Submandibular Duct Ligation in Rats: A Surgical Model of Induced Salivary Hypofunction for Cell Based Therapeutic Applications

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Abstract

Educational Objective: At the conclusion of this presentation, the participants should be able to compare the effects of salivary duct ligation of the rodent salivary gland and assess a rodent disease model for use in salivary gland tissue engineering.

Objectives: To create an animal model of salivary gland hypofunction suitable for testing pre-clinical salivary cell replacement therapies.

Study Design: Prospective intervention animal study.

Methods: 36 adult male rats were randomized to age/sex matched control (C), permanent submandibular duct ligation (SDL-p), or temporary SDL (SDL-t) groups. SDL-p and SDL-t animals underwent ligation of the right submandibular gland. SDL-t animals underwent surgical reversal 10 weeks post-ligation. All animals underwent induced salivary flow volume collection pre-ligation, 10 weeks post-ligation, and 4, 8, and 12 weeks post-ligation reversal. Under general anesthesia, animals were placed on a decline ramp, and saliva was collected for 15 minutes (Figure 2). Saliva volumes were recorded.

Results: Pre-ligation saliva volumes were not statistically different between C, SDL-p, or SDL-t groups (p>0.05). A statistically significant decrease in salivary flow volumes (p<0.05) and acinar cell density (p<0.05) was observed in SDL-p and SDL-t animals when compared to controls. No statistically significant difference in salivary volumes was observed between SDL-p and SDL-t animals (p>0.05).

In SDL-p and SDL-t animals, gross glandular atrophy without compensatory contralateral hypertrophy was observed. Key histopathologic findings in SDL-p and SDL-t animals were identical and included fibrosis and acinar cell loss with ductal preservation.

Conclusions: Temporary ligation of a single submandibular gland causes durable salivary hypofunction and corresponding acinar cell loss in a rat when compared to C and SDL-p animals. Despite persistence of the ductal system, regeneration of functional salivary tissue did not occur in SDL-t animals. These data show that SDL-t is a suitable model for testing cell replacement strategies in rats.

Introduction

Radiation therapy for head and neck cancer results in salivary gland cell loss and xerostomia (Figure 1). There is no cure for this condition. Engineering of replacement salivary tissue has been hindered by lack of a reproducible, clinically relevant animal disease model. We aimed to develop a small animal surgical model of salivary hypofunction for salivary cell replacement applications.

Methods and Materials

All animal procedures were performed under an approved Wake Forest University Animal Care and Use Committee protocol.

Thirty-six athymic nude rats were used in this study. Animals were divided equally into 3 groups: Control (C), Permanent Ligation (SDL-p), & Temporary Ligation with Reversal (SDL-t).

Saliva Collection (C, SDL-p, SDL-t): Saliva collections were performed prior to submandibular duct ligation, 10 weeks post-ligation, and 2, 4, 8, and 12 weeks post ligation reversal. Under general anesthesia, animals were placed on a decline ramp, and saliva was collected for 15 minutes (Figure 2). Saliva volumes were recorded.

Hematoxylin and eosin stained sections of SDL-p and SDL-t glands, showed significant acinar loss, fibrosis, vacuolization, honeycombing, and ductal preservation, compared to normal rat controls. These changes were histologically comparable to irradiated human salivary tissue (Figure 5, Figure 6).

Conclusions

Temporary salivary duct ligation (SDL-t) in a rat produces significant acinar cell loss with ductal preservation and durable salivary hypofunction.

An in-vivo duct ligation model of submandibular acinar cell loss achieves a histologic picture that is comparable to irradiated human salivary glands.

Unilateral, temporary submandibular duct ligation is technically feasible and reproducible as an in vivo model for testing salivary tissue engineering strategies.

Future Goals

Compare the degree of salivary hypofunction and acinar cell loss seen in rats undergoing temporary ligation of one versus both submandibular glands.

Qualitative analysis of saliva obtained from ligated and unligated salivary glands in a rat.

Results

All animals that underwent ligation (SDL-p, SDL-t), had statistically significant decrease in saliva volume output per animal weight, at all time points when compared to pre ligation (internal control) and C animals (Figure 4).

Animal groups were then sacrificed at 2, 4, 8, and 12 weeks post - reversal for histopathology assessment. Tissue blocks were embedded in paraffin, sectioned, and stained. H&E staining was used to assess acinar cell density, fibrosis, vacuolization, honeycombing, and ductal preservation.

Statistical Analysis: Statistical analysis was performed using Two-Way ANOVA method with GraphPad Prism 5.01 Software (QuickCalcs, GraphPad Software, San Diego, CA). Immunohistochemistry showed reduction in phenotypical and functional salivary cell markers in SDL-p and SDL-t glands, when compared to controls (Figure 8).

Figure 1: Clinical representation of xerostomia with findings of dry fissured tongue (left) and corresponding histopathologic findings of acinar cell loss, fibrosis, and ductal preservation in an irradiated human salivary gland (right).

Figure 2: Saliva Collection Method

Salivary Duct Ligation (SDL-p, SDL-t): Twenty-four animals underwent general anesthesia with isoflurane. The right submandibular duct was isolated, a sterile polyethylene tube placed around the duct, and two metal clips placed around the tube and duct. The left submandibular gland and duct remain undisturbed and were used as internal controls (Figure 3).

Reversal of Salivary Duct Ligation (SDL-t): At 10 weeks post ligation, animals were placed under general anesthesia, and the temporary ligation site on the right submandibular duct was identified and isolated. The metal clips and polyethylene tube were removed. The left submandibular gland and duct were not disturbed.

Tissue Analyses: Bilateral submandibular glands and ducts were harvested at 2, 4, 8, and 12 weeks post-ligation reversal. Histological assessment was performed with light microscopy. Acinar cell counts and immunohistochemical cell characterization for amylase, p63, ck 8-18, and aquaporin 5 were performed.

Statistical Analysis: Statistical analysis was performed using Two-Way ANOVA method with GraphPad Prism 5.01 Software (QuickCalcs, GraphPad Software, San Diego, CA).