Dendrimers as Vehicles for Intravitreal Drug Delivery

These nanoparticles can selectively localize within activated retinal microglia, ferrying drugs to fight inflammation.

REVIEWED BY RAYMOND IEZZI, MD, MS; AND RANGARMANUJAM M. KANNAN, PHD

Dendrimers, synthetic nanoscale, "tree-like" globular macromolecules characterized by well-defined branching architecture, have been of interest in recent years as potential vehicles for delivery of drugs and imaging agents.[1-8] Dendrimers are comparable in size to small molecules, such as hemoglobin (3–10 nm), and represent the possibility of a new approach to drug delivery. Certain types of polyamidoamine (PAMAM) dendrimers have been shown to be noncytotoxic and are cleared intact through the urine.

Of interest for some ophthalmic applications is the intrinsic targeting ability of PAMAM dendrimers to localize in cells associated with neuroinflammation.[9] In the development of therapies for inflammatory conditions, this "self-targeting" characteristic may permit the design of therapeutic dendrimer-drug conjugates that will seek out inflammation without the need for a targeting moiety.

COMMON PATHWAY

Chronic, progressive forms of retinal degeneration including age-related macular degeneration (AMD) and retinitis pigmentosa (RP) share the characteristic of vision loss due to photoreceptor cell death. Although these diseases are genotypically distinct, they share final common pathways of photoreceptor cell death and may provide targets for drug therapies that could address a variety of neurodegenerative diseases.[10]

One such target is neuroinflammation in the outer retina that is mediated by activated microglia. Photoreceptor cell damage in the outer retina can result from the release of cytotoxic proteins and reactive oxygen species by activated microglia. Dying cells then trigger more microglial activation, leading to an amplifying cycle of neuroinflammation and resulting in massive photoreceptor degeneration. In the Royal College of Surgeons (RCS) rat model of retinal neurodegeneration, our group has demonstrated significant apoptotic and necrotic cell death due to activated microglia.[14]

No known effective treatments currently exist for dry AMD or RP. Steroids have been shown to exhibit neuroprotective and antiinflammatory properties in the retina,[15,16] and there has been considerable interest in recent years in the development of extended-delivery modalities to supply steroid to the posterior segment. Our group previously performed animal work evaluating a durable drug-delivery implant that provides chronic intravitreal infusion of fluocinolone acetonide (FA). In RCS rats, we found that low-dose intravitreal sustained delivery of FA was neuroprotective, preserved retinal outer nuclear layer morphology, electroretinogram (ERG) amplitudes, and reduced retinal neuroinflammation.[13]

There has been limited exploration of the use of dendrimers for ophthalmic drug-delivery applications. Vandamme and colleagues[17] assessed the use of PAMAM dendrimers topically and found that they improved the bioavailability of their cargo drugs and induced no cytotoxicity. Grinstaff et al[18,19] have developed dendrimer-based biodegradable sealants for ophthalmic surgical applications. Marano and coworkers[20] assessed the use of dendrimer-oligonucleotide conjugates to inhibit laser-induced choroidal neovascularization in rats and found that they were able to reach the retinal pigment epithelium (RPE) and improve efficacy over free nucleotides.

We recently explored the use of hydroxyl-terminated PAMAM dendrimers as vehicles for sustained intravitreal delivery of FA to treat inflammation in the outer retina.[10] FA was chosen for this work in order to allow eventual comparison of this mode of delivery with commercially available nonbioerodible intravitreal FA implants. In this work we relied on the intrinsic tendency of PAMAM dendrimers to selectively localize in inflammatory tissue, rather than on the use of an active targeting ligand.

RESULTS

Dendrimer-FA conjugates containing approximately 15% of payload drug were prepared. The conjugate was readily soluble in buffer and saline solution, unlike FA itself, which is poorly water-soluble. In order to assess

uptake of the nanoparticle, conjugates of the dendrimer with fluorescein isothiocyanate (FITC) were also prepared.

In the RCS rat, enhanced uptake of dendrimer-FITC was seen in the outer retina. The RPE demonstrated enhanced fluorescence in retinas that received injections of the dendrimer-FITC conjugate, compared with those that received free FITC injections. This distinction was still seen at 10 days after injection.

These data suggest that cells with high endocytotic rates, such as activated microglia and RPE, experienced enhanced uptake of the dendrimer-FITC conjugates. In addition, dendrimer conjugates were not cleared from the retina as rapidly as free FITC.

The same injections were also given to healthy Sprague Dawley rats. It is important to note that the pattern of uptake of dendrimer-FITC was very different from that of the RCS rats. No significant accumulation and retention of dendrimer-FITC was seen in the healthy rats.

Regarding pharmacodynamic efficacy, the dendrimer-FA conjugate performed significantly better than free drug in providing neuroprotection, preserving photoreceptor health, and attenuating neuroinflammation over a period of 30 days.

One intravitreal injection of 1 µg of FA conjugated to 6 µg of the dendrimer arrested retinal degeneration, preserved photoreceptor outer nuclear cell counts, and attenuated activated microglia, and this effect persisted for a full month. Dendrimer-FA conjugate significantly reduced the number of microglia within retinal photoreceptor layers compared with buffer solution and free FA. Furthermore, this effect was apparent even at a 10-fold lower dose (0.1 µg).

CONCLUSIONS

This recent work at Wayne State University, Mayo Clinic, and The Wilmer Eye Institute at Johns Hopkins showed that dendrimers demonstrate enhanced uptake in RCS rat retinas undergoing active neuroinflammatory and other neurodegenerative processes. The pattern of enhanced uptake was not seen in the retinas of healthy rats receiving the same injections.

This observation suggests that dendrimer-drug conjugates could be an effective means of prolonging the residence time of drugs in areas of active neuroinflammation, thereby enhancing pharmacodynamic efficacy, specifically targeting inflamed tissues, reducing the overall amount of drug needed, and potentially reducing drug side effects.

Although activated microglia are not the root cause of either AMD or RP, they play key roles in mediating the inflammatory response in these conditions. Their pathophysiologic role seems to be shared among multiple types of retinal degenerative disease. Thus, it stands to reason a therapy that targets activated microglia may in turn serve to address the inflammatory component of multiple forms of retinal disease. The efficacy reported here suggests that even though microglia are not directly involved, targeted attenuation of these cells can have significant positive consequences.

The ability of PAMAM dendrimers to localize only within activated microglia may allow investigators to develop systems for sustained delivery of antiinflammatory drugs to the retina. We used FA in our work because it has previously been shown to slow photoreceptor cell loss and suppress inflammation in animal models of retinal degeneration.[12,13] The drug is also commercially available in Europe in a sustained-release intravitreal implant (Alimera Sciences).

As development of sustained-delivery methodologies for treatment of retinal diseases continues, we believe that the unique intracellular targeting and sustained-release properties of dendrimer-based nanodelivery technology will play an important role. ■

Raymond Iezzi, MD, MS, is an Associate Professor of Ophthalmology specializing in medical and surgical diseases of the retina and vitreous at the Mayo Clinic in Rochester, MN. He may be reached at Iezzi.Raymond@mayo.edu.

Rangaramanujam M. Kannan, PhD, is a Professor of Ophthalmology at the Wilmer Eye Institute, Johns Hopkins Medicine. He may be reached at krangar1@jhmi.edu.

1. Tomalia DA. In quest of a systematic framework for unifying and defining nanoscience. *J Nanopart Res*. 2009;11(6):1251-1310.
2. Florence AT, Hussain N. Transcytosis of nanoparticle and dendrimer delivery systems: evolving vistas. *Adv Drug Deliv Rev*. 2001;50 Suppl1:s69-89.
3. Kobayashi H, Brechbiel MW. Dendrimer-based MRI macromolecular contrast agents: characteristics and applications. *Mol Imaging*. 2003;2(1):1-10.
4. Lee CC, MacKay JA, Frechet JM, Szoka FC. Designing dendrimers for biological applications. *Nat Biotechnol*. 2005;23(12):1517-1526.
5. Kambhampati S, Kannan RM, Dendrimer Nanoparticles for Ocular Drug Delivery. *J Ocular Pharm and Therapeutics*. 2013;29(2):151-165.
6. Hayder M, Poupot M, Baron M, et al. A phosphorus-based dendrimer targets inflammation and osteoclastogenesis in experimental arthritis. *Sci Transl Med*. 2011;3(81):81ra35.
7. Menjoge AR, Kannan RM, Tomalia DA. Dendrimer-based drug and imaging conjugates: design considerations for nanomedical applications. *Drug Discov Today*. 2010;15(5e6):171-185.
8. Sarin H, Kanevsky AS, Wu H, , et al. Effective transvascular delivery of nanoparticles across the blood-brain tumor barrier into malignant glioma cells. *J Transl Med*. 2008;6:80.
9. Dai H, Navath RS, Balakrishnan B, et al. Intrinsic targeting of inflammatory cells in the brain by polyamidoamine dendrimers upon subarachnoid administration. *Nanomedicine (Lond)*. 2010;5(9):1317-1329.
10. Iezzi R, Guru BR, Glybina IV, Mishra MK, Kennedy A, Kannan RM. Dendrimer-based targeted intravitreal therapy for sustained attenuation of neuroinflammation in retinal degeneration. *Biomaterials*. 2012:33:979-988.
11. Tso MO, Zhang C, Abler AS, Chang CJ, Wong F, Chang GQ, et al. Apoptosis leads to photoreceptor degeneration in inherited retinal dystrophy of RCS rats. *Invest Ophthalmol Vis Sci*. 1994;35(6):2693-2699.
12. Glybina IV, Kennedy A, Ashton P, Abrams GW, Iezzi R. Intravitreous delivery of the corticosteroid fluocinolone acetonide attenuates retinal degeneration in S334ter-4 rats. *Invest Ophthalmol Vis Sci*. 2010;51(8):4243-4252.
13. Glybina IV, Kennedy A, Ashton P, Abrams GW, Iezzi R. Photoreceptor neuroprotection in RCS rats via low-dose intravitreal sustained-delivery of fluocinolone acetonide. *Invest Ophthalmol Vis Sci*. 2009;50(10):4847-4857.
14. Zeng HY, Zhu XA, Zhang C, Yang LP, Wu LM, Tso MO. Identification of sequential events and factors associated with microglial activation, migration, and cytotoxicity in retinal degeneration in rd mice. *Invest Ophthalmol Vis Sci*. 2005;46(8):2992-2999.
15. Wenzel A, Grimm C, Seeliger MW, et al. Prevention of photoreceptor apoptosis by activation of the glucocorticoid receptor. *Invest Ophthalmol Vis Sci*. 2001;42(7):1653-1659.
16. Kompella UB, Bandi N, Ayalasomayajula SP. Subconjunctival nano- and microparticles sustain retinal delivery of budesonide, a corticosteroid capable of inhibiting VEGF expression. *Invest Ophthalmol Vis Sci*. 2003;44(3):1192-1201.
17. Vandamme T, Brobeck L. Poly(amidoamine) dendrimers as ophthalmic vehicles for ocular delivery of pilocarpine nitrate and tropicamide. *J Control Release*. 2005;102(1):23-38.
18. Velazquez A, Carnahan MA, Kristinsson J, Stinnett S, Grinstaff MW, Kim T. New dendritic adhesives for sutureless ophthalmic surgical procedures: in vitro studies of corneal laceration repair. *Arch Ophthalmol*. 2004;122(6):867-870.
19. Grinstaff MW. Designing hydrogel adhesives for corneal wound repair. *Biomaterials*. 2007;28(35):5205-5214.
20. Marano RJ, Toth I, Wimmer N, Brankov M, Rakoczy PE. Dendrimer delivery of an anti-VEGF oligonucleotide into the eye: a long-term study into inhibition of laser-induced CNV, distribution, uptake and toxicity. *Nat Gene Ther*. 2005;12(21):1544-1550.