Use of localized gene transfer to develop novel treatment strategies for the salivary component of Sjögren's syndrome

Kok, M-R.

Citation for published version (APA):
Summary and discussion
SUMMARY AND DISCUSSION

Sjögren’s (SHOW-grins) syndrome (SS) is an autoimmune disorder predominantly affecting females. It is regarded the second most common rheumatic disease (1,2). The disorder usually affects the exocrine glands, like the salivary gland, but can also affect other tissues and organs. These patients can sometimes be recognized by carrying around a bottle of water to alleviate their dry mouth. Presently there is no cure for SS. The currently available prescription drugs can improve the symptoms of the dry mouth, but they do not cure the disease. The lack of sufficient saliva can cause considerable discomfort and morbidity, leading to rampant tooth decay, oral fungal infections, and difficulty swallowing and speaking. The lack of effective treatment is unsatisfactory for most patients, so there is a clear need for developing new treatment strategies for SS.

Gene therapy has been used to treat monogenetic or single protein deficiency diseases by replacing the faulty gene with a functional copy of that gene. In addition, gene therapy has now been used to target polygenetic diseases such as cancer, metabolic disorders and autoimmune diseases. Our knowledge of gene therapy and human disorders has greatly expanded in the past decade, creating the possibility to develop gene transfer as novel treatment strategy for a multitude of diseases. However, we need to be cautious using this potentially elegant treatment method in the human body. In 1999 Jesse Gelsinger died at the University of Pennsylvania, where he was enrolled in a phase I clinical trial evaluating a adenoviral gene construct. This not only led to a more critical evaluation of the safety and toxicity of adenoviral vectors, but also caused significant doubt in the entire gene therapy field (3,4). Important information about optimal vector type, stability of expression and safety are crucial for safe and successful treatment using gene therapy. In summarizing this thesis I will address several of these aspects and propose a new model for the screening of potential therapeutic targets in SS.

PART ONE: (CHAPTER 2) GENE THERAPY AND ITS POTENTIAL FOR TREATMENT OF SJÖGREN’S SYNDROME.

Although the pathogenesis of SS is largely unknown, the presence of an infiltrating lymphoid focus within the gland seems to be associated with a dysregulation of normal cytokine gene expression by salivary epithelial cells.

In trying to develop an approach for management of these patients, we have relied on more general immunomodulatory targets as well as strategies utilized to manage other autoimmune diseases, notably rheumatoid arthritis (RA). The recent and very successful development of “biologicals” for RA and the indication that cytokines may play an important role in the pathogenesis of SS as well justifies adoption of this strategy (5). Modulation of dysfunctional salivary epithelial cells with cytokines, such as IL10,
or inhibition of TNF-α could restore the imbalance between Th1-like cells and Th2-like cells within the gland. To circumvent the need for repetitive protein injections, discomfort for patients and the need for possible life-long treatment, gene transfer might be a good alternative to protein-based therapeutics.

The natural ability of the salivary glands to produce large quantities of proteins which can be secreted both in the saliva and in the blood stream, as well as the fact that salivary glands are affected organs in SS, makes them ideal candidates for local gene transfer. Recombinant AAV2 vectors are currently the vector of choice for the treatment of SS. They lack a potent immune response, as observed after local rAd5 delivery (6) Moreover, wild type AAV2 is not associated with any known pathology.

The Non Obese Diabetic (NOD) mouse model, an established model for type I diabetes, develops an exocrinopathy, characterized by diminished salivary flow that is age and gender dependent. The latter characteristic, which is lacking in all other SS animal model to date, makes it the most promising candidate for the development of a pre-clinical testing model for SS.

PART TWO: USE OF ADENO-ASSOCIATED VIRAL VECTORS FOR GENE THERAPEUTICS

**Gene transfer to the salivary gland**

Local gene transfer to the salivary glands is quite straightforward. The mice are anesthetized, their mouths opened, and thereafter a hair-thin cannula is inserted into the orifices of the submandibular gland in the floor of the mouth. The vector is delivered by connecting a syringe to the cannula and infusing (retrogradely) the vector under a surgical microscope. This procedure is a modification of the one used in clinical practice for obtaining contrast radiographs of salivary glands (sialograms). Chapter 3 shows the capability of the (mouse) salivary glands to produce a biologically active protein, after administration of a low amount of an AAV vector encoding human erythropoietin (hEPO) for 54 weeks (longest time studied). Hematocrit levels were similarly increased. We hypothesized that vector dissemination would be minimal, due to the fact that the salivary gland is well encapsulated. Vector DNA was detected only within the targeted salivary glands. This strongly suggests that mouse salivary glands are capable of long term expression of a biologically active protein with minimal vector dissemination after gene transfer using rAAV2.

**Immune response after rAAV2 delivery**

Treatment of chronic diseases including SS, may require long-term or even life-long expression of the disease-modifying therapeutic protein. This might be achieved by AAV gene therapy. The observation of extended transgene expression after local salivary...
gland delivery of rAAV2 vectors could result from the relatively modest immune response elicited by these vectors. Theoretically stable life-long expression after infection with a non-integrating vector may require readministration of the transgene containing vector. Unlike the response to use of adenoviral vectors, cytotoxic T-cell responses to the AAV capsid or transgene product have been rarely observed (7,8). rAAV2 vectors typically contain a transgene cassette in which all wild-type coding sequences (Rep and Cap genes) are replaced, with only the AAV2 inverted terminal repeats (ITRs) being present. Thus, the transgene product is the only source of non-self antigen besides the input capsid. In Chapter 4 studies of host innate and adaptive immune responsiveness were performed over a 56-day period. No significant innate immune response was observed; moreover, salivary flow was not altered after vector infection. Significant T cell-dependent humoral immune responses, characterized by neutralizing antibodies (NAB) titers were detected in serum of mice following rAAV2 vector administration. Successful vector re-administration was not possible using the same serotype vector. Since these NABs did not show cross-reactivity, repeated administration was possible with an alternative serotype (rAAV4).

Testing of two alternative serotypes of AAV (AAV4 and AAV5)

After the observation that successful readministration was possible changing serotypes, we investigate the tropisms, transduction efficiencies, and antibody response to AAV vectors based on AAV serotypes 2, 4, and 5 in chapter 5.

Recombinant adeno-associated virus vectors based on serotype 2 (rAAV2) can successfully direct transgene expression in salivary gland cells. The capsids of serotypes 4 and 5 are distinct from rAAV2 and from each other, suggesting that they may direct binding and entry into different cells due to their different binding receptors.

Local administration of the vector resulted in efficient transduction of salivary epithelial cells, with AAV4 and AAV5 producing 2.3 and 7.3 times more activity compared with AAV2. Like AAV2, AAV5 primarily transduced striated and intercalated ductal cells. AAV4 transduction was evident in striated and intercalated ductal cells, as well as convoluted granular tubules. In conjunction with chapter 4, neutralizing antibodies found in the serum of injected animals were serotype specific and there was no cross reactivity found between the NABs produced by the injected animals. This data provide suggest that changing serotypes may be the key to successful re-administration. Furthermore, because of differences in receptor binding and transduction pathways the other serotypes may have improved utility as gene transfer vectors in the salivary gland.
PART THREE: TREATMENT OF THE SALIVARY COMPONENT OF SJÖGREN'S SYNDROME

For studying the therapeutic impact and the feasibility of our proposed preclinical model for SS we tested the model in chapter 6. Female NOD mice, were injected with rAAV vectors encoding either hIL10 (rAAVhIL10) or a control vector (rAAVLacZ). These vectors were delivered by retrograde infusion to their submandibular glands, either at age 8 weeks (before onset of sialadenitis) or at 16 weeks (after onset of sialadenitis, a situation comparable to treating patients). As a systemic treatment control separate mice received intramuscular delivery of rAAVhIL10 at each time point. Both submandibular and intramuscular delivery of vector led to low circulating levels of hIL10. The long-term (20 week) salivary function was about three times higher in animals that were given the gene directly in their glands when compared to animals receiving control vector. Systemic delivery of the gene improved salivary flow only in the late treatment group. Inflammatory infiltrates in submandibular glands were significantly reduced in mice treated with rAAVhIL10 in the salivary gland, but not in mice receiving an intramuscular injection or control vector. In addition, after submandibular rAAVhIL10 delivery NOD mice exhibited significantly lower blood glucose and higher serum insulin levels than all other groups, indicating some systemic benefit of this treatment. Importantly, this study validates the use of the NOD mouse model as a pre-clinical model for SS. It also identifies hIL10 to be a possible target candidate for treatment of SS.

Chapter 7 contains a pilot study, investigating the effect of local gene transfer to the salivary gland using cDNA encoding soluble TNF-α receptor.

Evidence suggests that tumor necrosis factor-α, may play an important role in the pathogenesis of SS. However, systemic treatment of patients using soluble TNF-α receptor recombinant protein treatment was not successful (9,10). We hypothesized that this could be due to the lack of sufficient levels of TNF-α blocking agent in the salivary glands. Using the tools described in chapter 6, rAAVTNFR, was injected in the salivary gland as active treatment compared with either saline or rAAVLacZ (both negative controls), or rAAVhIL10 (positive control) as control vectors. Mice received vector at age 8 weeks, before onset of sialadenitis and were sacrificed at week 16. Mice treated with rAAVTNFR or rAAVhIL10 exhibited a significant increase in salivary flow rate. In contrast, mice receiving either saline or rAAVLacZ had a decline in salivary flow over time. The mononuclear cell infiltrates were significantly reduced in animals treated with the either sTNFR or the hIL10 gene compared to animals receiving saline.

Despite the overall improvement in salivary function and histologic appearance after rAAV-mediated hIL10 or sTNFR cDNA delivery to submandibular glands of NOD mice, we found a decrease of mouse TNF-α in the sTNFR injected mouse, however we did not find the hypothesized clear cut local alteration in cytokineprofiles (increase in Th2 versus
Summary and discussion

Th1 cytokines) in treated salivary glands. Thus, the mechanism by which this approach inhibits inflammation in the salivary gland and preserves saliva flow still remains to be fully elucidated.

CONCLUDING REMARKS AND FUTURE RESEARCH

Viral gene therapy for the treatment of human disease has caused both commotion and excitement. The commotion follows after the rollercoaster ride of the past few years of successes and serious adverse events. Excitement follows because viral vectors, in particular adeno-associated viruses, are believed to be an elegant and promising treatment option. During the past few years the field of gene transfer became more self-critical, and careful in addressing safety, tropism and vector-host interactions. This thesis is only the start for developing a potentially safe and effective gene therapeutic vector in the treatment of SS.

The salivary gland holds great promise as a delivery site for gene-based therapies. However, for successful local treatment several issues need to be addressed. Most important are vector-host interactions, currently addressed in the laboratory. Outside the scope of this thesis, but equally important, is the need to have proper tools for gene regulation and tissue specificity.

As for the treatment of SS, future studies will seek to provide more insight into the molecular changes that result in preserved salivary function.
REFERENCES


