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Biological evaluation of dendrimers based on melamine

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The synthetic strategies used to prepare dendrimers based on melamine are reviewed. The ability to control the number and positions of functional groups within third generation dendrimers has been established. Strategies for post-synthetic modification of peripheral groups and the biological consequences of such modifications in vitro *and* in vivo *are described. The results of all biological inquiries of these systems to date (August 2004) are presented including studies of cytotoxicity, antigenicity, hemolytic potential, acute and subchronic toxicity and the reduction of drug toxicity.*

Key words: Dendrimer – Drug delivery – Polymer therapeutics – Vaccine – Synthesis.

The ability to control shape, size and composition of dendrimers to arrive at a single molecule or narrowly dispersed family of molecules serves as both the motivation for the exploration of these vehicles as well as the preoccupation of chemists engaged in this field. Compared with the progress made with linear polymers, liposomes, and polymeric micelles, the use of dendrimers as drug delivery vehicles is far from being realized [1]. Yet these spherical, perfectly-branched polymers are curious and promising targets for drug delivery and other medical applications as evidenced by the first clinical trials of dendrimers as either a prophylactic anti-viral or MRI contrast agent [2]. The globular and often hydrophobic nature of the core of the dendrimer offers harbor for hydrophobic drugs while the wealth of peripheral groups are amenable for manipulation for drug attachment or the attachment of ligands or other groups [3]. As discrete covalent constructs that result from designed and controlled stepwise syntheses, the biological challenges that these vehicles will ultimately face including absorption, distribution, metabolism, excretion and toxicity can be systematically probed at the molecular level to search for solutions. These challenges are problems for chemistry: the molecular design criteria for a polymeric drug delivery vehicle are not known and dendrimers offer opportunities to address these areas of basic inquiry.

Biological evaluations of dendrimers have been reported with studies of commerically available polyamidoamine (PAMAM) and poly(propyleneimine), commonly referred to by the core group as either DAE or DAB. While much of this work has been performed *in vitro*, a few *in vivo* studies have been described. To summarize this literature, the following lessons can be learned:

Lesson 1: Dendrimers can carry drugs. There are numerous reports of dendrimer-drug complexes [4]. In a few cases, the complex results from covalent attachment of the drug through a biolabile bond (i.e. a hydrazone) [5]. More common are examples of noncovalent interactions attributed to hydrophobic interactions, hydrogen bonding and/or acid-base chemistry. Generally, within a dendrimer class (i.e. PAMAM) the amount of drug solubilized is directly proportional to the molecular weight of the dendrimer. Many of these examples rely on commercially available dendrimers (PAMAM) with derivatized surface groups and common cancer drugs including doxorubicin [6], methotrexate [7], 5-fluorouracil [8] or taxol [9]. In most cases, the number of solubilized drugs/dendrimer ranges from 5-25 depending on the system with the typical number being < 10. Taxol represents an interesting target of inquiry given the current requirement that it is administered in Cremophore EL: often the solubility of taxol in dendrimer is 200 x increased over that in water using dendrimers with low generation numbers (3-5).

Lesson 2: Dendrimers appear to be effective for in vitro *drug delivery.* A number of groups have shown that dendrimer/drug complexes are efficacious *in vitro*. Fréchet's polyester-based dendrimer bearing the anticancer drug doxorubicin attached via an acid-sensitive hydrazone linkage consistently showed lower toxicity than free drug [6]. Similarly, Kopeček's starlike HPMA copolymer showed similar trends in that covalently attached doxorubicin was effective against human ovarian A2780 cells *in vitro* [10]. Free doxorubicin, however, was more effective that the HPMA-DOX conjugates. Finding a balance between what is probably a reduced bioavailability and affording the requisite conditions and time for *in vivo* delivery will require effort.

Lesson 3: The surface groups of the dendrimer greatly affect its biocompatibility. The meaning of "surface" is illusive, and whether these sites are always on the surface of the globular dendrimer is debated and probably case-dependent. Irrespective of where surface groups are, what holds true is that there are a lot of them. A typically third generation dendrimer (MW ~10,000 Daltons) might have anywhere between 12 and 64 groups depending on the system and nomenclature. Cationic surface groups are typically toxic *in vitro* [11] and *in vivo* [12]. Surface modification of these surface groups to yield anionic or neutral dendrimers reduces toxicity [13]. The community currently appears to be moving towards poly(PEGylated) architectures. For example, PEGylation can afford protection against

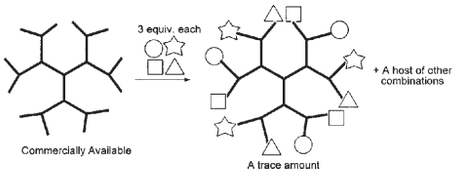
toxicity both *in vitro* and *in vivo* with mice being challenged up to 1.3 g/kg by intravenous administration resulting in 100% survival [6].

Lesson 4: Biodistribution studies suggest that dendrimers pass through the liver. Biodistribution data is sparse and the first human data from the clinical trials of the lysine-based MRI contrast agent, Gadomer-17 from Schering, are eagerly awaited. In mice, PAMAM dendrimers – both cationic and anionic – primarily distribute to the liver with the cationic species being rapidly cleared from circulation irrespective of route of administration [14]. Anionic PAMAM dendrimers exhibit somewhat longer circulation times (15-40% of the recovered dose in the blood after 1 h). Comparable to anionic dendrimers, investigations of neutral polyester-based dendrimers show them to be rapidly cleared from circulation and distributed to the liver when given by i.v. administration [6]. Recent studies by Nigavekar *et al*. suggest that changes in the charges of the dendrimer's surface groups can have profound implications on distribution levels, but not on the site of distribution [15]. Both cationic and neutral PAMAM dendrimers primarily partitioned to the lungs, liver and kidneys while modest levels were found in the heart, pancreas and the spleen consistent with high circulation volumes. Biotinylated-PAMAM dendrimers labelled with ^{125}I were rapidly cleared from circulation showing preferential distribution to both the liver and kidneys following i.v. administration [16]. PEGylated dendrimers have also been shown to accumulate in the liver [6].

These lessons hold true in our work, and will too, presumably, for other designer dendrimer systems [17]. While some of these lessons can be learned in cell culture, the next set of challenges will require animal models. These challenges are being addressed with a subset of these systems. Professor Ruth Duncan's laboratory at the Welsh School of Pharmacy in Cardiff has reported on PAMAM and DAB/DAE dendrimers, often in collaboration with other laboratories more synthetically inclined. As a result, the most thorough cross-architecture comparisons usually can be found in this work. Professors Donald Tomalia of Central Michigan University and James Baker of the University of Michigan lead groups focusing on PAMAM dendrimers. Professors Jean Fréchet of Berkeley and Frank Szoka of the University of California San Francisco focus primarily on polyesters [6] and polyethers and hydrids thereof [18], the most biocompatible-looking materials of these architectures. Professor Bert (E.W.) Meijer's group at the Eindhoven University of Technology in the Netherlands is responsible for much of the inquiry into poly(propyleneimine) dendrimers [14]. The versatility of triazine chemistry leads us to offer this fourth class as candidates for additional inquiry. This versatility affords structural complexity and an opportunity to use synthesis to counter biological challenges.

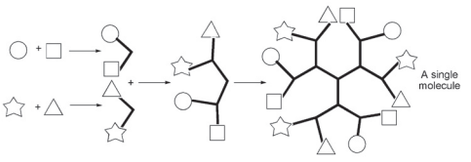
I. STRATEGIES FOR STRUCTURAL COMPLEXITY

There are two general strategies to accomplish structural complexity. The first pathway (*Equation 1*) relies on statistically decorating dendrimers with a series of groups to provide a mixture of products. The advantages to this strategy include the use of dendrimers that are often commercially available, product mixtures that are often narrowly dispersed, and rapid access to large numbers of compositionally diverse mixtures of dendrimers. Reducing synthetic demands can greatly accelerate the investigation of biological challenges to these vehicles, but dispersity could ultimately cloud clear resolution of structure-activity trends.



Eq. 1

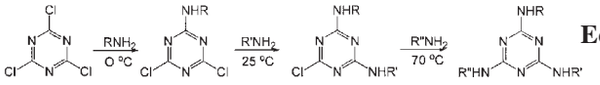
The second strategy to accomplish structural complexity increases the burden of synthesis by preparing single molecules from elementary building blocks (*Equation 2*). The result is a single complex target with which a biological challenge can be probed. While monodispersity offers great advantage over polydispersity in dissecting the molecular basis for a given property, an inability to prepare the required number of molecules would preclude the observation of a trend.



Eq. 2

II. DENDRIMERS BASED ON MELAMINE

These challenges aside, our approach relies on this second strategy. Our choice of targets has rested with triazines linked by diamines thus yielding dendrimers based on melamine (triaminotriazines) [19]. We sacrifice natural building blocks such as amino or hydroxy acids in favor of a more synthetically versatile system. This choice gambles that in the end – should we be fortunate enough to surmount the numerous hurdles to get there – the vehicle will be either excreted or inert to the recipient. The underlying tenet is that structural complexity will lead to functional utility. Triazine chemistry can be exploited to overcome many of the challenges of synthesis. The advantage of triazine chemistry lies in the sequential substitution of the triazine ring (*Equation 3*). Temperature and nucleophilicity can be exploited to generate trisubstituted triazines wherein each substituent is unique.



Eq. 3

The multistep strategy that is employed is convergent in the "dendrimer" sense. That is, we typically start with the peripheral groups, and through iterative reactions of diamines and triazines, build "convergently" towards the core. The results of this stepwise synthesis is a dendrimer like that shown in *Chart 1* where a wealth of different peripheral groups are presented [20]. These groups are orthogonal protecting groups: selective removal provides sites for post-synthetic manipulation.