhanced anti-bacterial activity was contributed by the dendrimers which might favor the interaction of sulfonamide with its target or help the drug to penetrate into bacteria by disturbing the cell membrane. Similar results were obtained in this respect when other anti-microbial agents such as quinolones [34] and sulfadiazine [35] were used as the model drugs (Table 1). Unfortunately, *in vivo* PD and PK data, which are essential to evaluate the clinical effectiveness of these dendrimer–drug complexes, were limited at current stage. Camptothecins, a class of well-established anti-cancer drugs with extremely low aqueous solubility, were successfully encapsulated in a biocompatible G4.5 carboxylate-terminated polyester dendrimer to prepare dendrimer–drug complexes [29,36]. All the complexes exhibited a much lower half-maximal inhibitory concentration (IC_{50}) on cancer cells than free camptothecins (1.2- to 7.1-fold of activity depending on different cancer cell lines, see Table 1). Cellular uptake levels of the complexes

Table 1
The effect of dendrimers on the therapeutical activity of drugs bound to them

Drugs	Dendrimers	Interaction pattern	Goal	Activity ratio of complex and free drug	References
Sulfamethoxazole	PAMAM	Complexation	Anti-bacterial	4–8	[33]
Prulifloxacin	PAMAM	Complexation	Anti-bacterial	2	[34]
Nadifloxacin	PAMAM	Complexation	Anti-bacterial	1	[34]
Sulfadiazine	PAMAM	Complexation	Anti-toxoplasmic	10^4–10^7	[35]
Camptothecins	Polyester dendrimer	Complexation	Anti-cancer	1.2–7.1	[29]
Pilocarpine nitrate	PAMAM	Complexation	Miotic	1–1.4[a]	[37]
Tropicamide	PAMAM	Complexation	Mydriatic	1–1.3[a]	[37]
Ibuprofen	PAMAM	Conjugation	Anti-inflammatory	2[b]	[54]
Paclitaxel	PAMAM	Conjugation	Anti-cancer	10	[57]
Methotrexate	PAMAM	Conjugation	Anti-cancer	3–24	[13]
Doxorubicin	Polyester dendrimer	Conjugation	Anti-cancer	0.02–0.21	[40]
Doxorubicin	Bow-tie dendrimer	Conjugation	Anti-cancer	<0.1	[58]
Cisplatin	PAMAM	Conjugation	Anti-cancer	-10^{-3}	[18]
Methotrexate	Folate–PAMAM	Conjugation	Anti-cancer	4	[59]
Methotrexate	Folate–PAMAM	Conjugation	Anti-cancer	Decreased	[60]

[a] The activity ratios for pilocarpine nitrate and tropicamide are obtained as the ratios of maximum miotic or mydriatic activity of the two drugs.
[b] The activity ratio of ibuprofen is obtained as the ratio of minimum response time for prostaglandin suppression; the activity ratios of other drugs are obtained as the ratios of half-inhibitory concentrations of the drugs.

by MCF-7 cells were significantly higher (up to 16-fold) than that of the free drug formulations. In addition, efflux measurements of the drug formulations in cancer cells showed that the presence of dendrimers increased the drug retention time within the cells. These results suggested that the EPR effect of dendrimer-based drug formulations also exists in cell level studies, which is in accordance with animal studies. Similarly, when pilocarpine nitrate and tropicamide were used as guests to different generations of PAMAM dendrimers [37], the miotic and mydriatic activities of their complexes on rabbit eyes were significantly enhanced compared to that of free drugs (Table 1). The presence of PAMAM dendrimers significantly increased the duration of miotic effect of pilocarpine nitrate and the bioavailability of both drugs. The improved effect of the dendrimer–drug formulations might be contributed to the greatly enhanced penetration of the drugs through the cornea favored by dendrimers, the perfect bioadhesive properties of PAMAM dendrimers, and the sustained release of drugs from the globular scaffolds of dendrimers.

It is worth noticing here that although numerous dendrimer–drug complexes showed enhanced or similar drug activities compared to free drugs, this rule is not universally applicable. An exceptional example is that when G4–G6 amine-terminated PAMAM dendrimers were used as vehicles of camptothecins [32], the anti-cancer activities of the dendrimer–drug complexes were reduced though significantly increased solubility of the drugs was obtained (data not shown). This is because an accelerated hydrolysis of camptothecins from an active lactone form to an inactive carboxylate form was found after dendrimers added to the camptothecin systems (Fig. 1). Interestingly, pilocarpine nitrate also has such lactone and carboxylate balance in aqueous medium but without decreasing its miotic activity after its complexation with dendrimers [37]. More attentions should be paid to such drugs that have conformational changes when varying the pH conditions of the drug solutions.

Before considering the effect of dendrimers on the *in vivo* PD and PK behaviors of drugs complexed to them, we should evaluate the biodistribution of dendrimers themselves. Tritium labeled G5 PAMAM dendrimers were rapidly cleared from the blood via the kidney after 1 day of intravenous injection [38]. ^{125}I-Labeled PAMAM dendrimers were found to be mainly accumulated in the liver (60%) and only 1% of the injected dose was still in the blood after 1 h of intravenous injection [39]. In contrast, carboxyl-terminated and PEGylated dendrimers exhibited a much longer circulation time in the blood [40], suggesting that the biodistribution problem of dendrimers can be resolved by altering the functional groups of dendrimers or modifying the periphery of dendrimers with biocompatible molecules.

As most of the non-steroidal anti-inflammatory drugs (NSAIDs) are hydrophobic molecules with carboxyl groups, cationic dendrimers (amine-terminated) might be all-purpose carriers for these drugs. Now, NSAIDs are the mostly investigated drugs in dendrimer-based DDS and the handful families of drugs whose PD and PK data are available at current stage (Table 2). Aspirin, indomethacin, flurbiprofen, ketoprofen, ibuprofen, naproxen, diflunisal, diclofenac sodium, and piroxicam [10,28,41] were successfully encapsulated or complexed into/with amine-terminated PAMAM or poly(propylene imine) (PPI) dendrimers (PAMAM and PPI dendrimers are now commercially available dendrimers and are the most investigated dendrimers) by several research groups. In the case of flurbiprofen [42], the PAMAM dendrimer–drug complex formulation showed an inflammatory inhibition of 75% at 4 h and maintained above 50% up to 8 h after intravenous injection, while the free drug exhibited an inhibition above 50% only in the first 4 h of injection. The bioavailability of flurbiprofen was also increased by a factor of 1.09 and the mean residence time of the drug in the blood was 3.07 times longer in the presence of dendrimers, compared to that of free drug (Table 2). In the case of indomethacin [43], intravenous administration of the PAMAM dendrimer–indomethacin complex showed a two-compartment PK profile. The area under curve $[AUC]_{0-24\ h}$ of the complex was 1.91 times higher and mean retention time of the complex was also 1.91 times longer than that of free indomethacin. The targeting efficiency of the complex in the inflamed regions was 2.29 times higher compared to free indomethacin. It is interesting that PAMAM dendrimers prolonged the retention of drug at the inflamed site though the existing of lymphatic drainage may decrease the retention of dendrimers. Transdermal delivery of PAMAM

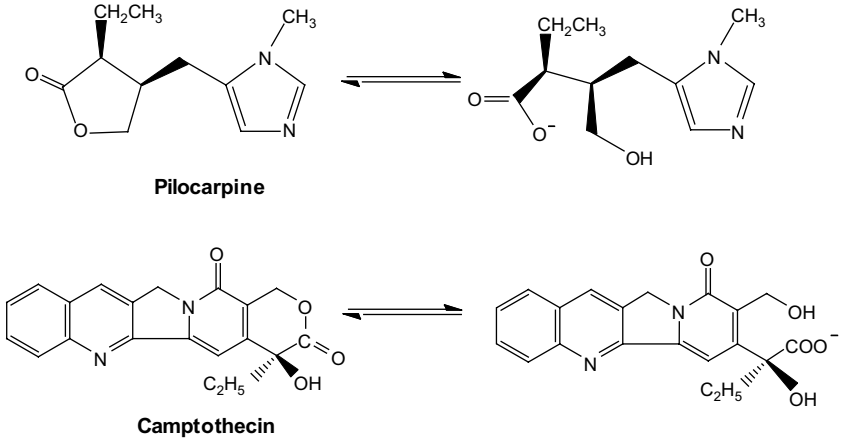

Fig. 1. Hydrolysis of camptothecin and pilocarpine from lactone forms to carboxylate forms.

Table 2
The effect of dendrimers on the pharmacodynamic (PD) and pharmacokinetic (PK) behaviors of drugs complexed to them

Drugs	Dendrimers	Administration route	[AUC] value ratio of complex and free drug	Effective therapeutic time of complex and free drug	References
Flurbiprofen	PAMAM	Intravenous	1.09	8 h/4 h	[42]
Indomethacin	PAMAM	Intravenous	1.91	–	[43]
Indomethacin	PAMAM	Transdermal	2.38	4 h/0 h	[44]
Ketoprofen	PAMAM	Oral	1.17	5.5 h/1.5 h	[46]
Ketoprofen	PAMAM	Transdermal	2.73	7 h/2 h	[47]
Diflunisal	PAMAM	Transdermal	2.48	6 h/1 h	[47]
Indomethacin	PAMAM	Intraperitoneal	1.43	–	[48,49]
Indomethacin	Folate–PAMAM	Intraperitoneal	1.19–1.78	–	[48,49]
Primaquine phosphate	PPI	Intravenous	1.87	–	[50]
Primaquine phosphate	Galactose–PPI	Intravenous	2.59	–	[50]
5-Fluorouracil	PAMAM	Intravenous	0.97	–	[51]
5-Fluorouracil	PEG–PAMAM	Intravenous	0.71	–	[51]

dendrimer–indomethacin complex exhibited a distinct PD and PK profile from the intravenous route [44]. The effective inhibition of paw edema (over 50% of inhibition) could be maintained from 2 h up to 6 h of complex transdermal administration, while administration of the same amount of free drugs was inactive during the whole experimental period. PK data showed that the presence of dendrimer increased the bioavailability of indomethacin by a factor of 2.38, reflecting the same trend as in the PD studies (Table 2). In the case of ketoprofen, oral delivery of the complex showed an effective anti-nociceptive activity period of 0.5–6 h, while the activity of pure drug was absent after 2 h of administration [45,46]. The bioavailability of ketoprofen was increased by a factor of 1.17 in the presence of G5 amine-terminated PAMAM dendrimer. It is interesting that the release rate of ketoprofen in blood appears to be much higher than the *in vitro* release rate, which may be contributed to the degradation of the complex in the acidic conditions in stomach. In a separate study, transdermal delivery of the G5 PAMAM dendrimer–ketoprofen complex showed a prolonged PD profile (1–8 h for the complex versus 4–6 h for the free drug) [47]. PK studies revealed that the bioavailability of ketoprofen was 2.73 times higher for the complex than that of the free drug. Similar results were obtained when diflunisal was used as the model drugs in this administration route [47] (Table 2). Since the over-expression of folate receptors on the surface of activated synovial macrophages taken from rheumatoid arthritis patients, NSAIDs complexed to folic acid conjugated dendrimers may lead to site-specific delivery and reduced systemic problems of these drugs. Indomethacin loaded PAMAM dendrimers and folate-conjugated PAMAM dendrimers exhibited distinct PD and PK behaviors after intraperitoneal injection [48,49]. The bioavailability of indomethacin for the folate-conjugated dendrimer complex (21.38 mol folic acid per mol dendrimer) was 1.78 and 1.25 times higher than that of free drug and dendrimer–indomethacin complex, respectively. The mean retention time of the folate containing complex was 1.81 and 1.23 times longer than that of free indomethacin and the non-folate complex. Besides NSAIDs, other drugs such as primaquine phosphate [50] and 5-fluorouracil [51] also showed significantly improved PD and PK behaviors when complexed to dendrimers. Compared to normal drug injections, the bioavailability of primaquine phosphate, a liver schizonticide, was increased by factors of 2.59 and 1.87 when it was complexed to galactose-conjugated PPI dendrimer and non-modified PPI dendrimer, respectively [50]. 5-Fluorouracil encapsulated in PAMAM dendrimer or PEGylated PAMAM dendrimer showed much increased mean retention time and much decreased maximum drug concentration in blood after intravenous administration [51]. Although the AUC value of the PEGylated drug complex was lower than that of non-PEGylated drug complex and pure drug itself, the sustained release profile of 5-fluorouracil from the complex by both *in vitro* and *in vivo* studies suggested an increased bioavailability of 5-fluorouracil.

Based on the examples described above, we suggest that dendrimers increase the activities of many drugs complexed to them by favoring them to penetrate through cell membrane and interact with their targets. These promising materials with excellent bioadhesive property also prolonged the retention of the drugs within the cells, tissues or organs after the complexes were captured. That is why drugs complexed to dendrimers exhibited a sustained release profile but without decreasing their activity or therapeutic efficacy. PD and PK results of the formulations indicated that the presence of dendrimers can increase the drug bioavailability, prolong the retention time of the drugs at their active regions, and optimize the therapeutic efficacy of these drugs. Although the PD and PK behaviors of some dendrimer–drug complexes were reported to be not suitable for clinical trails, we can resolve this problem by either administrating these complexes in some local administration routes (such as transdermal delivery, ocular delivery, mucosal delivery and nasal delivery) or modifying the surface of dendrimers with targeting moieties (such as folic acid and galactose) or biocompatible molecules (such as PEG chains) to alter the PD and PK profiles of these complexes.

3. Drugs conjugated to dendrimers

Generally, drugs conjugated to polymeric carriers can achieve decreased non-specific toxicity, enhanced therapeutic efficacy, sustained drug release profile, optimized drug

biodistribution, increased circulation time in blood, and targeted drug delivery [4,10,12]. Large numbers of functional groups on the outer shell of dendrimers are responsible for high reactivity and expected to conjugate with a series of bioactive molecules (such as therapeutic agents, targeting moieties, imaging chemicals, and biocompatible molecules) [26]. Drugs covalently conjugated to the periphery of dendrimers can lead to a much slower release rate from the polymer matrixes and much more influenced the PD and PK behaviors by the properties of the dendrimers, compared to those encapsulated in the interior cavities by hydrophobic interactions and loaded on the surface of dendrimers by electrostatic interactions [26,52].

Dendrimer–drug conjugates with a high drug payload can rapidly penetrate into cells and localize in the cytoplasm or nucleolus, which is in accordance with the phenomena obtained with dendrimer–drug complexes [53–55]. The PAMAM dendrimer–ibuprofen conjugate linked via ester bond was able to hydrolyze inside the cells and exhibited a superior prostaglandin suppression at short time scales (30 min for the conjugate versus 1 h for free ibuprofen, Table 1), indicating higher drug activity for the conjugate [54]. PAMAM dendrimer–penicillin V conjugate showed a comparable anti-bacterial activity to free penicillin though the conjugate exhibited excellent sustained release behavior [56]. Hydroxyl-terminated PAMAM dendrimer–paclitaxel conjugate through a succinic acid linker showed significantly increased anti-cancer activity compared to free paclitaxel (10-fold, Table 1) [57]. Though both the dendrimer–paclitaxel and PEG–paclitaxel conjugates displayed the similar release profile and could penetrate through the membrane of cancer cells, the dendrimer-based conjugate had a much higher anti-cancer activity than the PEG-based one (250-fold), suggesting dendrimers increase the uptake level of paclitaxel while PEG molecules decrease the uptake by increased molecular weight of the drug. Dendrimer (carboxyl-terminated)–methotrexate conjugate linked via amide bond was 3- and 8-fold more potent than free methotrexate on methotrexate-sensitive (CCRF-CEM) and resistant (CEM/MTX) cell lines, respectively [13]. The conjugate was even 24-fold more active than free methotrexate on another methotrexate-resistant cell line (CHO). It is interesting that the dendrimer–methotrexate conjugate formed by the linkage of carboxyl groups in methotrexate to amine groups of dendrimer was not as active as free methotrexate towards both methotrexate-sensitive and resistant cell lines, indicating different intracellular release profiles of methotrexate from the conjugates.

Conjugation of drugs to dendrimers can increase the activity of the attached drugs by EPR effect or other featured properties of dendrimers, but this rule was not true for all the dendrimer–drug conjugates. Polyester dendrimer–doxorubicin conjugate via a hydrazone linkage showed decreased anti-cancer activity by factors of 4.84, 8.74, and 58 towards B16F10, MDA-MB-435, and MDA-MB-231, respectively [40]. The dendritic architecture used to construct the conjugate was composed of biocompatible molecules such as PEG chains and 2,2-bis(hydroxymethyl) propanoic acid, indicating that the dendrimer kept doxorubicin escaping from the polymer scaffold in a controlled release profile but without increasing the uptake level of the drug. Most importantly, the circulation time of dendrimer–doxorubicin in blood was significantly increased compared to free doxorubicin (half-life in blood: 72 min for conjugate and 8 min for free drug), suggesting an increased bioavailability of doxorubicin during intravenous administration of the conjugate. Similarly, when doxorubicin was conjugated to a bow-tie dendrimer through a pH-sensitive hydrazone linker, the obtained dendrimer–doxorubicin conjugate was over 10-fold less toxic than free doxorubicin in C-26 colon carcinoma cells [58]. After the conjugate and free doxorubicin were injected into BALB/c mice bearing C-26 tumors, the tumor uptake of the conjugate was 9-fold higher than that of the free doxorubicin; all the mice survived during the 60-day experiment for the conjugates, while no one was cured during this period when the animals were treated with free doxorubicin at its maximum tolerated dose or doxorubicin–free dendrimer. The remarkable anti-cancer activity of the conjugate was contributed to EPR effect of the biocompatible dendrimer and the ability of dendrimer to modulate the PK behavior of attached doxorubicin. Not limited to the dendrimer–doxorubicin conjugates, carboxylate-terminated PAMAM dendrimer–cisplatin conjugate was also much less toxic than free cisplatin when incubated with CCRF, COR L23 and B16F10 cells (200- to 500-fold depending on the cell line, Table 1) [18]. After the intraperitoneal injection of dendrimer–cisplatin conjugate or cisplatin into mice bearing tumor (B16F10 or L1210), C57 mice treated with the conjugate at 15 mg/kg displayed much reduced toxic death, decreased body weight, and increased *T*/*C* (%) value compared to the control groups and those treated with free cisplatin at its maximum tolerated dose. The dendrimer–cisplatin conjugate administrated by intravenous injection also showed a 5- to 50-fold (depending on dose) higher cisplatin concentration in the tumors than can be achieved for free cisplatin at its maximum tolerated dose, indicating a much increased bioavailability by the animals and enhanced selectivity to accumulate in tumors (EPR effect).

Dendrimer–drug conjugation greatly changes the PD and PK behaviors of the bound drug. It prolongs the circulation time of the drug in blood and selectively accumulates in tumor tissues by the EPR effect. Of course, methods for targeted delivery of dendrimer–drug conjugates to the tumors are not limited to the passive targeted delivery. An active targeted delivery system based on dendrimer–drug conjugate can be achieved by modifying targeting moieties to the conjugate scaffold, which will lead to directly targeting the conjugates to tumor vasculatures, tissues and cells. The PD and PK behaviors of the dendrimer–drug conjugates are discussed to evaluate the designs of these nanodevices. Methotrexate is the most used model drug in this aspect. In a early study, methotrexate conjugated to G5 PAMAM dendrimer–folate conjugate through ester linkage showed a much increased anti-cancer activity (4-fold) on KB cells compared to equimolar concentrations of free methotrexate, while the methotrexate–dendrimer–folate conjugate through amide linkage was less toxic than the free drug, suggesting that the distinct cytotoxicity of the conjugates was contributed to different intracellular drug release profiles [59]. Unfortunately, the anti-cancer activity of

the methotrexate–dendrimer conjugate without folate molecules was not tested by the authors to further partition the contributions of active targeting effect of folate molecule and EPR effect of the dendrimer scaffold to the enhanced drug activity. In a separate study, the methotrexate–dendrimer–folate nanodevice exhibited a time-dependent and dose-dependent inhibition of cell growth on KB cells and a specific targeting behavior in cancer cells that overexpress folate receptors [60,61]. Surprisingly, the anti-proliferative activity of the folate containing conjugate on KB cells was not as high as that of free methotrexate. When this conjugate as well as free methotrexate was administrated to SCID mice bearing KB xenografts by intravenous injection, the conjugate was more effective in tumor growth delay than equimolar dose of free methotrexate and as effective as a high dose of free methotrexate (more than 4-fold of methotrexate) [38]. Nearly 40% of the animals treated with the bifunctional conjugate survived during 99 days of trial, while all the animals treated with equimolar amount of methotrexate died before 70 days of trail. The methotrexate–dendrimer–folate nanodevice also showed much more effective anti-cancer activity than dendrimer–methotrexate conjugate and could prolong the mean survival time of animals treated with dendrimer–methotrexate conjugate by at least 38 days.

Generally, the PD behavior and biodistribution of dendrimer–drug conjugates were greatly influenced by the properties of dendrimers including dendrimer component, surface charges and functional groups. The *in vivo* parameters of the conjugates such as site specificity, degradation rate, plasma circulation time and cytotoxicity can be altered by modifying the surface of dendrimers with targeting moieties and biocompatible molecules. When designing such a dendrimer–drug conjugate for therapeutic purpose, more attention should be paid to the intracellular release profile of the drug from the polymer. If the dendrimer–drug conjugate is stable inside the cells, pH or enzyme cleavable linkers should be employed to resolve the problem.

4. Complexes or conjugates?

Altogether, the complexation of drugs to dendrimers via hydrophobic encapsulations or electrostatic interactions usually preserves the chemical integrity and pharmacological properties of drugs, while covalent attachment of drugs to the surface groups of dendrimers through chemical bonds offers the opportunity for a better control over drug release than that can be achieved by simple encapsulation/electrostatic complexation of drugs into/with the dendrimers [26,52]. Covalent conjugation is more suitable for passive or active drug targeting but decreases the aqueous solubility of the formulations compared to dendrimer–drug complexes. A recent study compared the release kinetics of methotrexate from the folate–dendrimer–methotrexate conjugate and folate-dendrimer–methotrexate complex [25]. The results revealed that the dendrimer–drug conjugate was more suitable for targeted drug delivery, while the complex which can improve the solubility of methotrexate in aqueous solutions was identical to free methotrexate on cytotoxicity towards cancer cells.

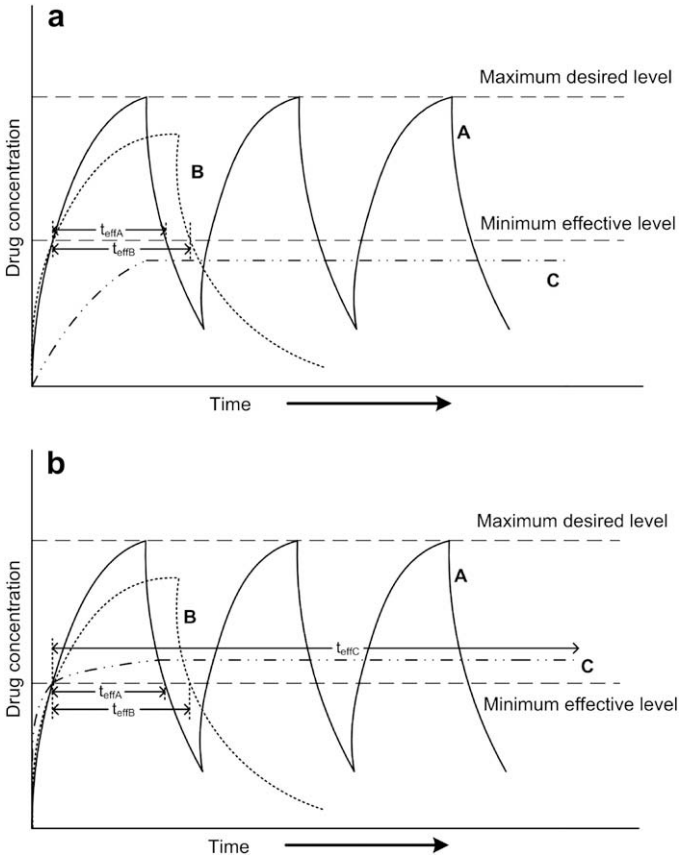

Fig. 2. Potential pharmacokinetic profiles of (A) traditional dosing, (B) formulations of drug–dendrimer complexes, and (C) drug–dendrimer conjugates. In part figure (a), the order of the effective therapy time (t_{eff}) is: $t_{effB} > t_{effA} > t_{effC} = 0$; in part figure (b), the order is $t_{effC} > t_{effB} > t_{effA}$. In both (a and b), the orders of the bioavailability of the three formulations are C > B > A.

Dendrimer-based DDS are in their infancy and the PD and PK data of them are still limited at current stage. We need to conduct more systemic investigations and collect more data on the PD and PK behaviors of the complexes and conjugates. A question proposed here is that ''Which drug formulation is our real need: the complex formulation or the conjugation formulation?'' Of course both the dendrimer–drug complex and the conjugate provide much increased bioavailability compared with free drugs. However, does bioavailability of a drug represent the benefits we can get from the delivery system? As shown in Fig. 2, we think the drug formulation which provides the longest effective therapy at a fixed dose is our best choice. We are now conducting experiments on comparing the relationship between the bioavailability and effective therapeutic time of dendrimer–drug complexes and conjugates at different dosing and animal models.

5. Conclusions and perspectives

The biomedical significance of dendrimer-based DDS (both dendrimer–drug complexes and conjugates) described in this mini-review is only just the beginning to emerge. Compared to other polymeric DDS (HPMA and PEG), dendrimer-based

drug formulations are relatively new concepts, but they have already exhibited several attractive features [2,11]. The already-established studies on the use of dendrimers for delivery of therapeutics indicate that these promising polymers will continue to play a significant role in the design of new DDS in the future. Despite significant promise of dendrimer-based drug formulations in cell culture and animal level studies, final applications of these therapeutics agents in humans are still very challenging. The most important issue that prevents dendrimer-based DDS from broad success at pre-clinical level is the biodistribution of dendrimers [12,26]. It is still a challenge to prepare dendrimer-based drug formulations with relatively low clearance and long plasma half-time in the blood, though the framework of dendrimers was always non-toxic polymers. Future efforts in this field should be directed to the development of dendritic scaffolds with long half-lives (PD and PK) and perfect degradation ability (health risk) in the blood. Of course, more clinical PD and PK studies on these promising drug formulations are urgently needed to permit the validation of appropriate PK models that can be used in the future to assist in the optimization of clinical protocols and improve the DDS design.

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