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):
General introduction
INTRODUCTION

In 1933, the Danish ophthalmologist Henrik Sjögren described in his thesis the clinical and histologic findings in 19 women, with dry mouth and dry eyes. Bloch et al outlined in 1965 the clinical features of the currently recognized syndrome (1).

Sjögren's syndrome refers to the clinical syndrome characterized by a particular form of dry mouth (xerostomia) and dry eyes (keratoconjunctivitis sicca, KCS) that result from systemic autoimmune disease with lymphocytic infiltration of the salivary and lacrimal glands. Classified as an autoimmune disorder of the exocrine glands, Sjögren's syndrome has disabling effects because of the prominent exocrine involvement, manifesting as daily discomfort of dry eyes and dry mouth. Many patients have to reduce their level of activities or even resign from their job. Currently only secretagogues have been approved for the alleviation of these exocrine symptoms. This array of treatments is unsatisfactory for most patients, so there is a need for developing new treatment strategies for Sjögren's syndrome. This thesis will investigate the possibility of using local viral vector mediated gene transfer to the salivary glands as a potential treatment.

CONTENT OF THIS THESIS

Part one: background
Sjögren's syndrome is generally regarded as the second most common rheumatic disorder; the incidence of Sjögren's syndrome is exceeded only by rheumatoid arthritis (2). Although the complex nature of this syndrome is still not well understood, activation and migration of T-lymphocytes and B-lymphocytes to the glands perpetuates immune responses, which may damage salivary gland function (3,4). The development of new treatment strategies using local delivery of viral vectors, requires a proper pre-clinical model for testing. In chapter 2 pre-clinical models, a novel technique for administering vectors to the salivary glands, and possible therapeutic targets are described by reviewing the relevant literature.

Part two: use of adeno-associated viral vector for gene therapeutics
Gene therapy is a promising clinical application of gene transfer. The current therapeutic transgenes are mostly aimed at correcting systemic single-protein deficiency disorders. Liver, lung or muscle are widely used sides for vector delivery. However, infection of major organs can pose a serious safety concern, while muscle is not a tissue physiologically intended for protein secretion. The relatively noninvasive manner of gene transfer to the salivary glands, by retrograde ductal delivery of the vector, is a procedure similar to standard clinical practice for obtaining sialograms (contrast radiographs of salivary glands) (5). Salivary glands are capable of secreting protein both in the blood stream and the gastrointestinal tract. Local gene therapeutics using disease-modifying transgenes
could not only be beneficial to alleviate or correct the local pathology, but also decrease systemic manifestation of disease. Earlier studies have shown robust levels of transgene expression after salivary gland instillation of serotype 5 adenoviral (rAd5) vectors. A major disadvantage of rAd5 vectors for use in chronic diseases is the short duration of transgene expression after rAd5 mediated gene transfer (6). In chapter 3 the duration of expression and biological activity of the transgene product after local salivary gland delivery of recombinant AAV serotype 2 (rAAV2) vectors is investigated.

Treatment of chronic diseases such as Sjögren's syndrome, requires long-term or even life-long expression of the disease-modifying therapeutic protein. In contrast to rAd5 only mild immune responses have been reported after rAAV 2 deliveries. 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 (7,8). Route of administration, dose and the encoded transgene are important factors (9). In the absence of stable transgene expression, it is desirable to be able to readminister vectors. Immune responses after salivary gland delivery of rAAV2 vectors are examined, and the possibility of vector readministration is evaluated in chapter 4.

The Parvoviridae family contains several nonpathogenic members, the Adeno-associated AAVs. AAV are helper virus dependent for replication (10). No replication in vivo has as yet been described. Several distinct serotypes are described, the most extensively studied serotype being AAV2. rAAV2-based vectors can transduce both dividing and non-dividing cells in vitro and in vivo. Other isolates of AAV have demonstrated distinct tissue tropisms compared to AAV2. In chapter 5 tissue tropism and transduction efficiency of two alternative serotypes of AAV (AAV4 and AAV5) are compared with AAV2 following retrograde delivery to the submandibular gland of mice.

**Part three: treatment of the salivary component of Sjögren’s Syndrome**

The non-obese diabetic (NOD) mouse is considered a model for Sjögren’s syndrome. Spontaneous autoimmune sialadenitis and loss of salivary flow are hallmarks of the female NOD mouse. The onset of sialadenitis in these mice is around 12 weeks of age. Chapter 6 outlines a strategy for screening local immunomodulatory genes in a pre-clinical model. Mice received vector either before or after onset of the sialadenitis. Delivery of vector after onset of disease is a situation comparable to treatment of patients in the clinic. In particular chapter 6 investigates the effect of an rAAV2 vector encoding either a therapeutic transgene, hIL10 or a control (β-galactosidase). Both vectors were delivered to the submandibular gland by retrograde ductal instillation and systemically to the muscle. Disease modifying effects were not only monitored locally, but also systemically.
The presence of cytokines during the formation and proliferation of focal mononuclear lymphocytic infiltrates in the glands has been investigated and evidence suggests that proinflammatory cytokines, tumor necrosis factor (TNF) in particular, may play an important role in the pathogenesis of Sjögren's syndrome (11,12). Systemic treatment of patients using either soluble TNF-α receptor recombinant protein or a monoclonal antibody directed at TNF-α was not as successful as in rheumatoid arthritis patients (13,14). This could be due to the lack of sufficient levels of TNF-α blocking agent in the salivary glands. Chapter 7 investigates the effect of local gene transfer to the salivary gland using cDNA encoding a soluble TNF-α receptor.

In the final chapter 8, the findings of this thesis are summarized and discussed. The important implications and limitations of the ongoing development of gene transfer for clinical purposes, and directions for further research will be highlighted.

**SCOPE OF THIS THESIS**

1. Recognition of the best currently available pre-clinical model and identification of the best possible cytokine or cytokine receptor target for the local treatment of Sjögren's syndrome using local gene transfer mediated by rAAV2 vectors.
2. Investigation of salivary gland biology after local rAAV retrograde delivery in mice
   a. Duration of expression
   b. Dissemination of vector
3. Examination of local and systemic immune responses, and the possibility of vector readministration after local rAAV2 delivery encoding different transgenes in mice.
4. Analysis of tissue tropism and transduction efficiency of two alternative serotypes of AAV (AAV4 and AAV5) in comparison with AAV2 following retrograde delivery to the submandibular glands of mice.
5. Testing of two possible therapeutic targets for clinical efficacy using gene transfer (hIL10 and sTNF-α receptor) in a pre-clinical Sjögren's syndrome model.
REFERENCES


