A MODEL FOR MUSCLE REGENERATION IN THE SOFT PALATE

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Introduction

Children with a cleft in the soft palate have difficulties with speech, swallowing, and sucking. These patients are unable to separate the nasal from the oral cavity; a phenomenon known as velopharyngeal dysfunction. Surgical repair is required in order to close the defect and to reconstruct the muscle sling of the m. levator veli palatini, the major muscle of the soft palate. It ensures optimal function and normal speech development. However, about 10% to 30% of the treated patients still have inadequate velopharyngeal function. Scar formation and defective regeneration hamper the functional recovery of the muscles after cleft palate repair. Therefore, the aim of this study is to investigate the anatomy and histology of the soft palate in rats, and to establish an in vivo model for muscle regeneration after surgical injury.

Material and Methods

Approval of the research protocol was obtained from the local Board for Animal Experiments in accordance with Dutch laws and regulations (RU-DEC 2011-125). 14 male adult Sprague Dawley rats were used. The animals were divided into three groups: Group 1 (n = 4) and 2 (n = 2) to investigate the histology of the soft palate respectively, and group 3 (n = 8) for surgical wounding of the soft palate. In the last group, two animals were used as control, and the wound area was evaluated after 7 and 56 days using (immuno)histochemistry for muscle markers (AZAN staining. Pax 7, MyoD, MyoG, and ASMA).

Results

Our study shows that the anatomy and histology of the soft palate muscles of the rat are largely comparable to those of humans. Two main areas were identified in the soft palate, an anterior and a posterior area. The anterior area is characterized by a thick layer of salivary glands, which is covered by a layer of oral (bottom) and nasal mucosa (top). The oral surface is covered by a keratinized stratified squamous epithelium, while the nasal surface is covered by a pseudostratified ciliated columnar epithelium. The oral mucosa contains a thick collagenous submucosa (Figure 1).

All animals survived surgery and showed no weight loss. After seven days, all wounds showed complete clinical healing. After 7 days, granulation tissue and initial regeneration of muscle fibers and salivary glands was observed. After 56 days, more collagen was present and only few myofibers had regenerated. Myofibroblasts were not present in the muscle tissue of the controls or at 56 days after wounding but only in blood vessels and salivary glands (Figure 2).

At 7 days the wounds showed large numbers of myofibroblasts, and more activated SatCs (Pax7-, MyoD- and MyoG-positive) in regenerating muscle fibers than in control muscles. At 56 days no myofibroblasts were present anymore (figure 3).

Conclusion

We show that this model is suitable to study muscle regeneration in the rat soft palate, and allows the development of novel adjuvant strategies to promote muscle regeneration after surgery. This will offer new perspectives for the treatment of CLP patients, and for various other conditions in which the regeneration of head muscles is compromised.