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Recovery of salivary epidermal growth factor in parotid saliva following parotid sparing radiation therapy: a proof-of-principle study

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Background. Although radiation therapy (RT) causes permanent xerostomia, parotid-sparing radiation therapy (PSRT) ensures recovery of saliva quantity over time. Salivary epidermal growth factor (EGF) is produced primarily by parotid glands.
Objectives. The aim of this study was to determine whether salivary EGF can be detected in parotid saliva after PSRT and whether protein secretion is time dependent.
Study design. Salivary EGF concentration (pg/mL) was determined by enzyme-linked immunosorbent assay in stimulated parotid saliva before RT and at 3, 6, and 12 months after RT from 22 patients with head and neck cancer treated with PSRT.
Results. Saliva samples were from 17 men and 5 women (age ranges 23-70 years and 46-71 years, respectively). At 6 months after RT, EGF concentration was 407 pg/mL lower than at baseline ($P = .045$). Twelve months after PSRT, parotid glands produce substantial amounts of EGF and other proteins, eventually approximating pre-RT levels, with recovery of salivary function.
Conclusions. This proof-of-principle study shows that even proteins in picogram quantities, such as EGF, can be detected in saliva after PSRT. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:64-70)**

Salivary glands are highly sensitive to radiation. In fact, salivary gland hypofunction and xerostomia is the most common oral complication of radiation therapy (RT) for head and neck (HN) cancer and the most commonly cited reason for reduced quality of life.[1-4] Salivary flow rates are reduced significantly after a dose of 10-15 Gy delivered to most of the gland volume.[5-7] In addition to decreased saliva volume, RT also causes changes in saliva consistency, buffering capacity, and pH.[8,9]

The parotid glands produce ~60%-65% of the stimulated whole saliva.[1] At the University of Michigan, from 1994 to 1997, the first clinical trial of parotid-sparing radiation therapy (PSRT) was conducted. Conformal radiation therapy was used to spare portions of the parotid glands during radiation therapy for head and neck (HN) cancer.[10-14] This and subsequent studies by our team and other investigators have demonstrated that sparing of the parotid glands is possible, using conformal and 3-dimensional (3DRT) techniques, including intensity-modulated radiation therapy.[10-18] There has been an exponential increase in the adoption of this treatment in the USA over the past 5 years.[19]

During and immediately after PSRT, stimulated parotid flow decreases dramatically. However, salivary

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flow primarily of stimulated saliva, from glands receiving reduced radiation, recovers over time up to 2 years after the end of treatment.[13,16] This is associated with gains in quality of life.[17,18] Patients with limited or no salivary flow can have trouble eating, swallowing, and speaking.[1] In addition to improvement in quality of life, recovery of salivary flow also improves oral health.[20] Saliva has many functions, including mucosal repair, dental remineralization, antimicrobial action, buffering, and lubrication.[14] These functions are mediated by different components of saliva, including cytokines and glycoproteins. Therefore, an important consideration is the quality of recovered saliva in this patient population. However, until now the quality of saliva produced after PSRT has not been assessed.

To evaluate the quality of saliva, it must first be determined if proteins that are secreted in pg/mL amounts can be detected in parotid saliva after 3DRT. Salivary epidermal growth factor (EGF) was selected as a representative protein for analysis in the present proof-of-principle study. This protein was chosen because it is known to be produced by parotid glands and plays an important role in oral mucosal wound healing and maintenance.[21,22] Furthermore, there is evidence that levels of salivary EGF fluctuate during conventional radiation therapy and are associated with severity of radiation mucositis.[23-25] With conventional RT, salivary studies can be challenging, because salivary flow significantly decreases or ceases completely after treatment. In contrast, salivary glands receiving <26 Gy using PSRT demonstrate an initial salivary flow decrease during and immediately after RT that gradually increases in the months after the end of treatment.[13,16] The objectives of the present study were to determine whether salivary EGF can be detected in stimulated parotid saliva after 3DRT and whether protein secretion is time dependent.

MATERIAL AND METHODS

Study population

Subjects were enrolled in protocol UMCC-9427, "Partial parotid sparing using 3-dimensional planning in patients undergoing bilateral neck radiation for head and neck cancer," at the University of Michigan Comprehensive Cancer Center from 1994 to 1997. This study has previously been described.[10,11,13,14] The experimental protocol, consent form, and the present retrospective analysis of the deidentified saliva samples collected under the protocol were approved by the University of Michigan Institutional Review Board for the protection of human subjects.

3D radiation treatment planning

All patients underwent immobilization and full 3D treatment planning as previously described.[10,12,14] The goal of the treatment planning was full exclusion of the contralateral parotid gland at the side of the neck least involved with the tumor. All patients were treated with continuous conventional fractionation and received 1.8-2.0-Gy fractions, 1 fraction per day, 5 fractions per week, for 7 weeks.[10,12,14]

Parotid saliva collection and flow measurements

Bilateral parotid saliva was collected before radiation treatment and at 3, 6, and 12 months after completion of RT over a course of time from 1994 to 1997. Subjects were instructed to refrain from eating, drinking, smoking, and oral hygiene for a minimum of 90 minutes before saliva collection. All saliva was collected between 8:00 and 12:00 a.m. to control for circadian variation in salivary gland function.[26] Parotid saliva was collected from both parotid gland orifices using a Carlson-Crittenden cup.[14,27,28] Salivary flow was stimulated by applying 2% citric acid swabbed onto the anterior dorsolateral surfaces of the tongue at 30-second intervals for 2 minutes for equilibration. This was followed by a 2-minute collection period during which gustatory stimulation was maintained.[14] After saliva collection, the volumes of all saliva samples were determined gravimetrically on an analytical balance, assuming a specific gravity of 1.0. Saliva samples were aliquoted and stored at −80°C until protein analysis. An aliquot was thawed before use for this study.

EGF assays

Right and left stimulated parotid saliva samples were pooled for each subject at each time point. This was done because of limited saliva production by irradiated glands. EGF concentration was determined using an enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, MN) according to the manufacturer's instructions. Briefly, this is a sandwich assay in which a 96-well plate is precoated with monoclonal antibody specific for human EGF. Quantification is by calorimetric detection. Several known concentrations of recombinant human EGF were used as standards. The standard curve range was 3.9-250 pg/mL. Standards and the samples to be quantified were run in parallel on the same plate. The samples were diluted to fall within the range of the standards. In initial studies, these dilutions were determined for saliva samples. For diluted samples, the dilution factor was considered when calculating the final concentration.

Total protein concentration was determined by the Bradford assay (BioRad Laboratories, Hercules, CA).[29] Bovine serum albumin was used as a standard accord-