REVIEW

Chitkara College of Pharmacy, Rajpura, Patiala, Punjab, India

Dendrimers and their pharmaceutical applications – a review

I. Singh, A. K. Rehni, R. Kalra, G. Joshi, M. Kumar

Received February 25, 2008, accepted February 27, 2008

Inderbir Singh, Chitkara College of Pharmacy, Chandigarh-Patiala National Highway, Rajpura – 140401, Patiala, Punjab, India
inderbirsingh2906@gmail.com

Pharmazie 63: 491–496 (2008) *doi: 10.1691/ph.2008.8052*

Dendrimers are hyperbranched macromolecules having tree like structure, consisting of a core molecule and alternating layers of monomers. They can be synthesized by divergent and convergent growth methods. During synthesis, properties like dendrimer size, molecular mass, surface group can be controlled and configured to the desired need. The ability of dendrimers to encapsulate and bind the guest molecule can be used for solubility enhancement, sustained release and drug delivery applications. In this review we tried to delineate the properties, synthesis methods and expound potential pharmaceutical applications along with toxicological considerations of dendrimers.

1. Introduction

The term dendrimer, from the Greek word "dendron" for tree and from the Greek suffix "mer" (segment) refers to a synthetic, three-dimensional molecule with branching parts. These compounds were first discovered in the early 1980's by Donald Tomalia and co-workers (Tomalia et al. 1985). Newkome et al. (1985) independently reported the synthesis of similar macromolecules and called them arborols from the Latin word "arbor" also meaning a tree. The terms *cascade molecule*, *arborescent polymers*, *hyperbranched polymers* are also used, but dendrimer is the best established one.

Dendrimers are stealth molecules that have many potential applications. By customizing and controlling dendrimer architecture these compounds can be used for drug delivery, diagnostic imaging and as carriers of genetic material. Dendrimers can easily move across biological membranes and they can store a wide range of metals, organic or inorganic molecules among their branches. Above all, most of these synthetic molecules do not trigger the immune system when injected or used topically, and have low cytotoxicity.

Dendrimers are built from a series of branches around an inner core, providing products of different generations and offer intriguing possibilities in this regard. They can be synthesized from almost any core molecule and the branches similarly constructed from any bi-functional molecules, while the terminal groups can be modified chemically to achieve charged, hydrophilic, or hydrophobic surfaces. Their dimensions are extremely small, having diameters (depending on generation) in the range of 2 to 10 nm; this means that they are authentic nanoparticles. They can be synthesized starting from the central core and working toward the periphery (in divergent synthesis), or in a "top-down" approach starting from the outermost residues (in convergent synthesis), or built up from component dendrons, either by their covalent attachment or by their self-assembly. The beauty of dendrimers is that they can be designed and synthesised for specific applications, as truly functional excipients. Until now, the most commonly studied system has been the family of PAMAM (poly-amidoamine) dendrimers (available commercially for research purposes). Several different kinds of dendrimers have been synthesized utilizing different monomers and some are commercially available.

Dendrimers are very much like ordinary organic molecules for the first three generations, as shown in Fig. 1. They are small and floppy without much consistent or specific three-dimensional structure. By G4 they are beginning to become spherical and to take on a preferred three-dimensional structure. By G5 they have a consistent and specific

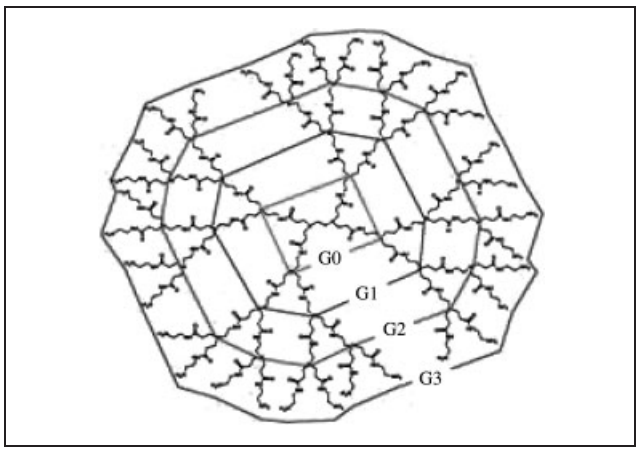


Fig. 1: Basic structure of dendrimer

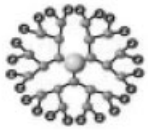
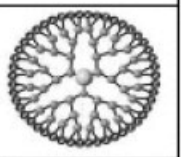
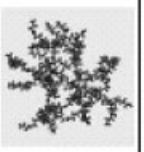
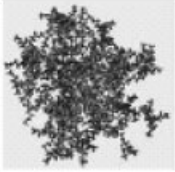
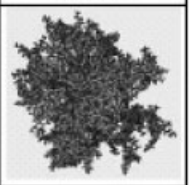
	G0	G1	G2	G3	G4
# of Surface Groups	3	6	12	24	48
Diameter (nm)	1.4	1.9	2.6	3.6	4.4
2D Graphical Representation					
3D Chemical Structure View					

Fig. 2:
2D and 3D representation of different dendritic generations

three dimensional structure. Beyond G5 they are highly structured spheres.

Dendrimers are branching molecules with the branching beginning at the core. Depending on the core, the dendrimer can start with 3 to 8 (or more) branches, with 3 and 4 being the most common number. Starting from the core, the dendrimer consists of long chains of atoms with a branch point about every half dozen atoms. At each branch point, the current chain of atoms becomes two chains of atoms. The molecular structure has the form of a tree with a great number of branches. Dendrimer diameters increase linearly per generation, whereas the number of surface groups increases geometrically with generation as shown in Fig. 2.

2. Composition

Dendrimers consist of a core molecule and alternating layers of two monomers. Each pair of monomer layers completes a shell and a generation. The core generally consists of an amine core, although sugars and other molecules can be used. All core molecules share the characteristic of having multiple reaction sites that are identical. Even the simplest core possible, ammonia (NH_3) has three amine reaction sites.

The core is mixed with an excess of the first monomer molecule that reacts with all of the core's reaction sites, giving rise to the first branches. This monomer molecule has two distinct reactive groups, one at each end. After one kind of end reacts, the other end will provide reaction sites for the next layer of the shell.

An excess of the second monomer, again a molecule which has two distinct reactive groups, one at each end, is reacted with this first layer to give the second layer and complete the first shell and the first generation. Each unreacted outer end of the second monomer provides a reaction site that can react with multiple molecules. This provides the branching and the reactions sites for the next shell.

Most of the dendrimers synthesized are polyamido molecules. At the outer surface of every full generation, the reactive groups are all amines. At the interface of the two layers making up the one shell of a generation, there is an amide linkage.

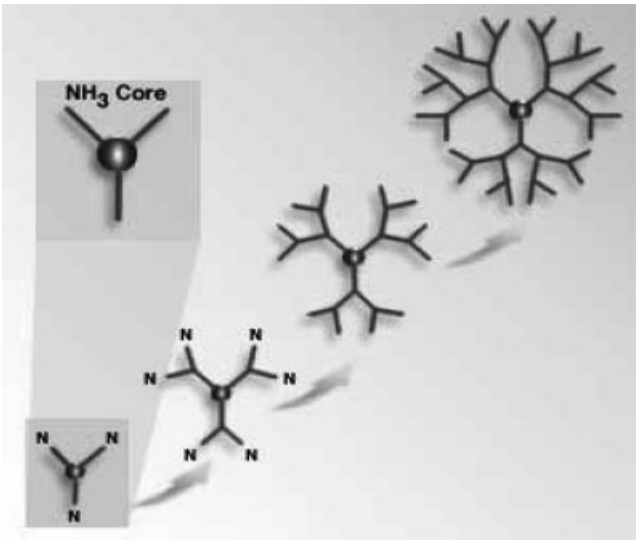


Fig. 3: Simple dendrimer structure with ammonia core

3. Properties

Unlike linear polymers, dendrimers are monodispersed macromolecules. The classical polymerization process can be specifically controlled to produce dendrimers of different sizes, whereas size and molecular mass of dendrimers can be specifically controlled during synthesis. Because of their molecular architecture, dendrimers show some significantly improved physical and chemical properties when compared to traditional linear polymers. In solution, linear chain polymers usually exist as flexible coils whereas dendrimers form a tightly packed ball that greatly affects their rheological properties. Dendrimer solutions have significantly lower viscosity than linear polymers (Frechet 1994). When the molecular mass of dendrimers increases, their intrinsic viscosity goes through a maximum at the fourth generation and then begins to decline. Such behavior is unlike that of linear polymers. For classical polymers the intrinsic viscosity increases continuously with molecular mass (Mourey et al. 1992).

The presence of many chain-ends is responsible for high solubility and miscibility and for high reactivity. Solubility of dendrimers is strongly influenced by the nature of surface groups. Dendrimers terminated in hydrophilic groups are soluble in polar solvents, while dendrimers having hydrophobic end groups are soluble in nonpolar solvents. The solubility of dendritic polyester showed remarkably higher solubility in tetrahydrofuran (THF) than that of analogous linear polyesters. Lower generation dendrimers which are large enough to be spherical but do not form a

tightly packed surface, have enormous surface areas in relation to volume (up to 1000 m^2/g) (Alper 1991). Dendrimers have some unique properties because of their globular shape and the presence of internal cavities. The most important one is the possibility to encapsulate guest molecules in the macromolecule interior. Meijer and co-workers (Jansen et al. 1994; Jansen and Meijer 1995) trapped small molecules like rose bengal or *p*-nitrobenzoic acid inside the 'dendritic box' of poly (propylene imine) dendrimers with 64 branches on the periphery. Then a shell was formed on the surface of the dendrimer by reacting the terminal amines with an amino acid (L-phenylalanine) and guest molecules were stably encapsulated inside the box. Hydrolysing the outer shell could liberate the guest molecules. The shape of the guest and the architecture of the box and its cavities determine the number of guest molecules that can be entrapped. Meijer's group described experiments in which they had trapped four molecules of rose bengal or eight to ten molecules of *p*-nitrobenzoic acid in one dendrimer.

4. Synthesis

Dendrimers are generally prepared using either a divergent method or a convergent one (Hodge 1993). There is a fundamental difference between these two construction concepts.

In the *divergent methods*, dendrimer grows outwards from a multifunctional core molecule. The core molecule reacts with monomer molecules containing one reactive and two dormant groups giving the first generation dendrimer. Then the new periphery of the molecule is activated for reactions with more monomers. The process is repeated for several generations and a dendrimer is built layer after layer. The divergent approach is successful for the production of large quantities of dendrimers. Problems occur from side reactions and incomplete reactions of the end groups that lead to structural defects in the dendritic molecule. To prevent side reactions and to force reactions to completion, a large excess of reagents is required in each step. Moreover elaborative purification steps are required for achieving the desired product. The overall yield is considerably small with this method of dendrimer synthesis. One of the highlighted advantage of this method is the ability to modify the surface of the dendrimer molecule. By changing the end groups at the outermost generation, the overall chemical and physical properties of the dendrimer can be configured to specific needs.

The *convergent method* was developed as a response to the weaknesses of the divergent synthesis. In the convergent approach, the dendrimer is constructed stepwise, starting from the end groups and progressing inwards (Hawker and Frechet 1990). When the growing branched polymeric arms, called *dendrons*, are large enough, they are attached to a multifunctional core molecule. This method basically involves attachment of the outermost functional groups to an inner generation and the attachment of the inner generations to the core. The structural units before the final attachment to the core are called "wedge." Usually, three to four wedges attach to the core. Each wedge can have different functional groups at the periphery.

The convergent method has some advantages over the divergent method.

- It is relatively easy to purify the desired product.
- The occurrence of defects in the final structure is minimized.

It becomes possible to introduce subtle engineering into the dendritic structure by precise placement of functional groups at the periphery of the macromolecule.

However, the convergent approach does not allow the formation of high generations because steric problems occur in the reactions of the dendrons and the core molecule.

The first synthesised dendrimers were polyamidoamines (PAMAMs) (Alper 1991). They are also known as *starburst* dendrimers. The term '*starburst*' is a trademark of the Dow chemicals Company. Ammonia is used as the core molecule. In the presence of methanol it reacts with methyl acrylate and then ethylenediamine is added:

$$NH_3 + 3\,CH_2CHCOOCH_3 \rightarrow N(CH_2CH_2COOCH_3)_3 \quad (1)$$

$$N(CH_2CH_2COOCH_3)_3 + 3\,NH_2CH_2CH_2NH_2 \rightarrow N(CH_2CH_2CONHCH_2NH_2)_3 + 3\,CH_3OH \quad (2)$$

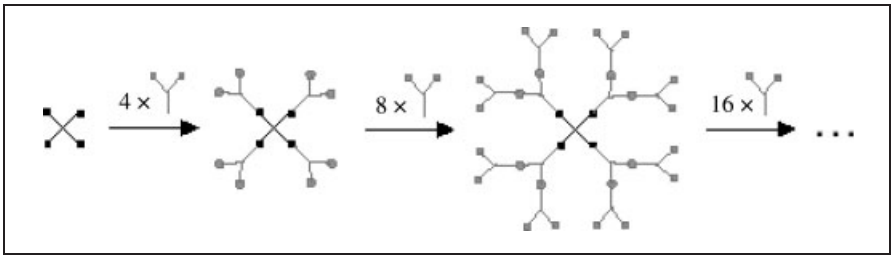


Fig. 4:
Divergent growth method for dendrimer synthesis

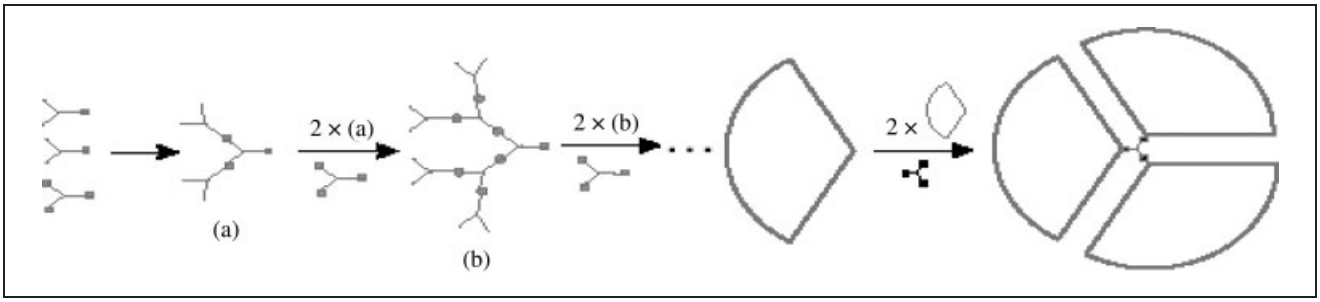


Fig. 5: Convergent growth method for dendrimer synthesis

At the end of each branch there is a free amino group that can react with two methyl acrylate monomers and two ethylenediamine molecules. Each complete reaction sequence results in a new dendrimer generation. The half-generation PAMAM dendrimers (e.g., 0.5, 1.5, 2.5) possess anionic surfaces of carboxylate groups. The number of reactive surface sites is doubled with every generation. The mass increases more than twice. The molar mass of the dendrimer can be predicted mathematically:

$$M = M_c + n_c \cdot \left[M_m \cdot \left(\frac{n_m^G - 1}{n_m - 1} \right) + M_t \cdot n_m^G \right] \qquad (3)$$

where: M_c is the molar mass of the core, M_m the molar mass of the branched monomer, M_t the molar mass of the terminal groups, n_c the core multiplicity, nm the branch-juncture multiplicity, G the generation number. The increase of the number of dendrimer terminal groups is consistent with the geometric progression:

$$Z = n_c \cdot n_m^G \qquad (4)$$

Nowadays dendrimers are commercially available. Dendritech™ (U.S.A.) manufactures PAMAM dendrimers. They are based on either an ethylenediamine (EDA) core or an ammonia core and possess amino groups on the surface. They are usually sold as a solution in either methanol or water. DSM (Netherlands) has developed the production of poly(propylene imine) dendrimers. They are currently available under the name Astramol™. Butylenediamine (BDA) is used as the core molecule. The repetitive reaction sequence involves Michael addition of acrylonitrile to a primary amino group followed by hydrogenation of nitrile groups to primary amino groups (Weener et al. 1999).

5. Applications

5.1. Dendrimers as drug carriers

Dendrimers, due to their controllable size and monodispersity, can act as excellent carriers for a wide range of molecules, which can be encapsulated in the interior of the dendrimer or interact with the dendrimer's terminal groups. The guest molecule, usually lipophilic, interacts with the dendrimer core via apolar or Van der Waals forces and is entrapped in its internal cavities. Dendrimers can be exploited as drug carriers for modifying drug properties that may include solubility enhancement, drug protection, controlled release, targeted drug delivery and many more.

5.1.1. Solubility enhancement

The entrapment of a guest molecule with in the dendritic architecture or interaction with the terminal groups approaches can be used to modify the solubility limitations of poorly soluble drug candidates. Dendrimer-mediated solubility enhancement mainly depends on factors such as generation size, dendrimer concentration, pH, core, temperature, and terminal functionality. Added advantage in solubilization can be achieved considering these factors. Ionic interaction, hydrogen bonding, and hydrophobic interactions are the possible mechanisms by which a dendrimer exerts its solubilizing property (Gupta et al. 2006).

Bhadra et al. (2005) showed solubility enhancement of artemether up to three to fifteen times depending on concentration, generation and type of dendritic micelles of MPEG (Methoxy polyethylene glycol) 2000 and 5000. They found dendritic carriers to form stable micelles at 10–30 μg/ml (lower CMCs) depending on generation and type of MPEG used. Increased the stability of the drug and also prolonged the release of artemether upto 1–2 days *in vitro* was also reported.

Devarakonda et al. (2005) used full generation amine terminated PAMAM dendrimers for enhancing the solubility of niclosamide, which is a practically water insoluble drug. Dendrimer solubility enhancement was compared with solubility enhancement by cyclodextrins using the same drug. They reported higher equilibrium stability constants and complexation efficiency of dendrimers compared with cyclodextrins, showing more stable complexation with dendrimers.

Milhem et al. (2000) compared solubility of the hydrophobic drug ibuprofen in an aqueous solution of polyamidoamine (PAMAM) G4 dendrimer and sodium dodecyl sulphate (SDS). The PAMAM G4 dendrimer solution significantly enhanced the solubility of ibuprofen compared to 2% SDS solution. It was found that the solubility of ibuprofen in dendrimer solution was directly proportional to dendrimer concentration and inversely proportional to temperature.

5.1.2. Controlled release

Drug entrapment capability of by dendritic nanostructures can be explored for controlled and/or sustained drug delivery. Various factors like dendritic generations, types of functional group with in the dendrimer core, terminal functional group, nature and structure of host, pH, and temperature can effect the drug release from dendrimers.

Kumar et al. (2007) studied PEGylated poly (propylene imine) dendritic architecture for the delivery of an anti-tuberculosis drug, rifampicin. The PEGylation of the system was found to have increased their drug-loading capacity, reduced their drug release rate and hemolytic toxicity. The systems were found suitable for prolonged delivery of rifampicin.

Na et al. (2006) investigated the potential of polyamidoamine (PAMAM) dendrimers as drug carriers of ketoprofen by *in vitro* and *in vivo* studies. The *in vitro* release of ketoprofen from the drug-dendrimer complex was significantly slower compared to pure ketoprofen. The authors concluded that PAMAM dendrimers might be considered as a potential drug carrier of ketoprofen with a sustained release behavior under suitable conditions.

Yang and Lopina (2005) used a polyamidoamine and polyethylene glycol (PEG)-containing semi-interpenetrating network (IPN) for extending the release of venlafaxine with an idea to reduce the multiple daily administration of the drug. The effect of PEG concentration and molecular weight was studied and discussed for an optimal controlled release.

5.2. Dendrimers as vectors for gene delivery

One of the major obstacles to the widespread clinical application of gene therapy has been the lack of appropriate vectors for efficient, non-toxic and safe gene transfer. Viral vectors such as adenovirus and retroviruses are efficient at mediating gene transfer, however, their immunogenic and pro-inflammatory nature limits their applicability. In addition there are potential safety concerns. Although non-viral vectors, such as liposomes, are less toxic and immunogenic, they are considerably less efficient than many of the viral vectors. Recently it has been shown that efficient gene delivery can be mediated to a variety of cell types using dendrimers. Numerous reports have been published describing the use of amino-terminated PAMAM or PPI dendrimers as non-viral gene transfer agents, enhancing the transfection of DNA by endocytosis and, ultimately, into the cell nucleus (Eichmen et al. 2000). A major advantage of dendrimers for *in vivo* applications is their ability to protect DNA from the action of DNAase found in serum.

PAMAM dendrimers have also been tested as genetic material carriers (Bielinska et al. 1996; Kukowska-Latallo et al. 2000). They are terminated in amino groups, which interact with phosphate groups of nucleic acids. This ensures consistent formation of transfection complexes. A transfection reagent called SuperFect™ consisting of activated dendrimers is commercially available. Activated dendrimers can carry a larger amount of genetic material than viruses. SuperFect–DNA complexes are characterised by high stability and provide more efficient transport of DNA into the nucleus than liposomes. The high transfection efficiency of dendrimers may not only be due to their well-defined shape but may also be caused by the low pK of the amines (3.9 and 6.9). The low pK permit the dendrimer to buffer the pH change in the endosomal compartment (Haensler and Szoka 1993).

5.3. Dendrimers as drugs

Dendrimers of various structures have recently been tested as bioactive compounds exhibiting, in some cases, high biological activity. A number of, usually anionic, dendrimers have been shown to exhibit antiviral activity. Having approximately the size of a virus, dendrimers are designed to bind multivalently either to the host cell surface or to the viral components via electrostatic forces, inhibiting infection at the stage of viral entry to the cell. The dendrimer works both as an inhibitor of virus entry and in the late stages of virus replication (Gong et al. 2002).

Activity has been shown against HSV, RSV and HIV while Starpharma's Vivagel™, a vaginal dendrimeric formulation against HIV is curreny entering clinic trials phase II (Gong et al. 2005; Gazumyan et al. 2000; McCarthy et al. 2005). Dendrimers have also been shown to exhibit antimicrobial activity. Typically these dendrimers have cationic surface groups, usually lysine residues, which interact with the heavily negatively charged prokaryotic membranes destabilizing them, leading to lysis of the bacterial cell (Klarjnet et al. 2006).

5.4. Dendrimers as diagnostic tools

Dendrimeric technology provides a valuable methodological tool in diagnostics, with applications in the construction of contrast agents for imaging and in various bioassays. Dendrimers are being widely investigated with

relation to magnetic resonance imaging as they can be specifically designed to carry ionic contrast agents (Gd^{3+}, Mn^{2+}, Mn^{3+}) by chelation or covalent interactions while diminishing their toxicity and controlling their biodistribution (Langereis et al. 2006). The first commercial dendrimer-based contrast agent was Gadomer-17, which was developed by Schering^{AG}, and is a polylysine-DTPA dendrimer with Gd^{3+} ions on the periphery. In microarray and ELISA bioassays, dendrimers are being used as tools for the increase of the sensitivity of the assays by enhancing the signal generation due to multivalent binding. Dendrislides^{TM} from Genopole, are dendrimer-based DNA chips for oligonucleotide detection and highly amplified labeling of PCR products for microarray detection.

6. Toxicological considerations

Biological properties of dendrimers are crucial because of the growing interest in using them in biomedical applications. "Cationic" dendrimers (e.g., amine terminated PAMAM and poly (propylene imine) dendrimers that form cationic groups at low pH) are generally haemolytic and cytotoxic. Their toxicity is generation-dependent and increases with the number of surface groups. PAMAM dendrimers (generation 2, 3 and 4) interact with erythrocyte membrane proteins causing changes in protein conformation. These changes increase with generation number and the concentration of dendrimers. The interactions between proteins and half-generation PAMAM dendrimers (2.5 and 3.5) are weaker. Anionic dendrimers, bearing a carboxylate surface, are not cytotoxic over a broad concentration range. Incubation of human red blood cells in plasma or suspended in phosphate-buffered saline with PAMAM dendrimers causes the formation of cell aggregates. No changes in aggregability of nucleated cells such as Chinese hamster fibroblasts were observed (El-Sayed M et al. 2002).

The cytotoxicity of dendrimers has been reported to be related to molecular weight and charge density. Anionic PAMAM dendrimers (G2.5 and G3.5) exhibited lower cytotoxicity than cationic PAMAM dendrimers (G2, G3 and G4). Low-generation cationic dendrimers (G2) showed lower cytotoxicity than higher-generations (G3, G4) (Jevprasesphant et al. 2003). Fischer et al. (2003) reported the cytotoxicity and haemolytic effects of a variety of water-soluble polycations used for gene transfection. G3 PAMAM dendrimers were found to be the most biocompatible of the polycationic polymers studied [poly(ethylenimine) (600–1,000 kDa), poly(L-lysine) (36.6 kDa), poly(diallyldimethylammonium chloride) (54 kDa) and poly(vinyl pyridinium bromide) (17.9 kDa)], and did not cause haemolysis at a concentration of 10 mg/ml after 1 hour incubation. It was suggested that the rigidity and globular structure of PAMAM dendrimers make cellular surface attachment (and consequently cellular damage) more difficult compared to more flexible linear or branched polymers.

Malik et al. (2000) examined the abilities of cationic and anionic dendrimers to cause red blood cell (RBC) haemolysis. RBC haemolysis displayed charge, concentration, generation and time dependence; cationic PAMAM dendrimers (G3 and G4) were haemolytic above concentrations of 1 mg/ml, whilst anionic PAMAM dendrimers (G1.5 and G3.5) and poly(ethylene oxide) (PEO) grafted carbosilane (CSi-PEO) dendrimers were non-haemolytic up to concentrations of 2mg/ml after 1 hour incubation.

Roberts et al. (1996) found no evidence of behavioural changes or weight loss after *in vivo* toxicity experiments with G5 dendrimer in mice (with exposure to dendrimer ranging from 7 days to 6 months). In addition, no evidence of immunogenicity was found with PAMAM dendrimers.

An *in vitro* cytotoxicity study by Neerman et al. (2004) showed a decrease in Clone 9 cell viability to 20% after incubation with melamine-based cationic dendrimers at a concentration of 0.1 mg/ml. No mortality (after 12 h) or renal damage (after 48 h) was observed when mice were injected with 160 mg/kg of dendrimers. However, acute liver damage was observed when dendrimer doses of 40 mg/kg were administered for 48 h. Similar results were found in subchronic studies in which 2.5–40 mg/kg of dendrimers were administered at 3-week intervals over a 6-week period. Surface modification of dendrimers has been shown to influence their toxicity.

Luo et al. (2002) compared the cytotoxicity of G5 PAMAM dendrimers to PEGylated G5 PAMAM dendrimers and showed that the cytotoxicity of PEGylated dendrimers was significantly lower than that of unmodified dendrimers. The PEGylation of G4 PAMAM dendrimer conjugates with surface attached 5-fluorouracil (5-FU-G4) was also found to reduce the haemolytic effect compared with parent dendrimers32.

Brazeau et al. (1998) have demonstrated that binding plasmid DNA to dendrimers led to a 3-fold decrease of dendrimer-induced myotoxicity, due to dendrimer charge neutralisation following binding.

Jevprasesphant et al. (2003) demonstrated the decrease in cytotoxicity of cationic PAMAM dendrimers (G3 and G4) by surface conjugation with lauroyl chains. IC_{50} values of PAMAM dendrimer conjugates (with six lauroyl chains) were approximately 7-fold higher than those for parent PAMAM dendrimers. The reduction in cytotoxicity may be explained by the decrease or shielding of the overall positive charges on cationic PAMAM dendrimers by the attached chains.

7. Conclusion

Control on dendritic structure related parameter like size, shape, branching length, density and surface functionalities, has widened the horizon of the pharmaceutical applicability of dendrimers. Moreover, because of the unique structure and number of possible applications, researchers are trying to expose dendrimers in the field of biomedicine. However, dendrimers have still to cross the regulatory and toxicological hurdles for their pharmaceutical acceptance. Days are not far away when pharmaceutical industry will open-handedly acknowledge the versatile applicability of dendrimers.

Acknowledgements: The authors are grateful to Dr. Madhu Chitkara, Director, Chitkara Institute of Engineering and Technology, Rajpura, Patiala, India and Dr. Ashok Chitkara, Chairman, Chitkara Educational Trust, Chandigarh, India for support and institutional facilities.

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