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Dendrimer-based Drugs as Macromolecular Medicines

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Introduction

Nature makes extensive use of the multiple interactions between large molecules such as proteins, DNA, and RNA. This is because they are capable of receptor–ligand interactions by a process called polyvalency. This process entails many small, consecutive, and cooperative interactions between two molecules and it results in well-defined biological interactions. Exploiting this mechanism to make new medicines from synthetic molecules has now become the subject of intense study. Until recently, progress had been limited by the difficulties in preparing structurally defined macromolecules. One class of synthetic macromolecule that can be made with structural precision is the *dendrimers*. These hyperbranched molecules are frequently drawn as circular molecules. They can be prepared with a defined molecular weight and with a defined number of surface end-groups that have the potential to interact with other molecules. So far, their biomedical applications have been limited to use in drug delivery. It has been shown recently how new and unique polyvalent macromolecules can be made and used as immuno-modulators and as anti-angiogenic drugs (Shaunak *et al.*, 2004): this short review looks at the underpinning principles behind this exciting development and the many potential applications it offers in the treatment of disease.

Dendrimers with cationic (i.e. amine) end-groups are toxic after repeated intravenous use or topical ocular application. In contrast, anionic (i.e. carboxy) dendrimers

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Abbreviations: CD, cluster of differentiation; DNA, deoxyribonucleic acid; FACS, fluorescence-activated cell sorting; FGF, fibroblast growth factor; HSPG, heparan sulfate proteoglycan; IC_{50}, 50% inhibitory concentration; LPS, lipopolysaccharide; MIP, macrophage inflammatory protein; RNA, ribonucleic acid; SIRS, systemic inflammatory response syndrome; TLR, toll-like receptor; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

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are not toxic (Malik *et al.*, 2002). For example, cationic dendrimers cause substantial changes to red blood cell morphology at 10 μg/ml, whilst anionic dendrimers have no such effect at 2000 μg/ml. The Shaunak team set out to determine whether the polyvalent receptor–ligand interactions between carbohydrates and proteins that affect many of these important aspects of cell surface mediated immuno-regulation could be manipulated to make new chemical entities (Clonigner, 2002). They examined whether these interactions could be pharmacologically manipulated by using anionic dendrimers as the scaffold on which to spatially configure carbohydrates so that they are capable of interacting with cell surface receptors in a polyvalent receptor–ligand manner. The new drugs needed to possess multiple and co-operative receptor binding properties. Previous attempts to pharmacologically manipulate these interactions with other macromolecules (e.g. synthetic linear polymers) had failed because of their structural heterogeneity, and because most of the synthetic macromolecules evaluated adversely activated complement and coagulation pathways. Efforts to examine glycosylated dendrimers include completely glycosylated substrates (Page and Roy, 1997), dendrimers with hydrophobic interiors that can aggregate (Thoma *et al.*, 2002), and oligosaccharide dendrimer conjugates (Turnbull *et al.*, 2002). The most recent studies have been aimed at determining the physicochemical characteristics of those glycosylated dendrimers whose biological properties are mediated by polyvalent receptor–ligand interactions (Pavlov *et al.*, 2001).

Immuno-modulatory dendrimers

Shaunak and colleagues conjugated the aminosaccharide glucosamine to an anionic dendrimer precursor with 64 end-groups using water-based chemistry. This dendrimer is known as a generation 3.5 anionic polyamidoamine dendrimer. Generation 3.5 anionic dendrimers were used because they are not toxic to mice at 95 mg/kg, and because they have a therapeutically useful pharmacokinetic profile (Malik *et al.*, 2002). The percentage loading of glucosamine on the dendrimer was 14% and the final molecular weight was 13 600 Daltons. No toxicity was observed with human T cells, macrophages, dendritic cells, or endothelial cells.

Critically, dendrimer glucosamine did not alter the spontaneous release of pro-inflammatory chemokines or cytokines from resting lymphocytes, macrophages, or dendritic cells. Remarkably, dendrimer glucosamine was found to prevent lipopolysaccharide (LPS)-induced release of the pro-inflammatory chemokines macrophage inflammatory protein (MIP)-1α [CCL3], MIP-1β [CCL4], and interleukin (IL)-8 [CXCL8], and the pro-inflammatory cytokines tumour necrosis factor (TNF)-α, IL-1β and IL-6 from macrophages and dendritic cells without having a cytotoxic effect (*Figure 16.1*). The release of large amounts of proinflammatory mediators is the main cause of the morbidity and mortality that is seen in patients with severe bacterial infections. The 50% inhibitory concentration (IC_{50}) of dendrimer glucosamine was 92 μg/ml [6.8 μM]. Its immuno-modulatory activity was reversible. Of considerable significance was the observation that the addition of dendrimer glucosamine to the cells *after* LPS still reduced the release of these proinflammatory mediators.

Importantly, dendrimer glucosamine did not interfere with LPS-induced maturation of dendritic cells, as determined by fluorescence-activated cell sorting (FACS)

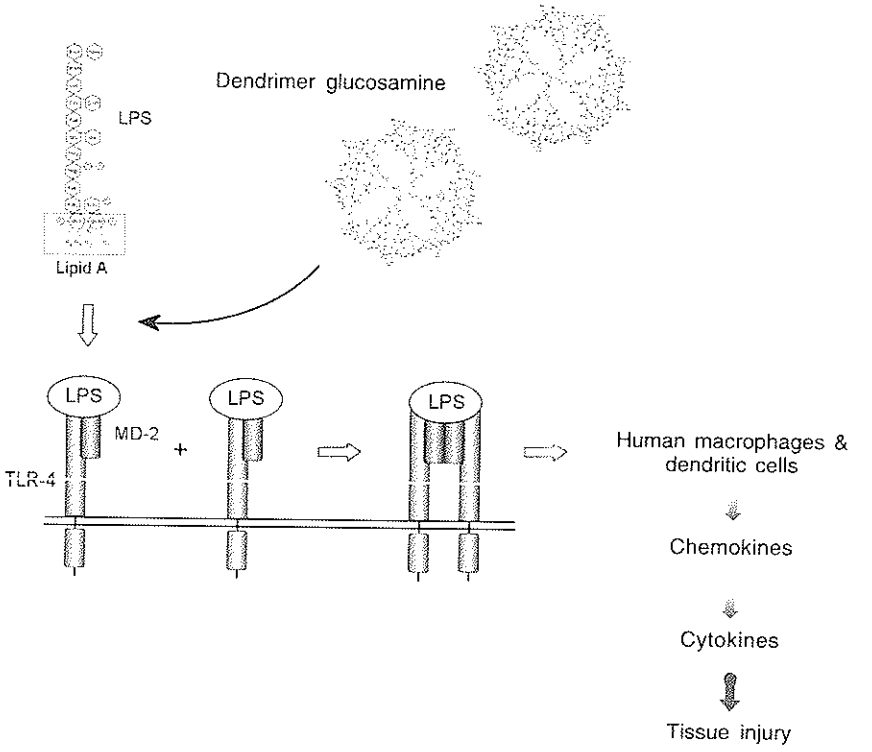

Figure 16.1. Dendrimer glucosamine inhibits lipopolysaccharide (LPS)–Toll-like receptor (TLR) 4 mediated pro-inflammatory chemokine (MIP-1α, MIP-1β, IL-8) and cytokine (TNF-α, IL-1β, IL-6) release.

analysis for CD25, CD80, CD83, and CD86 expression, or with the production of β-interferon; i.e. the physiological function of these cells was not impaired. Taken together, these dose-dependent effects were consistent with dendrimer glucosamine having an inhibitory effect on the Toll-like receptor (TLR) 4 that mediates LPS-induced stimulation of dendritic cells and macrophages. This is an important new property for a synthetic macromolecule because dendritic cell chemokine and cytokine secretion is central to immunological cells to orientate and amplify specific components of the immune response and thereby ensure resolution of the tissue injury that occurs after infection or trauma.

Anti-angiogenic dendrimers

A second dendrimer-based molecule was made and studied using glucosamine 6-sulfate. The sulfated glucosamine was covalently conjugated to a generation 3.5 anionic dendrimer. The percentage loading of glucosamine 6-sulfate on the dendrimer was 14% and its molecular weight was 14 000 Daltons. Unlike other sulfated macromolecules (e.g. dextrin 2-sulfate, fucoidan), dendrimer glucosamine 6-sulfate had no anticoagulant or heparin-like or complement activating activity when it was added to fresh whole blood. The molecule inhibited new blood vessel formation in

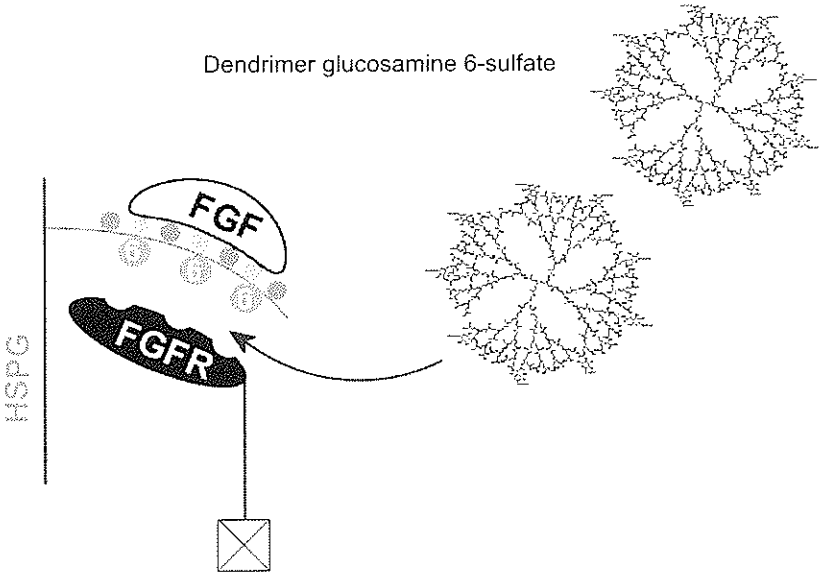

Figure 16.2. Dendrimer glucosamine 6-sulfate inhibits FGF-2 mediated proliferation of human endothelial cells. Fibroblast growth factor (FGF), fibroblast growth factor receptor (FGFR), heparan sulfate proteoglycan (HSPG). Intracellular signalling: the X in the box means that cell surface receptor mediated signalling has been inhibited.

a human Matrigel assay and in a human placental angiogenesis assay. Fibroblast growth factor (FGF)-2 mediated proliferation of human endothelial cells was inhibited by 50% at 50 μg/ml [3.6 μM], whilst having no effect on vascular endothelial growth factor (VEGF) mediated proliferation of these cells. Compounds with anti-angiogenic activity increasingly are being recognized as having a novel, therapeutic role in the treatment of inflammatory conditions.

The rationale for using two molecules acting in synergy

Shaunak and colleagues went on to postulate that these two novel anionic dendrimer conjugates, each with defined immuno-modulatory and anti-angiogenic properties, could be used safely and synergistically to prevent scar tissue formation after surgery (Shaunak *et al.*, 2004). Although pattern recognition receptors on dendritic cells and macrophages have been studied primarily because of their role in the identification of exogenous molecules such as LPS, it has become clear that the same innate immune mechanisms can be triggered also by surgically mediated tissue injury (Paterson *et al.*, 2003). Shaunak and colleagues also postulated that the earliest events that mediate these pro-inflammatory responses would be expected to be generic to several inflammatory diseases. The considerable advantage of testing this hypothesis after a controlled surgical procedure was the availability of a clinically validated animal model and a clear biological endpoint by which to determine success or failure. In this context, the *in vitro* results suggested that inhibition of pro-inflammatory chemokine and cytokine release from dendritic cells and macrophages would be sufficient to interrupt the inflammatory, proliferative,

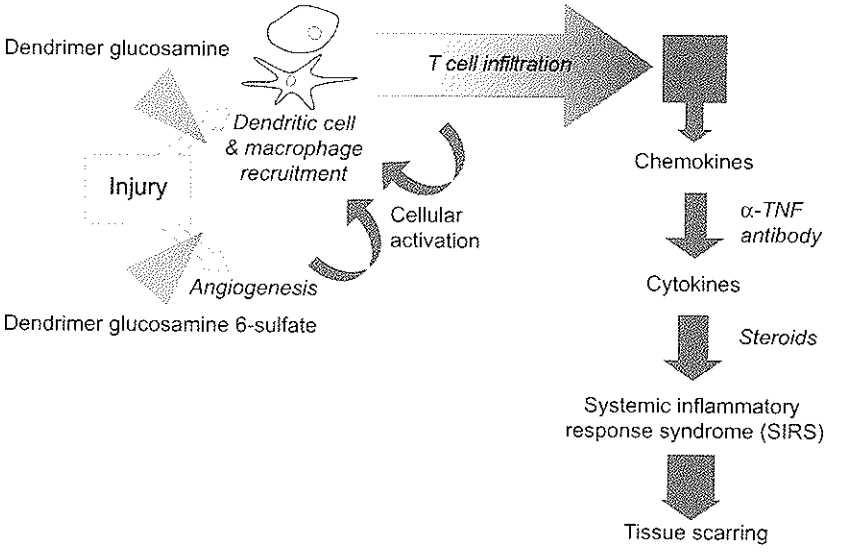

Figure 16.3. Pathogenic mechanisms responsible for the systemic inflammatory response syndrome and scar tissue formation.

maturation and remodelling phases of wound healing that lead to excessive scar tissue formation. The anti-angiogenic activity would ensure that the recruitment of immunologically activated cells was reduced to a minimum.

The dendrimer glucosamine 6-sulfate conjugate had an inhibitory effect on the three most important hallmarks of angiogenesis in man; i.e. (1) FGF-2 receptor mediated endothelial cell proliferation and migration (*Figure 16.2*); (2) capillary sprouting by normal endothelial cells; and (3) new blood vessel formation in a model of cell migration and microvessel formation (Pye *et al.*, 1998; Schlessinger *et al.*, 2000). Evaluation of anti-angiogenic activity in adult animals is difficult because endothelial cells proliferate very slowly, if at all, under normal conditions. However, once a pathological stimulus such as surgical trauma intervenes, the pro-inflammatory and pro-angiogenic chemokine IL-8 activates endothelial cells, increases adhesion molecule expression, and promotes infiltration by inflammatory cells. These include antigen-activated CD4+ T lymphocytes, which stimulate macrophages to produce TNF-α, IL-1, and IL-6. This completes a positive feedback loop that leads to endothelial cell migration, division, and re-organization.

The research team went on to use the combination of dendrimer glucosamine and dendrimer glucosamine 6-sulfate to synergistically inhibit these early and critical stages of the pro-inflammatory and pro-angiogenic cascades (*Figure 16.3*). The preferential accumulation of dendrimer-based conjugates in tissues containing inflammatory cells had the added advantage of amplifying this pharmacological synergy.

Evaluation in a clinically validated animal model

Subconjunctival scarring after glaucoma filtration surgery was chosen as the animal model in which to test these hypotheses because: (1) the surgical intervention is

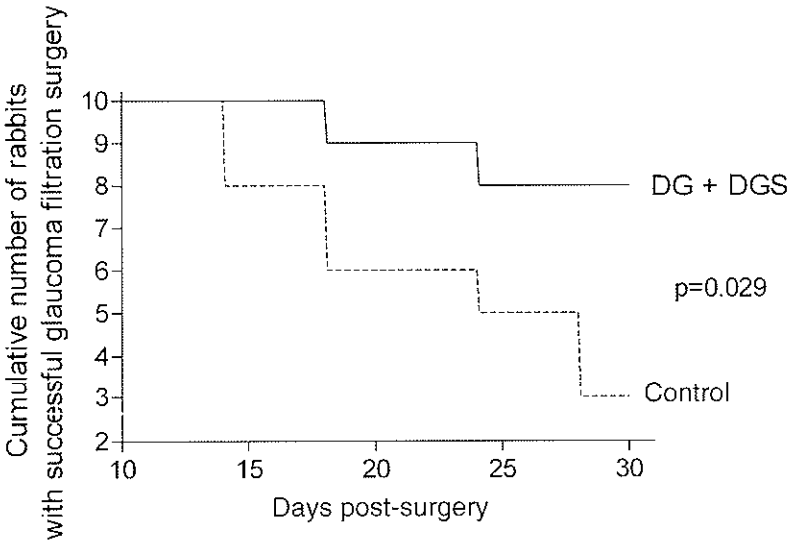

Figure 16.4. The combination of dendrimer glucosamine (DG) and dendrimer glucosamine 6-sulfate (DGS) increased the long-term success of the glaucoma filtration surgery from 30% to 80% (P = 0.029).

precisely defined; (2) it is one of the most aggressive models of excessive scar tissue formation available; and (3) surgical failure is due to excessive scar tissue formation and is caused by the inflammatory responses described. This animal model is better validated for scar tissue formation in humans than any other model of scarring; drug efficacy in this model has previously led to two new treatments that were effective in patients undergoing glaucoma surgery, and to treatments for other scarring situations in humans (Mead *et al.*, 2003). The post-surgical scarring that occurs is due to a persistent inflammatory and angiogenic response that promotes fibroblast proliferation. Using validated endpoints, it was shown that the combination of dendrimer glucosamine and dendrimer glucosamine 6-sulfate increased the long-term success of the surgery from 30% to 80% ($P = 0.029$, *Figure 16.4*). No clinical, haematological, or biochemical (including blood glucose) toxicity was seen, and there were no local or systemic bacterial, viral, or fungal infections in 30 rabbits treated over 30 days. Histopathological examination of the eyes from rabbits that were successfully treated with dendrimer glucosamine and dendrimer glucosamine 6-sulfate showed minimal scar tissue formation compared to placebo treated animals. In addition, there was no evidence of a persistent inflammatory or neo-angiogenic response in successfully treated rabbits at day 30. Clinical validation of this model means that drugs that work in this animal model should also work in humans.

New horizons

This study is the first demonstration that simultaneous targeting of pro-inflammatory mediators and neo-angiogenesis with synthetic, narrow molecular

weight, water-soluble macromolecules that are neither toxic nor immunogenic can safely and synergistically prevent scar tissue formation after surgery. The polyvalent and co-operative cell surface receptor–ligand interactions that lead to TLR4 and fibroblast growth factor receptor mediated signalling play an important role in the pathogenesis of inflammation and angiogenesis, respectively, in many diseases. Simultaneous targeting of these pathways at the level of the cell surface could have important clinical applications in several areas of medicine. For example, it is becoming clear that the molecular mechanism responsible for the LPS–TLR4 mediated syndrome of bacterial sepsis is also responsible for mediating the systemic inflammatory response syndrome in other shock-related diseases (Johnson *et al.*, 2004). Therefore, molecules that target these immunological pathways could be used to reduce the mortality associated with SIRS in patients after surgical procedures, burns, acute pancreatitis, and in bacterial sepsis. Further and detailed studies in appropriate and clinically validated models for each disease will be required now to determine the broader applicability of this new therapeutic approach.

References

CLONIGNER, M.J. (2002). Biological applications of dendrimers. *Current Opinion in Chemical Biology* **6**, 742–748.

JOHNSON, G.B., BRUNN, G.J. AND PLATT, J.L. (2004). An endogenous pathway to systemic inflammatory response syndrome (SIRS)-like reactions through Toll-like receptor 4. *Journal of Immunology* **172**, 20–24.

MALIK, N., WIWATTANAPATAPEE, R., KLOPSCH, R. *ET AL.* (2002). Dendrimers: relationship between structure and biocompatibility *in vitro*, and preliminary studies on the biodistribution of ^{125}I-labelled polyamidoamine dendrimers *in vivo*. *Journal of Controlled Release* **65**, 133–148.

MEAD, A.L., WONG, T.T.L., CORDEIRO, M.F., ANDERSON, I.K. AND KHAW, P.T. (2003). Evaluation of anti-TGF–β_2 antibody as a new postoperative anti-scarring agent in glaucoma surgery. *Investigative Ophthalmology and Visual Science* **44**, 3394–3401.

PAGE, D. AND ROY, R. (1997). Synthesis and biological properties of mannosylated starburstpoly(amidoamine) dendrimers. *Bioconjugate Chemistry* **8**, 714–723.

PATERSON, H.M., MURPHY, T.J., PURCELL, E.J. *ET AL.* (2003). Injury primes the innate immune system for enhanced toll-like receptor reactivity. *Journal of Immunology* **171**, 1473–1483.

PAVLOV, G.M., ERRINGTON, N., HARDING, S.E., KORNEEVA, E.V. AND ROY, R. (2001). Dilute solution properties of lactosylated polyamidoamine dendrimers and their structural characteristics. *Polymer* **42**, 3671–3678.

PYE, D.A., VIVES, R.R., TURNBULL, J.E., HYDE, P. AND GALLAGHER, J.T. (1998). Heparan sulfate oligosaccharides require 6-O-sulfation for promotion of basic fibroblast growth factor mitogenic activity. *Journal of Biological Chemistry* **273**, 22936–22942.

SCHLESSINGER, J., PLOTNIKOV, A.N., IBRAHIMI, O.A. *ET AL.* (2000). Crystal structure of a ternary FGF–FGFR–heparin complex reveals a dual role for heparin in FGFR binding and dimerization. *Molecular Cell* **6**, 743–750.

SHAUNAK, S., THOMAS, S., GIANASI, E. *ET AL.* (2004). Polyvalent dendrimer glucosamine conjugates prevent scar tissue formation. *Nature Biotechnology* **22**, 977–984.

THOMA, G., KATOPODI, A.G., VOELCKER, N., DUTHALER, R.O. AND STREIFF, M.B. (2002). Novel glycodendrimers self-assemble to nanoparticles which function as polyvalent ligands *in vitro* and *in vivo*. *Angewandte Chemie International English Edition* **41**, 3195–3198.

TURNBULL, W.B., KALOVIDOURIS, S.A. AND STODDART, J.F. (2002). Large oligosaccharide glycodendrimers. *Chemistry – A European Journal* **8**, 2988–3000.