Pharmaceutical applications of dendrimers: promising nanocarriers for drug delivery

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1. ABSTRACT

Dendrimers are new artificial macromolecules which have the structure like a tree. They are hyperbranched and monodisperse three-dimensional molecules with defined molecular weights, large numbers of functional groups on the surface and well-established host-guest entrapment properties. Recently, dendrimers have successfully proved themselves as promising nanocarriers for drug delivery because they can render drug molecules a greater water-solubility, bioavailability, and biocompatibility. In this review, recent progress in the pharmaceutical applications of dendrimers as delivery systems for drugs, particularly, the non-steroidal anti-inflammatory, anti-microbial/anti-viral and potent anti-cancer drugs is discussed. Three possible interaction mechanisms between dendrimers and drug molecules are presented. In addition, the pharmacodynamic and pharmacokinetic properties of both dendrimer/drug complex and dendrimer-drug conjugation after their administration to animals are evaluated.

2. INTRODUCTION

Dendrimers are new artificial tree-like macromolecules that consist of three distinct components: a central core, repeated branches and terminal functional groups (1, 2) (Figure 1). Distinct from traditional linear polymers, they are monodisperse macromolecules with unique molecular weights and well-defined numbers of peripheral groups due to the step-by-step synthesis strategy (3). They can employ either small organic molecule or polymer as structural component. Thus, the choice of central core and repeated unit is of great importance to determine the molecular weight, size, shape, density, polarity, flexibility, and solubility of dendrimers (4).

Generally, there are two approaches used to synthesize dendrimers: the divergent method and the convergent one (Figure 2). Tomalia et al. first reported the successful, well-characterized synthesis of poly(amidoamine) (PAMAM) dendrimers by using the divergent method in the early 1980s (5). Later in 1990,
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Figure 1. Schematic representation of a dendrimer structure. Reproduced with permission from (2).

Figure 2. Two different strategies of dendrimer synthesis. Top: divergent strategy, bottom: convergent strategy. Reproduced with permission from (60).
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Hawker et al. proposed the convergent method for dendrimer construction (6). The divergent method starts from a central core and extends toward the surface, while the convergent one uses a top-down approach and starts from the periphery of dendrimers. Currently, commercially available dendrimers, both PAMAM and poly(prolenemine) (PPI), which have been most widely used in biomedical fields, were synthesized by using the divergent method (7) (Figure 3).

It has been reported that significant conformational changes occur when dendrimers reach a specific generation (a variable factor according to the dendritic structure, for PAMAM dendrimers with an ethylenediamine core, this factor equal to 4). Low-generation dendrimers (G0–G3) have ellipsoidal shapes but no interior characteristics, whereas the high-generation dendrimers (G4–G10) have roughly spherical shapes and well-defined interior cavities (4). Besides dendrimer generation, the conformation of dendrimers also depends on the solution conditions. Welch and Muthukumar (8), using Monte Carlo simulations, reported that the conformation of PAMAM dendrimer was tunable from the dense core pattern to the dense shell one by manipulation of the salt concentration or pH value of the dendrimer solution (Figure 4). Another key characteristic where traditional linear polymers and PAMAM dendrimers exhibit distinct properties is their viscosity behavior. The viscosity of linear polymers increases with the increase of molecular weight of the polymers, while PAMAM dendrimers exhibit linear relationships at lower generation range, maximum at their conformational change points and followed by a decrease in viscosity at higher molecular weight (9).

PAMAM dendrimers are one of the most studied dendritic polymer families today. They are nano-scale molecules in that they range from 1.1 nm to 12.4 nm in diameter for generation 1 (G1) through generation 10 (G10)
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Figure 5. Several mechanisms for dendrimer-drug interactions. Reproduced with permission from (18).

(4, 10, 11). PAMAM dendrimers were reported to possess perfect solubility in a large number of solvents, especially in water. Their high solubility in these solvents leads to rapid dissolution and provides various approaches to characterize their structures (4, 12). Non-polar cavities in PAMAM dendrimers in combination with their hydrophilic exterior surface make them capable of encapsulating hydrophobic drug molecules and ensure their applications as solubility enhancers of these hydrophobic agents (13). These non-covalent inclusions offer a variety of physicochemical advantages over the free drug molecules including the possibility of enhanced water solubility and drug stability (14). Moreover, large numbers of functional groups (such as amine groups, carboxyl groups and hydroxyl groups) on the outer shell of PAMAM dendrimers are responsible for high reactivity and expected to conjugate with a series of bioactive molecules (15). Drugs or other guest molecules can be loaded either in the hydrophobic cavities, or can be attached to the functional groups on the surface (Figure 5). As the absorption and distribution of encapsulated/conjugated drugs depend on the properties of dendrimers, in vivo parameters of the dendrimer-drug formulation such as site specificity, degradation rate, plasma circulation time and toxicity can be altered by modifying the surface functional groups of dendrimers (9). These specific features of dendrimers together provide the availability of dendrimers to deliver bioactive agents to specific diseased sites, consequently minimizing drug systemic toxicity (16-18). They make dendrimers reliable alternatives to traditional polymers as novel biocompatible drug carriers (19). This review will highlight recent progress in the pharmaceutical applications of dendrimers with particular references to PAMAM dendrimers as delivery systems for NSAIDs (26).

3. DELIVERY OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS BY DENDRIMERS

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently used drugs in the world, primarily for symptoms associated with osteoarthritis and other chronic musculoskeletal conditions (20). Also, they reduce the mortality from colon cancer by about half and constitute the prototypical colon cancer chemopreventive agents (21). However, the oral administration of NSAIDs is limited by their significant toxicity. They may cause a vast variety of clinical side effects, including gastrointestinal side effects (such as dyspepsia, gastrointestinal bleeding, and even perforation), renal side effects and some additional side effects (such as hypersensitivity reactions and distinct salicylate intoxication) (22). Also, the poor solubility of NSAIDs restricts their use in parenteral applications and presents a major challenge during drug formulation. The side effects of NSAIDs and their poor solubility have prompted intensive efforts to identify safer alternatives. For example, in order to improve the aqueous solubility of NSAIDs, addition of surfactants such as sodium dodecyl sulphate. The approximate number of ibuprofen molecules attached to each G4 PAMAM dendrimer was calculated to be 41. The effect of pH value of dendrimer solution on the solubility of ibuprofen indicated that the enhancement was due to electrostatic interactions between dendrimers and ibuprofen molecules. Kolhe et al. reported that the maximum number of ibuprofen associated with each G4 PAMAM dendrimer was 78 (28). Since the number of primary amine groups on the surface of each G4 PAMAM was 64, it could be concluded that ibuprofen molecules can be either encapsulated in the interior cavities by hydrophobic interactions or attached on the surface by electrostatic interactions. FT-IR and NMR results of the dendrimer-drug complexes suggested that the carboxylate ion of ibuprofen predominantly formed a stable complex with the amine

3.1. Non-covalent complexation of NSAIDs

In early studies, Milhem et al. investigated the potential of PAMAM dendrimers as drug carriers of hydrophobic drugs as exemplified by ibuprofen (27). They found that PAMAM dendrimers could significantly enhance the solubility of ibuprofen compared to traditional surfactants such as sodium dodecyl sulphate. The approximate number of ibuprofen molecules attached to each G4 PAMAM dendrimer was calculated to be 41. The effect of pH value of dendrimer solution on the solubility of ibuprofen indicated that the enhancement was due to electrostatic interactions between dendrimers and ibuprofen molecules. Kolhe et al. reported that the maximum number of ibuprofen associated with each G4 PAMAM dendrimer was 78 (28). Since the number of primary amine groups on the surface of each G4 PAMAM was 64, it could be concluded that ibuprofen molecules can be either encapsulated in the interior cavities by hydrophobic interactions or attached on the surface by electrostatic interactions. FT-IR and NMR results of the dendrimer-drug complexes suggested that the carboxylate ion of ibuprofen predominantly formed a stable complex with the amine
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groups of cationic dendrimers. To study the strength and stability of the complexes, in vitro release studies were conducted in RPMI 1640 culture medium and deionized water. The results showed that release of ibuprofen from the dendrimer-drug complex was much slower than pure ibuprofen formulation. The release rate depended on the pH condition and salt concentration of the dendrimer solution. The uptake of dendrimer-drug complex by A549 cells were found to be much more rapidly than pure ibuprofen suggesting that PAMAM dendrimers were able to enhance the cell entry rate of ibuprofen.

As most of the NSAIDs are hydrophobic molecules with carboxyl groups, dendrimers are probably all-purpose carriers for these drugs. Further studies on applications of dendrimers in this aspect are not limited to in vitro solubility and release studies, but also extend to in vivo pharmacodynamic and pharmacokinetic studies in different routes (intravenous, transdermal and oral) of drug administration. Encapsulation of indomethacin by PAMAM dendrimers was studied by Chauhan et al. (29). The solubility of indomethacin in the presence of dendrimers followed the Higuchi's $A_s$ solubility profile (drug solubility increases linearly at lower dendrimer concentrations and exhibits a negative deviation from the linearity at higher dendrimer concentrations). The decreased solubility from prediction was presumably due to self-association of dendrimer at higher concentrations. In vivo biodistribution of dendrimer-indomethacin complex after intravenous administration showed enhanced effective indomethacin concentration (2.29 times compared to that of the free drug) in the inflamed regions. Although the lymphatic drainage existed, PAMAM dendrimers still prolonged the retention of indomethacin at the inflamed site. In a separate study, the transdermal ability of PAMAM dendrimers was proposed by using indomethacin as a model drug (30). The results showed that the steady-state flux of indomethacin from the dendrimer-drug complex formulation was significantly enhanced compared to that from the pure drug suspension. The effective drug concentration could be maintained for 24 h in the blood after transdermal administration of the dendrimer-drug formulation. Pharmacodynamic activity of these formulations, which is based on the inhibition rate of paw volume in carrageenan-induced rat paw edema model, reflected the same trend as in the pharmacokinetic studies. These data indicated that dendrimer-drug based transdermal drug delivery system (TDDS) was effective and might be a safe and efficacious method for treating various diseases in clinical trials. Recently, Asthana et al. evaluated the in vitro and in vivo behaviors of PAMAM dendrimers as drug carriers of another NSAID, flurbiprofen (31). The solubility of flurbiprofen increased linearly with the increase in dendrimer concentration and attained its maximum value at neutral pH conditions. The in vitro release behavior of the dendrimer-flurbiprofen complexes displayed initial rapid release followed by the delayed release of the drug. The authors explained that it might be due to that the attachment of flurbiprofen to dendrimers via electrostatic interactions released the drug earlier, while the encapsulation of drug molecules within the hydrophobic cavities of dendrimers delayed the release rate. The in vivo efficacy study on dendrimer-flurbiprofen formulations showed 75% inhibition at 4 h after intravenous administration and maintained above 50% until 8 h. It was concluded that PAMAM dendrimers could prolong the delivery of the drug at the inflamed region and allow the drug to provide effective pharmacological action by selectively inhibiting cyclooxygenase, and hence optimize the therapeutic efficacy of the drug by reducing its side effects. Wiwattanapatapee et al. reported that both G3 and G2.5 PAMAM dendrimer were able to enhance the solubility of piroxicam (32). The enhancement in the presence of carboxyl group terminated dendrimer (G2.5) was due to electrostatic interactions between piroxicam and the carboxyl groups while that in the presence of G3 amine group terminated dendrimer was due to the increase of pH value by the cationic dendrimer. Cheng et al. studied the solubilization behavior of four NSAIDs (ketoprofen, naproxen, ibuprofen and diflunisal) in PAMAM dendrimers solutions (33). The effects of dendrimer concentration, generation on solubilization as well as pH-dependent studies were investigated. Solubilities of NSAIDs in dendrimer solutions increased in an approximately linear manner with the increase in dendrimer concentration. Higher generation dendrimers at higher pH conditions were able to solubilize more NSAIDs. The solubility enhancement was greater in amine-terminated dendrimer solutions than in the corresponding generation of ester-terminated ones. These observations are evidence of that the solubility enhancement of NSAIDs is due to electrostatic interactions between the surface amine groups of PAMAM dendrimers and the carboxyl groups of NSAIDs and to hydrophobic interactions between the interior cavities of dendrimers and hydrophobic drug molecules (34). After the in vitro solubility data have been collected, the potential of PAMAM dendrimers as oral drug carriers of NSAIDs (ketoprofen, naproxen, ibuprofen and diflunisal) mediated by PAMAM dendrimers (36). In vitro permeation studies performed on excised rat skins indicated that PAMAM dendrimers significantly enhanced the steady-state flux of both drugs, compared to the drug suspensions without PAMAM dendrimers. A possible explanation for this transdermal-enhancing effect was that dendrimers increased the water-solubility of NSAIDs and cationic dendrimers with primary amine groups on the surface could conceivably alter the skin barrier function and enable dendrimer-drug complexes to pass through. Anti-nociceptive studies using the acetic acid-induced writhing model showed that the anti-nociceptive activity (inhibit rate >50%) of the pure drug was absent after 3 h of oral administration, while that of dendrimer-drug complex was maintained until 8 h. These results revealed that dendrimer-NSAID complexes with sustained release behavior could be used as novel oral drug formulations (17). Recently, the authors investigated the transdermal delivery of NSAIDs (ketoprofen and diflunisal) mediated by PAMAM dendrimers (36). In vitro permeation studies performed on excised rat skins indicated that PAMAM dendrimers significantly enhanced the steady-state flux of both drugs, compared to the drug suspensions without PAMAM dendrimers. A possible explanation for this transdermal-enhancing effect was that dendrimers increased the water-solubility of NSAIDs and cationic dendrimers with primary amine groups on the surface could conceivably alter the skin barrier function and enable dendrimer-drug complexes to pass through. Anti-nociceptive studies using the acetic acid-induced writhing model in mice also showed a prolonged pharmacodynamic profile for the dendrimer-drug complex after transdermal administration. Moreover, blood drug level studies revealed that the drug bioavailability was 2.73 times higher for the dendrimer-ketoprofen complex and 2.48 times higher for the
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Figure 6. Chemical structures of PAMAM dendrimer and dendrimer–naproxen conjugates.

dendrimer-diflunisal complex, respectively, than that in the pure drug suspensions. The plasma NSAIDs concentrations in general agreed with the pharmacodynamic profile in mice. These results suggested that PAMAM dendrimers could effectively facilitate skin penetration of NSAIDs and might have the potential applications for the development of new transdermal formulations (17).

Site-specific delivery of NSAIDs to the inflamed site is the best choice to increase the drug efficacy and overcome the systemic problems caused by the side effects of NSAIDs. Since it has been discovered that functionally active folate receptors are present in the activated synovial macrophages taken from rheumatoid arthritis patients, Chandrasekar et al. developed the folate-PAMAM dendrimer conjugates for targeted delivery of anti-arthritic drugs using indomethacin as a model drug (37, 38). Increasing folate content of the conjugates increased indomethacin encapsulation efficiency but decreased the in vitro release rate of indomethacin from the complexes. The indomethacin blood concentration profile of the conjugates showed a rapid distribution behavior followed by slow elimination. The area under curve, half-life and residence time of indomethacin in inflamed paw was higher for folic acid-dendrimer conjugates compared to unconjugated dendrimer. Among the synthesized conjugates, the ones with more folic acid molecules had better indomethacin encapsulation properties (39). NSAIDs including 5-amino salicylic acid (5-ASA), mefenamic acid and diclofenac were employed as model drugs. These hydrophobic molecules gained enhanced solubility in aqueous medium after being trapped into the interior cavities of the synthesized dendrimers.

3.2. Covalent conjugation of NSAIDs

The presence of large numbers of functional groups on the surface of dendrimers makes them suitable for the covalent conjugation of numerous NSAIDs with relevant functional groups. In this case, NSAIDs can be covalently attached to the surface of dendrimers and released from the conjugates via chemical or enzymatic cleavage of hydrolytically labile bonds. Wiwattanapatapee et al. designed 5-ASA modified PAMAM dendrimers and studied their colonic delivery behavior (40). The drug was bound to dendrimers using two different spacers containing azo-bond, p-aminobenzoic acid and p-aminohippuric acid. It is well-known that the release of drug molecules from prodrugs containing azo-bond is triggered by enzyme azoreductase in the colon. When the synthesized dendrimer-5-ASA conjugates attend to the colonic region in the body, the amount of drug released from the conjugates significantly increased. Also, the cleavage of azo-bond in the conjugates (45% for p-aminobenzoic acid containing conjugates and 57% for p-aminohippuric acid containing conjugates in 24 h) were found to be much slower than that in low molecular weight prodrugs such as sulfasalazine (80% in 6 h). This result was explained by a decrease of enzyme cleavability to the azo-bond due to the highly branched structure of dendrimers. Thus, these conjugates possessed colon-specific properties and could prolong the delivery of 5-ASA. Najlah et al. synthesized a series of G0 PAMAM dendrimer-naproxen prodrugs for potential enhancement of drug solubility, stability and bioavailability (41). Naproxen was conjugated to PAMAM either directly by an amide bond or by ester bonds using either L-lactic acid or PEG as a linker (Figure 6). The direct
Figure 7. Chemical structure of a dendritic salicylic acid prodrug. Reproduced with permission from (44).

linkage of naproxen to PAMAM resulted in a stable prodrug under various pH conditions, while the ester linked conjugates were slowly hydrolyzed in human plasma with 20% release of naproxen after 16 h. In a separate study, the synthesized dendrimer-naproxen prodrugs were proposed to enhance the transepithelial permeability of naproxen (42). The prodrugs were non-toxic to Caco-2 cells and showed a significant enhancement in the permeation of naproxen. They could act as promising nano-carriers for the oral delivery of low solubility or stability drugs such as naproxen. Kolhe et al. prepared a high drug payload nanodevice by covalent conjugation of ibuprofen to a G4 PAMAM dendrimer with 64 hydroxyl groups on the surface (43). The dendrimer-ibuprofen conjugate was found to enter into A549 cells rapidly and localize predominantly in the cytoplasm. Moreover, the conjugates exhibited superior prostaglandin suppression at short time scales, indicating higher activity for the conjugates. Therefore, the dendrimer-ibuprofen conjugates ensured a high local drug level at the inflammatory site and potentially overcame the systemic problems of free ibuprofen. Tang et al. attached salicylic acid onto the surface of well-defined HO (G1-G3) dendrimers (Figure 7) and characterized the structures and polydispersity of the salicylate dendritic prodrugs by NMR and GPC (44). The dendrimer-drug conjugate displayed in vitro degradation lag time of several days and thereby would be useful for sustained delivery of NSAIDs such as salicylic acid.

Like most of the drugs, the encapsulation of NSAIDs within hydrophobic cavities or absorption of drugs to the surface of dendrimers via electrostatic interactions preserves the chemical integrity and pharmacological properties of NSAIDs, while covalent attachment of
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Figure 8. Potential pharmacokinetics of (A) traditional dosing, (B) formulations of drug/dendrimer complexes, and (C) drug-dendrimer conjugates.

Figure 9. Dendrimer-like PEO glycopolymers with anti-inflammatory properties. Reproduced with permission from (46).

NSAIDs 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 (9, 45) (Figure 8). Moreover, covalent conjugation allows tissue targeting and controlled delivery as the drug-dendrimer conjugates diffuse slower than the free drug in the body and might be absorbed in specific interfaces (17). Naturally, a problem may arise as a consequence of coupling large numbers of the drugs to the dendrimer surface by covalent conjugation, i.e., the insolubility of the resultant product. This problem can often be resolved through the concomitant attachment of soluble agents such as short PEG chains (15, 17).

Recent researches have demonstrated that heparin exhibits anti-inflammatory properties by mediating blockade of L-selectin and P-selectin via sulfate-dependent interactions, Rele et al. reported a dendrimer-like PEO glycopolymers exhibited anti-inflammatory properties (46) (Figure 9). The glycopolymers inhibited inflammatory cell recruitment to a similar degree compared to heparin but avoided the concurrent anticoagulant effects of heparin in clinical applications. These results provided an easily accessible route to carbohydrated-based compounds with anti-inflammatory activities.

4. DELIVERY OF ANTI-MICROBIAL AND ANTI-VIRAL DRUGS BY DENDRIMERS

Microbial and viral infections remain major causes of morbidity and mortality in hospitals around the world (47). Although numerous of antibiotics have been introduced in the 20th century, antibiotic resistance decreases the therapeutic efficacy of these drugs because of accumulation and acceleration of resistance during clinical
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Figure 10. Antimicrobial activities of different formulations of prulifloxacin.

administration. Also, it usually takes decades to develop a new anti-microbial or anti-viral drug. Therefore, the development of new formulations of existed anti-microbial and anti-viral agents is a promising method to overcome such limitations today.

4.1. Non-covalent complexation of anti-microbial and anti-viral drugs

Sulfonamides are widely used anti-microbial drugs in various bacterial infections including enteric and urinary tract, and respiratory tract (48). They are preferred due to the ease of administration and wide-spectrum of anti-bacterial activity. However, the clinical use of sulfonamides is limited mostly due to their extremely low solubility in water, rapid elimination in blood, low level of association to plasma proteins and several side effects (49). The poor solubility of sulfonamides restricts their use in topical and parenteral applications. Ma et al. studied the interactions between PAMAM dendrimers and sulfonamides using sulfamethoxazole (SMZ) as a model drug (50). The results showed that the aqueous solubility of SMZ was approximately proportional to dendrimer concentration. The in vitro release of SMZ from dendrimer-SMZ complex was much slower than that from pure SMZ formulation. Interestingly, when equal amounts of free SMZ and dendrimer-SMZ complex are tested in microbiology studies, the complex was definitely more potent than free SMZ against Escherichia coli (E. coli) (a 4 or 8-fold increase in anti-bacterial activity). The authors presumed that the enhanced anti-bacterial activity was contributed to the dendrimers which might favor the interaction of the drug with its target or help SMZ to penetrate through the bacterial membrane. Although further investigations are necessary in this respect, the in vitro results are very promising as they indicate that appropriate complexation with dendrimer can increase the effectiveness of SMZ.

Quinolones, another expanding class of clinically established potent antibiotics, whose accidental discovery occurred in the early 1960s, were very important in addition to the antibiotics that we had already developed (51). Although quinolones are well absorbed when orally given, they can still cause side effects such as stomach pain or upset, nausea, vomiting, and diarrhea (52). In some situations, quinolones should be given by intravenous injection for more serious infections. However, such compounds exist mainly in their zwitterionic form owing to the acid/base interaction between the basic nitrogen of the piperazine and the carboxylic acid group. Such interaction also determines the low aqueous solubility of these compounds at pH close to 7 (53). Cheng et al. investigated the potential of PAMAM dendrimers as drug carriers of quinolones (nadifloxacin and prulifloxacin) by aqueous solubility and anti-bacterial activity studies (54). The results showed that the aqueous solubilities of both nadifloxacin and prulifloxacin were significantly increased by PAMAM dendrimers. The increased solubility was proposed due to (1) electrostatic interactions between amine groups of dendrimers and carboxyl groups of quinolones, (2) hydrophobic interactions between the cavities and hydrophobic guest molecules and (3) hydrogen bond formations between tertiary amines in cavities of dendrimers and the atoms of quinolones. Nadifloxacin and prulifloxacin still exhibit their strong antimicrobial activities in the presence of PAMAM dendrimers (Figure 10). This work demonstrated that encapsulation/complexation quinolones into/with dendrimers led to excellent solubility of these drugs and similar anti-bacterial activity with pure drugs themselves. Now, the authors are in the process of conducting pre-clinical testing to evaluate these effective dendrimer-anti-bacterial drug complexes.

Recently, Prieto et al. employed G4 and G4.5 PAMAM dendrimers as biocompatible vessels of antitoxoplasmic agent sulfadiazine (SDZ) (55). Both dendrimers could efficiently entrap SDZ molecules into dendrimer interior cavities. G4.5 dendrimer and its SDZ complex showed no toxicity on Vero and J774 cells, while G4 dendrimer and its SDZ complex were toxic on these cells. The G4.5-SDZ complex decreased the infection of Toxoplasma gondii against Vero cells to 25–40%, while G4-SDZ complex produced a highest infection decrease of 60%. These in vitro preliminary results demonstrated that dendrimer-SDZ complexes might be used as a potential candidate for antitoxoplasmic therapy in clinical trials. Similarly, Bhadra et al. investigated the applications of PEGlated poly (lysine) dendrimers as drug carriers of a clinical well-established anti-malarial drug-artemether (56). Devarakonda and coworkers enhanced the solubility of an anthelmintic drug-niclosamide by using PAMAM dendrimers (57).

4.2. Covalent conjugation of anti-microbial and anti-viral drugs

Yang and coworkers conjugated penicillin V to G2.5 and G3 PAMAM dendrimers through a PEG spacer by amide or ester bond (58). Although the dendrimer-penicillin conjugates exhibited perfect controlled release behavior, the anti-microbial activities of the conjugates were found to be comparable to that of pure penicillin. Tam et al. described the synthesis and bioactive properties of anti-microbial dendrimeric peptides employing the poly (lysine) dendrimer and potent broad-spectrum antibiotics (tachyplesins) (59). The dendrimeric peptides were broadly effective against bacteria and fungi. The potency and activity of the dendrimeric peptides, depending on their size and charge on the surface, were comparable to tachyplesins. The multi-valency peptide dendrimers could amplify cationic charges and hydrophobic clusters as generation of dendrimer increases, and hence appeared to
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Figure 11. Schematic representation of an anti-bacterial dendrimer against E. coli. Reproduced with permission from (60).

Figure 12. Schematic representation of the highly mannosylated dendrimeric ligands. Reproduced with permission from (68).

be desirable in the design of membranolytic peptides for biochemical applications. These results indicated that incorporating peptide antibiotics in dendrimeric architectures presented an attractive approach for developing novel biocides in clinical practice.

The bio-adherence of toxins produced by bacteria to surface ganglioside receptors of epithelial cells can result in the induction of microbial infections and other serious diseases (60). Use of oligosaccharides to bind toxins prevents them from acting at the cell surface receptor sites. Large numbers of functional groups on the outer shell of dendrimers are expected to conjugate with a series of bioactive molecules such as oligosaccharides (61). Thus, once the cell surface receptors are well established, these modified dendrimers could be used to neutralize relevant toxins. Thompson and Schengrund synthesized oligosaccharide derivated dendrimers and evaluated their biological properties (62). The functionalized dendrimers exhibited effective inhibition of adherence of cholera toxin to cell surface, and had no effect on cell viability. Nishikawa and coworkers developed a series of carbosilane dendrimers in which trisaccharides of globotriaosyl ceramide (a receptor for Stxs) were variously oriented on the surface (63). The trisaccharide modified dendrimers could specifically bind to Stx with high affinity and inhibit the incorporation of the toxin produced by E. coli O157:H7 into target cells. In vivo studies showed that the functionalized dendrimers could strongly reduce the Stx-caused brain damage and fully suppress the lethal effect of Stx after its intravenous administration along with Stx to mice. The authors concluded that the modified dendrimers were effective therapeutic agents against Stx-producing E. coli infections. Besides the oligosaccharides, specific peptides were also reported to prevent toxins such as diphtheria toxin and staphylococcal enterotoxin B from adhering to the human cell surface. These peptides might enhance their anti-bacterial activity after multi-covalently conjugated to dendrimers.

Another anti-bacterial mechanism of the functionalized dendrimers is to attach to and thereby damage the bacterial membrane, causing bacterial lysis. Chen et al. designed quaternary ammonium modified PPI dendrimers as effective anti-microbial agents against Gram positive and Gram negative bacteria (64-66) (Figure 11). The anti-bacterial activity of these dendrimer biocides depended on generation of the dendrimer, length of the hydrophobic chains and counteranion. The anti-bacterial activities of dendrimer-based biocides exhibited a parabolic dependence on molecular weight, while traditional polymer biocides displayed a bell-shape dependence. The dendrimer biocides with bromide anions were found to be more potent than that with chloride anions. However, these potent biocides suffer from a general drawback in that they are high-cost and not easy for commercial purpose. Subsequently, Chen et al. investigated the interactions between dendrimer biocides and bacterial membranes (67). The anti-bacterial process might involve three stages: (1) cationic dendrimers associate with the negatively charged membrane of bacterial via electrostatic interactions, replace the surface ions such as calcium and magnesium, bind to the negatively charged phospholipids and thereby cause a enhanced permeability of the membrane; (2) further added dendrimer biocides denature the membrane proteins, penetrate through the phospholipids and lead to leakage of potassium ions; (3) more dendrimer biocides further destabilize the membrane structure and finally lead to a complete disintegration of the bacterial membrane corresponding to a bactericidal effect. N. Nagahori et al. prepared a serious of mannose terminated dendrimers and assayed their inhibitory potency by using a binding assay (68) (Figure 12). These dendritic architectures can strongly inhibit adhesion of E. coli to horse blood cells in a haemagglutination assay and were proposed to be used as promising antibacterial agents.

In contrast, dendrimers with anti-viral activity always have anionic functional groups on the outer shell and exhibit their anti-viral activity by competing with negatively charged cell surface for binding of virus (60, 69). Witvrouw and coworkers synthesized polyanionic (polysulfonate) dendrimers and evaluated their antiviral
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Figure 13. Schematic representation of an anti-viral dendrimer against human immunodeficiency virus. Reproduced with permission from (60).

Figure 14. Schematic representation of an anti-viral dendrimer against Respiratory Syncytial Virus. Reproduced with permission from (60).

activity (70) (Figure 13). Phenylidicarboxylic acid (BRI6195) and naphthylsulfonic acid (BRI2923) dendrimers were able to inhibit the replication of human immunodeficiency virus (HIV) in MT-4 cells at low concentrations. Both BRI2923 and BRI6195 showed non-toxic to MT-4 cells at even the highest concentrations tested. However, BRI2923 could permeate into MT-4 cells while BRI6195 could not. They exhibited their antiviral activity by interfering with both virus adsorption and reverse transcriptase/integrase steps in the virus replicative cycle. The authors concluded that polyanionic dendrimers with high cell-penetrating properties was necessary to inhibit the replication cycle of HIV at intracellular stages.

Gong et al. evaluated the anti-virus efficacy, mechanism of action and toxicity profile of a polyanionic dendrimer SPL2999 against herpes simplex virus (HSV) (71). Results showed that SPL2999 could be used as an inhibitor for early stage of HSV adsorption to cells and later stages of HSV replication by interfering with the reverse transcriptase and/or integrase enzymes. Bourne et al. prepared a series of poly (lysine) dendrimers with anionic groups (such as naphthyl sulfonate residues) on the surface and evaluated their in vitro activity against HSV (72). All of the compounds were active against type 1 and type 2 HSV in the cytopathic effect inhibition assay. The activity required the antiviral dendrimers be administrated before exposing to HSV. In vivo studies showed that the most effective antiviral dendrimer BRI2999 prevented HSV from infecting when applied half an hour before the virus given. Therefore, it is possible for these bioactive dendrimers to be developed as novel drugs in the vaginal delivery routes to prevent human from HSV infections in the future.

Most viruses can recognize receptors that bind to a cellular surface component as a targeting mechanism (60). Blocking the interactions between viruses and cellular surface receptors can lead to a lower cell-virus infection probability and thereby prevent viral infection. Influenza A viral infection begins by binding hemagglutinin glycoproteins on the viral envelope to sialic acid on the cell membrane. Landers et al. synthesized sialic acid modified PAMAM dendrimers and evaluated their antiviral activity (73). In vitro and in vivo studies showed that dendrimer-sialic acid conjugates could inhibit the H3N2 subtype stains of influenza A at low concentrations and completely prevent the influenza A caused pulmonary infections. However, the inhibition or prevention was restricted to this influenza subtype.

Surprisingly, a dendrimer-like compound (RFI-641) with an amide surface exhibited potent and selective inhibition of respiratory syncytial virus (RSV) (74) (Figure 14). The anti-viral mechanism might be due to hydrogen bonding interactions between the viral fusion protein and the dendrimer amide groups, which inhibit virus binding and fusion with cells. Modifications on the aromatic residues of the dendrimer significantly decrease the anti-viral activity and viral selectivity of RFI-641. These results provided us a new opportunity to design RSV inhibitory agents.

5. DELIVERY OF POTENT ANTI-CANCER DRUGS BY DENDRIMERS

Cancer therapy remains the major challenge in clinical practice today. An attractive approach to fight against cancer is delivering anticancer drugs to tumor by targeting cancer cells or their vasculature (75). It can enhance the cancer therapeutic effect and prevent normal cells from the toxic side effects associated with chemotherapy. It is not surprising that single drug molecules often act poorly at these tasks, thereby requiring the use of therapeutic cocktails in clinical practice (75). Macromolecular carriers have shown promise in combining aspects of an ideal targeted drug delivery system. They can be employed either by the enhanced permeability and retention (EPR) strategy or as polymeric platforms with the help of a targeting moiety for selective delivery (16). These carriers must be biocompatible, highly soluble, stable, and have the ability to load large amount of anti-cancer drugs
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5.1. Non-covalent complexation of anti-cancer drugs

Camptothecin (CPT), a clinical well-established anti-cancer drug isolated from *Camptotheca acuminate*, has an extremely low solubility in aqueous medium, which presents a major challenge during the drug formulation in clinical trials. Cheng *et al.* studied the potential of PAMAM dendrimers as drug carriers of CPT (76). Results showed that the aqueous solubility of CPT (both CPT-carboxylate form and CPT-lactone form) was significantly increased in the presence of PAMAM dendrimers (Figure 15). Very recently, Morgan *et al.* reported the encapsulation of CPTs (10-hydroxy-CPT and 7-butyl-10-amino-CPT) by a biocompatible polyester dendrimer (77, 78). The polyester dendrimer composed of glycerol and succinic acid could significantly enhance the solubility, anti-cancer activity and cellular uptake of CPTs. Efflux measurements in MCF-7 cells showed that the polyester dendrimer increased CPT retention time in the cell when being used as CPT vehicles. Both results suggested that dendrimers are promising nanocarriers in CPT clinical administration.

Bhadra *et al.* used G4 PAMAM dendrimer and PEG-5000 to synthesize PEGylated dendrimers, which were applied as potential drug carriers of 5-fluorouracil (5-FU) (79). PEG at the peripheral portions of dendrimers increased drug encapsulation and complex stability but decreased the release rate of 5-FU from the complex. After intravenous administration of different formulations of 5-FU (equivalent 5-FU in each formulation) to rats, the maximum drug concentration (C_max) from free drugs, non-PEGylated dendrimers, and PEGylated dendrimers, was 200-220, 21-23, and 6-7 µg/mL, respectively. The blood level of 5-FU was still detectable up to 12 h after the PEGylated dendrimer-5-FU formulation was administrated. In an earlier study, Tripathi *et al.* modified the surface of PAMAM dendrimers with fatty acid and converted them to unimolecular micelles in water (80). Phospholipids were further coated on the surface of dendrimer grafts. The obtained dendritic structures were then used to encapsulate 5-FU and employed as potential oral drug carriers (Figure 16). *In vivo* pharmacokinetic parameters and bioavailability were determined from the blood concentration of 5-FU performed in albino rats. The dendrimer-drug formulation was found to be much more bioavailable than the free drug after oral administration.

Khopade *et al.* investigated the effect of PAMAM dendrimer on entrapment and release of methotrexate (MTX) from liposomes (81). Dendrimers were able to entrap more MTX molecules in liposomes and slow down the release rate of MTX from liposomes due to electrostatic interactions between dendrimers and the acidic drugs. Papagiannaros and coworkers prepared PAMAM dendrimer-doxorubicin (DOX) complex and attached the complex to liposomes with a high incorporation efficiency (more than 90%) (82). Results suggested that liposomes composed of lipids and dendrimer-drug complex could easily modulate the release rate and cytotoxicity of DOX by altering the system components. Kojima *et al.* evaluated the potential application of PEGylated PAMAM dendrimers as drug carriers of anti-cancers such as MTX and DOX (83) (Figure 17). The ability of PEGylated

[Figure 15. Solubility of CPT (CPT-lactone and CPT-carboxylate) in the presence of G5 PAMAM dendrimers.]

[Figure 16. Schematic representation of a dendrimer device for the entrapment of 5-FU. Reproduced with permission from (80).]

[Figure 17. Synthesis of PEG conjugated PAMAM dendrimers. Reproduced with permission from (83).]
dendrimers to encapsulate drug molecules increased with the increasing dendrimer concentration, generation and molecular weight of PEG. PEG2000 modified G4 PAMAM dendrimer which exhibits the highest ability was able to entrap 6.5 molecules of DOX and 26 molecules of MTX inside a dendrimer. Khopade and Caruso prepared hollow microcapsules by depositing poly (styrenesulfonate) and G4 PAMAM dendrimer multi-layers on melamine formaldehyde colloid particles using the layer-by-layer self-assembly strategy (84). The microcapsules were successfully used to control the loading and release of DOX.

Ooya et al. used PEG4000 modified polyglycerol dendrimers to increase the water solubility of taxol (85). The solubility of taxol was increased by 10000-fold in the presence of PEGylated dendrimers. The results indicated that dendrimers with PEG chains were promising drug carriers for both oral and parenteral delivery of taxol and permit the development of novel delivery systems for other hydrophobic anti-cancer drugs. In a separate study, Ooya et al. investigated the complex structures of G4 and G5 polyglycerol dendrimer-taxol complex by NMR studies and found that the aromatic rings and methyne groups of taxol were surrounded by the dendrimer (86). Benito and coworkers synthesized cyclodextrin-dendrimer conjugates with mannosyl ligand on the dendrimer surface and investigated their solubilization capability using docetaxol as a guest molecule (87). This study put forward the design of efficient nanodevices with targeting ability to tumor cells based on dendritic polymers and cyclodextrin.

5.2. Covalent conjugation of anti-cancer drugs

Malik et al. synthesized conjugates of G3.5 PAMAM dendrimers with cisplatin, a potent anti-cancer drug with non-specific toxicity and poor solubility (88). The conjugates showed increased solubility, decreased systemic toxicity and enhanced permeation and retention properties. Intravenous administration of these conjugates to mice bearing B16F10 tumors was able to selectively accumulate cisplatin in solid tumors (50-fold increase compared to that achieved after intravenous administration of cisplatin at its maximum tolerated dose).

Padilla De Jesus et al. evaluated the application of various dendritic architectures composed of 2,2-bis (hydroxymethyl) propanoic acid as biocompatible drug carriers to improve the therapeutic efficiency of anti-cancer drugs such as DOX (89). DOX was conjugated to the dendrimers via a hydrazone linkage. In vitro studies showed that the cytotoxicity of dendrimer-DOX conjugates was reduced because of the slow release of DOX from the conjugates. The half-life of dendrimer-DOX in blood was significantly increased compared to free DOX. The results suggested that the polyester dendrimers were highly water soluble, non-toxic and biocompatible polymers and promising nanocarriers in the development of new anti-cancer delivery systems. Very recently, Lee et al. prepared DOX-functionalized bow-tie dendrimers and evaluated their anti-cancer activity in mice bearing C-26 colon carcinomas (90) (Figure 18). The biodegradable polyester dendrimer could enhance the blood circulation time of DOX through optimizing the dendrimer size or component, increase the solubility of DOX through modifying the surface of dendrimer with short PEG chains, and alter the DOX release rate through the use of pH-sensitive hydrazone linkers. The conjugate was much more biocompatible (more than 10 times) than free DOX in C-26 cancer cells. However, the in vivo anti-cancer activity of the conjugate was significantly increased compared to free DOX and was similar to that of a FDA approved anti-cancer formulation-Doxil.

Gurdag et al. prepared PAMAM dendrimer-MTX conjugates through two different strategies and evaluated their anti-tumor activity in both MTX-sensitive and MTX-resistant cell lines (91). The conjugates showed significantly improved cytotoxicity in MTX-resistant cells compared free MTX. Zhuo et al. synthesized a series of dendritic polymers (up to G5.5) with a cyclic core
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(1,4,7,10-tetraazacyclododecane) and acylated the surface of dendrimers to enhance their solubility in water (92). The dendrimer-5-FU conjugates were prepared by reacting acylated dendrimers with 1-bromoacetyl-5-FU (Figure 19). Release rate of 5-FU from the conjugates was significantly slower than that from the free drug formulation and greatly influenced by dendrimer size. Thus, the slow release of 5-FU from macromolecular carriers will reduce the toxicity of 5-FU and make the designed dendrimers potentially useful carriers for anti-cancer drugs. Khandare et al. compared the delivery efficacy and anti-cancer activity of PAMAM dendrimer-taxol conjugate and PEG-taxol conjugate (93). The results showed that both PEG and PAMAM dendrimer improved the bioavailability of taxol. The anti-cancer activity of PEG-taxol conjugate was significantly decreased (25-fold) compared to the free taxol, while anti-cancer activity of dendrimer-taxol with succinic acid as a spacer was much higher (10-fold) than that of free drug (Figure 20). These results suggested that PAMAM dendrimers represented promising nanocarriers for intracellular delivery of anti-cancer drugs.

During these years, increasing interest has been attracted to the application of dendrimers as targeting carriers in cancer therapy. It was well established that the conjugation of special molecules such as antibodies, small molecules or peptides with targeting properties to dendrimers allows the targeting ability of dendrimers, ensuring preferential distribution of the conjugation in a certain tissue (94). These special molecules include sugar (95), folic acid (96), specific antibody (97), specific peptide (98) and epidermal growth factor (99). Nowadays, researchers in this field are still searching for a more promising targeted delivery system based on dendrimers and such targeting molecules (17).

Several researches have investigated the ability of folate conjugated dendrimers as a targeting delivery system of anti-cancer therapeutics or imaging agents by in vitro and in vivo studies. Wiener et al. firstly reported the targeting delivery dendrimer-chelates to tumors and tumor cells via specific folate and folate receptor interactions (100). Kono and coworkers synthesized dendrimers with
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Figure 20. In vitro cytotoxicity of free paclitaxel, PEG-paclitaxel and paclitaxel-dendrimer conjugates. Reproduced with permission from (93).

Figure 21. Synthetic scheme for multi-functional PAMAM dendritic nanodevices. Reproduced with permission from (106).

Figure 22. Schematic representation of G5 PAMAM dendrimer functionalized with FITC, FA and MTX. Reproduced with permission from (96).

Konda et al. evaluated the specific targeting of folate-dendrimer MRI contrast agents to folate receptors expressed in ovarian tumor xenografts and compared the biodistribution of the conjugates in mice with folate-receptor positive and negative tumors (102-104). Quintana et al. designed a nanodevice based on anti-cancer drug (MTX), targeting agent (folate) and PAMAM dendrimer (105). The biological function of the nanodevice in KB cells that overexpress folate receptors showed rapid, specific and highly selective binding of the conjugate to tumor cells (100-fold over the free MTX). Majoros and coworkers synthesized a multi-functional dendritic nanodevice containing folate, FITC, MTX and PAMAM dendrimer and characterized its structure by GPC, NMR, HPLC and UV spectroscopy (106) (Figure 21 and Figure 22). In a separate study, Thomas et al. studied the cellular uptake, cytotoxicity and targeting efficacy of the multi-functional dendritic nanodevice (96). The nanodevice exhibited a time-dependent and dose-dependent inhibition of cell growth in KB cells and a specific targeting behavior in cancer cells that overexpress folate receptors. These results suggested that the development of dendrimers as a new nano-platform for cancer targeting was a promising way to decrease the non-specific cytotoxicity of anti-cancer drugs and to overcome some forms of drug resistance caused by free drugs. Patri et al. compared the release kinetics of MTX from the folate-dendrimer-MTX conjugate and folate-dendrimer/MTX complex (107). The results suggested that the stable conjugate was more suitable for targeted drug delivery, while the complex which can improve the solubility of MTX in aqueous solutions was identical to free MTX on cytotoxicity in cancer cells. Majoros et al. synthesized folate-dendrimer-FITC-taxol conjugate and evaluated its targeted delivery efficacy to specific cancer cells (108) (Figure 23). Kukowska-Latallo et al. intravenously injected multi-functional conjugates into immunodeficient mice bearing KB xenografts (109). Biocompatibility of these materials was found from animal weight examination and histopathology of the liver, spleen and kidney after administration of these conjugates. Folate-dendrimer-MTX conjugate was found to be much more effective than free MTX as well as dendrimer-MTX conjugate in this study. The survival period of mice from the groups administrated with the conjugate could be prolonged by at least 30 days. In a recent work by Choi and coworkers, DNA assembled PAMAM dendrimer clusters were designed for cancer cell targeting. Folate conjugated dendrimer and imaging agent/drug conjugated dendrimer were linked together via complementary oligonucleotides. In vitro targeting studies of the DNA-linked dendrimer clusters indicated specific binding to KB cells (75, 110, 111). Besides PAMAM dendrimers, poly (L-glutamic acid) dendrimers and folate conjugates were employed as biodegradable drug carriers of anti-cancer drugs (112) (Figure 24). Very recently, Hong et al. studied the binding avidity of folate-dendrimer-FITC conjugates towards folate-binding protein by qualitative measures (SPR and flow cytometry studies) (113). The data obtained in the study demonstrated that the improved biological targeting by the conjugates is mainly due to the multi-valent enhancement of dissociation constants between the folate and folate-binding protein, but not due to the enhanced rate of endocytosis.
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Figure 23. Schematic representation of taxol-dendrimer-folate conjugate. Reproduced with permission from (108).

Figure 24. Schematic representation of dendrimer functionalized with folate and dye molecules. Reproduced with permission from (112).
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The use of monoclonal antibodies (MoAbs) for the delivery of radionuclides for therapeutic purposes requires a very high density. As a result, a large number of radionuclides should be attached to each antibody molecule. The high density of surface functional groups and multi-valency of the dendrimer offer an extraordinary interfacial and functional advantage for this purpose (16). Singh et al. coupled several antibodies to PAMAM dendrimers and found that the antibodies retained their stability and immunological binding after coupling (114, 115). Kobayashi et al. synthesized monoclonal antibody-dendrimer-radioelement conjugates with high specific activity and with minimal loss of immunoreactivity (116). Qualmann et al. investigated the interactions between boronated poly(L-lysine) dendrimer-antibody conjugate and specific antigen by electron spectroscopic imaging (117). Wu et al. modified dendrimers with bi-functional metal chelators and monoclonal antibody (118). Both DTPA- and DOTA-dendrimer-antibody conjugates without loss of anti-body immunoreactivity could easily been labeled with $^{90}$Y, $^{111}$In, $^{212}$Bi. Kobayashi et al. evaluated the in vivo biodistribution of $^{111}$In and $^{90}$Y labeled dendrimer-1B4M-DTPA and its conjugate with anti-Tac monoclonal antibody (119). The antibody linked dendrimer-1B4M-DTPA conjugate was also successfully used as MRI contrast agents for the detection of alterations of tumor vessel permeability induced by radiation (120). Thomas et al. investigated the in vitro targeting behavior of anti-body conjugated dendrimer-FITC conjugates by flow cytometry and confocal microscopy and concluded that dendrimer-antibody conjugates are suitable platforms for targeted drug delivery into antigen expressing cells (121). Similarly, Patri and coworkers tested the targeting abilities of J591 antibody and dendrimer conjugates for targeted prostate cancer therapy (122). Shukla et al. investigated the targeting efficacy of anti-Her2 antibody-dendrimer conjugates to Her2 specific tumors via cell and animal studies (123). Barth et al. prepared a boronated PAMAM dendrimer, and then the antibody, which is directed against the murine B16 melanoma, was conjugated to the synthesized boronated dendrimer for boron neutron capture therapy (BNCT) (97) (Figure 25). The data obtained from in vivo distribution of $^{125}$I-labeled conjugates after intraperitoneal administration demonstrated that PAMAM dendrimer has a propensity to localize in the liver and spleen. The conjugates showed strong reactivity with several human glioblastoma and melanoma cell lines and could be used to target boronated dendrimers to brain tumors for BNCT (124). Recently, the same research group evaluated the intratumoral administration of monoclonal antibody modified boron-dendrimer conjugate (125). The mean boron concentration in rats bearing F98 EGFR gliomas was 92.3 ug/g after 24 h of intratumoral administration, while that of boron-dendrimer conjugate without a monoclonal antibody was 6.7 ug/g. The mean survival time of rats administrated with the conjugate was 45 d, compared to 25 d for the controls (126). These results indicated that the antibody-conjugated dendrimer targeting system was attractive for future studies and promising for clinical applications. Yang et al. investigated the targeting...
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Figure 26. Schematic representation of dendrimer-porphyrin conjugate for photodynamic therapy. Reproduced with permission from (16).

delivery of boronated dendrimer-monoclonal antibody L8A4 to receptor-positive gliomas (127). The results demonstrated that the molecular targeting of gliomas using either boronated-dendrimer-monoclonal antibody conjugate alone or combination with boronophenylalanine were efficient for chemotherapy. Wu et al. evaluated the targeting delivery of boronated-dendrimer and anti-epidermal growth factor monoclonal antibody (cetuximab, IMC-C225) conjugates for BNCT (128). MTX was delivered into receptor positive brain tumors by using dendrimer- cetuximab as a drug delivery vehicle (129). These results together suggested that the antibody functionalized dendrimers can be successfully employed in cancer chemotherapy and cancer diagnosis.

Epidermal growth factor (EGF) was evaluated as the targeting ligand in dendrimer-based targeting delivery systems by several authors (99, 130, 131). Yang et al. synthesized bornated dendrimer and EGF conjugate and administrated the conjugate to animals by means of convection enhanced delivery (CED) (130, 131). After 24 h of administration of this multi-functional conjugate to rats bearing F98EGFR gliomas and F98WT (wild type) receptor negative tumors, 47.4% of initial dose was localized in F98EGFR gliomas compared to 12.3% in F98WT receptor negative tumors. The mean survival time of rats receiving the conjugate was 53 d, compared to 31 d for irradiated controls. In an earlier study, Yang et al. evaluated the in vivo behavior of bornated dendrimer-EGF conjugate in intratumoral and intravenous administration routes (99). The intratumoural route was found to be more suitable in this targeting delivery system than the intravenous one. The accumulation of dendrimer in the liver and spleen from intratumoral injection was lower than that from intravenous injection, while the tumor localization of dendrimer was significantly increased from 1.3% of initial dose (intravenous injection) to 16.3% (intratumoral injection). These data indicated that dendrimer-EGF conjugate was an effective vessel for delivering anti-cancer agents to EGFR-positive brain tumors by intratumoral administration.

Recently, we developed a targeted cancer drug delivery system based on biotin-dendrimer conjugates (132). Biotin, a member of the vitamin family (Vitamin H), is a growth promoter of cell. Its content in cancerous tumors is significantly higher than in normal tissue. Rapid proliferation of cancer cells may require extra biotin, and the cell surface receptors specific for biotin may be over-expressed on many different types of cancer cells. Our results demonstrated that dendrimer and biotin conjugates were able to increase the uptake of anti-cancer drugs in tumor cells.

Another alternative cancer treatment in clinical trials is photodynamic therapy which involves the administration of a light-activated photo-sensitizing drug that selectively accumulates in diseased tissues (16). Dendrimers with globular shapes, polyvalent characters and EPR effects have already proved themselves suitable in such applications to overcome the skin phototoxicity, poor selectivity for tumor tissues, poor solubility in water and poor penetration into solid tumors of currently used photosensitizers (16) (Figure 26).
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6. DELIVERY OF OTHER DRUGS BY DENDRIMERS

During these years, dendrimers have proved themselves as biocompatible vessels of drugs not limited to non-steroidal anti-inflammatory drugs, anti-bacterial and anti-viral drugs and anti-cancer drugs, but also many other hydrophobic drugs as well as hydrophilic drugs which are too sporadic to summarize. As a necessary part, some typical applications of dendrimers as promising carriers by non-covalent complexation or covalent conjugate are listed in this section.

Dendrimers as promising carriers for delivery systems (138). Pilocarpine nitrate and tropicamide were proposed to load primaquine phosphate (a liver schizonticide) by Bhadra et al. (135). The results of drug biodistribution after intravenous administration of these formulations to rats showed that the primaquine phosphate mainly distributed in blood (18.5 ± 0.89% of initial dose) after 2 h of administration for free primaquine formulation, 25.7 ± 2.89% of initial dose of drug was found in liver and 21.8 ± 0.89% drug concentration was found in blood for uncoated PPI dendrimer-primaquine formulation, and 50.7 ± 5.9% of the primaquine was retained in liver whereas 7.8 ± 0.76% drug concentration was found in blood for galactose coated dendrimer-primaquine formulation. These results indicated that galactose coating could endue the dendrimers with effective targeting ability and reduce the hematological toxicity and hemolytic toxicity.

Wang et al. reported the utilization of polyhydroxyalkanoate (PHA) and G3 PAMAM dendrimers as novel transdermal carriers of tamsulosin hydrochloride, an antagonist of alpha-receptors in the prostate (136). When PAMAM dendrimer was co-administered with the PHA matrix, the penetration amount of tamsulosin in the dendrimer-containing PHA matrix was 24.0 μg/cm²/d while that in the dendrimer-lacking PHA matrix was 15.7 μg/cm²/d (the required amount of this drug in clinical trials is 20 μg/cm²/d). To clarify the mechanism of this enhancement effect by cationic dendrimers, the authors accidentally found that tamsulosin hydrochloride formed crystal in the cationic dendrimer/drug formulation by X-ray diffraction and hence suggested that crystallization reduced the drug diffusion direction and improved a highly ordered orientation (137).

Vandamme and co-workers have reported the use of PAMAM dendrimers as ophthalmic vehicles in ocular delivery systems (138). Pilocarpine nitrate and tropicamide were employed as model drugs for miotic and mydriatic activity tests, respectively. The results obtained from the miotic and mydriatic activity tests on rabbits indicated that the pharmacological activities of pilocarpine nitrate and tropicamide were much greater when they were co-administrated with PAMAM dendrimers. The eye drops containing PAMAM dendrimers were also found to have a prolongation of miotic/mydriatic activity and an improved bioavailability of pilocarpine nitrate and tropicamide. The authors explained that the increased bioavailability of drugs was contributed to (1) the host-guest relationship between dendrimers and drug molecules and (2) the bioadhesive properties of PAMAM dendrimers.

D’Emanuele et al. synthesized the propranolol-PAMAM dendrimer conjugate and investigated the transport route of the conjugate across Caco-2 cell monolayers (139). The authors concluded that PAMAM dendrimers could penetrate though the intestinal epithelium by paracellular and transcellular pathways. Therapeutic use of dendrimer-propranolol conjugate for reducing the effects of intestinal P-glycoprotein on drug absorption could improve the oral bioavailability of propranolol and many other orally administrated drugs. Yang and coworkers covalently attached an anti-depressant drug (venlafaxine) to a G2.5 PAMAM dendrimer via a hydrolysable ester bond to overcome the problem of poor patient compliance due to the multiple daily administrations (140). The conjugate displayed a sustained release of venlafaxine over a period of 5 days and hence could simplify the drug administration schedule.

Shaunak et al. used carboxylated PAMAM dendrimer (G3.5) to synthesize dendrimer-glucosamine (DG) and dendrimer-glucosamine 6-sulfate (DGS) conjugates with immuno-modulatory and anti-angiogenic properties, respectively (141). These two conjugates were used together in a validated and clinically relevant rabbit model of wound healing after glaucoma filtration surgery to increase the long-term success of the surgery and prevent scar tissue formation. The results showed that combination sub-conjunctival treatment with DG and DGS increased the long-term success of the surgery from 30% to 80% and inhibited the pro-inflammatory and pro-angiogenic responses in these animals. Rabbits treated with DG and DGS showed minimal scar tissue formation compared to placebo-treated animals. Furthermore, no clinical, hematological, or biochemical toxicity, or microbial infections were found in all animals during the whole experimental period. The results suggested that ocular administration of DG and DGS might be effective, reasonable, and safe routes in clinical practice.

Besides these drugs, many other bioactive molecules such as vitamins (142) and hormones (143, 144) were attached or conjugated to dendrimers to act as nanodevices in tissue targeting or engineering. They can bind to their specific receptors and regulate cellular functions after its binding. Of course, dendrimers are promising tools for delivering these drugs in clinical trials and overcome the disadvantages (poor solubility, stability, bioavailability or patient compliance) of these bioactive agents.

7. CONCLUSIONS AND FUTURE PERSPECTIVES

Dendrimers are expected to play a key role in biomedical fields in the 21st century. They provide uniform
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platforms for drug attachment and have the ability to encapsulate or bind drugs through several mechanisms such as physical encapsulation, electrostatic interaction and covalent conjugation. The encapsulation/complexation of drug molecules into/with dendrimers can be widely used in different fields of biomedicine (17). They are useful additives in drug formulations for increasing the solubility, stability, bioavailability, cellular uptake, targeting ability and patient compliance of the administrated drugs, and for decreasing the drug resistance and irritation. Scientists in this field are now in the process of conducting preclinical tests to evaluate the potential of dendrimers as biocompatible and promising nanocarriers for various drug molecules. The issue that should be addressed before the application of dendrimers in human is the bio-distribution properties of dendrimers. It still remains a major challenge to synthesis dendrimers with long blood circulation time. This problem might be resolved by modifying dendrimer structures with biocompatible molecules such as PEG chains.

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**Abbreviations:** Poly (amidoamine) PAMAM; poly (prolenemine) PPI; non-steroidal anti-inflammatory drugs NSAIDs; transdermal drug delivery systems (TDDS); poly (ethylene glycol) PEG; 5-amino salicylic acid 5-ASA; sulfamethoxazole SMZ; sulfadiazine SDZ; human immunodeficiency virus HIV; herpes simplex virus HSV; respiratory syncytial virus RSV; enhanced permeability and retention EPR; Camptothecin CPT; 5-fluorouracil 5-FU; methotrexate MTX; doxorubicin DOX; monoclonal antibodies MoAbs; Epidermal growth factor EGF; convection enhanced delivery CED; polyhydroxyalkanoate PHA; dendrimer-glucosamine DG; dendrimer-glucosamine 6-sulfate DGS

**Key Words:** Dendrimers, Nanocarrier, Non-Steroidal Anti-Inflammatory Drugs, Anti-Microbial Drugs, Anti-Viral Drugs, Anti-Cancer Drugs, Review

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