Journal of Oral Biosciences 56 (2014) 38–42

Contents lists available at ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Review

Salivary gland gene therapy: Personal reflections

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ARTICLE INFO

Article history:
Received 20 August 2013
Received in revised form
2 October 2013
Accepted 4 October 2013
Available online 18 November 2013

Keywords:
Oral biology
Gene transfer
Xerostomia
History

ABSTRACT

The purpose of this paper is to describe, in somewhat familiar terms, the personal experiences I had trying to develop a clinically useful gene therapy for dry mouth over an approximately 20-year period. That research journey, which reached fruition in a recently completed Phase I clinical trial, was nurtured by my long career spent at the US National Institutes of Health and, in particular, by working within its hospital, The Clinical Center, the largest research hospital in the world. Through this paper, I wish to transmit several important lessons that I learned on my journey, which I believe will be applicable broadly across oral biology.

Contents

For many years, I have been interested in what I consider the failure of dental education to recognize the importance of biology and medicine to dental practice, as well as to embrace both fully in planning for the future of the dental profession [1,2]. Indeed, I have long believed that biology would provide dentistry, and thus dental patients, with the profession's next “fluoride-like” advance [3]. While the latter remains to be achieved, I still find that dentistry and oral biology, in much of the world, is separated from mainstream biomedical science.

Why has this “artificial” separation of dentistry from medicine and biomedical science been important to me? The answer is that I think dentistry would profit enormously from a full reengagement with both. Unlike most of my colleagues in dentistry and oral biology, I spent my professional career embedded in mainstream biomedical science. I was especially fortunate to work from January 1982–October 2011 at the US National Institute of Dental and Craniofacial Research (NIDCR), and within the National Institutes of Health (NIH)'s Clinical Center, the largest research hospital in the world. In that environment it was impossible not to be stimulated by the endemic level of biological innovations and applications being translated to the patient's bedside. As a young dental scientist I was most impressed by leading physician–scientists at the Clinical Center who broke from traditional clinical approaches, i.e., who thought “outside the box” of convention, and used biology to develop new ways to help their patients.

I hope through this paper that I can transmit some of the lessons I learned, and applied to my work in gene therapy, to the readership of the Journal of Oral Biosciences. I am particularly grateful to the Journal, as well as its Editor, Prof. Hayato Oshima, and the Japanese Association for Oral Biology, for the opportunity to do this, because I believe the general lessons I transmit will be widely applicable, or at least generally useful to consider, for translational investigators in each of the specific scientific disciplines encompassing oral biology.

The concept of transferring genes for therapeutic purposes was first discussed in the 1960s [see comments in 4]. However, it was then a technically impossible goal to achieve, i.e., molecular biology and its abundance of experimentally useful tools did not exist. As a real life example, consider that in 1972 I took a graduate course in nucleic acid biochemistry while studying for my PhD. In that course there was no mention of restriction endonucleases or reverse transcriptase, because both were only just beginning to be

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1349-0079/$ - see front matter
http://dx.doi.org/10.1016/j.job.2013.10.001

studied, while the concept of the polymerase chain reaction was completely unknown. By 1990, armed with such tools, gene therapy had become a reality, in humans [5], after being demonstrated in various animal disease models [e.g., 6–9].

In the late 1980s, in addition to my laboratory studies on salivary gland signal transduction, I was involved in clinical studies focused on the salivary gland hypofunction (dry mouth) that results from therapeutic radiation for head and neck cancers and which occurs as a sequela of Sjögren's syndrome. While my colleagues, most notably Philip Fox, and I had experienced some success in developing a pharmacological treatment for dry mouth, i.e., the per os use of pilocarpine [10,11], such a treatment was only useful for patients with a sufficient amount of functional salivary acinar (fluid secreting) tissue remaining. This represented perhaps one-third, or less, of patients with either condition. As a result, I increasingly grew frustrated at my inability to offer our research patients any relief or, more important personally, any experimental ideas that might eventually lead to their future relief. Consequently, I searched the biomedical science literature for possible novel approaches for the treatment of dry mouth. I became aware of the potential of gene therapy, i.e., the transfer of an oligonucleotide (at the time either a true gene or a cDNA) for therapeutic purposes, in particular because of the research then being done on using gene transfer to the lung to correct the principle pulmonary defect in cystic fibrosis [12,13]. As I knew from a past post-doctoral fellowship, the lungs are an epithelial tissue with many biological similarities to salivary glands [14,15]. In 1990, essentially all proposed uses of clinical gene therapy were either for single gene mutations, e.g., in-born errors such as cystic fibrosis, for which there was no existing treatment, or for cancers that were refractory to conventional therapy, such as malignant melanoma [16]. However, I reasoned that the idea of using gene therapy was a worthwhile consideration "even for a quality of life" disorder such as dry mouth, since there were so many patients who were unable to benefit from the available pharmacological therapies. Accordingly, I began to plan a strategy for accomplishing it [17].

One major personal difficulty was that I had no training in either molecular biology or virology, and I recognized that elements of both disciplines were likely going to be essential to developing a gene therapy for dry mouth. To overcome this hurdle, I did four things, each of which proved to be instrumental in achieving the end result. First, I took a 4-day course in molecular biology, so that I at least would become more familiar with its language and techniques. Secondly, I took a 6-month sabbatical in the laboratory of a good friend at NIH, so I could practice those techniques and become comfortable with their applications. Next, I sought a major collaborator with bona fide expertise in gene therapy, and I was very lucky, because one such person, Dr. Ronald G. Crystal, then at the National Heart, Lung and Blood Institute at NIH, also had been one of my post-doctoral mentors [14,15]. Finally, I was successful in recruiting a very talented, dentist/then new PhD (Brian O'Connell; now a professor in the School of Dental Science at Trinity College Dublin) to join this as yet unproven endeavor as a post-doctoral fellow. The tools of molecular biology were part of his PhD thesis and his contributions while in our department were absolutely critical to demonstrating the feasibility of gene transfer to salivary glands [18]. His entire first year at NIH was spent in Dr. Crystal's laboratory and, thereafter, he brought all of the required molecular biology and virology tools back to our department. Indeed, his initial success allowed me to recruit another new PhD post-doctoral fellow (Christine Delporte; now a professor in the medical school of the Free University of Brussels). She specifically began testing the gene therapy strategy that I had formulated for radiation (IR)-induced dry mouth, as well as demonstrated proof of concept in irradiated rats [19].

There were several key questions that I thought were necessary to answer in the development of a salivary gland gene therapy for a dry mouth: (i) who were the most appropriate patients to treat; (ii) what would be the most efficient method to target the gene to the greatest number of epithelial cells in a damaged salivary gland; (iii) how would a gene be delivered; and (iv), most importantly, what gene would be used? The answers to those questions came as a result my past clinical experience, some reasonable hypothesizing, and a good deal of luck. The answer to question (i), as implied above, was to focus on patients experiencing IR-induced dry mouth. It actually was a simple decision for, unlike patients with Sjögren's syndrome, who experience a chronic disease with the continued lymphocytic infiltration of their salivary glands, once an IR regimen is completed patients suffer no further insult to their gland tissue. The answer to question (ii) also was fairly straightforward; cannulation of a targeted parotid gland and retrograde infusion of the gene suspended in an appropriate buffer, much the same as would be done for a contrast X-ray of the gland (sialogram). The answer to question (iii) followed from my reading of the existing gene therapy literature. There were two basic means to transfer a gene, with or without a viral vector, and the literature at that time was clear that use of a viral vector was markedly more efficient at gene transfer than non-viral methods. The answer to question (iv) also came from the literature, and its timing was pure good fortune. As I was putting the gene therapy strategy together, I was at a loss over what gene could be used; I was unaware of any existing gene with the therapeutic potential for "repairing" a dry mouth. In late 1991, a good friend and colleague told me that Preston and Agre has just published a paper in the *Proceedings of the National Academy of Science USA*

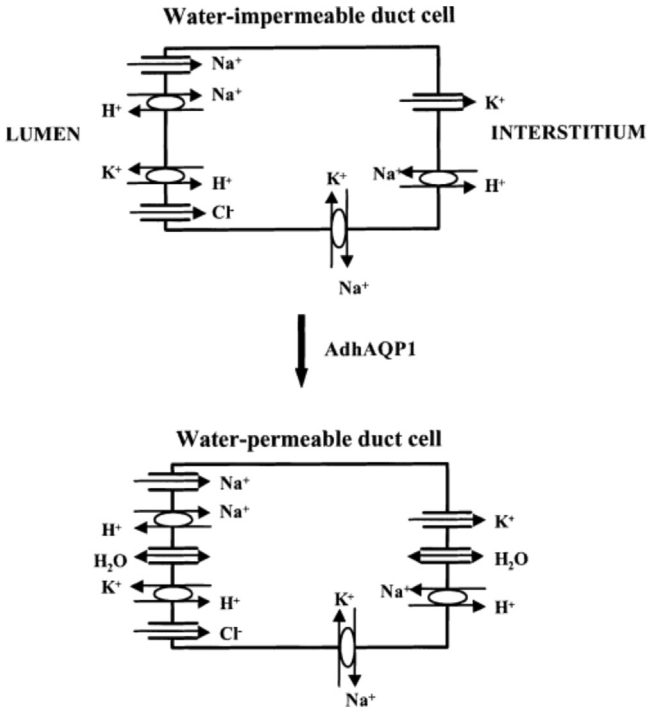

Fig. 1. Schematic diagram of hypothesized process by which AdhAQP1 facilitates fluid secretion from irradiated salivary glands. Surviving duct cells are presented in a simplified form, with only ion channels and ion transporters depicted (top). The lumen is to the left of the cell shown, and the interstitium is to the right. The cell is shown as water impermeable. After transduction of this cell with AdhAQP1 (bottom), the water channel aquaporin-1 is inserted into the apical and basal membranes providing a pathway by which water can flow in response to an osmotic gradient. We have hypothesized that this gradient would be generated by movement of K^+ and HCO_3^- into the lumen, i.e. lumen > interstitium. The figure is based on the experiments presented in Delporte et al. [19]. .
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