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Salivary glands and gene therapy: the mouth waters

R Zufferey and P Aebischer

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Adding an endocrine function to the naturally exocrine salivary glands is one of the latest remarkable successes of gene therapy. The saliva is a mixture of serous and mucous secretions produced by multiple glands surrounding the oral cavity. In salivary glands, histologists distinguish two functional compartments: the secretory units called acini and a system of branching ducts converging in each gland into one single large secretory duct. All cells making this secretory apparatus are polarized and have a direct access to the acinar or ductal lumen. This anatomical structure, which resembles the many branches and the trunk of a tree, explains that the apical pole of each glandular cell is accessible for gene delivery by a minimally invasive procedure. The opening of the main duct in the oral cavity is canulated and gene delivery vectors, viral or nonviral, are infused by a retrograde injection.

The first successful gene delivery to salivary glands was reported 10 years ago.[1] Retrograde injection of adenoviral vectors in rat submandibular glands resulted in the transduction of both proximal ductal cells and distal acinar cells. When the transgene encoded a secretory protein, α1-antitrypsin, the gene product was detected in the saliva, indicating exocrine secretion. Therefore, the procedure was initially presented as a promising way to treat salivary glands disorders and deliver proteins to the mouth and upper gastrointestinal tract. The next major development was the recognition that, in the same setting, some gene products were at least partially secreted into blood. First, in 1997, human kallikrein was detected in rat plasma after salivary glands transduction.[2] The first demonstration of a systemic biological effect due to the human growth hormone gene product released from rat salivary glands followed in 1998.[3] These studies showed that gene delivery to salivary glands might not be limited to the treatment of salivary gland disorders, but might be an attractive approach to cure certain cases of major systemic pathologies such as diabetes or hemophilia, to mention only a few examples. However, several limitations had to be overcome before this prospect could become a reality.

First, transgene expression after transduction by adenoviral vectors was short-lived. This could be explained by the strong inflammation induced by the early generation of these vectors. A systematic comparison of most of the transduction methods available was therefore conducted. Recombinant proteins, naked DNA and liposomes are disappointing, and efficiency of liposomes is less than 1% of what is obtained with recombinant adenoviruses.[4–6] Adding DNAse inhibitors to naked DNA resulted in significant improvements,[7] but still the efficiency of nonviral methods is rather discouraging. Among viral vectors, simple retroviruses are useless in these rarely dividing cells.[8] Although lentiviruses do not require cell division, they are poorly efficient.[9] This low efficacy may be related to the lipidic envelope that may be sensitive to the lipases present in the saliva. Alternatively, the vesicular stomatitis virus glycoprotein (VSV G) might not mediate vector entry through the apical pole: pseudotypes carrying other fusion proteins, especially the Ebola Zaire glycoprotein, should be tested.[10] Other viruses such as herpes simplex and vaccinia are more efficient than lentiviral vectors, but local lymphocytes infiltrations is induced by these vectors.[8] Long-term gene expression without local inflammation is obtained with recombinant AAV vectors.[11–12] Only serotype 2 has been tested so far, but other serotypes deserve attention. Interestingly, AAV vectors seem to transduce exclusively ductal cells. Whether this is an advantage or a limitation remains to be established. Nevertheless, AAV appears at the moment as the vector of choice for gene delivery to salivary glands.

The second problem was related to the polarized secretion of proteins expressed ectopically in the salivary glands. Both exocrine (apical) and endocrine (basolateral) secretory pathways are active in glandular cells and most ectopically expressed proteins follow both pathways, albeit at different rates. The rules governing this protein traffic are not yet well understood. For the human growth hormone, the exocrine pathway is predominant resulting in a saliva:serum ratio of 20. On the contrary, the endocrine secretion is predominant for α1-antitrypsin.[13] A breakthrough came recently with the observation that human erythropoietin (hEPO) is secreted by ductal cells mostly if not exclusively into blood with a concentration in the saliva below the detection threshold. In an elegant study published recently in the Proceedings of the National Academy of Sciences, Voutekakis *et al* were able to demonstrate systemic hEPO secretion from mouse submandibular salivary glands at potentially therapeutic levels for a whole year.[12] Although hEPO was used, no major immune reaction against the transgene product occurred.

It seems that two key decisions led Voutekakis *et al* to their success: they chose the right vector, AAV, and the right transgene for basolateral secretion, hEPO. Can we expect to see hEPO production by salivary glands entering soon in clinical trials for diseases requiring hEPO administration such as chronic renal failure or β-thalassemia? There are reasons to remain cautiously optimistic. First, there was no immune response against hEPO in mice. However, two recent papers report alarming data on the induction of an autoimmune response against autologous EPO in monkeys.[14–15] In some animals of the first study, the rhesus EPO was secreted from the airways epithelium, a setting similar to the secretion by salivary glands.[14] Pre-

viously, the same rhEPO secreted from the trachea had not been immunogenic in mice, but it turned out to be surprisingly immunogenic in an autologous setting. Future studies will probably analyze more deeply the immunologic issues associated with EPO administration, especially the influence of the dose and the production site. Also more studies investigating protein traffic in the ductal and acinar cells of salivary glands and possible ways to control it are now warranted. Fusion of EPO, directed toward blood, to growth hormone, directed toward saliva, would be a logical experiment to do. Much more must be learned before the approach of Voutekakis *et al* can be translated in a clinical setting. Their success with EPO should encourage the testing of many more gene products (eg factor IX). In spite of all the hurdles, the smell from the kitchen makes already the mouth water. ■

Correspondence: R Zufferey and P Aebischer are at the The Institute of Neurosciences, Swiss Federal Institute of Technology Lausanne, EPFL, Switzerland.
E-mail: romain.zufferey@epfl.ch, patrick.aebischer@epfl.ch
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