DENDRIMERS IN DRUG DELIVERY

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ABSTRACT

Dendrimers have received considerable attention as drug delivery carrier in recent years. Their safe, nontoxic and biocompatible nature makes them suitable for site specific as well as prolonged drug delivery carriers. Various products for the treatment of sexually transmitted diseases and cancers based on dendrimer technology are available in regulated markets. The investigation over dendrimers has not been fully explored yet. The present review focuses the structure, physico-chemical properties, types, characterization and applications of dendrimer in pharmaceutical field.

KEYWORDS : *Dendrimers, site specific drug delivery, physico-chemical properties*

Introduction

Dendrimers are a unique class of polymers which have particle size in the nanometer range and display properties different from the traditional linear polymers. Dendritic molecules are repeatedly branched species that are characterized by their structural perfection. They are classified into a low molecular weight species (dendrimers and dendrons) and a high molecular weight species (dendronized polymers, hyperbranched polymers, and brush-polymers). Currently, there are some dendrimer-based formulations available in world market. VivaGel™ (Starpharma) is available as a topical microbicide to prevent sexually transmitted disease. Alert Ticket™ is formulated by the US army Research Laboratory which is used to detect anthrax. There are thousands of published patents since the discovery of dendrimers in 1978 which claims the potential of dendrimers for overcoming many drug delivery challenges such as cytotoxicity, tumor targeting, and hydrophobicity.

History and origin

The name "Dendrimer" is derived from the Greek word "δενδρον"/*dendron*, meaning "tree". Some time also called as arborols and cascade-molecules. Dendrimers were first synthesized by a divergent method of synthesis by Vogtle in 1978. Denkewalter and coworkers at Allied Corporation synthesized polylysine dendrimers in 1981. Dendrimers were synthesized and characterized by Donald Tomalia et al at Dow chemical in 1983. It was then synthesized using a convergent method by Frechet. A lot of research has already been done by studying the various properties and applications of dendrimers but a lot of researchers still consider it to be in its initial stages (18).

Design and structure

Dendrimers may be spheroid or nanostructures that are capable of carrying molecules encapsulated in the void spaces or attached to the surface. Factors which determine the size, shape and reactivity are determined by the generation and chemical composition of the core and surface functionalities. They have very low polydispersity and usually have a size in the range of 1-10 nm. Proper control over size, shape, and terminal functional groups make dendrimers an invaluable system of delivering drugs (31). A typical dendrimer (Fig.I) consists of three basic components: (a) a *central core* (b) *repeat units,* which determines the microenvironment of the interior and in turn the solubilization ability of the dendrimer; and (c) the *terminal groups* (periphery).

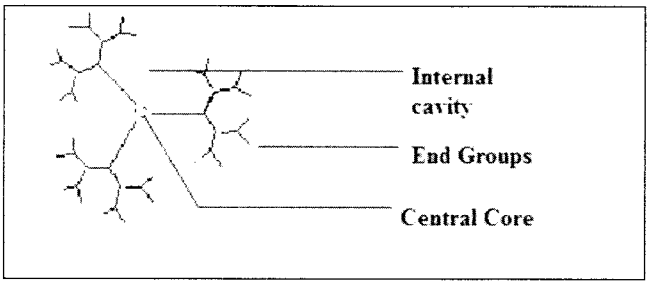

Figure I. A Typical Dendrimer

Synthesis

During the synthesis of dendrimers, monomers together lead to a monodisperse polymer, tree-like, or generational structure. Methods for the synthesis of dendrimers can be categorized as divergent and convergent method of synthesis. The former involves the assembly of the molecule from the core to the periphery and the latter from the outside to termination at the core.

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Divergent method

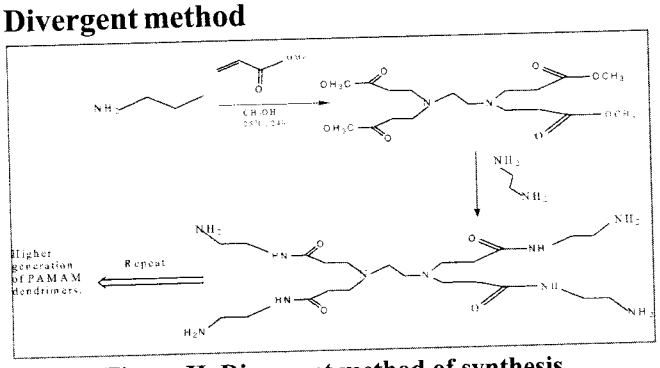


Figure II. Divergent method of synthesis

In this method 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 (Fig.II). The periphery now reacts with new monomers to form higher generations of dendrimers (12, 15).

Convergent method

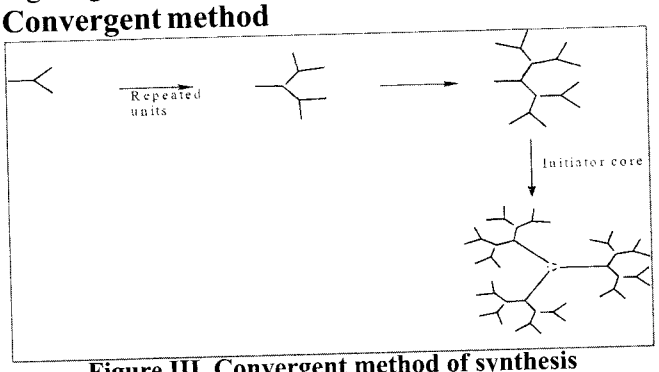


Figure III. Convergent method of synthesis

The convergent method involves starting from end groups and coming towards the central core (Fig.III). The growing branched polymeric arms are attached to multifunctional core molecule when they have grown large enough. The first dendrimers synthesized were polyamidoamine (PAMAM) dendrimers. The half-generation PAMAM dendrimers (e.g., 0.5, 1.5, 2.5) possess anionic surfaces of carboxylate groups where the number of reactive surface sites is doubled with every generation (18).

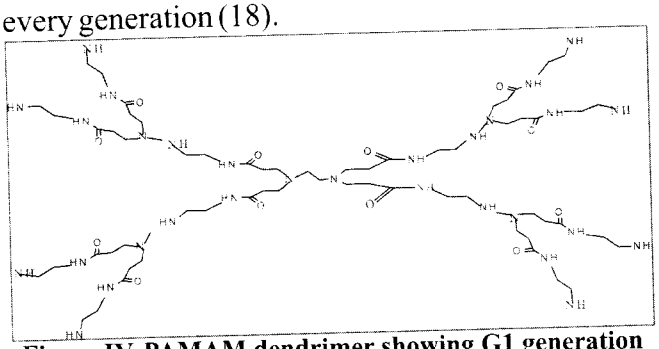


Figure IV. PAMAM dendrimer showing G1 generation

PROPERTIES

The properties of dendrimers are mainly influenced by the types of functional groups on its surface. They show better physical and chemical properties than linear polymer due to their molecular structure. Intrinsic viscosity is found to be less in case of dendrimers when compared to linear polymers (18).

Solubility

The peripheral functional groups decide the solubility of the dendrimer molecules. The dendrimer with hydrophilic end group is soluble in water whereas a dendrimer with hydrophobic end group is soluble in non aqueous solvents. It is theoretically possible to design a water-soluble dendrimer with internal hydrophobicity, which would allow it to carry a hydrophobic drug in its interior. Dendrimers have the unique property of having a globular shape and a lot of internal cavity available for drug entrapment (18).

Monodispersity

Systematic synthesis and proper purification of dendrimers leads to very low polydispersity as characterized by using HPLC, size exclusion chromatography, transmission electron spectroscopy (TEM), and mass spectroscopy. The dendrimers synthesized by convergent method are found to have very high monodispersity (17).

Size and shape

The diameter of dendrimer increases as the generation increases. The size of the PAMAM G1 dendrimer was found to be around 1.1 nm and a G10 dendrimer had a size of about 12.4 nm. Lower generation dendrimers have ellipsoidal shape and higher generation dendrimer have a spherical (18).

Cytotoxicity

Dendrimer cytotoxicity depends upon the central core but is also greatly affected by the peripheral groups on its surface. Dendrimers having – NH_2 end groups have cytotoxic properties which are usually found to be concentration or generation dependent (6). Malik et al observed effect of PAMAM dendrimers with amine terminal groups and carboxylate terminal groups on different cell lines. They confirmed the fact that dendrimers with amine terminal groups show hemolytic effect at concentrations as low as 10 microgram/ml (21).

TYPES OF DENDRIMERS

Liquid Crystalline Dendrimers

A liquid crystalline substance is one which shows mesophormism i.e. it exists in a state of matter intermediate between liquid and solid. Liquid crystalline dendrimers (LCD) containing silicone are the most studied and the most important type because they have excellent chemical and thermal stability (13).

Tecto dendrimers

Tecto dendrimers or partial shell filled core shell dendrimers are prepared by arranging nucleophillic or electrophillic PAMAM dendrimer shell reagents around nucleophillic or electrophillic core reagents. These almost resemble the traditional PAMAM dendrimers and show similar properties. Tomalia *et al* have synthesized such tecto dendrimers and compared their properties with the methods of synthesis of traditional dendrimers (29).

Chiral dendrimers

Angiolini *et al* synthesized and studied properties of chiral azoaromatic dendrimers. These three dimensional photoresponsive structures are used for optical storage of memory and holographic recording (2).

PAMAM dendrimers

The PAMAM dendrimer has an ethylene diamine or an ammonia core. It is extensively commercialized as Starburst PAMAM dendrimers by Dendritech. The generation levels of a PAMAM dendrimer range from 1 to 10 having a diameter in the range of 1.5 nm to 14.5 nm. PAMAM dendrimer are widely used by researchers all over the world to solubilize insoluble drug molecules such as nifedipine and NSAID's like flurbuprofen (12).

The above mentioned are major types of dendrimers. Other types of dendrimers include ampiphillic dendrimers, micellar dendrimers and hybrid dendrimers (12).

DENDRIMER AS A DRUG VEHICLE

Due to the presence of multiple external groups for attachment of drug molecules, dendrimers act as an excellent vehicle for carrying various drug moieties. Their inert and nontoxic nature makes them a perfect carrier for drug molecules with high drug loading capacity. One of the major applications of the dendrimers is to delay or sustain the release of a drug molecule. For example, the *in vitro* release of ibuprofen from dendrimers was found to be slower compared to free drug. Also the cell permeability of drug dendrimers complex was found to be better compared to unconjugated drug (9). Drug molecules are conjugated with dendrimers in one of the three methods such as simple encapsulation, electrostatic interactions, and covalent conjugation

Electrostatic interaction

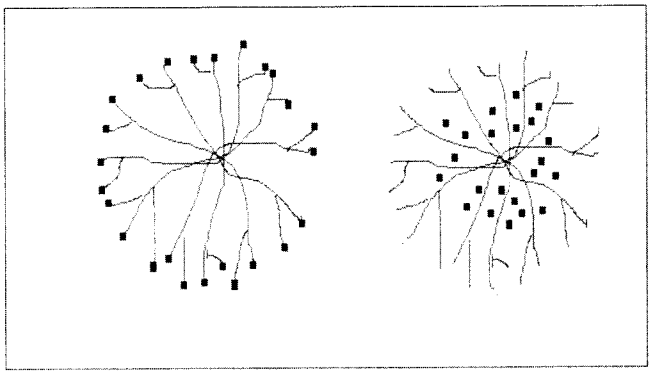
Weakly acidic drugs like ibuprofen and ketoprofen get conjugated with the peripheral functional groups via electrostatic interaction. The PAMAM dendrimers have been found to have higher amine units per volume compared to the traditional polymers thereby providing more sites for drug attachment (30) Fig 5a.

Simple encapsulation

The internal cavity of a dendrimer has hydrophobic properties, which helps to encapsulate water insoluble drug. Also, nitrogen and oxygen present in the internal cavity form hydrogen bonds with the drug (Fig 5b).

Covalent conjugation

As the name suggests, the drug gets covalently linked to the peripheral functional groups and the release of drug takes place via enzymatic degradation.



(a) (b)

Figure V (a). Electrostatic attraction (b) Simple encapsulation

APPLICATION OF DENDRIMERS IN DIFFERENT DRUG DELIVERY SYSTEMS

Intravenous drug delivery system

The intravenous (IV) route is the most simple and an easy route for a drug to reach systemic circulation. This route is used so as to avoid the first pass effect and to attain a faster response. The

major barrier to use of a drug via this route is its solubility. Dendrimers can be used to enhance the solubility of these drugs. Administration of dendrimer through the IV route is safe and nontoxic. More than 60% of cationic dendrimer gets accumulated in the liver and slow rate of clearance is observed in the case of anionic dendrimers. Anti cancer drugs like cisplatin and 5-FU if administered using dendrimer show detectable blood levels for 12 hrs (9). Fernandez et al used first generation dendrimer to deliver anti-chagasic agents intravenously. They found dendrimers to be a perfect carrier for such type of drugs since the dendrimers showed absolutely no hemolytic effect and did not produce damage in cellular membrane (15). Dendrimers are also used for gene therapy. Administration of surface modified PPI [Poly (Propyleneimine)] dendrimer complexed with DNA resulted in liver targeted gene expression (5).

Oral drug delivery system

The major problems associated with the oral dosage of drugs are first pass effect and insolubility of hydrophobic drugs. Dendrimers as described before do help in increasing the solubility of hydrophobic drugs. Dendrimers offer a very good protective coating of drug to minimize the effect of enzymes. Dendrimers can act as potential candidate for control release formulation because they can encapsulate drug into their core region (9,11).

Transdermal drug delivery system

Transdermal route of administering drugs has several advantages such as bypassing first pass effect, sustained release of drug, and better patient compliance. However, only a very small amount of drug can be delivered due to the barrier provided by the outmost layer of skin namely stratum corneum. To enhance the penetration of drugs through the skin many organic solvents are used which may cause dermatotoxicity and/or immune response. PAMAM dendrimers were found to not only increase solubility but also increase the penetration of hydrophobic drugs. PAMAM dendrimers administered along with polyalkanoate were found to increase the penetration of model drug tamsulosin hydrochloride in comparison to when it was administered alone (12). The same effect was observed for various NSAID's like ketoprofen and ibuprofen (9,14).

Dendrimer for targeted drug delivery

Ovarian cancer cells show over expression of folte, Vit B12 and biotin receptors. Yellepeddi *et al* made use of this fact and synthesized biotinylated PAMAM G4 dendrimer and found that these dendrimers showed better selectivity than the non biotinylated dendrimers for ovarian cancer cell lines (OVCAR-3). Patri et al prepared methotrexate and PAMAM dendrimer conjugates by two different methods and compared the cytotoxicity and selectivity towards cancer cell lines (25). The drug dendrimer conjugate was prepared by encapsulating the hydrophobic drug inside the hydrophobic interior of the PAMAM dendrimer. The second method used to prepare the conjugate was by coupling the drug covalently onto the surface of the dendrimer. It was found that covalently conjugated drugs were better for targeted drug delivery than by encapsulating drugs in the interior of dendrimer (25). Agarwal *et al* synthesized dextran conjugated dendrimers and found that they also showed better targeting than free drug alone (1). Cisplatin administered along with PAMAM dendrimer was found to have slower release, lower toxicity and higher accumulation in cancerous cells (33). Choi et al conjugated dendrimers to different biofunctional moieties such as folic acid which were linked to complementary DNA to form molecules capable of targeting to the cancerous cells overexpressing the folate receptors (10,23).

Sustained release of drugs using dendrimer technology

Cheng et al prepared ketoprofen- PAMAM dendrimer complex and compared the in vitro release profile of this complex with the release profile of ketoprofen alone. It was found that the drug dendrimer complex showed sustained release of ketoprufen thereby also showing a prolonged effect when given in vivo to model mice (9). PAMAM dendrimer conjugated with sulfamethoxzole were prepared by Ma et al and tested for the in vitro release, solubility enhancers and anti bacterial activity. It was found that the drug dendrimer complex showed sustain release and better solubility profile. Anti bacterial activity was also found to be increased when given in combination with dendrimer (20).

Controlled release drugs using dendrimer technology

Asthana *et al* used flurbiprofen as a model drug along with PAMAM dendrimer to prove that dendrimers can be used to deliver drugs parenterally and have controlled or delayed release (1). Self immolative dendrimers are a new and exciting class of dendrimers in which all the drug molecules conjugated with the dendrimers get released due to a cleavage caused by an enzyme substrate present in the periphery of the dendritic moiety. For example, a tumor associated enzyme can be used as target for the self immolative dendrimers. The trigger i.e. the reaction between the enzyme substrate and the enzyme causes a cascade of self destructive reactions leading to separation of the components of the dendrimer-drug complex (31). Shabat was able to synthesize anti cancer drugs like doxorubicin and campothecins. Catalytic antibody was used as the triggering enzyme. This reaction between the substrate and the enzyme starts a cascade of reactions (26).

Dendrimer encapsulated nanoparticles (DEN's)

These are prepared by first forming a complex between the dendrimer and a metal. Then this complex is reduced by treating with a reducing agent and then the dendrimer encapsulated nanoparticles are collected by precipitation followed by centrifugation and dialysis. DENs reside within the voids of the dendrimers (22). DEN's are useful as homogeneous or heterogeneous catalyst. Homogeneous catalysis reactions include hydrogenation and Stille carbon coupling (24).

characterization of dendrimers

A dendrimer is characterized by the following methods:

NMR

NMR can be carried out to analyze structural changes taking place at the peripheral functional groups. The dendrimer and its conjugation with other drugs can be characterized by ^{1}H NMR and ^{13}C NMR spectroscopy. The amide bond formation between G0 PAMAM dendrimer and lactic acid was confirmed by an amide carbonyl peak at 182 ppm, while there was no such peak observed in the normal peak of G0 PAMAM dendrimers (23).

Fluorometric assay

Sharma *et al* devised a fluorometric method to analyze PAMAM dendrimers using 8-anilino-1-naphthalene-sulfonic acid as dye. The dye showed an increase in fluorescence after conjugating with the dendrimers. The fluorescence intensity showed a linear relationship with dendrimers concentration (27).

Infrared (IR)

The presence of a G0 PAMAM dendrimer was confirmed by Najlah et al. An N-H stretching between 3282- 3264 cm^{-1} and an amide stretching band was observed between 1667 and 1654 cm^{-1} (23).

Size exclusion chromatography

Size exclusion chromatography is carried out usually to calculate molecular weight of the dendrimer. The retention time changes in the SEC can be used to detect structural changes or defects in the dendrimers (8).

Reverse phase HPLC

HPLC analysis is carried out to confirm purity of the dendrimer and also to detect structural changes. It can also be carried out to detect the functional groups present in the dendrimer solution, polydispersity and solubility (6).

Miscellaneous

Atomic force microscopy and near field scanning optical microscopy are used to take visual images of the dendrimer loaded with drug molecules. It is also used to determine the total dendrimer volume (6).

CONCLUSION

To summarize, delivery of hydrophobic as well as hydrophilic drugs can be achieved by using these biodegradable and biocompatible dendrimers. Dendrimers not only increase the solubility but also increase the stability of drugs. Monodispersity and nanomolecular size range of dendrimer drug complex has provided a reason to reinvestigate the delivery of various hydrophobic drugs which could not be administered before. One of the major advantages of dendrimer includes its use via the transdermal route. The dendrimers were found to increase the permeability of many drugs like the NSAID's. The stability and the plasma concentration were also found to be increased when compared to drugs administered without the dendrimer. Drugs can be incorporated into the

dendrimer by simple encapsulation, electrostatic interaction or by forming covalent bonds. Dendrimer are non toxic and can be used by any route. They can also be used for sustain release or control release of drugs. Conjugated with genetic materials they are also used in gene therapy. One of the most important applications of dendrimer is in the field of anticancer therapy. PAMAM dendrimers conjugated with folic acid have proven to be useful for administering several anti cancer drugs like 5- FU and cisplatin.

These encouraging results provide reasons for further research into dendrimers to develop better and safe drug delivery systems.

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