Clinician's Corner

Reducing the Risk of Hydroxychloroquine Toxic Retinopathy

by Michael F. Marmor, MD, Byers Eye Institute, Stanford University, Palo Alto, and Ronald B. Melles, MD, Kaiser Permanente, Redwood City Medical Center, Redwood City, California

Hydroxychloroquine (HCQ) is commonly used for its anti-inflammatory properties in the treatment of Sjögren's, because it is well-tolerated and rarely needs to be discontinued for adverse systemic reactions. However, HCQ may cause irreversible damage to the retina, especially if taken in high doses or if used for many years. We studied nearly 2,500 patients who were on HCQ for more than five years, which gave us the opportunity to make new observations about the risk factors for retinal toxicity and recommendations to reduce the risk. Overall, we found that retinal toxicity is not as rare as once believed (7.5% in our population), but this number reflects a population of long-term users, of which many were being given too high a dose. We'll show

Times of Drought: A Study of the Oral Microbial Ecosystem Under Conditions of Hyposalivation

by David A. Relman, MD, Thomas C. and Joan M. Merrigan Professor, Depts of Medicine, and of Microbiology & Immunology, Stanford University; and Chief of Infectious Diseases, Veterans Affairs Palo Alto Health Care System Palo Alto, California, and Diana Proctor, PhD Candidate, Relman Lab Dept of Microbiology & Immunology, Stanford University, Palo Alto, California

Over the past 50 years, we've seen an exponential increase in the number of published articles pertaining to the human microbiome—defined as the genetic coding capacity of the microbes, known as the microbiota, that live in and on our bodies. This striking surge in research on the human microbiome reflects our current fascination with these bacteria and their interactions with us, but it belies the rich and expansive history of the field. The field of human oral microbiology, in particular, reaches back over 300 years. In the present article, we first describe the history of the field and then outline two major lessons that we, as researchers, have taken away from this history.
Prospects for Genetic Intervention in the Salivary Glands of Sjögren’s Patients

by Michael J. Passineau, PhD, Director, Gene Therapy Program, Allegheny Health Network, and Associate Professor, Drexel University College of Medicine Pittsburgh, Pennsylvania

Editor’s Note: Dr. Passineau recently completed his SSF Innovative Research Grant project entitled, “Ultrasound-assisted gene transfer of IL17R:Fc to the salivary glands as a gene therapy for Sjögren’s Syndrome.” The Sjögren’s Syndrome Foundation depends largely on donations to support its research grants program and especially acknowledges the generous contributions from the Leach family and the Galewood Foundation who have supported Innovative Concept Research Grants and key medical and scientific initiatives since 2008 and 2009 respectively.

In the past decade, genetic intervention, or gene therapy, has transitioned from a promising research topic to a mainstream medical tool in select applications. Broadly defined, genetic intervention describes the use of genetic material (DNA or RNA) as a drug in order to manipulate the cellular programming of somatic cells. As such, genetic intervention is a method for acting on information gained from the complimentary sciences of human genomics, genetics, and tissue proteomics.

The genetic basis of Sjögren’s disease (SD) is not yet clear, although genome-wide association studies strongly suggest a polygenetic etiology that shows both similarities and differences relative to other autoimmune diseases. Until a systemic mechanistic understanding of SD is assembled, there is great therapeutic potential in focusing on the cellular programming of the salivary gland. While only one manifestation of this complex disease, SD-induced xerostomia constitutes a major portion of the disease burden most patients experience with SD. Further, minor salivary gland biopsies are a standard element of disease diagnosis in SD, providing the opportunity to directly examine the molecular biology of the minor salivary glands. These studies have provided important insights into potential genetic targets for therapy that might be applied to the major salivary glands.

Genetic intervention in the salivary glands is highly evolved and has very recently shown irrefutable clinical therapeutic efficacy. The first successful gene transfer to the salivary gland was shown by Bruce Baum and Ron Crystal in 1991. Over the next fifteen years, the National Institute of Dental and Craniofacial Research (NIDCR) Gene Therapeutic Branch, led by Bruce Baum, refined the technology and built preclinical data sufficient for the initiation of the first human gene therapy clinical trial involving gene transfer to the salivary gland in 2006. The xerostomia targeted by this gene therapy clinical trial was not SD-associated per se, but the reporting of successful reversal of both objective hyposalivation and subjective xerostomia in this patient cohort establishes the powerful principle that genetic intervention in the human salivary gland is safe, feasible, and therapeutically efficacious.

Looking forward from this milestone, there are two key challenges to achieving a similar result in patients suffering from Sjögren’s. First, the vector technology needed to deliver the therapeutic transgene must be considered and refined. Second, it is not yet clear which genetic target(s) will be of most therapeutic value locally within the salivary gland. Research aimed at answering these two questions require different sets of knowledge and skill, and both must proceed in parallel in order for genetic intervention to become a mainstream element of the therapeutic armamentarium available to clinicians who treat SD.

Vector Technology

The first challenge facing any gene therapy application is a means of efficiently and selectively delivering the gene drug to the target tissue. In the salivary gland, selectivity is achieved by retrodental infusion of the vector into either the parotid or submandibular ducts, whereby the vector can perfuse the organ through the ductal labyrinth while (in theory) preventing the vector from breaching the epithelial layer and spreading systemically. The simple technique of retrodental infusion means that salivary gland gene therapy could ultimately become an outpatient procedure performed in dental offices and also makes the salivary gland in many ways an ideal organ for research aimed at optimizing gene delivery techniques before applying them to more challenging delivery paradigms involving internal, solid organs (e.g. heart or liver).

Viral vectors are relatively efficient means of delivering gene drugs, and viral vectors have historically formed the basis of gene therapy research and clinical trials. In the salivary gland, Adenovirus (Ad) and Adeno-associated virus (AAV) have both been shown to efficiently delivery gene drugs, but AAV appears to infect only ductal, not acinar cells. Ad robustly infects..."
all cell types in the salivary gland, and Ad was the vector used to deliver the Aquaporin-1 transgene in the human gene therapy clinical trial at NIH that was mentioned previously. (It is referred to as “AdAQP1.”)

The challenge with viral vectors, and Ad in particular, is the host immune response elicited against the vector, and in particular the MHC Class II-mediated cellular immune response against cells infected with Ad vectors. This host response leads to rapid clearance of therapeutic transgenes in animal models, although questions remain as to whether this phenomenon occurs in the salivary glands of humans, with clinical observations of unexpectedly extended functional improvements in some humans treated with the AdAQP1 vector. Regardless of interspecies differences in host response to Ad vectors in the salivary gland, it seems problematic to imagine using viral vectors as agents for gene therapy in SD due to concerns regarding exacerbation of the existing inflammatory state of the salivary gland.

Our group has utilized ultrasound-assisted gene transfer or “sonoporation” as a non-viral means of delivering gene drugs to the salivary glands of both rodents and swine with results comparable to Adenovirus. This technique relies upon the association of naked DNA drugs with ~2mm microbubbles (currently approved for human intravascular use as ultrasound contrast agents) and the destruction of these microbubbles with a high energy ultrasound beam focused on the salivary gland. Microbubble destruction releases fluid-phase shock waves that produce transient pores in the cell membrane that allow transit of the gene drug into the cell. Remarkably, this technique does not appear to damage the salivary gland or elicit extracellular immune response.

Another approach to genetic intervention in the salivary gland is so-called “nanoparticles,” synthetic nanoscale structures capable of carrying genetic material through the cell membrane. For technical reasons, these nanoparticles are quite efficient at delivering silencing RNAs of various configurations but are far less efficient when delivering DNA transgenes. The upshot is that nanoparticles are excellent agents for gene “knockdown” but poor choices for delivery of therapeutic transgenes. An excellent application of nanoparticles for genetic intervention in the salivary gland has recently been described by Catherine Ovitt and coworkers.

**Molecular Targets**

With several viable vector systems now available for genetic intervention in the salivary gland, a strong impetus exists for identifying molecular targets for gene “knockdown” or “knock-in” to disrupt or reverse the pathophysiological cascade in Sjögren’s. Numerous reviews exist detailing the multitude of molecular candidates associated with SD in human biopsies and animal models, but more research is needed to elucidate cause and effect and to distill research down into actionable gene targets. At present, a unifying pathophysiological cascade(s) in Sjögren’s remains elusive and the SD research community finds itself with a powerful new weapon in the form of genetic intervention but no clear target upon which to aim.

Animal models have contributed to our understanding of Sjögren’s, but it is now fair to ask whether animals should remain the major substrate for research into the molecular basis of SD. With the rapid evolution of “omic” technologies, the scientific value that can be extracted from a piece of tissue as small as a labial gland
biopsy is orders of magnitude greater than it was even a decade ago. If animal models are not capable of fully simulating the complex systemic/glandular interactions in SS, vigorous efforts should be made to collect, bank, and disseminate human salivary gland biopsies from both SD and non-SD patients. In particular, these efforts should be focused on determining whether there are unifying principles of SD in every patient, or whether the disease is in fact many different autoimmune processes with common clinical manifestations. If the latter, genetic intervention gives great reason for hope toward a cure, as genetic intervention is almost endlessly versatile, with very few restrictions on which gene targets can be manipulated.

It is also important to bear in mind that the risks of salivary gland gene therapy are thought to be much less than in other applications of gene therapy. Ultimately, genetic intervention in human patients is the only way to determine the validity of a gene therapy strategy. In Sjögren’s, the salivary gland is dysfunctional and rarely improves. Further, the salivary gland is not critical to life, and the additional risk to a patient with a gland that is already dysfunctional is modest. As has been discussed, genetic intervention in the salivary gland is well-isolated to the gland itself, presenting very low risk of off-target effects. In the aggregate, these factors should lower the risk/reward ratio of gene therapy clinical trials in the salivary glands of SD patients and perhaps lower the regulatory hurdles.

**In Conclusion**

Genetic intervention in the salivary glands is feasible, safe, and effective in humans. Vector technologies continue to evolve, and non-viral methodologies appear poised for clinical testing. Salivary glands are but one focus of this complex disease, but for many patients, salivary gland dysfunction is the chief complaint and therapeutic options are limited. Until the initiation and systemic propagation of Sjögren’s is completely understood, the Sjögren’s community should redouble efforts to identify molecular targets for genetic intervention in the salivary gland. Above all, -omic analyses of human salivary gland biopsies and bioinformatic synthesis of this data is needed in order to direct the gene therapist in the design of human clinical trials to fully exploit the enormous progress that has been made in the technological feasibility of salivary gland genetic intervention.

**References**