

Parotid gland function after radiotherapy

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Parotid gland function after radiotherapy

Oorspeekselklierfunctie na radiotherapie

(met een samenvatting in het Nederlands)

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Aan mijn ouders

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Contents

1. General introduction	9
2. CT-based parotid gland location: implications for preservation of parotid function	19
3. The influence of clinical factors on human stimulated parotid flow rate in cancer and other patients	27
4. Quantitative dose-volume response analysis of changes in parotid gland function after radiotherapy in the head-and-neck region	39
5. Scintigraphic assessment of early and late parotid gland function after radiotherapy for head-and-neck cancer: a prospective study of dose-volume response relationships	59
6. Preservation of the rat parotid gland function after radiation by prophylactic pilocarpine treatment: radiation dose dependency and compensatory mechanisms	75
7. A comparison of mean parotid gland dose with measures of parotid gland function after radiotherapy for head-and-neck cancer: implications for future trials	89
8. General discussion	99
9. Summary	113
Samenvatting	117
Dankwoord	121
Curriculum Vitae	125

CHAPTER

1

General introduction

Head-and-neck cancer accounts for approximately 4% of all malignancies in the Netherlands. This means that of the newly diagnosed tumors per year 2350 occurred in the head-and-neck region¹. Over the last 20 years there has been an increase in the number of patients with head-and-neck carcinoma². The incidence of head-and-neck cancer in women is 3 to 4 times lower than in men. Prognosis varies considerably according to the anatomical site: the best 5-year survival rates are seen for cancer of the larynx (more than 70%)³. Together with surgery, radiotherapy is the main treatment for malignant tumors in the head-and-neck region. Approximately 50% of all new cases of invasive head-and-neck cancer received radiation therapy⁴. Most radiotherapy for head-and-neck cancer is administered by high-energy (megavoltage) external beam irradiation using a linear accelerator. External beam irradiation has developed from conventional techniques using standard beam portals based upon bony structures to three-dimensional conformal radiotherapy. Three-dimensional conformal techniques permit more selective delivery of radiation to defined targets in the head-and-neck. The target volume can be defined on a planning CT scan in three dimensions. To achieve a higher target dose conformity a new technology called intensity-modulated radiotherapy has been developed⁵. The main variables in a course of external beam radiotherapy are the number of fractions, the dose per fraction, the total dose given, and the overall duration of treatment time. Radiation-oncologists endeavor to employ a combination, which will achieve the maximum tumor control with the minimum normal tissue damage. In patients with head-and-neck cancer, radiation treatment often involves the tissues of the oral cavity and the major and minor salivary glands, either because of the site and extension of the primary tumor or due to the path of lymphatic spread. Exposing the salivary glands to radiation often results in salivary gland hypofunction and changes in saliva composition, leading to a number of acute and long-term complications. As the prognosis for localized head-and-neck cancer is relatively favorable, the oral and dental status of the patient should be considered with great care.

Salivary (hypo) function

The average daily flow of whole saliva varies between 0.5 and 1.0 L in normal physiological conditions⁶. The major salivary glands (parotid, submandibular, and sublingual) account for more than 90% of the salivary flow. The remainder fluid is produced by the minor salivary glands, found in the lower lip, tongue, palate, cheeks and pharynx. During unstimulated flow the relative contributions of the different salivary glands are 20% from parotid, 65% from submandibular, 7-8% from sublingual, and less than 10% from numerous minor glands⁷⁻⁹. Stimulation changes the percentages, with the parotid glands contributing more than 50% of total salivary secretions. The parotid secretion is much richer in water than the more viscous and mucin-rich submandibular/sublingual glands¹⁰⁻¹². Saliva is a valuable oral

fluid that is critical for the preservation and maintenance of oral health (**Table 1**). Patients undergoing radiation treatment for their disease often experience a variety of problems as a consequence of difficulties in maintaining these functions. Patients may describe a subjective feeling of dryness of the mouth. They complain about a burning sensation and thirst. The oral mucosa, the lips, and the mucosa of the tongue can have a dry, atrophic or fissured aspect. Oral functioning is hindered because of insufficient lubrication of the mucosal surfaces. Moreover, chewing and swallowing is difficult because of insufficient moistening of food by saliva. Patients may experience difficulty in wearing dentures because of increased viscosity and

Table 1¹¹

Functions of saliva

Fluid	Cleansing of the oral cavity
	Solubilisation of food substances and taste compounds
	Dilution of detritus
	Lubrication of oral mucosa
	Bolus formation
	Facilitation of:
	-taste perception
	-mastication
	-swallowing
	-speech
Solutes	Protection of teeth
	Acid neutralisation (buffering actions)
	Saturated calcium phosphate concentration
	Participation in formation of enamel pellicle
	Protection of oral mucosa
	Mucosal coating
	Antimicrobial defense
	Digestive actions
	Initial digestion of starch and lipids

reduced flow. Many patients suffer from nocturnal discomfort. They are awakened by a serious dryness of the mouth or have to get up frequently because of polyuria due to polydipsia throughout the day. Impaired salivary gland function is not only a cause of considerable discomfort to patients but is also a major cause of the dental problems that occur following radiotherapy. The shift in oral flora towards cariogenic bacteria, the reduced oral clearance, and the changed saliva composition may result in rapidly progressing radiation caries, along with a greater incidence of periodontal infections¹³. These factors are compounded by the tendency of patients

to take frequent high carbohydrate meals as a result of the dryness of mouth and alteration of taste perception. Saliva is an essential factor in quality of life: without saliva, the patient will suffer from persistent discomfort, what will have a negative influence on his/her social life. The most effective way to gain an appreciation of the variety of roles played by saliva in humans and its importance to well being is to sample the complaints of people with salivary dysfunction. They are miserable: "My mouth and throat are dry, rough and sticky. I'm hoarse: it is so hard to talk. I can't wear my dentures, my mouth is always sore. I have to sip fluids frequently so my tongue won't stick to the sides or roof of my mouth. Eating is difficult and sometimes impossible. Food sticks to my mouth and teeth. I can't tell the position of food in my mouth. My mouth often feels numb. I have difficulty tasting and have to add more salt and sugar to my food. My fillings are falling out and my teeth are crumbling away"¹⁴.

Quantification of xerostomia and salivary gland function

Evaluation of effects of radiotherapy on salivary gland function may comprise subjective rating of oral symptoms, or objective measures such as flow rate measurements.

Subjective responses must be considered in order to obtain a complete understanding of the impact of xerostomia on the quality of life. Quality of life is fundamentally a subjective measure and should be self-assessed by the patients. Patients themselves reported lower scores and more side effects compared with the assessment made by clinicians¹⁵. Several head-and-neck cancer specific quality of life instruments have been conducted and validated¹⁶⁻²¹. All these kind of instruments contain a number of questions covering various aspects of head-and-neck related quality of life. Quality of life as assessed by patients, and toxicity in general are not always strongly related. After radiotherapy in head-and-neck cancer patients, it is not clear if the subjective feeling of a dry mouth is related to a significant reduction in salivary flow. Therefore, it is essential to measure saliva secretion to get more insight in the functioning of the salivary glands after the course of therapy.

Whole saliva, the product of the major and minor salivary glands, can be collected or saliva can be collected from individual glands. Saliva flow is termed unstimulated when no exogenous stimulus is used and is termed stimulated when secretion is promoted by mechanical, gustatory or pharmacological means²².

Several methods have been employed to collect whole saliva. These include draining, in which the subject bends the head forward and after an initial swallow allows saliva to drip off the lower lip into a graduated cylinder or preweighed container; spitting, in which the subject spits out every 60 seconds; sucking, in which saliva is sucked continuously from the floor of the mouth with a suction tube and allowed to accumulate in a collection vessel; and swabbing, in which preweighed absorbent swabs are inserted in the mouth and removed for weighing at the end of the collec-

tion period. The last two methods introduce some degree of stimulation. Stimulated whole saliva can be collected using any of the above procedures after stimulation. Pharmacological stimulation with drugs such as pilocarpine is rarely used. Gustatory stimulation with citric acid or mechanical stimulation by the chewing of paraffin wax or rubber bands is usually employed. The value of collecting stimulated saliva is that it provides information about the secretory capacities of the glands. Acid is the most potent gustatory stimulus but, since it is rapidly diluted and buffered with saliva, it must be renewed frequently. In practice, a few drops of about 2-5% citric acid are usually placed on the subject's tongue at regular intervals from 30 to 60 seconds. The main advantage of mechanical stimulation is that the stimuli used are inert and thus do not add anything to the saliva. More recently a measurement of the weight loss of a candy held in the mouth for a fixed period of time was described to determine salivary output²³.

Various methods have been developed for the collection of saliva from individual salivary glands. Unstimulated or stimulated saliva can be collected but it takes a considerable amount of time to collect without stimulation. Parotid saliva can be collected by intra oral cannulation of the duct or more conveniently by the use of suction cups placed over the openings of Stensen's duct. Carlson and Crittenden first described such a device in 1910. Numerous modifications of the original device have been made and the device is now most commonly referred to as the Lashley cup after one of its modifiers. Submandibular saliva is more difficult to obtain and cannulation of the ducts is difficult. However a silicone rubber device that fits into the floor of the mouth has been described to collect from both submandibular glands simultaneously. This device can be used to collect sublingual saliva at the same time. With these methods gustatory stimulation can be applied without contamination of the collected saliva. An alternative and simple technique is to block off secretion from the parotid glands and collect mixed submandibular and sublingual saliva by pipette from the floor of the mouth²⁴. Minor salivary gland secretions can be collected by pipette from the inner aspect of the lips or soft palate or by absorption on filter paper of known weight.

When flow of saliva is stimulated, the nature of the stimulus is particularly important, acid being the most potent gustatory stimulus. In this way it can easily be found out whether sufficient saliva is secreted and to what extent stimulation of the salivary flow is possible.

Another test available for investigation of salivary gland function is salivary gland scintigraphy using ^{99m}Tc-pertechnetate²⁵⁻²⁷. ^{99m}Tc-pertechnetate is actively trapped and concentrated in intralobular ductule cells with subsequent ductal epithelium secretion and discharge into the excretory ducts²⁸. Following the intravenous injection of ^{99m}TcO₄ with the patient in supine position under a gamma camera with high-resolution collimators, views of the head and hence the salivary glands are obtained. Imaging is commenced at the time of injection and sequential frames

are acquired. Salivary excretion can be induced by local stimulation e.g. by ingestion of citric acid. For analysis of data, regions of interest are selected over parotid (and submandibular) glands and corresponding time-activity curves are created. In this way the major salivary glands can be examined simultaneously and continuously over a period of time. This technique has gained widespread acceptance in evaluating a variety of salivary glandular disorders, and its usefulness to evaluate salivary function after radiotherapy has been demonstrated in patients with head-and-neck malignancies²⁸⁻³¹.

Radiation therapy

Depending on the extent and location of the planning target volume, a rapid decrease of the salivary flow rate is observed during the first week of treatment. Already in the literature of the 1970s, early and marked falls in the secretion of saliva were described when the major salivary glands were included in the treatment fields³²⁻³⁴. These marked early effects may persist in the period after irradiation and impaired salivary gland function following radiotherapy is a major cause of late morbidity following the treatment of head-and-neck cancer with radiotherapy. Unstimulated and stimulated whole salivary flow can be demonstrated to be reduced even up to 25 years following irradiation^{35,36}. Parotid glands included in the treatment volume have been shown to have markedly reduced stimulated flow rates several years after treatment^{37,38}. This concerns largely clinical observations and in most studies no pretreatment assessment or internal control but comparison with control non-irradiated glands from other patients or volunteers was used. There are only a few studies where saliva has been collected serially before, during, and for extended periods of time after radiotherapy to determine objectively if recovery can occur. Moreover, the large variability in flow rates within individuals has complicated the establishment of standard base rate values³⁹.

It is generally agreed that the final degree of irradiation-induced hyposalivation depends on the dose of radiation delivered and on the volume of the salivary tissue included in the radiation field^{8,40}. However, there are still only a limited number of accurate studies on dose-volume response relationships, especially in patients irradiated with conventional techniques. Studies from the past are difficult to compare, because the dose to the glands and the irradiated gland volumes were not assessed or estimated from simulation films^{38,41-45}. Furthermore, in some studies dose-volume values were correlated with whole saliva measurements, reflecting the flow of all major and minor salivary glands^{33,35}.

Aim and outline of the thesis

The aim of this thesis was to objectively and subjectively evaluate parotid gland function before the initiation of the treatment, and after radiation therapy and correlate these observations to the dose distributions of the parotid glands.

First, the importance of the knowledge of the position of the parotid gland was stated (chapter 2). Chapter 3 involves the effects of clinical variables on parotid flow rate. An accurate description of relationships between radiation dose, volume irradiated and response concerning the parotid glands by measuring stimulated flow rate using Lashley cups is given in chapter 4. Chapter 5 offers a quantitative dose-volume response analysis using nuclear scintigraphy as a method to evaluate the function of parotid glands after radiotherapy. An animal model was used to study the effectiveness of a prophylactic pilocarpine treatment to preserve parotid gland function in chapter 6. In chapter 7 the most adequate parameter to measure the consequences of reducing the parotid gland dose is determined. In the last chapter, a general discussion is provided.

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CHAPTER

CT-based parotid gland location: implications for preservation of parotid function

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Abstract

Purpose

The position of the parotid gland in relation to surrounding structures was investigated.

Methods

Sixty-five patients with head-and-neck tumors were prospectively evaluated. Parotid position was determined using beams eye views of CT images projected on simulator films. Distances between the different borders of the parotid gland and surrounding bony marks were quantitatively assessed.

Results

The parotid gland volume ranged from 12.9 cm³ to 46.4 cm³. The distance between the cranial border of the parotid gland and the tuberculum anterior of the atlas ranged between 0.7 and 4.8 cm. The position of the parotid gland was unaffected by the angle of the mandible.

Conclusions

The size and position of the parotid gland varies largely among patients. As the extent of radiation-induced salivary dysfunction depends on the volume of the gland tissue exposed, CT-based simulation of radiation fields is necessary.

Introduction

Radiotherapy of the head-and-neck region is known to cause changes in salivary secretion, as a result of radiation damage to the salivary glands^{3,15,17,18}. The radiation field, specifically the volume of gland tissue exposed, is an important factor affecting the development of oral dryness^{2,9-12}. As the normal parotid gland may show a four-fold variation in volume between individuals^{6,7,13}, it is important to exactly determine the position of the gland. In most studies the relative degree of inclusion of the salivary glands in the radiotherapy field was derived from the assumed position of the salivary glands in relation to the surrounding bony structures visible on the simulation and portal films⁸⁻¹². In more recent studies computed tomography (CT)-based treatment planning was assessed^{4,5,13}. Yet, conventional three-field technique using bony structures to define radiation field borders is still common practice for target localization of head-and-neck tumors¹. Therefore, the purpose of this study was to investigate the variability in the position of the parotid glands in relation to surrounding structures.

Methods

Sixty-five patients treated with radiotherapy for various malignancies of the head-and-neck were studied. None of the patients received previous radiotherapy or surgery of the parotid glands, or suffered from malignancies or other diseases of the parotid glands. All patients were treated with radiation therapy without induction or concomitant chemotherapy. Twenty-six patients had surgical resections followed by postoperative radiotherapy.

Patients were treated predominantly with 6 MV X-rays from a linear accelerator using isocentric techniques. In the majority of patients opposing lateral neck fields were used to cover the target volume. The supraclavicular regions were treated with an anterior field using independent collimators with half beam blocking^{16,19}. Patients were immobilized using individually designed facial shells for reproducible positioning with the neck extended as far as possible. Lateral and anterior set up marks were placed on the patient's shell. Contrast enhanced CT imaging of the irradiation area including whole major salivary glands was performed in the treatment position with the immobilization device using 3-mm thick slices. Accurate alignment of position was obtained using midline and lateral lasers. The marks were made visible on the CT images by using radio-opaque marker balls. The CT data were transferred to the computerized planning system by optical disc. The location of the parotid glands was outlined on the axial CT slices and reviewed with a radiologist whose expertise is head-and-neck imaging. Treatment plans were individually designed for each patient. When treatment fields were designed using orthogonal radiographs, reconstruction of these fields on the CT slices took place using the patient's set up marks.

Right and left lateral beams eye views of the parotid glands were printed and projected onto the (right and left) simulator films. The projected distances on the film between the cranial, caudal, anterior and posterior borders of the parotid glands and the center of the tuberculum anterior of the atlas were measured. Also, the angle at which the mandible meets the high cervical vertebrae was measured.

Results

The variation between the individuals in the size of the parotid glands was large: the gland volumes ranged from 12.9 cm³ to 46.4 cm³ with a mean of 26.4 cm³. Differences between the volume of the left gland and the right gland in the same patient were distributed from 0 cm³ to 15 cm³ (mean 1.17 cm³). The mean total length of the parotid glands was 5.8 cm (range 3.0-8.1 cm).

The distances between the cranial border of the parotid glands and the tuberculum anterior of the atlas (TAA) ranged between 0.7 and 4.8 cm. There was also a wide range of distances between the caudal, anterior and posterior border of the parotid glands and the TAA. All these distances are described in **Table 1**. Differences from 0.0 up to 2.0 cm (mean cm) were seen between the extension of the parotid

Table 1 Median and range of distances between different borders of the parotid glands and the tuberculum anterior of the atlas (cm).

	Mean distance	S.D.	Range of distance
Cranial	2.77	0.85	0.70 – 4.80
Caudal	2.98	0.83	1.30 – 6.10
Ventral	1.74	0.69	0.10 – 3.20
Dorsal	2.05	0.63	0.20 – 3.80

gland of the right and left sides in the same individual.

There was a considerable variability in the angle of the mandible. The mean angle of the mandible was -4.2° with a range of -42° to 21° . The angle of the mandible was significantly lower in patients who had surgical resection. However, there was no relationship between the mandible angle and the position of the parotid gland.

Discussion

The radiotolerance of human parotid glands has not been well defined³. The level of the upper border of the radiation field has shown to be a critical factor when using parallel-opposed lateral fields to the upper head-and-neck region. It is generally assumed that more than 50% of the parotid glands has to be excluded from

the treatment portals to prevent subjective dryness-related complaints in most patients¹¹. Yet, reliable data on the actual volume of a particular salivary gland irradiated in relation to surrounding structures are not available in literature. The parallel opposed two-field technique or the three-field (anterior and bilateral) irradiation techniques are commonly used techniques in head-and-neck cancer^{16,19}. Nowak assessed the variation in the routinely applied treatment portals in case of elective neck irradiation¹⁴. Major variations in the size and shape of the lateral opposed portals selected to treat similar lymph node regions were seen. This could result in substantial variation in the position of the cranial boundaries of the treatment portals at the level of the parotid gland.

In this study, a large individual difference in the volume of parotid glands was demonstrated from the measurements of CT images. Quantitative measurements of the anatomical relationship between the parotid gland and the TAA were made in a large group of patients. The TAA is a central bony structure visible on simulation and portal films and showed to be a good reference point for the different borders of the radiation field. A large variation in the position of the parotid glands was noticed. In most studies the volume of the salivary glands included in the radiotherapy fields was calculated by comparing the extent and borders of the radiation fields with the assumed position of the glands⁸⁻¹². Bony structures are often used to define the borders of the radiotherapy fields¹. However, as shown in this study, because there is a considerable variability in size and position of the parotid gland between patients, it is inaccurate to assess the part of the parotid gland irradiated assuming a certain position with respect to bony structures. Even in the same patient there is variation in position between the left and right parotid gland. These differences may have consequences for the treated gland volume and, therefore, with the degree of xerostomia.

The movement of the neck of the patients was impaired by previous surgical procedures. However, the angle of the mandible did not influence the position of the parotid gland expressed as difference in distances between TAA and parotid gland borders.

Conclusion

The volume of the parotid gland exposed to the radiation field is one of the most important factors that determine the severity of parotid gland damage. Our results indicate that, to ensure adequate information about the position of the parotid gland, CT-based visualization is necessary.

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CHAPTER

The influence of clinical factors on human stimulated parotid flow rate in cancer and other patients

Judith M. Roesink

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Abstract

Reduced salivary secretion can produce a wide variety of complaints, having a negative impact on the daily life of a patient. Multiple causes of salivary hypofunction are described, however, there are no consistent data about the influence of clinical parameters on parotid gland function. We studied a group of patients with head-and-neck malignancies before treatment with radiotherapy. We used Lashley cups to collect stimulated parotid saliva simultaneously from both parotid glands. Sizes of the parotid gland, gender, age, tobacco and alcohol consumption, and tumor characteristics were related to the function of the parotid gland. A considerable variability in parotid output was found with a range of 0.03-1.66 mL/min (mean 0.34 mL/min). None of the variables were correlated with parotid flow. These results are important, especially when evaluating effects of radiation on parotid gland function.

Introduction

Normal salivation is an essential component of oral health due to its important contributions to the oral defense mechanisms and digestive function. Reduced salivary secretion may produce a wide variety of complaints including dry and burning sensations of the mouth, progressive carious destruction of the teeth, frequently occurring oral infections, as well as difficulties in chewing, swallowing, speaking, and sleeping. Salivary gland dysfunction may be caused by many factors. A large number of physiological and pathological processes can cause a reduction of the salivary flow. The most severe forms of hyposalivation are observed after the radiation of malignant tumors in the head-and-neck region, in Sjögren's syndrome and after the use of certain drugs¹. Although numerous studies have investigated the effects of physiological changes on salivary flow rate, conflicting results have been reported^{2,3}.

The purpose of this study was to evaluate several parameters on salivary function of the parotid gland after stimulation in a group of 108 patients before treatment with radiotherapy for head-and-neck malignancies.

Methods

Patients

Data presented in this report are based on the evaluation of 108 patients (82 male and 26 female) to be treated with primary or postoperative radiotherapy for various malignancies in the head-and-neck region. These patients ranged in age from 24 to 83 years (mean 57 years). Information on tobacco and alcohol consumption was obtained on each participant. Almost two-third of the patients (70) in this study used tobacco with 35 patients (32%) using 20 or more cigarettes a day. The alcohol intake varied from zero to 20 units a day (mean 3.3 units). Fifty-one patients underwent surgery before the salivary flow measurements. None of the patients received previous radiotherapy or surgery of the parotid glands, or suffered from malignancies or other diseases of the parotid glands. Patients were excluded if they were taking any medication known to affect salivary gland function. A course of chemotherapy in the past was not allowed. The main locations of the tumors were as follows: larynx (45), floor of mouth/oral cavity (19), oropharynx (16), nose/nasal cavity (8) and nasopharynx (3). If patients had evidence of (p)N2c-(p)N3 disease (TNM staging system 1997) or distant metastatic disease they were not included in the study. A WHO-performance status of 0-1 was required for entrance into the study. Informed consent was obtained from each patient.

Saliva collection

Parotid saliva flow rates were measured before radiotherapy. Stimulated parotid saliva was collected simultaneously from both parotid glands with Lashley cups, which were placed over the orifice of Stenson's duct. Stimulation of saliva flow was achieved by applying three drops of a 5% acid solution to the mobile part of the tongue every 30 s and collection was carried out for 10 min. The volume of saliva was measured in tubes by weight assuming the specific density of parotid saliva to be 1 g/mL. The flow rate for each gland was expressed in milliliters per minute (mL/min). Most samples were collected between 12:00 and 17:00. No oral stimulus was permitted for 60 min before saliva collection. In more than 90% of the cases saliva was collected by one of the two authors to prevent high inter-observer variability.

Parotid volume

Parotid gland volume was measured using contrast enhanced CT imaging. The CT images of the irradiation area included whole major salivary glands in the radiation treatment position using 3-mm thick slices. Patients were immobilized using individually designed facial masks. The CT data were transferred to the computerized radiotherapy planning system (Plato External Beam Planning RTS 1.7, Nucletron B.V., Veenendaal, the Netherlands). The location of the parotid glands was outlined on the axial CT slices and parotid gland volume was calculated.

Statistical analysis

Data were analyzed with the SPSS program (version 9.0). Differences between left and right parotid flow rate within one patient resulted, for the whole group of patients, in a scatter around zero values. Therefore, to correlate parotid gland function with the clinical parameters gender, age, smoking habits, consumption of alcohol and tumor characteristics, the mean of left plus right parotid gland flow rate for each patient was used. Because flow rates were not normally distributed log transformation was used to improve normality. A linear regression analysis was performed and the correlation coefficients were determined. *T*-tests were used with a criterion of $p < 0.05$ for significance.

All figures and the table present untransformed data, indicative of the measured parotid flow rates.

Results

When the patient's anatomy of the cheek was considerably altered after operation, technical difficulties developed. This made the attachment of the cup difficult and the cup dislodged when the patient made the finest movement. Therefore, samples of 174 (in 93 patients) instead of 216 parotid glands could be taken. The patients had a mean parotid salivary secretion rate of 0.34 mL/min. A considerable variability in

parotid output was found with a range of 0.03–1.66 mL/min, S.D. 0.28 mL/min. There was a large variation in the size of the parotid glands, ranging from 12.9 cm³ to 46.6 cm³ (mean 25.5 cm³, S.D. 7.7 cm³). The secretory rate of the parotid gland was not related to the size of the gland. **Figure 1** illustrates the relationship between parotid flow rate and the volume of the parotid salivary gland.

The mean of left plus right parotid gland flow rate could be assessed in 81 patients. As shown in **Figure 2**, no difference in basal flow rates between genders was observed. Average values for males and females were nearly identical (0.33 and 0.36 mL/min, respectively). Across the age range studied, we observed no age-related pattern in the flow rates responses. No reduction of stimulated parotid flow rate with increased age was found (**Figure 3**). An evaluation of stimulated parotid flow rates, according to tobacco use, revealed no influence of smoking habits on the salivary flow. Also, there were no differences in flow rate related to the consumption of alcohol. Tumor characteristics, like the location of the tumor, T-status and N-status, and surgery preradiation were not correlated with the saliva output (**Table 1**).

Figure 1 The distribution of stimulated parotid flow rates, expressed as mL/10 min according to parotid gland volume. Each data point is the flow rate of an individual gland.

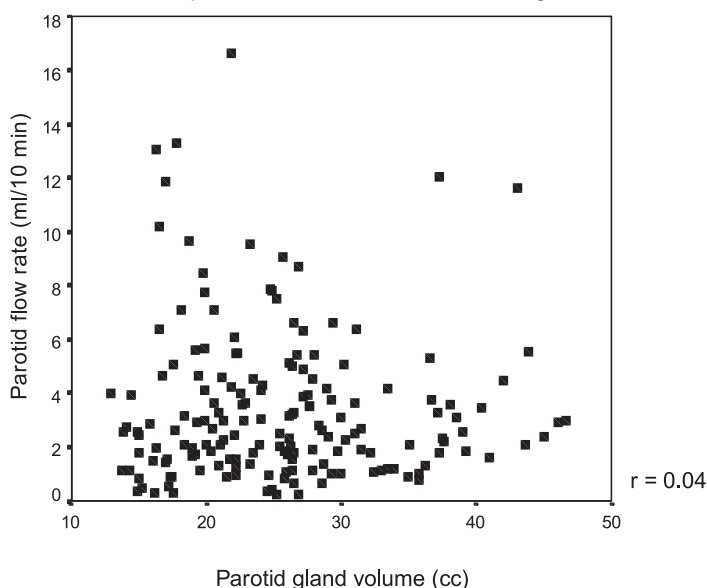


Figure 2 A boxplot showing the distribution of stimulated parotid flow rates, expressed as mL/10 min according to gender.

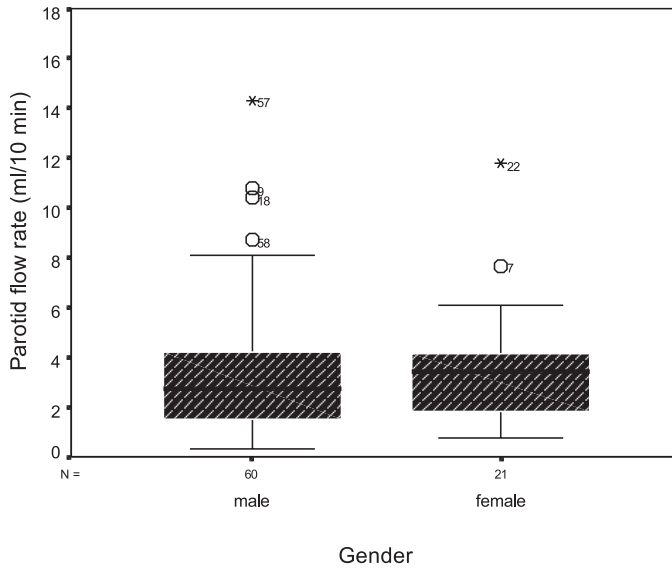


Figure 3 The distribution of stimulated parotid flow rates, expressed as mL/10 min according to age. Each data point is the mean stimulated flow rate of left plus right parotid gland of one patient.

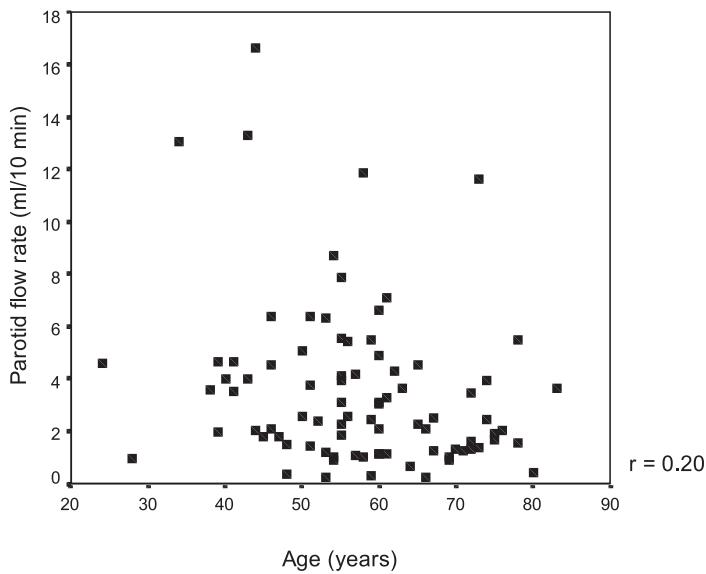


Table 1

	Parotid flow rate			p
	Mean	N	SD	
Patient characteristics				
<i>Tobacco use (number of cigarettes per day)</i>				
0	3.03	31	2.05	n.s.
<10	2.58	10	3.03	
10-20	2.90	15	1.66	
>20	4.66	25	3.44	
<i>Alcohol use (number of units per day)</i>				
0	3.57	16	2.16	n.s.
1	3.62	13	3.15	
2	4.78	13	3.34	
3	2.18	13	1.00	
4	2.59	9	2.74	
5	3.48	7	0.76	
≥ 6	4.06	8	4.32	
Tumor characteristics				
<i>Tumor location</i>				
Larynx	3.33	34	2.21	n.s.
Floor of mouth/oral cavity	5.26	10	4.50	
Oropharynx	3.58	11	3.78	
Nose/nasal cavity	2.66	7	1.35	
Nasopharynx	3.10	3	0.54	
Other	2.99	16	1.80	
<i>T-status</i>				
T1	4.62	12	3.41	n.s.
T2	3.46	31	2.90	
T3	3.30	8	1.71	
T4	4.02	8	4.03	
Tx	1.60	2	0.13	
Not applicable/recurrent	2.76	20	1.33	
<i>N-status</i>				
No	3.38	43	2.57	n.s.
N1	3.61	9	3.14	
N2	4.77	10	4.78	
Not applicable/recurrent	2.86	19	1.29	
<i>Surgery preradiotherapy</i>				
Local	3.25	14	2.13	n.s.
Loco-regional	4.76	21	4.06	
No surgery	2.92	46	1.80	

Discussion

Many studies have reported that normal salivary flow rates encompass a broad range of values. This was also a prominent finding in the present study. We observed a considerable variation in the range of the stimulated parotid salivary flow rates with a mean value and high standard deviation in general agreement with previous reports^{4,5,6}. This great amount of variability has also been demonstrated in healthy individuals^{7,8,9}, for which variations in stimulated output exceeded 40%. Maximum flow rate of parotid saliva was shown to be proportional to gland size by Dawes¹⁰. However, this conclusion was drawn from parotid gland volume measurements by a sialographic technique. The volume of each parotid gland was calculated from the mean of the areas in cm² of the two tracings of the radiographic outline of the gland. There is a considerable variability in size and position of the parotid gland between patients. So, it is important to exactly determine the position and thereby the volume of the gland¹¹. In our study, exact measurements of parotid gland volume were assessed using CT data. Parotid glands were outlined on 3-mm thick axial CT slices and were reviewed with a radiologist whose expertise is head-and-neck imaging. With a range of 3.0-8.1 cm of the total length of the parotid glands, at least 10 slices per patient were outlined. Using these precise measurements of CT images, a large individual difference in the volume of parotid glands was demonstrated. However, no correlation was found between the parotid output and the size of the parotid glands.

Overall, the results of this study demonstrate that, with respect to stimulated parotid flow rate, there are apparently no physiological or clinical parameters that can significantly change parotid gland function among patients with different head-and-neck malignancies. There is a suggestion from literature data that especially age may play a role in salivary secretions. Significant age-related decreases in the secretion rates of parotid saliva were observed^{2,12}. However, others have not confirmed this conclusion^{3,13-16}. We found that stimulated parotid output, in about 100 patients with a broad range of age, was independent of age. An aging population may have an increasing incidence of salivary gland dysfunction caused by local and systemic disease, immunologic disorders and also by the side effects of hundreds of medications^{1,17}. In this study, however, patients were not allowed to use any known medication to induce xerostomia or salivary hypofunction. Also, none of the patients suffered from malignancies or other diseases of the parotid gland. Yeh², showed that especially in the oldest age range (>75 years old) the greatest decline in stimulated parotid saliva occurred. In the non-medicated subgroup no significant age-related decline was found. However, values for both the whole population and the non-medicated subgroup were very similar, and the greatest decrease occurred in the oldest age group. Although these authors reported about a larger group of subjects at an epidemiological level, the distribution of the oldest age group was comparable with our data, with the smallest proportion of the

patients (8.3%) in the group of >75 years old. Gender was not a prognostic factor for stimulated parotid flow rate in agreement with the results of several other reported studies describing both patients and healthy subjects^{3,5,7,14,16,18}. Information about tobacco and alcohol consumption in relation to human parotid flow rate is hardly available. In agreement with earlier reports³, we found no differences existing between non-smokers and patients who smoked. Alcohol abuse did not correlate with stimulated parotid flow in our study. Regarding the consumption of alcohol, more than 70% reduction in the flow rate of stimulated parotid saliva in alcoholic subjects was reported¹⁹. However, one week of abstinence resulted in near normalization of the parotid output.

Literature about the effects of tumor characteristics on parotid flow rate is scarce and so far not conflicting with our results. For instance, a pre- and postsurgical evaluation of individuals who underwent full mouth tooth extractions revealed no differences in parotid flow rate²⁰.

Because of the location of the primary tumor and regional lymph nodes, the salivary glands of most head-and-neck patients are in the fields of radiation. When the parotid glands are within the radiation field, salivary dysfunction develops immediately in a radiation dose and volume dependent matter. When evaluating effects of radiation on parotid output it is important to know if clinical variables could further explain the variation in saliva flow rate. In our study, for the stimulated parotid flow rate, no physiological or clinical variables including sex, age, smoking habits and alcohol use, parotid volume, tumor location, T- and N-stage, or preradiotherapy surgery were correlated with the saliva output.

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CHAPTER

4 Quantitative dose-volume response analysis of changes in parotid gland function after radiotherapy in the head-and-neck region

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Jan J. Battermann

Abstract

Purpose

To study the radiation tolerance of the parotid glands as a function of dose and volume irradiated.

Methods

One hundred-eight patients treated with primary or postoperative radiotherapy for various malignancies in the head-and-neck region were prospectively evaluated. Stimulated parotid flow rate was measured before radiotherapy and 6 weeks, 6 months, and 1 year after radiotherapy. Parotid gland dose-volume histograms were derived from CT-based treatment planning. The normal tissue complication probability model proposed by Lyman was fit to the data. A complication was defined as stimulated parotid flow rate < 25% of the preradiotherapy flow rate.

Results

The mean stimulated preradiotherapy flow rate of 174 parotid glands was 0.34 mL/min. The mean flow rate reduced to 0.12 mL/min 6 weeks postradiotherapy, but recovered to a mean flow rate of 0.20 mL/min at 1 year after radiotherapy. Reduction in postradiotherapy flow rate correlated significantly with mean parotid dose. No threshold dose was found. Increasing the irradiated volume of parotid glands from 0%-40% to 90%-100% in patients with a mean parotid dose of 35-45 Gy resulted in a decrease in flow ratio from, respectively approximately 100% to less than 10% 6 weeks after radiation. The flow ratio of the 90%-100% group partially recovered to 15% at 6 months and to 30% at 1 year after radiotherapy. The normal tissue complication probability model parameter TD_{50} (the dose to the whole organ leading to a complication probability of 50%) was found to be 31, 35 and 39 Gy at 6 weeks, 6 months and 1 year postradiotherapy, respectively. The volume dependency parameter n was around 1, which means that the mean parotid dose correlates best with the observed complications. There was no steep dose-response curve ($m = 0.45$ at 1 year postradiotherapy).

Conclusions

This study on dose/volume/parotid gland function relationships revealed a linear correlation between postradiotherapy flow ratio and parotid gland dose and a strong volume dependency. No threshold dose was found. Recovery of parotid gland function was shown at 6 months and 1 year after radiotherapy. In radiation planning, attempts should be made to achieve a mean parotid gland dose at least below 39 Gy (leading to a complication probability of 50%).

Introduction

Exposing the parotid glands to radiation may result in reduced flow of saliva. Without adequate parotid gland function an individual may experience severe impairment of oral health. The relative contribution of the parotid to the whole saliva increases with the level of stimulation. Stimulated parotid flow is clinically important: the lubricating properties of the saliva help to soften food, aid in the formation and swallowing of a food bolus, facilitate speech, and take part in a mechanical cleansing effect. Moreover, saliva acts as a solvent and is thus essential for taste perception¹⁻⁶. Stimulation with citric acid (the most potent gustatory stimulus) provides information about the secretory capacities of the glands⁷.

There is general agreement that fully irradiated parotid glands receiving doses higher than 60 Gy produce permanent salivary damage without recovery of function. It has also been confirmed that salivary flow is reduced dramatically in patients whose parotid glands receive modest doses, such as 30-50 Gy⁸⁻¹². However, what dose reduction is sufficient to preserve substantial salivary flow has not been thoroughly studied. There has been widespread speculation that recovery can be expected when a portion of the parotid gland is not irradiated. However, patient data available on parotid gland function are difficult to compare, because the volume of irradiated salivary tissue is either not exactly defined, very variable, or unknown^{10,13-17}. Because the normal parotid gland may show a fourfold variation in volume among individuals¹⁸⁻²¹, it is important to determine exactly the position of the glands. Yet in most studies, the relative degree of inclusion or exclusion of salivary glands in the radiotherapy field was determined by consulting simulation films. In more recent studies using CT-based treatment planning to reduce the dose of the parotid glands, the tolerance to irradiation has not been well defined^{19,22-26}. Moreover, in most of these studies, only a small group of patients has been studied. To our knowledge, only one recent study determined dose-volume response relationships using CT data²³.

We prospectively investigated the functional alterations in the parotid glands in a large group of patients treated with radiotherapy for head-and-neck cancers, to establish more precisely the radiation tolerance of the glands as a function of the total dose and volume irradiated.

Methods

Patients

One hundred-eight patients treated with primary or postoperative radiotherapy for various malignancies in the head-and-neck region were included. In 93 patients, it was possible to collect samples of stimulated parotid saliva before the start of radiation treatment. Characteristics of the patient population are shown in **Table 1**.

None of the patients received previous radiotherapy or surgery of the parotid glands or suffered from malignancies or other diseases of the parotid glands. No use of any medication known to affect salivary gland function was allowed. All patients treated with radiation therapy with induction or concomitant chemotherapy were excluded, because this may influence parotid function²⁷. If patients had evidence of (p)N_{2c}-(p)N₃ (TNM staging system 1997) or distant metastatic disease, they were not included in the study. A World Health Organization performance status 0-1 was required for entrance into the study. Informed consent was obtained from each patient.

Table 1 Patient and tumor characteristics (n = 108)

<i>Mean age (range)</i>		57	(24-83) yr			
<i>Gender</i>						
	Female	26	(24%)			
	Male	82	(76%)			
<i>Tumor site</i>						
	Larynx	45	(42%)			
	Floor of mouth/oral cavity	19	(17%)			
	Oropharynx	16	(15%)			
	Nose (nasal cavity)	8	(7%)			
	Nasopharynx	3	(3%)			
	Hypopharynx	2	(2%)			
	Unknown primary	1	(1%)			
	Other	14	(13%)			
<i>Surgery preradiotherapy</i>						
	Local	18	(17%)			
	Local + regional	33	(30%)			
	No	57	(53%)			
<i>Stage (TNM staging system)</i>						
	T1	14	(17%)	No	59	(69%)
	T2	42	(49%)	N1	11	(13%)
	T3	15	(18%)	N2a	2	(2%)
	T4	12	(14%)	N2b	13	(15%)
	Tx	2	(2%)	N2c	1	(1%)
	Not applicable/recurrent	23			22	

Saliva collection

Saliva flow rates were measured before radiotherapy and 6 weeks, 6 months, and 1 year after radiotherapy. Stimulated parotid saliva was collected from both parotid glands simultaneously with Lashley cups, which were placed over the orifice of Stenson's duct. Stimulation of saliva flow was achieved by applying three drops of a 5% acid solution to the mobile part of the tongue every 30 s, and collection was carried out for 10 min. The volume of saliva was measured in tubes by weight, assuming the specific density of parotid saliva to be 1 g/mL. The flow rate for each gland was expressed in milliliters per minute (mL/min). Most samples were collected between noon and 5 pm. No oral stimulus was permitted for 60 min before saliva collection. To prevent high interobserver variability in more than 90% of cases saliva was collected by only two of the authors.

Radiation treatment planning

Patients were treated predominantly with 6-MV X-rays from a linear accelerator using isocentric techniques. The irradiation delivered varied with the diagnosis, according to generally accepted treatment strategies. There were no limitations concerning the field arrangements or dose schedules. In the majority of patients, opposing lateral neck fields were used to cover the target volume. The spinal cord was shielded at 40-46 Gy, and electron beams were used to boost the posterior neck region. The supraclavicular regions were treated with an anterior field using independent collimators with half-beam blocking^{28,29}.

Before the start of treatment, patients were immobilized in the supine position using individually designed facial masks for reproducible positioning. Lateral and anterior setup marks were placed on the patient's mask to define the isocenter. Contrast-enhanced CT imaging of the irradiation area, including whole major salivary glands, was performed in the treatment position with the immobilization device using 3-mm-thick slices. Accurate alignment of position was obtained using midline and lateral lasers. The marks were made visible on the CT images using radiopaque markers. The CT data were transferred to the planning system (Plato External Beam Planning RTS 1.7, Nucletron B.V., Veenendaal, the Netherlands) by optical disc.

The locations of both parotid and submandibular glands were outlined on the axial CT slices. Treatment plans were individually designed for each patient. When treatment fields were designed using radiographs, reconstruction of these fields on the CT slices took place using the patient's setup marks. Dose distributions were calculated using a pencil beam convolution algorithm. The information from the calculated dose distribution was condensed into dose-volume histograms (DVHs). Separate DVHs were generated for right and left parotid glands. Portal films achieved verification of the treatment plan.

Daily fractions of 2 Gy were given 5 days per week. Fourteen patients received al-

tered fractionations because of their participation in an institutional protocol^{30,31}. Prescribed target doses were 46-50 Gy for the clinically negative, undissected neck that was at risk for microscopic metastatic disease; 50-70 Gy for postoperative tumor beds or dissected neck sites, depending on the results of the pathologic review of the operation specimen; and 66-70 Gy for tumors treated primarily with radiation. Six patients with Hodgkin's disease or non-Hodgkin's lymphoma received a total dose of 40 Gy. A continuous course of radiotherapy was delivered in all patients. Only one patient interrupted radiation for 14 days in the fourth week of treatment, because of a stomach perforation.

Normal tissue complication probability model

The data were fit to the normal tissue complication probability (NTCP) model proposed by Lyman³². With this model, the effects of both radiation dose and volume of the gland irradiated on the probability of radiation-induced changes in parotid gland function were quantitatively established. The model is a sigmoid dose-response relationship described by the following cumulative normal distribution:

$$NTCP = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^t \exp(-t^2 / 2) dt \quad (1)$$

where

$$t = \frac{D - TD_{50}(v)}{m * TD_{50}(v)} \quad (2)$$

$$v = V / V_{ref} \quad (3)$$

V_{ref} is the total volume of the parotid gland, and $TD_{50}(v)$ is the dose resulting in 50% probability of a complication for uniform irradiation of the partial volume v . The parameter m describes the slope of the dose-response curve. Furthermore, the model assumes a power law relationship for tolerance dose and irradiated volume, as follows:

$$TD(1) = TD(v) * v^n \quad (4)$$

The parameter n accounts for the volume effect of an organ. It is close to 1 if partial sparing of the organ reduces the complication probability (as in liver, lungs) and is close to 0 if partial irradiation induces dysfunction (as in esophagus, spinal cord). Equation 2 in this model requires input of a single parotid gland dose. The multistep DVH has to be reduced to a single number (e.g., an effective volume at a fixed reference dose). Therefore, the DVH representing the nonuniform irradiation of a parotid gland was reduced to a DVH representing uniform irradiation of an effective volume V_{eff} to the maximum dose D_{max} in the original histogram³³. Each step of the histogram ΔV_i at dose D_i is assumed to follow a power law relationship (Compare with Eq. 4):

$$V_{eff} = \sum_i \Delta V_i \left[\frac{D_i}{D_{max}} \right]^{\frac{1}{n}} \quad (5)$$

The transformed histogram is assumed to have the same complication probability as the original histogram. The values V_{eff} and D_{max} are substituted in V (Eq. 3) and D (Eq. 2).

Inputs to the model are the transformed DVHs and the salivary flow ratios. We used a maximum likelihood estimator to find the best fit of the model to the data, and thus the estimates for the parameters m , TD_{50} and n ^{34,35} are as follows:

$$M(m, TD_{50}, n) = \sum_p Ln [NTCP_p(m, TD_{50}, n)] + \sum_q Ln [1 - NTCP_q(m, TD_{50}, n)] \quad (6)$$

where the sums over p and q run over glands for which complications occurred and did not occur, respectively. We regarded a posttreatment parotid flow ratio $< 25\%$ as a complication, according to our data (not shown) and the RTOG/EORTC Late Effects Consensus Conference³⁶. The function M is maximal if the calculated NTCPs for glands with flow ratio $< 25\%$ are large (close to 1) and if the calculated NTCPs for glands with flow ratio $> 25\%$ are small (close to 0). The model parameters TD_{50} , m and n were iteratively adjusted until the maximum of the log likelihood function M was found. For these parameter values, the model has the greatest probability of describing the pattern of complications correctly. For a detailed description of maximum likelihood estimation applied in the field of clinical complication data, see Jackson *et al.*³⁵. Confidence limits were calculated using the profile likelihood technique, which is the same maximization method, but now with one parameter fixed³⁷. The differences in log likelihood M_k with k fitted parameters and log likelihood M_{k-1} with $k-1$ fitted parameters is asymptotically distributed as $\chi^2/2$ with 1

degree of freedom. Thus 95% profile likelihood confidence limits of a parameter are determined by varying that parameter around the optimal value and repeating the maximization process until the minimum and maximum parameter values are found for which:

$$M = M_{\max} - \frac{1}{2} \chi_1^2(0.05) = M_{\max} - 1.92 \quad (7)$$

where M_{\max} is the maximum value of the log likelihood function when all parameters are fitted. The profile likelihood technique to calculate the confidence limits is described in more detail elsewhere³⁷.

Results

Initial flow rate

Technical difficulties developed when the patient's anatomy of the cheek was considerably altered after operation. This made the attachment of the cup difficult, and the cup dislodged when the patient made the finest movement. Therefore, preradiotherapy samples could be taken from 174 parotid glands, instead of 216. The patients had a mean parotid salivary secretion rate of 0.34 mL/min (range 0.03 – 1.66 mL/min, SD 0.28 mL/min) before therapy. No difference in basal flow rates was observed between genders. Age and preradiation surgery were not significantly correlated with the saliva output. The variation among individuals in the size of the parotid glands was large; parotid glands ranged from 12.9 cm³ to 46.6 cm³ (mean 25.5 cm³). The secretory rate of the parotid gland before radiation was not related to the size of the gland.

Effects of irradiation on flow rate

The mean stimulated preradiotherapy flow rate of 174 parotid glands in 93 patients was 0.34 ± 0.28 mL/min (mean ± SD). The mean stimulated salivary flow rate reduced to 0.12 ± 0.16 mL/min at 6 weeks postradiotherapy and recovered to 0.20 ± 0.22 mL/min at 1 year postradiotherapy (**Table 2**).

Flow reduction depended on parotid gland dose. A significant correlation between the ratios of the 6 weeks postradiotherapy and preradiotherapy flow rates and the mean dose D_{mean} was observed (**Figure 1**). Similar results were obtained 6 months and 1 year after radiotherapy. **Table 2** gives the linear regressions of flow ratio f in percentage and the parotid gland dose D_{mean} in Gy; the correlation coefficient r and the number of glands and patients are also evaluated. During the evaluation period, glands were lost because of (progressive) disease and inability to tolerate citric acid, and because patients were lost to follow-up. These losses to follow-up

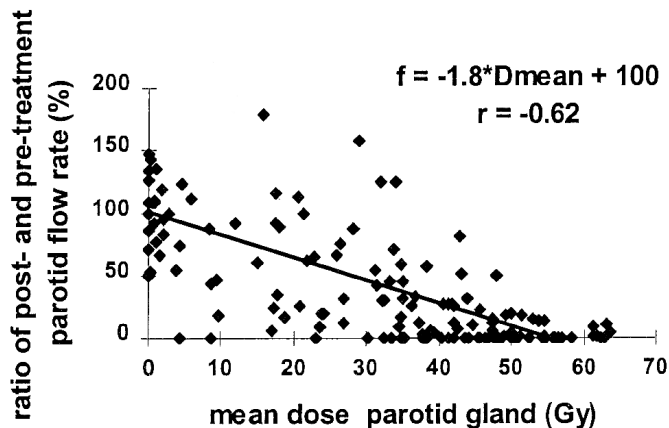
did not influence the results, since the average flow values and the linear regression curves did not differ for the subset of 95 glands with saliva flow measurements before radiotherapy and 6 weeks, 6 months, and 1 year after radiotherapy. The same holds for the parameters of the NTCP model (see below).

Table 2 The stimulated parotid flow rates (mean value \pm 1 standard deviation of the mean) and linear regression and correlation coefficients of postradiotherapy flow ratio f (%) at different timings after radiotherapy and parotid gland dose D_{mean} (Gy).

Timing	Parotid flow (mL/min)	Regression	r	No. of patients	No. of glands
Pre-RT	0.34 ± 0.28				
6 weeks post-RT	0.12 ± 0.16	$f = -1.8 \cdot D_{\text{mean}} + 100$	-0.62	86	154
6 months post-RT	0.17 ± 0.21	$f = -2.8 \cdot D_{\text{mean}} + 157$	-0.50	72	127
1 year post-RT	0.20 ± 0.22	$f = -2.7 \cdot D_{\text{mean}} + 155$	-0.55	66	123

The last columns show the numbers of patients and glands that were evaluated for the regression analysis.

Figure 1 Stimulated parotid flow rate as a function of mean parotid gland dose 6 weeks after radiation. The flow rates are expressed as percentage of the preradiotherapy flow rates for each parotid gland.



The volume effect

The radiation treatment portals for the different primary tumor sites included a wide range of parotid gland volumes irradiated to a mean dose of approximately 40 Gy, due to the prescribed dose for the majority of primary fields of 46 to 50 Gy (**Figure 2**). For a large number of parotid glands, coverage by the treatment portals was more than 90%, and mean doses ranged from 35 to 65 Gy, the latter being the dose resulting from primary and boost fields (**Figure 2**). To study dose and volume effects, we identified seven groups of patients and calculated the mean flow ratios at 6 weeks, 6 months, and 1 year postradiotherapy. Postradiotherapy saliva flow decreased with increasing fraction of the parotid gland irradiated to a dose between 35 and 45 Gy (**Figure 3**). The saliva flow decreases further if more than 90 % of the parotid gland is irradiated to doses larger than 45 Gy. **Figure 3** also shows recovery of parotid gland function 6 months and 1 year postradiotherapy.

Figure 2 Distribution of partial volumes of parotid glands irradiated to a mean dose.

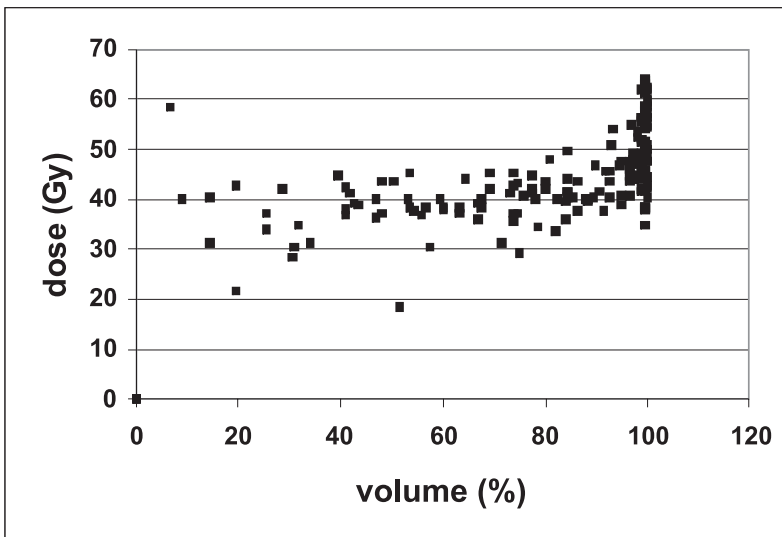
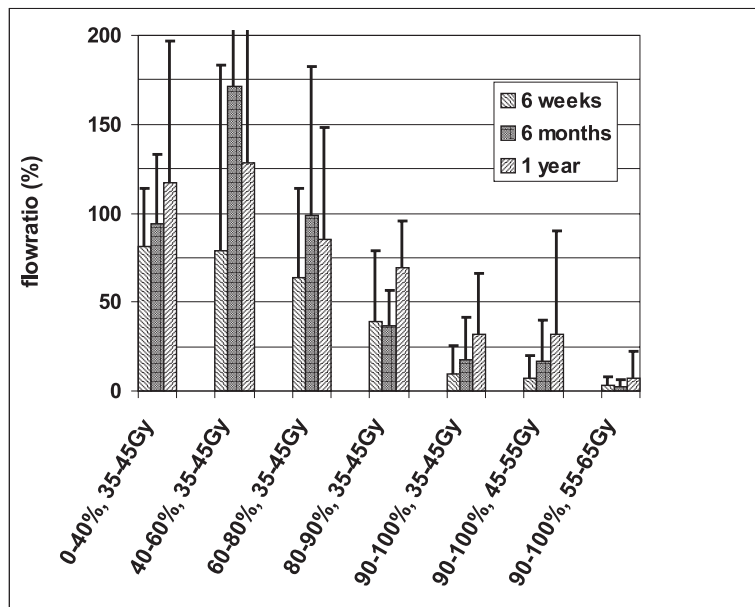


Figure 3 The flow ratios at 6 weeks, 6 months, and 1 year postradiotherapy as a function of irradiated parotid gland volume and dose. The flow rates are expressed as percentage of the preradiotherapy flow rates for each parotid gland.



Normal tissue complication model

Fitting of the probability model to the data using the maximum likelihood estimator resulted in n values equal to or larger than 1, indicating a large volume effect. It was decided to fix the value of n at 1 or, in other words, to represent the effective parotid gland dose by the mean dose. Consequently, the maximum likelihood function M was maximized by adjusting the parameters TD_{50} and m . The function M for the data at 6 weeks postradiotherapy is shown in **Figure 4** and has a maximum for $TD_{50} = 31$ Gy and $m = 0.54$. **Table 3** gives the estimates for the parameters and the 95% confidence intervals for the data at 6 weeks, 6 months, and 1 year postradiotherapy. The corresponding NTCP curves at 6 weeks, 6 months, and 1 year postradiotherapy are depicted in **Figure 5**, which also shows a tendency toward parotid gland function recovery in time. Once the parameters n , m and TD_{50} have been determined, iso-NTCP curves can be calculated as a function of the volumes irradiated uniformly by a reference dose (**Figure 6**). These curves are helpful in determining the tolerance dose to the parotid gland if it is partially irradiated.

The number of complications expected from the NTCP model was compared to the observed number of complications in glands for the various dose groups (**Table 4**).

The chi-squared value, calculated as the sum of the squares of differences between the observed and expected number of complications divided by the expected number of complications, amounted to 1.70. For 6 degrees of freedom and a 5% significance level the chi-squared statistic is 12.59, so the hypothesis that the complication model is an acceptable fit of the complication data cannot be rejected. Similar results were obtained for the data 6 months and 1 year postradiotherapy.

Figure 4 The function M (log likelihood) for the data 6 weeks postradiotherapy as a function of the parameters m and TD_{50} . The parameter n was set to 1, which means that an inhomogeneous parotid gland dose distribution was described by the mean dose. M is maximal for $TD_{50} = 31$ Gy and $m = 0.54$ (See also Table 4), which means that using the Lyman NTCP model, these parameter values give the best prediction of complication probability 6 weeks postradiotherapy.

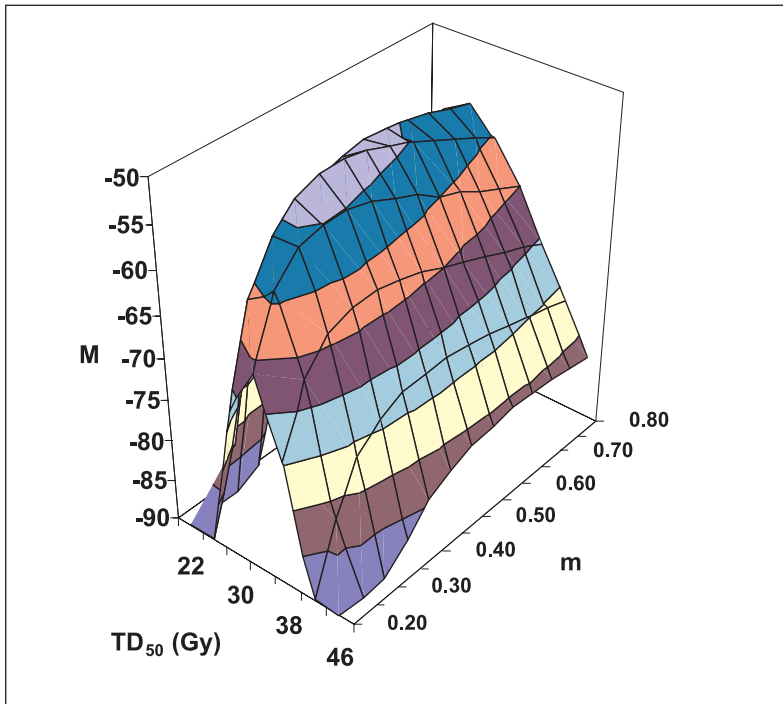


Table 3 The parameters TD_{50} and m and 95% confidence intervals estimated from the dose distribution and parotid flow data at 6 weeks, 6 months, and 1 year postradiotherapy.

Timing post-RT	TD_{50} (Gy)	95% CI	m	95% CI
6 weeks	31	26-35	0.54	0.40-0.78
6 months	35	30-40	0.46	0.34-0.66
1 year	39	34-44	0.45	0.33-0.65

Figure 5 Complication probability curves as a function of the mean parotid gland dose. The symbols indicate the NTCP at 6 weeks (diamonds), 6 months (triangles), and 1 year (circles) after radiotherapy. Complication is defined as stimulated parotid flow rate <25 % of the preradiotherapy rate.

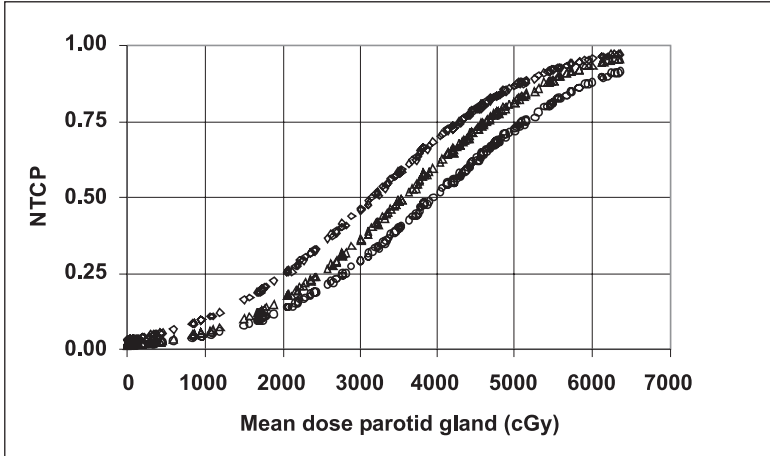


Figure 6 Iso-complication curves as a function of partial parotid gland volume V_{eff} irradiated to a reference dose D_{ref} . End point is <25 % flow at 1 year postradiotherapy with $TD_{50} = 39$ Gy, $n = 1$, and $m = 0.45$.

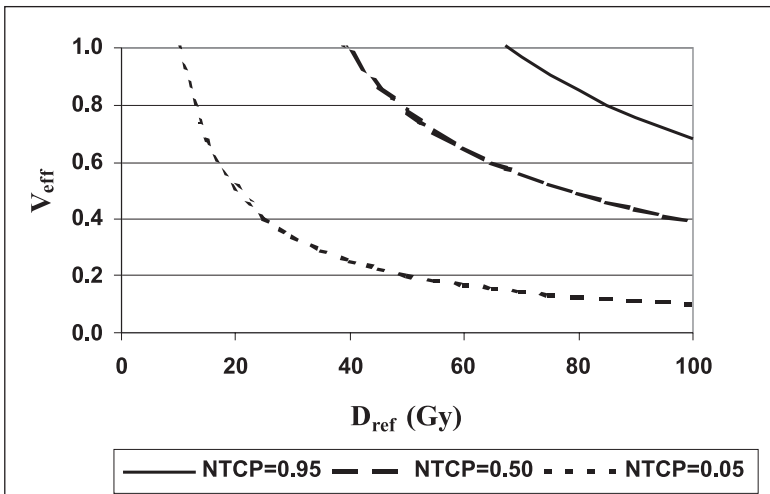


Table 4 Observed and expected numbers of complications in glands within a certain mean dose range.

Mean dose range (Gy)	No. of glands	No. of complications	
		Observed	Expected
0-10	34	3	2
10-20	11	3	2
20-30	17	5	6
30-40	31	16	18
40-50	36	28	29
50-60	20	19	19
60-70	7	7	7

Discussion

Radiation fields for tumors in the head-and-neck region frequently include the parotid glands. Exposing the salivary glands to radiation can result in reduction of salivary flow and change in saliva composition, leading to a number of clinical sequelae including xerostomia, difficulties in mastication and speech, changes of taste, increased risk of caries and oral infections, and altered nutrition^{1,4,38}. These severe side effects may have a negative effect on the quality of life of patients³⁹.

To evaluate the parotid function after radiation, subjective and objective parameters are described. Subjective parameters include assessment by questionnaires, visual analogue scales and patients' diaries. Objective parameters include unstimulated and/or stimulated salivary gland flow rates⁷. The value of collecting stimulated saliva is that it provides immediate quantitative information about the secretory capacities of the glands. Also, the effects of irradiation on the different constituents of saliva can be followed. Stimulated parotid saliva can be measured fairly easily using Lashley cups. However, we experienced that, especially in patients treated with surgery before radiotherapy, anatomic changes of the cheek made it difficult to place the Lashley cups and collect parotid saliva. Considerable variation is described in the normal range of stimulated parotid salivary flow rates. We observed a wide range of the secretory parotid rate before radiotherapy, with a mean value and high standard deviation in general agreement with previous reports^{8-11,25,26,40,41}. Because the range of normality is so broad, it was difficult to determine whether a particular patient has an abnormally low flow rate. Therefore, to control for differences in salivary flow rates among different individuals, each subject's parotid flow rates were converted to percentages of the individual baseline flow rates.

Previously, we showed that there is considerable variability in size and position of the parotid gland among patients²⁰. It was concluded that it might be inaccurate to assess the irradiated parotid gland volume from simulator films, as done in several

earlier studies^{10,11,40}. In this study, exact information about the degree of inclusion of the parotid glands in the radiotherapy fields was ensured using CT images. Because the data represent a large group of patients with different tumor locations, radiotherapy was performed with different field arrangements, especially with different cranial field borders. The amount of parotid gland tissue included in the radiotherapy field, and the radiation doses delivered to the individual parotids, varied considerably. In this way, accurate information could be obtained to assess dose/volume/effect relationships with a broad range in dose and volume of irradiated parotid glands. Using the planning CT scans, DVHs were deduced from the dose distribution in each individual gland. To judge the radiation damage for a given treatment, DVHs provide useful information. However, DVHs are not always easy to correlate with toxicity parameters like decreased parotid salivary flow rate, and therefore several mathematical models exist that derive NTCPs from DVHs. The most widely used model is the NTCP model developed by Kutcher and Lyman^{32,33}. This empiric model, which may be applied to any organ, including the parotid gland, was used in this study. We found this complication model an acceptable fit for our parotid flow data. For the flow rates measured at 6 weeks, 6 months, and 1 year after radiation, the number of expected complications was in agreement with the observed number of complications. Complication was defined as stimulated parotid flow rate <25% of the preradiotherapy flow rate according to the RTOG/EORTC Late Effects Consensus Conference.

In the current study, volume effects were clearly shown (see **Figure 3**). Parotid salivary flow after radiation decreased with increasing fraction of the parotid gland irradiated. The volume of tissue irradiated is an important factor determining the clinical tolerance of an organ⁴². Volume effects differ among various organs, depending on their structural organization, as well as on the migration characteristics of the target cells. The high sensitivity of, for example, parotid, kidney, or lung tissue may largely be related to the organ's high degree of compartmentalization into functional subunits: the acini, nephrons, and alveoli. Once all stem cells in such a subunit are sterilized, it is unlikely that repopulation from neighboring subunits will occur. In such a parallel organization of functional subunits, a large number can be inactivated without any consequence for the overall organ function. In the NTCP model, we found a high value for the volume parameter ($n = 1$), assuming a large volume effect related to parallel architecture of the parotid glands. In earlier studies, Lyman's model has been applied to a compilation of clinical tolerance data developed by Burman *et al.*⁴³. To determine the volume dependence parameter, n , a best clinical estimate was made using xerostomia as endpoint for the parotid gland. This led to a somewhat lower volume dependency of $n = 0.7$. The study of Eisbruch *et al.*¹² is the only other study that has tested the parameters of the Lyman model with accurate dose/volume/parotid gland function relationships. In that study, the same large volume dependency of $n = 1$ was described.

In accordance with clinical observations, our flow measurements indicated partial recovery of parotid gland function over time. This was also concluded from the TD_{50} values obtained from fitting the Lyman model. At 6 weeks, 6 months, and 1 year postradiotherapy, the TD_{50} values were 31, 35 and 39 Gy, respectively. Only a small number of authors prospectively investigated parotid gland function. In a paper by Franzén *et al.*¹¹, doses to parotid glands exceeding 64 Gy caused irreversible depression of function in the vast majority of parotid glands. A recovery of parotid flow rate was seen in several patients in this study, with parotid glands receiving doses less than 52 Gy. In a group of patients receiving 40-45 Gy, the secretion rates at 6 and 12 months after radiation were 42 % and 54 %, respectively, of the initial rate. In a group treated with 47-52 Gy, a mean salivary secretion rate at 6 and 12 months after the end of radiotherapy was calculated to be 21 % and 25 % of the initial values. However, no exact information about the volume of the parotid gland irradiated was given. No CT data were available in their study. Most studies describe dose/volume/effect relationships in general terms, because they stem from dose and volume estimations from simulator films. Data based on dose-volume histogram analysis using CT data are scarce. Our study is best comparable to the study by Eisbruch *et al.*¹², because both are based on CT data, dose-volume histogram analysis, and saliva flow measurements. Furthermore, in both studies the Lyman model was used as NTCP model, and the parameters of the model were found by the maximum likelihood estimation. Preradiotherapy and 1 year postradiotherapy saliva flow data were in agreement: 0.34 and 0.20 mL/min vs. 0.365 and 0.20 mL/min found by Eisbruch *et al.*¹² However, maximum likelihood estimation of the NTCP model resulted in different parameter values. For the same endpoint (less than 25% flow of baseline value at 1 year postradiotherapy), we found $TD_{50} = 39$ Gy and $m = 0.45$ versus $TD_{50} = 28.4$ Gy and $m = 0.18$ found by Eisbruch *et al.*¹² The dose-response curve obtained from our data was less steep, and the TD_{50} value was 10 Gy higher. These discrepancies may stem from differences in the underlying data; e.g., Eisbruch *et al.*¹² have few data in the range 30-40 Gy mean parotid gland dose, which is near our TD_{50} value. Furthermore, discrepancies may arise from uncertainties in the saliva flow measurements. We observed that the maximum likelihood estimation method is sensitive to uncertainties in the flow measurements; this is particularly relevant for the parameter m (the steepness of the dose complication curve for a fixed partial volume). Some authors found a significant saliva flow reduction induced by a relatively small parotid gland dose of 10 to 15 Gy^{9,24,40}. In another study comparing saliva flow of treated and spared glands, a dose of 20 Gy led to a 50% reduction in saliva flow^{23,25}. These studies also demonstrate that flow reduction occurs at low dose levels, which is contradictory to the threshold model proposed by Eisbruch *et al.*¹² Other investigators using CT data in their studies are encouraged to make a goal of drawing definite conclusions about dose-response curves for the parotid gland.

Figures 5 and 6 describe the complication probability curves after fitting the NTCP model to the clinical data. The iso-complication curves especially can be very useful in the clinic when parotid glands are partially irradiated. As shown in these curves, the different TD_{50} values were below the prescribed dose 46-50 Gy for subclinical disease. Because the parotid glands are located adjacent to the lymph nodes of Level II, they are often located partly inside the planning target volume. Because the consequences of the radiation-induced parotid gland injury are still difficult to manage, meticulous treatment planning and beam arrangement must be designed to spare as much of the salivary glands as possible. Dosimetric sparing of the contralateral parotid gland in patients requiring unilateral irradiation is feasible by using nonopposing conformal beams^{22,23}. In patients receiving bilateral neck irradiation, theoretical studies have reported improved dose distribution aiming at sparing the parotid function by applying intensity-modulation radiotherapy, generally in combination with inverse planning⁴⁴⁻⁴⁶. Precise definition of the areas of the neck treated electively, as well as position accuracy, is of crucial importance in these advanced treatment plans.

Another attempt to prevent decrease in parotid flow is the use of drugs to promote salivation. Administration of cholinergic sialogogue during radiation may have a beneficial effect on parotid gland function. Prophylactic pilocarpine treatment showed to attenuate radiation-induced loss of parotid gland function in an animal model⁴⁷. Small clinical trials demonstrated that concomitant use of pilocarpine during head-and-neck irradiation was associated with decreased posttreatment xerostomia⁴⁸⁻⁵⁰. The dose and the volume of the gland lying within the radiation portal seemed to be important parameters for the effectiveness of the drug. In our institute, the effect of prophylactic oral pilocarpine is currently being tested in a prospective Phase III clinical trial.

In conclusion, dose/volume/response relationships in the parotid glands were accurately described. A large volume dependency was found with no threshold effects for the mean parotid dose. Iso-complication curves calculated after fitting the NTCP model to the clinical data can assist the clinician in determining the tolerance dose of the parotid gland, especially if it is partially irradiated. We showed partial recovery of parotid gland function in time with TD_{50} values of 31, 35, and 39 Gy at 6 weeks, 6 months, and 1 year after radiation.

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CHAPTER

Scintigraphic assessment of early and late parotid gland function after radiotherapy for head-and-neck cancer: a prospective study of dose-volume response relationships.

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Abstract

Purpose

To investigate the value of scintigraphy as an indirect measurement of parotid function after radiotherapy (RT).

Methods

Ninety-six patients with primary or postoperative radiotherapy for various malignancies in the head-and-neck region were prospectively evaluated. Parotid gland scintigraphy was performed before RT and 6 weeks and 1 year after RT. The uptake, excretion fraction of the saliva from the parotid gland to the oral cavity (SEF), and the ratios of uptake and SEF after and before treatment were calculated. CT-based treatment planning was used to derive dose-volume histograms of the parotid glands. To establish the effects of both the radiation dose and the volume of the parotid gland irradiated, the normal tissue complication probability model proposed by Lyman was fit to the data.

Results

The mean maximal uptake of 192 parotid glands decreased significantly from 3329 counts (ct)-/s before RT to 3084 ct/s and 3005 ct/s at 6 weeks and 1 year after RT. The SEF before treatment was 44.7%. The SEF decreased to 18.7% at 6 weeks after RT, but recovered to a SEF of 32.4% at 1 year after RT. A significant correlation was found between the uptake 1 year after RT and the mean parotid dose. The reduction in post-RT SEF correlated significantly with the mean parotid gland dose. The normal tissue complication probability model parameter TD_{50} was found to be 29 and 43 Gy at 6 weeks and 1 year after RT, respectively, when a complication was defined as a posttreatment SEF parotid ratio of <45%.

Conclusions

The effects of radiation on parotid gland function using scintigraphy could well be established. A statistically significant correlation between the SEF ratio and the mean parotid dose was shown, with some recovery of function at 1 year after RT, comparable with the flow results. When direct flow measurements are not feasible, parotid scintigraphy appears to be a good indicator of gland function.

Introduction

After treatment of head-and-neck cancer, a significant deterioration of physical functioning and head-and-neck symptoms occurs¹. Irradiation (RT) of salivary glands during the treatment of head-and-neck cancer may lead to an alteration in the amount and quality of the saliva produced. Patients frequently report problems with teeth, dry mouth, sticky saliva, altered taste acuity, and dysfunction of speech, chewing, and swallowing.

The parotid glands consist entirely of serous acini producing a clear watery product virtually devoid of mucins. Under conditions of stimulation, these glands become the main contributors to salivary secretion. The loss of their watery product results in thick tenacious secretions.

As stated at the Late Effect Consensus Conference² the radiotolerance of the parotid glands, using gland function as the index of late injury has not well been defined. It has generally been assumed that >50% of the salivary glands must be excluded from the treatment portals to prevent subjective dryness-related complaints in most patients³. In general, no recovery of salivary flow rates was observed at cumulative doses >50 Gy^{4,5}. Only recently, data on the correlation between the actual volumes of the parotid gland irradiated, the dose applied to that gland, and the residual function of that gland became available in literature^{6,7}. In those studies, salivary gland function was assessed by flow measurements. Although major gland saliva collection of secretion from parotid gland orifices is the most widely used means of assessing secretory function, the variability in individual gland performance is great. Furthermore, we found that, especially in patients treated with surgery before RT, it was difficult to place the Lashley cups to collect parotid saliva owing to anatomic changes of the cheek⁶. Measurements of parotid dysfunction can also be performed with ^{99m}Tc-pertechnetate scintigraphy, which is a reproducible and minimally invasive test for quantitative evaluation^{8,9}. However, most studies on scintigraphy and radiation parameters used retrospective data or had with small group of patients¹⁰⁻¹³.

We prospectively investigated the functional alterations in the parotid glands in a large group of patients treated with RT for head-and-neck cancers using scintigraphy. Dose-volume response relationships were studied to provide insight into the severity of radiation injury to parotid gland tissue and the potential of the parotid gland to recover from this damage.

Methods

Patients

A total of 108 patients who had undergone primary or postoperative RT to the head-and-neck region for various malignancies were included. Owing to missed appointments, refusal (3 patients), and organizational problems, it was possible to

evaluate objectively parotid gland function using scintigraphy in 96 patients before the start of RT.

Patient characteristics are listed in **Table 1**. None of the patients had undergone previous RT or surgery of the parotid glands or had malignancies or other diseases of the parotid glands. No use of any medication known to affect salivary gland function was allowed. All patients treated with RT in combination with induction or concomitant chemotherapy were excluded, because this may influence parotid function¹⁴. If patients had evidence of stage N2c-N3 disease (TNM staging system 1997) or distant metastatic disease they were not included in the study. These patients were expected to have a great change of not surviving 1 year. A World Health Organization performance status of 0-1 was required for entrance into the study. Each patient provided informed consent.

Table 1 Patient and tumor characteristics (n = 96)

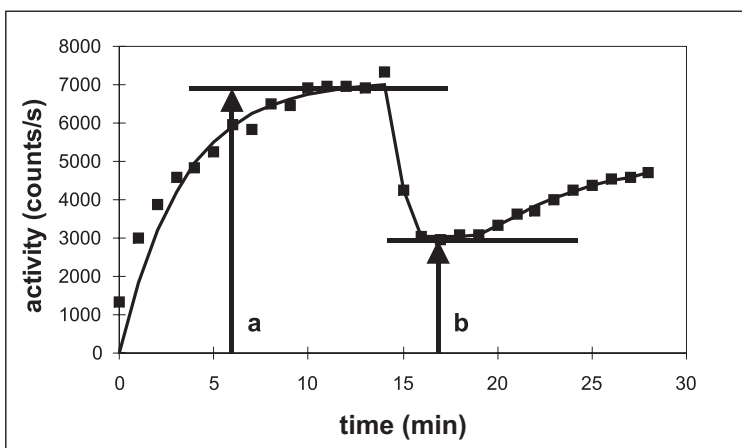
<i>Mean age (range)</i>	57 (28-83) yr
<i>Gender</i>	
Female	21 (22%)
Male	75 (78%)
<i>Tumor site</i>	
Larynx	40 (42%)
Floor of mouth/oral cavity	18 (19%)
Oropharynx	15 (16%)
Nose (nasal cavity)	6 (6%)
Nasopharynx	3 (3%)
Hypopharynx	2 (2%)
Unknown primary	1 (1%)
Other	11 (11%)
<i>Surgery before radiotherapy</i>	
Local	13 (14%)
Local + regional	31 (32%)
No	52 (54%)
<i>Stage (TNM staging system)</i>	
T1	14 (15%)
T2	39 (41%)
T3	13 (13%)
T4	10 (10%)
Tx	2 (2%)
Not applicable/recurrent	18 (19%)
No	53 (55%)
N1	11 (11%)
N2a	2 (2%)
N2b	13 (14%)
Not applicable/recurrent	17 (18%)

Salivary gland scintigraphy

Salivary gland scintigraphy studies were performed with the patient in the supine position under a γ -camera with high-resolution collimators. No oral stimulus was permitted for 60 minutes before imaging. After intravenous administration of 200 MBq ^{99m}Tc -pertechnetate, 60-s sequential frames (anterior view) were acquired and stored in the computer system. Fifteen minutes after injection, the patient was stimulated for salivary discharge by ingesting 5% citric acid. Five milliliters was administered orally onto the midline of the dorsum of the tongue via a syringe. The total study time was 30 min. For analysis of the data, regions of interest were drawn around the right and left parotid glands and the corresponding time-activity curves were created. Background correction was performed using the temporal region. The time-activity curves were fit to exponential functions.

The response to citric acid was evaluated on the basis of the variations in activity of the major salivary glands and subsequent accumulation in oral cavity. Salivary excretion factor (SEF) was quantified by determination of the maximal excretion activity per gland as a fraction of the maximal uptake (**Figure 1**). Time-activity curves made 6 weeks and 1 year after RT were compared with the values before treatment. Uptake and excretion response was analyzed per patient and subsequently per individual gland.

Figure 1 Typical example of pretreatment time-activity curve of salivary gland scintigram. Circles indicate parotid gland activity derived from the 60-s sequential frames. The line is fitted exponential curve. Salivary excretion fraction (SEF) is defined as $\text{SEF} = (a - b)/a$.



RT planning

The treatment technique has been extensively described⁶. In brief, all patients were treated with megavoltage equipment using isocentric techniques. No limitations concerning field arrangements or dose schedules were used. In most patients, opposing lateral neck fields were used to cover the target volume. The supraclavicular regions were treated with an anterior field using independent collimators with half-beam blocking.

Contrast-enhanced CT imaging of the patients with immobilization using individually designed masks was performed. The salivary glands were delineated on the CT scans of the patients. Three-dimensional dose distributions in these glands and their associated dose-volume histograms (DVHs) were calculated by the treatment planning system (Plato External Beam Planning RTS 1.7, Nucletron B.V., Veenendaal, the Netherlands).

Normal tissue complication probability model

The data were fit to the normal tissue complication probability (NTCP) model proposed by Lyman¹⁵. Thus, the effects of both the radiation dose and the volume of the gland irradiated on the probability of radiation-induced changes in parotid gland function was quantitatively assessed. In brief, this model assumes that the probability of complications after uniform irradiation of a specified volume of the organ follows a sigmoid dose-response relationship. Three parameters are used in this model: n , m and TD_{50} . The parameter n accounts for the volume effect of an organ. It is close to 1 for an organ in which a small fraction of the organ can be damaged without a significant effect on the function of the whole organ and it is close to 0 if partial irradiation induces dysfunction. The parameter m describes the slope of the dose-response curve. The $TD_{50}(v)$ is the dose resulting in 50% probability of a complication for uniform irradiation of the partial volume v .

The model requires input of a single parotid gland dose. The multistep DVH has to be reduced to a single-step DVH with an effective partial volume irradiated uniformly by a reference dose. The transformed DVH is assumed to have the same complication probability as the original DVH.

The inputs to the model were the transformed DVHs and the parotid gland function. A maximum likelihood method was used to fit the model to the complication data and find the best values and confidence intervals for the model parameters. The model and methods have been described in more detail elsewhere^{6,7,16-19}.

Results

Initial uptake and excretion

The mean maximal uptake of the parotid glands ($n = 192$) before RT was 3329 ± 1675 counts (ct)/s (range 914-10656 ct/s). The SEF before RT had a normal distribution,

with a mean of $44.7 \pm 14.2\%$. A considerable variability in parotid output was found, with a range of 9.9–78.3%. One patient had no excretion after stimulation in one (left) parotid. No explanation was found for the absence of a response to citric acid. This measurement was not used in the data analysis. No differences were found in uptake and SEF between left and right parotid glands. To correlate parotid gland function with the clinical parameters of gender, age, smoking habits, consumption of alcohol, and tumor characteristics, the mean of the left plus right parotid gland uptake and SEF for each patient was used. No difference in basal uptake or SEF between genders was observed. The basal scintigraphic measurements were also independent of age. Alcohol consumption, tobacco use, tumor characteristics (tumor location, T stage, and N stage), and surgery before RT did not correlate with gland uptake or excretion response. No relationship between the volume of the parotid gland and the uptake of tracer or the secretory rate could be established.

Effect of RT on uptake and excretion

The RT portals for the different tumor sites included a wide range of parotid gland volumes. Therefore, a broad range in the mean parotid dose (**Figure 2**) was available for analysis of the effects of radiation on parotid gland function using the scintigraphy parameters.

An example of uptake and excretion curves before and 6 weeks and 1 year after RT, obtained by salivary gland scintigraphy is shown in **Figure 3**. Six weeks after RT, 176 parotid glands (92%) could be evaluated. At the 6-week point, 10 patients could not be evaluated because of progressive disease or missed appointments or because they were lost to follow-up. At 1 year after RT, more glands were unavailable for evaluation, mainly because of progressive disease. Therefore, at 1 year, 132 glands (69%) could be evaluated. The mean uptake was reduced from 3329 ± 1675 ct/s before RT to 3084 ± 1205 ct/s and 3005 ± 1557 ct/s at 6 weeks and 1 year after RT, respectively. The mean SEF before RT of 192 glands in 96 patients was $44.7 \pm 14.2\%$. Six weeks after RT, the SEF had decreased to $18.7 \pm 20.1\%$ and had recovered to $32.4 \pm 20.3\%$ at 1 year after RT.

The SEF ratio was used to evaluate the percentage of SEF lost after RT (6 weeks and 1 year) with respect to the baseline SEF. Eighty-six patients (172 glands) underwent scintigraphies before and 6 weeks after RT. The ratios of 64 patients could be established at 1 year. The parotid glands showed a decrease shortly after RT compared with their baseline value (ratio, 45.8%, range, 0–512.3%; SD, 63.5%). Late after treatment (1 year), a recovery of excretion function was seen (ratio, 73.7%, range, 0–445.2; SD, 62.9%).

The reduction in excretion was dependent on the parotid gland dose. A significant correlation between the 6-week and 1-year post-RT ratios and the mean dose was observed (**Figure 4**). Similar results were obtained for the correlation between uptake 1 year after RT and the mean parotid dose. No statistically significant

Figure 2 Range in mean dose of irradiated parotid glands. Bars represent frequencies of different mean doses in left and right parotid glands quantitatively assessed using CT data.

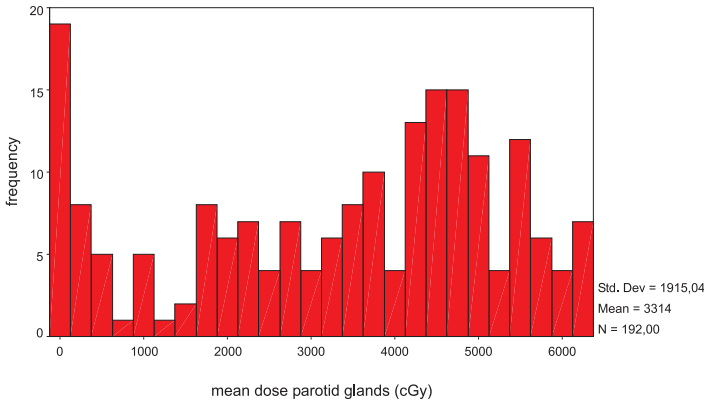
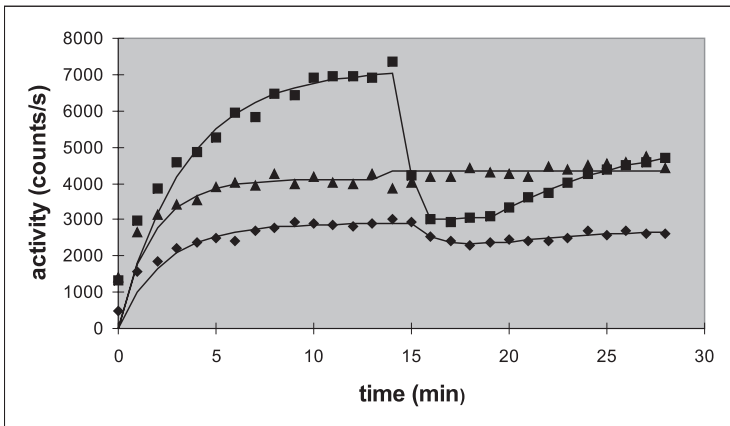


Figure 3 Typical example of uptake and excretion curves before (squares), 6 weeks (triangles) and 1 year (diamonds) after RT.



correlation was found between the mean parotid gland dose and uptake 6 weeks after RT.

Because the decrease in uptake may influence the excretion parameter SEF, a second excretion parameter was evaluated: the product of uptake and SEF (**Figure 5**). The correlation between radiation dose and the product of uptake and SEF was significant. The correlation coefficients were equivalent to the relation of dose and the parameter SEF.

Figure 4 Salivary excretion factor (SEF) as a function of mean parotid gland dose (A) 6 weeks ($R^2 = 0.3672$) and (B) 1 year ($R^2 = 0.1929$) after RT. SEF is expressed as percentage of pre-RT SEF for each parotid gland.

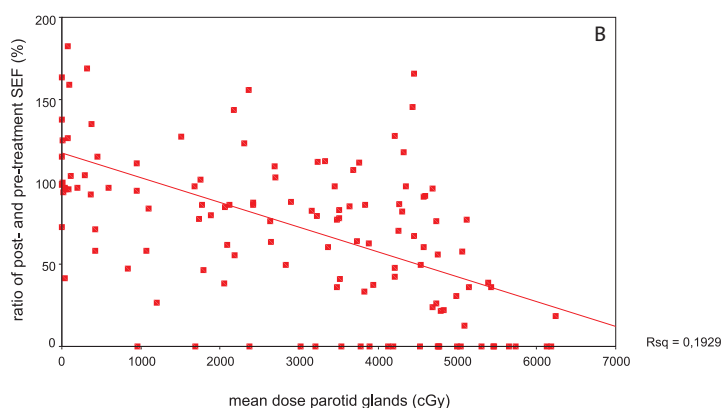
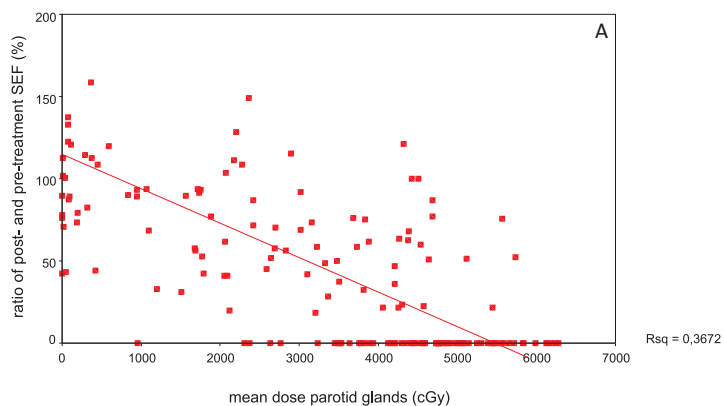
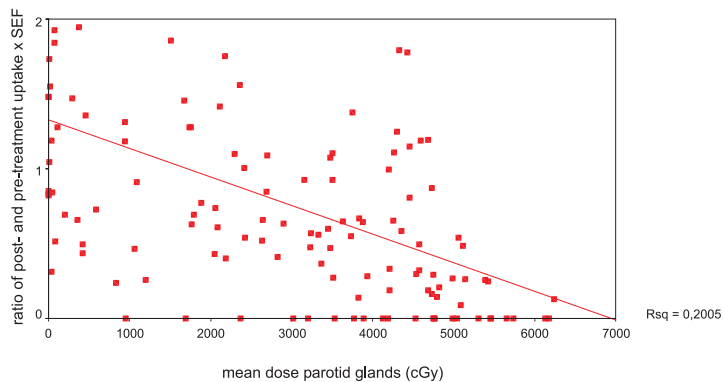


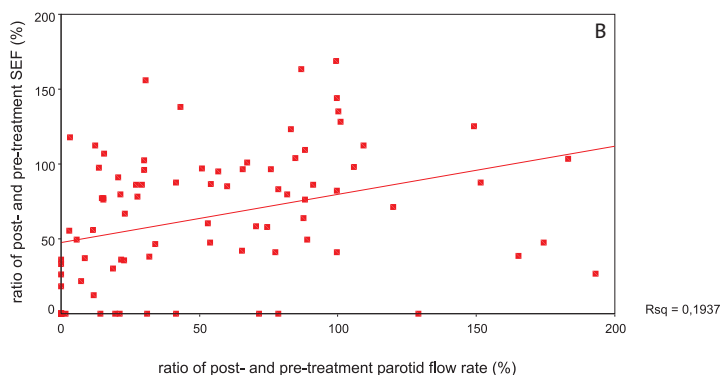
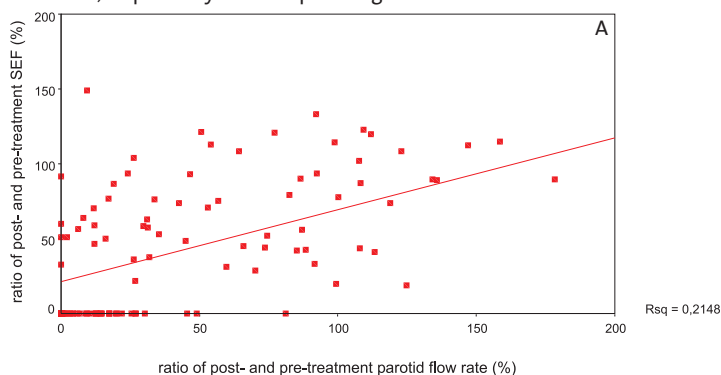
Figure 5 Product of uptake and salivary excretion factor (SEF) as a function of mean parotid dose 1 year after RT. Uptake x SEF expressed as percentage of pre-RT for each parotid gland ($R^2 = 0.2005$)



Normal tissue complication model

The salivary excretion fraction of the parotid glands correlated significantly with our direct measurements with Lashley cups⁶ (**Figure 6**). However, both the stimulated parotid flow rates and the SEF values showed a wide scatter in the data. Because scintigraphic data are still scarce and no data have been fit to NTCP models, no definition was available of a posttreatment SEF ratio that is regarded a complication. For the flow measurements, flow reduction to <25% of the pretreatment output is regarded as a complication (RTOG/EORTC Late Effects Consensus Conference 1995⁶). The NTCP model was fit to the scintigraphy data for different SEF ratio complication levels (**Table 2**). Fitting the probability model to the data using the maximum likelihood estimator resulted in n values ≥ 1 , indicating a large volume effect. Thereafter, it was decided to fix the value of n at 1 for subsequent analysis. The maximum likelihood function was maximized by adjusting the parameters

Figure 6 Correlation between stimulated parotid flow rates and salivary excretion factor (SEF) (A) 6 weeks ($R^2 = 0.2148$) and (B) 1 year ($R^2 = 0.1937$) after RT. Flow rates and SEF expressed as percentage of pre-RT flow rates and SEF, respectively for each parotid gland.



TD_{50} and m . **Table 2** gives the estimates for these parameters and the 95% confidence intervals for the data at 6 weeks and 1 year after RT. A complication defined as an SEF ratio of $<45\%$ gave comparable results in terms of TD_{50} with our flow data at 6 weeks and 1 year after treatment⁶. Recovery in terms of TD_{50} value was larger for the SEF ratio data than for the flow ratio data. Of 176 glands with scintigraphy data available 6 weeks after RT, 154 (88%) had SEF ratios of $<45\%$. One year after the treatment, 93 (70%) of 132 glands had stimulated output in terms of excretion fraction of $<45\%$. The corresponding NTCP curves for an SEF ratio of $<45\%$ at 6 weeks and 1 year after RT are shown in **Figure 7** and compared with the NTCP curves for a flow ratio of $<25\%$ at 6 weeks and 1 year after RT.

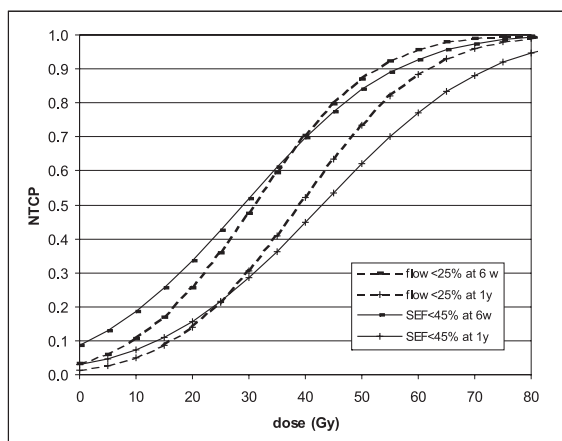
Table 2 TD_{50} and m and 95% confidence intervals estimated from dose distribution and parotid scintigraphy data at 6 weeks and 1 year after RT for different SEF ratio complication levels and comparison with flow ratio complication data.

End point	TD_{50} (95% CI) (Gy)		m (95% CI)	
	6 wk	1 y	6 wk	1 y
<i>SEF ratio (%)</i>				
<25	35(32-38)	52(47-63)	0.42(0.33-0.57)	0.42(0.32-0.63)
<35	33(30-37)	47(42-54)	0.48(0.40-0.67)	0.43(0.33-0.64)
<45	29(25-34)	43(37-51)	0.73(0.57-1.20)	0.53(0.42-0.75)
<55	25(22-29)	40(34-49)	0.77(0.60-1.3)	0.59(0.46-0.87)
Flow ratio* ($<25\%$)	31(26-35)	39(34-44)	0.54(0.40-0.78)	0.45(0.33-0.65)

* Data from Roesink et al.⁶

Abbreviations: TD_{50} = dose resulting in 50% probability of a complication; m = slope of dose-response curve; CI = confidence interval; SEF = salivary excretion factor

Figure 7 Complication probability curves as a function of the mean parotid gland dose. Solid curves from scintigraphy data, dashed curves from flow data (Roesink *et al.*⁶). Minus signs and plus signs indicate normal tissue complication probability (NTCP) curves at 6 weeks and 1 year after RT, respectively.



Discussion

During RT for head-and-neck tumors, acute normal tissue damage is observed in the oral mucosa, salivary glands, taste buds, and skin. Late changes can occur in all these tissues, as well as in muscles and bone². The consequences of irradiation-induced salivary gland injury are still very difficult to manage. The reduced salivary flow and altered salivary composition result in severe, clinically distressing complications^{2,20}. The high sensitivity of salivary and, especially, parotid gland tissue to radiation damage and the severity of the side effects make reduction of radiation injury to salivary gland tissue an important topic in head-and-neck oncology.

Among the various methods regarding dose-response studies, measurements of parotid flow rates with or without stimulation have been most widely performed. However, technical limitations in collecting saliva have been described⁶, and it has been shown that the flow rates have wide individual variations. Furthermore, under resting conditions or after treatment with high radiation doses, only small amounts or no saliva can be collected. Scintigraphic methods continue to play a role in the study of functional disorders²¹. They are able to detect minor impairment of glandular function. The parotid glands may be imaged and their function assessed using pertechnetate. The scan can supply additional features by following changes in the uptake and excretion function of the parotid glands. In this study, parotid gland function was assessed by scintigraphy with technetium pertechnetate immediately before RT and early (6 weeks) and late (1 year) after RT. Stimulation with citric acid was done to induce forced excretion of the parotid saliva, so that even a minor degree of functional damage could be detected. No technical difficulties were experienced. However, uptake and excretion parameters showed a broad range of normality before RT. Others have also reported that a wide range of SEF exists in patients before RT^{11-13,22}, with mean values and high standard deviations, comparable to our data. To control for differences in these scintigraphy parameters among different individuals, each subject's parotids uptake and excretion were converted to percentages of the individual baseline.

After RT, the degree of impairment of the parotid gland function varies, and the effects may be temporary or persist permanently. These variations appear to depend on both the radiation dose and the volume of the gland included in the radiation field^{2,6,7}. In radiation-induced injury of the parotid glands, the loss of secretory function and impairment of excretion may play a role in the onset of xerostomia in the period after RT¹⁰. Using salivary gland scintigraphy, both parenchymal function (uptake of technetium) and excretion function of both parotid glands could be quantified simultaneously. In this study, the mean maximal uptake values were impaired with time for 12 months. Only the uptake 1 year after RT correlated with the mean parotid dose. In accordance with clinical observations, a statistically significant correlation was found between the mean radiation dose to the parotid gland and its SEF, with recovery of secretion capacity late (1 year) after RT. This was also concluded

from the TD_{50} values obtained from fitting the Lyman model. With this model, the effects of radiation on parotid gland function could be evaluated accurately using DVHs derived from planning CT scans. A large group of patients was studied with a broad range in dose and volume of the irradiated parotid glands. DVHs provide useful information in predicting the effects of RT on normal tissues. Several mathematical models exist that derive NTCPs from DVHs. We used the widely used complication model by Kutcher and Lyman to correlate DVHs and parotid gland toxicity. This model helped us to predict the response of the parotid gland to nonuniform irradiation. When the complication was defined as an SEF ratio of <45%, the TD_{50} value was 29 Gy and 43 Gy at 6 weeks and 1 year after RT respectively. A complication was defined as an SEF ratio of <45% to have comparable TD_{50} values with our flow data. As far as we know, no other study using the NTCP model for parotid glands with complications stated by scintigraphic methods has been published. A small number of authors have prospectively studied the dose-response curves of parotid glands using scintigraphy. However, in these studies, only small group of patients could be evaluated and no NTCP models were used. In 25 patients, excretory function was affected in relation to the radiation dose for doses >30 Gy²³. Maes *et al.*¹¹ also used salivary gland scintigraphy as a parameter of parotid gland function. A group of 39 patients was evaluated. Comparable to our data, a correlation between SEF and mean dose, without a threshold dose, was found. Liem *et al.*²³ and VanAcker *et al.*¹² also reported that at early after RT the uptake phase was not affected.

In accordance with retrospective data from Valdés Olmos *et al.*¹⁰, our results could also be explained by the role of both the inability of pertechnetate to concentrate in the intralobular ductule cells (with subsequent ductal epithelium secretion) and impairment of the discharge into the excretory ducts. The initial damage of the parotid glands may be characterized by failure of gland excretion, and late irradiation effects might be associated with damage to the gland parenchyma and decreased trapping ability.

Earlier, significant correlations were found between parotid flow rates and scintigraphy parameters⁸. We found a correlation between the stimulated parotid flow rates⁶ and the SEF ratio 6 weeks and 1 year after RT. However, a wide scatter was shown. It appeared that the scatter in the scintigraphic data were comparable with our flow rate results. Therefore, parotid scintigraphy was not superior to direct flow measurements. However, in 19% (42 of 216) of the parotid glands, no saliva could be collected using the method with the Lashley cups before the start of treatment⁶. In nearly all ($n = 39$) of the parotid glands lost for flow measurement, nuclear scans were available for data analysis.

For both endpoints (parotid flow rates and scintigraphy parameters), the Lyman model was used as NTCP model, and the parameters of the model were found by maximum likelihood estimation. For both end points, we found no threshold dose, with even greater m (slope of the dose-response relationship) values for the scin-

tigraphy data. The parotid flow rates and excretion fraction improved 1 year after RT. **Figure 7** describes the complication probability curves after fitting the NTCP model to the clinical scintigraphy data. Improved dose distribution aiming at sparing parotid function by applying intensity-modulated RT (IMRT), is currently under investigation and clinically used. Various studies have shown the reduction of the dose to the parotid glands using IMRT²⁴⁻²⁶. It might, therefore, be possible to preserve the function of the glands without decreasing the dose in the target volumes. The iso-complication curves, especially, can be very useful in the clinic when parotid glands are partially irradiated, as in IMRT plans. Quantitative data on the relationship between the three-dimensional dose distributions in the parotid glands and their function are needed in evaluation of these IMRT plans to preserve salivary flow.

Conclusion

We accurately described the influence of RT for head-and-neck tumors on parotid gland function using scintigraphy. A significant correlation between the SEF ratio and the mean parotid dose was found. Partial recovery of function appeared with TD_{50} values of 29 and 43 Gy at 6 weeks and 1 year after RT, comparable with our flow data. However, the scatter in the data was the same for both methods. Parotid scintigraphy did not seem superior to direct flow measurements. If direct flow measurements are not feasible, scintigraphy appears to be a good alternative.

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CHAPTER

Preservation of the rat parotid gland function after radiation by prophylactic pilocarpine treatment: radiation dose dependency and compensatory mechanisms

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Abstract

Purpose

To study the ability of a prophylactic pilocarpine administration to preserve the rat parotid gland function after unilateral irradiation with graded doses of X-rays.

Methods

The right parotid gland of male albino Wistar rats was irradiated with single doses of X-rays (10-30 Gy, at 1.5 Gy min⁻¹). Pilocarpine (4 mg/kg) was administered intraperitoneally, 1 hour prior to irradiation. Saliva samples of both left and right parotid gland were collected by means of miniaturized Lashley cups 4 days before and 3, 7, 10 and 30 days after irradiation. The parotid salivary flow rate (μl/min) was used as a parameter for the assessment of parotid gland function.

Results

Our data confirm that a single prophylactic treatment of pilocarpine can attenuate radiation-induced loss of gland function. Surprisingly, the effect of pilocarpine was not restricted to the irradiated gland only. Pilocarpine also enhanced the flow rate in the contralateral, nonirradiated gland. The latter effect was found for all doses above 10 Gy and became apparent around 7 days after the radiation treatment. The effectiveness of pilocarpine to attenuate function loss in the irradiated gland decreased with increasing dose and was lost after single doses of 30 Gy.

Conclusions

Our data provide direct evidence that increasing the compensatory potential of the nondamaged gland, at least in part, underlies the “radioprotective effect” of pilocarpine in case of unilateral radiation. The ability of pilocarpine to ameliorate the early radiation-induced impairment of the parotid gland function in the irradiated may therefore be dependent on the remaining number of functional cells, and thus on the volume of the gland that lies within the radiation portal.

Introduction

Radiotherapy of tumors in the head-and-neck region frequently involves the salivary glands in the irradiated volume. Exposing the salivary glands to radiation can result in severe side effects having a negative impact on the daily life of the patient¹. The consequences of irradiation-induced salivary gland injury are still very difficult to manage. The use of mouth rinses, saliva substitutes, and gustatory stimulants are often abandoned and replaced by repeated water consumption, generally offering only short-term relief of symptoms².

The muscarinic receptor agonist pilocarpine has been shown to produce clinically significant benefits for the symptomatic treatment of postradiation xerostomia when administered chronically³⁻⁶. However, administration of pilocarpine to stimulate any residual function of the salivary gland after radiotherapy is useful to a limited extent: the gain in function ceases as soon as the administration of the sialogogue is stopped⁶⁻⁸. This means that patients have to use this sialogogue for the rest of their lives.

Interestingly, histological studies on rat salivary glands have shown that prophylactic treatment with sialogogues has some radioprotective potential^{9,10}. The radioprotective effect of sialogogues on the morphology of the rat salivary gland consisted of attenuation of the irradiation-induced reduction in number of secretory granules, injury to the mitochondria and cell membrane^{9,10}. We recently showed that pretreatment with pilocarpine also resulted in sparing of radiation-induced changes in rat salivary gland function¹¹. This is consistent with small clinical trials demonstrating that concomitant use of pilocarpine during head-and-neck irradiation was associated with decreased posttreatment xerostomia and that prolonged postirradiation use of pilocarpine was not always required¹²⁻¹⁵. It was speculated that the sparing effect of pretreatment with pilocarpine might be due to stimulation of salivary gland tissue outside the radiation portal¹³. This would suggest that the protective effectiveness of pilocarpine should decrease with increasing radiation dose and/or increasing irradiated salivary gland volume. Yet, the validity of this explanation is still completely unclear, especially in the light of our studies in rats that revealed a protective effect of a single pretreatment dose of pilocarpine against early function loss, even when both glands were completely irradiated¹¹. Therefore the effects of preirradiation treatment with a single dose of pilocarpine on rat parotid salivary gland function 0-30 days after X-rays were investigated in relation to radiation dose. Furthermore the effects of unilateral rat parotid gland radiation, using different radiation doses, on bilateral stimulated parotid saliva secretion were assessed to look at compensatory effects.

Methods

Animals

Male albino Wistar rats between 9 and 10 weeks old (bodyweight 260–280 g) purchased from Harlan CPB Rijswijk, the Netherlands were used (strain Hds/Cpb: WU). They were kept in polycarbonate cages (six rats per cage) under a 14:10-h light:dark cycle. The rats were housed for 1½ weeks prior to the experiments. Food (RMH-B, Hope Farms, Woerden, the Netherlands) and water were given *ad libitum*. All experiments were performed in agreement with the Netherlands Experiments on Animal Act (1977) and the European Convention for the Protection of Vertebrates Used for Experimental Purposes (Strasbourg, 18.III.1986). The animals weighed 262 ± 4 g at the start of the experiment (day –4).

Irradiation procedure

Prior to irradiation all rats were anesthetized by an intraperitoneal injection Ketalar 60 mg/kg and Rompun 2.5 mg/kg. In order to irradiate only the right parotid gland a tailor-made radiation portal was designed. This 6-mm-thick shield was positioned so as to permit direct unilateral parotid gland irradiation. Most of the right submandibular/sublingual and the complete left submandibular/sublingual and parotid region and oral cavity were excluded from the treatment portal. Sialography and *in vivo* determination of the location of the salivary glands were used to establish the biological variation in position of the right parotid gland. Bilateral parotid gland irradiation was performed, as previously, using a 6-mm lead shield with a portal of 2×5 cm² positioned over the body of the rat so that, except for the right and left parotid and both submandibular/sublingual glands, the body including the oral cavity was excluded from the radiation field¹⁶.

Dose distribution associated with the radiation portal was measured using X-ray film densitometry. To minimize radiation source size effects on the penumbra of the beam, the radiation portal was positioned close to the object. Tissue equivalent material was used to ensure the data to be most realistic. In this setup, the irradiated gland area received a dose of 90 to 100% in the center of the field, whereas at the edge of the field the dose dropped from 90 to 50% within 1 mm and to 5% within 3 mm. The dose gradient across the depth was less than 10%.

The gland area was irradiated with a single exposure to 10, 15, 20 and 30 Gy at 1.5 Gy min⁻¹ delivered by an X-ray machine (Mueller MG 300, Philips, Eindhoven, the Netherlands) operated at 15 mA, 200 kV (filters: 0.5 mm copper and 0.5 mm aluminium; Half Value Layer (HVL) = 1 mm copper). Dose rate was determined in air with a calibrated electrometer and ionization chamber combination (Keithleg 35040 + NE 2571). Control animals were anaesthetized but not irradiated. Estimates of biological equivalent doses with protracted radiotherapy using 5 fractions per week of 2 Gy each are respectively 16, 32, 50, and 100 Gy for single exposures of 10, 15, 20, and

30 Gy, using an α/β ratio of 10, as estimated by Franzen *et al.*,¹⁷ for rat parotid gland late effects on acinar cell number.

Treatments

One hour prior to the irradiation, four groups of animals were intraperitoneally injected with 4 mg/ml pilocarpine (pilocarpine hydrochloride, University Hospital Pharmacy, Groningen, the Netherlands) and irradiated with a single dose of 10, 15, 20, and 30 Gy. A fifth group was pilocarpine treated but not irradiated. Another four groups of animals were irradiated with 10, 15, 20 and 30 Gy without pretreatment with pilocarpine. The tenth group did not receive pilocarpine and was sham-irradiated. The rats were divided at random into the 10 groups. Ten animals per group were used. The dose of 4 mg/kg pilocarpine given 1 hour prior to irradiation was chosen since at this dose and time it was shown to give rise to good sparing of the parotid gland after bilateral irradiation¹¹.

Collection of saliva

The rats were anesthetized by an intraperitoneal dose of 60 mg/kg Brietal and intubated to minimize respiratory complications. Salivary gland function was determined by collecting parotid saliva samples under halothane/N₂O/O₂ anesthesia by means of miniaturized Lashley cups¹⁸. Both left and right parotid gland saliva samples were collected simultaneously. The cups were placed upon the orifices of both parotid glands. Saliva was collected for 30 min after stimulation with 2 mg/kg pilocarpine administered subcutaneously (given at $t = 0$ and $t = 15$ min). Two times 2 mg/kg pilocarpine with an interval of 15 minutes was used to be able to collect enough saliva after irradiation for accurate measurements. The second dose induces a similar amount of saliva secretion as the first dose, indicating a good recovery of gland function.

Right and left parotid saliva secretion was separately collected in preweighted ice-cooled plastic tubes. Saliva was collected 4 days before and 3, 7, 10, and 30 days after irradiation.

Sialometry

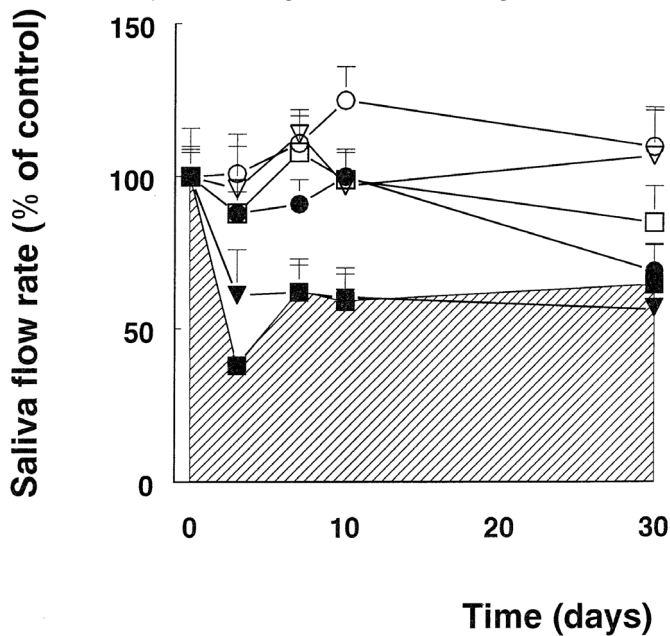
As a parameter for the assessment of parotid gland function, saliva flow rates were determined. The total volume of saliva secreted was estimated by weight assuming the specific gravity of saliva of 1.0 g/cm³. The salivary flow rate ($\mu\text{l}/\text{min}$) was calculated from the collecting time (min) and volume of saliva secreted (μl).

Statistical analysis

The sialometric responses were expressed as a percentage of the pretreatment. Furthermore, the sialometric data were expressed as area under the curve for 0-30, 0-7 and 7-30 days as the percentage change compared with sham-irradiated non-

treated controls. The area under the curve was calculated using the percentage of function (flow rate) as the ordinate and the time (days) as the abscissa (**Figure 1**). The results are expressed as means \pm SEM. The data were analyzed by a two-sided Student's *t*-test.

Figure 1 Changes in function of the irradiated (right) parotid gland (closed symbols) and nonirradiated (left) parotid gland (open symbols) following 10 (circles), 20 (triangles) and 30 Gy (squares) as a function of time after treatment. Data are expressed as a percentage of pretreatment control flow rate values and are the mean values (\pm SEM). The hatched area represents the area under the flow rate function curve used as a parameter for gland function in the Figures 2, 4, 5, and 6.



Results

Adverse effects and sialometry

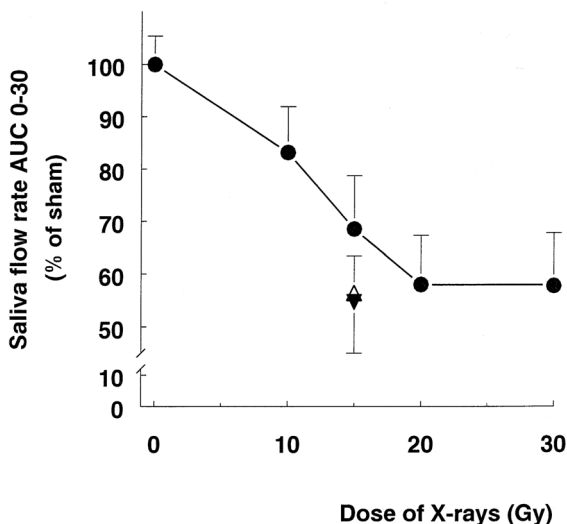
On average, one of six rats died during the experimental period (up to day 30) due to complications in the recovery from anesthesia or from pilocarpine application (obstruction of the endotracheal tube by bronchial secretions). In none of the cases did pseudomembranes or ulcers, which are known to interfere with the nutritional status, develop. No significant weight loss occurred throughout the experiment, except for a slight ($12 \pm 2\%$) weight loss at day 10 for the animals in which both the parotids were irradiated.

Effects of unilateral radiation on parotid saliva production

In contrast to our earlier studies^{11,19,20} in which both parotid and submandibular glands were irradiated, in this study only the right parotid gland was exposed to X-irradiation. Parotid saliva was collected separately from both glands. Salivary flow rates of two glands are plotted in **Figure 1**. Curves for 10, 20, and 30 Gy are depicted. The mean flow rate ($\mu\text{l}/\text{min}$) of the rats in the nontreated sham-irradiated control groups (day - 4) was 11.3 ± 0.4 for the right (irradiated) gland and 11.2 ± 0.6 for the left (control) gland. As can be seen, radiation caused a rapid, dose-dependent decline in saliva flow rates of the irradiated gland. Gland function was already impaired at day 3 after irradiation. Almost no recovery occurred up to 30 days postirradiation, irrespective of the radiation dose used. No significant effects were seen on the function of the nonirradiated, left gland during the time course of the experiment (**Figure 1**, open symbols).

To further illustrate the radiation dose dependency on the loss of salivary gland function, we plotted areas under the flow rate function curves (illustrated for the 30 Gy data by the hatched area in **Figure 1**) as a function of the radiation dose (**Figure 2**). The data show that there is a rapid dose-dependent function loss of the irradiated gland of doses up to 30 Gy. The extent of function loss of the right parotid gland after unilateral radiation of 15 Gy was similar to changes in the gland observed in using bilateral irradiation (**Figure 2**, open triangle). Also, the data are

Figure 2 Radiation dose-dependent loss of function of the parotid gland. Functional changes of the irradiated (right) parotid gland after unilateral radiation expressed as percentage decrease in the area under the curve (0-30 days) when compared to the sham-irradiated controls. The triangles show changes in the parotid gland function after bilateral radiation (open triangle: results from this study, closed triangle: results from previous studies (ref 11)). Data are the mean values (\pm SEM).



in good accordance with our previous studies^{11,16,20} performed with the same rat model but simultaneous collection of right and left parotid saliva (**Figure 2**, closed triangle).

Effects of preirradiation treatment with pilocarpine

As we have demonstrated before¹¹, pretreatment with pilocarpine can protect salivary glands against the detrimental effects of a single dose of 15 Gy using bilateral gland irradiation. This effect was confirmed using separate collection of saliva from both the left and right irradiated parotid gland (**Figure 3A**). Using unilateral irradiation of 15 Gy, we also observed that pretreatment with pilocarpine results in less damage to the function of the irradiated gland (**Figure 3B**, closed symbols). Interestingly, the pilocarpine treatment also resulted in flow rates above 100% in the left, nonirradiated gland (**Figure 3B**, open symbols). Pilocarpine had no effect on the function of either gland in sham-irradiated (0 Gy) animals (data not shown). At a dose of 30 Gy, the effect of pilocarpine on the function of the irradiated gland was not seen anymore (**Figure 3C**, closed symbols). Yet, in these unilaterally irradiated animals, the function of the nonirradiated gland was increased up to 120% above control values in contrast to those animals which were treated with 30 Gy alone without pretreatment with pilocarpine (**Figure 3C**, open symbols).

These data suggest that the ability of pilocarpine to attenuate radiation-induced loss of total function depends on the radiation dose and is—at least in part—due to compensatory mechanisms in the nonirradiated gland. This is further illustrated in **Figure 4**, in which again areas under the flow rate function curves (0-30 days after irradiation) were plotted as a function of the radiation dose. Pilocarpine reduced the loss of function of the irradiated gland after single doses of 15 and 20 Gy. At 30 Gy this effect was lost. Yet, for all doses used pilocarpine increased the flow rate in the nonirradiated, left gland.

When subdividing the observed response in acute (0-7 days) and early (7-30 days) effects, the pilocarpine-induced hyperfunctioning of the nonirradiated gland was not seen for the acute effects (**Figure 5A**). This effect was only apparent for the early effects, 7-30 days after the radiation treatment (**Figure 5B**). Thus the pilocarpine-induced compensatory response needs some time (>7 days) to develop. When the effects of pilocarpine on both irradiated and nonirradiated glands are combined, it can be observed that pretreatment with pilocarpine resulted in a preservation of total saliva production after radiation with doses up to 30 Gy (**Figure 6**).

Figure 3 Preservation of the rat parotid gland function after radiation by prophylactic pilocarpine treatment. Changes in parotid flow rate are expressed as a function of time after X-radiation. (A) 15 Gy bilateral radiation; (B) 15 Gy unilateral radiation; (C) 30 Gy unilateral radiation. Circles represent the effect of X-irradiation alone; triangles show the effects of radiation in rats pretreated with pilocarpine. Panel A: closed symbols represent the right, open symbols the left, irradiated gland. Panel B and C: closed symbols represent the right, irradiated gland, and open symbols the nonirradiated gland. Data are expressed as a percentage of pretreatment control flow rate values and are the mean values (\pm SEM).

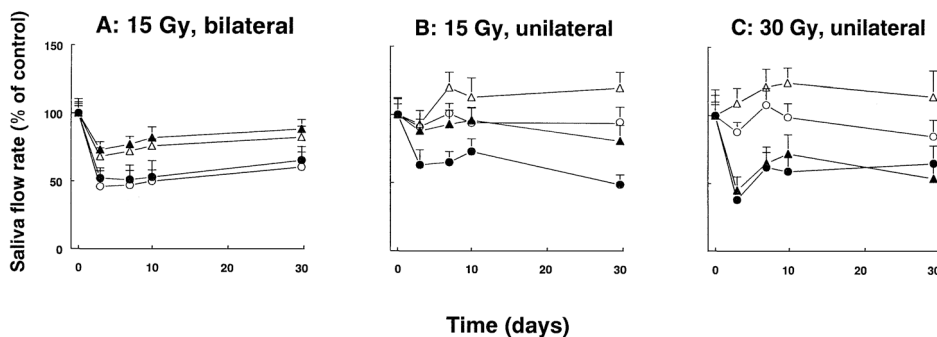


Figure 4 Radiation dose dependency of the capacity of pilocarpine to preserve the rat parotid gland function after radiation. Changes in parotid flow rate of the irradiated, right parotid gland (closed symbols) and nonirradiated, left parotid gland (open symbols) are expressed as a percentage of the flow rates in nonirradiated animals using the area under the curve (0-30 days) parameter as a function of radiation dose. Circles represent the effect of X-irradiation alone; triangles show the effects of radiation in rats pretreated with pilocarpine. Data are the mean values (\pm SEM).

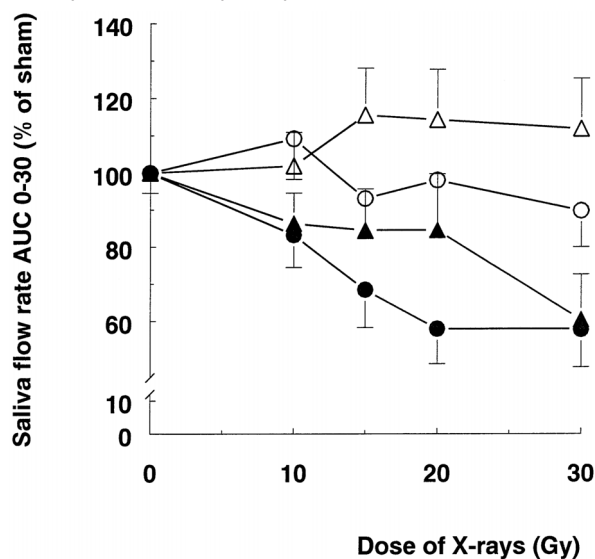


Figure 5 Time dependency of the capacity of pilocarpine to stimulate the function of the nonirradiated rat parotid gland after radiation of the contralateral gland. Changes in parotid flow rate of the nonirradiated, left parotid gland after irradiation of the contralateral, right gland with graded doses of X-rays. Data are expressed as a percentage of the flow rates in nonirradiated animals using the area under the curve parameter 0-7 days after irradiation (panel A: acute effects) or 7-30 days after irradiation (panel B: early effects). Circles represent the effect of X-irradiation alone; triangles show the effects of radiation in rats pretreated with pilocarpine. Data are the mean values (\pm SEM).

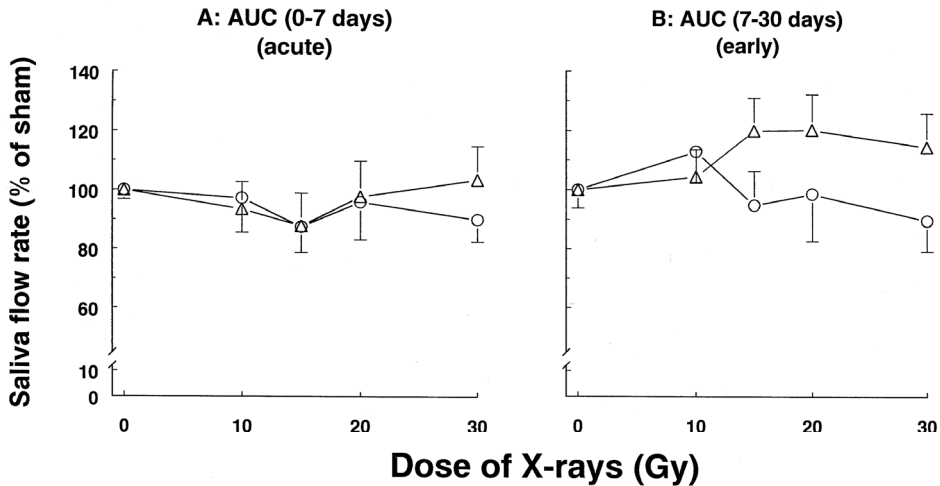
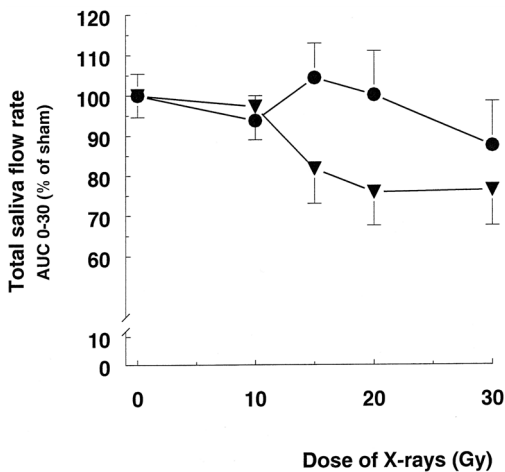


Figure 6 Overall effect of pilocarpine on total rat parotid gland function after radiation. Changes in the total saliva production of both the irradiated and contralateral, nonirradiated rat parotid gland after graded doses of X-rays. Data are expressed as a percentage of the flow rates in nonirradiated animals using the area under the curve (0-30 days) parameter. Triangles represent the effect of X-irradiation alone; circles show the effects of radiation in rats pretreated with pilocarpine. Data are the mean values (\pm SEM).



Discussion

This study is a refinement of previous ones on radiation effects on parotid gland function^{11,16,19,20} because of the use of unilateral irradiation and separate collection of the right (irradiated) and left (nonirradiated) gland. We were able to show a dose-dependent, acute function loss of the irradiated parotid gland. Since these treatments had no effects on the function of the nonirradiated (left) gland, it can be concluded that the function loss of the irradiated (right) gland is not due to indirect effects of anesthesia and/or radiation dose-related impairment of food intake as previously suggested by Nagler *et al.*²¹. Furthermore, our data confirm that a single prophylactic treatment of pilocarpine can attenuate radiation-induced loss of gland function¹¹. The effect of pilocarpine was not restricted to the irradiated gland only. Surprisingly, pretreatment with pilocarpine also enhanced the flow rate in the contralateral, nonirradiated gland, when compared to the nonpilocarpine-pretreated gland flow rate, an effect that became apparent at about 7 days after the radiation treatment. Pilocarpine had no effect on the flow rate of animals that were not irradiated at all (sham-treated controls). Therefore, the effect of pilocarpine is compensatory. In fact, stimulatory effects were only seen after doses of 15 Gy and above, indicating that a certain level of damage needs to be present before the stimulatory effects of pilocarpine on the nondamaged gland become apparent. Our data therefore provide the first direct evidence that pilocarpine induces compensation which at least in part underlies the “radioprotective” effect. So, rather than being a classical radioprotector, directly decreasing the effect of radiation on the cell, pilocarpine indirectly ameliorates radiation-induced effects on gland function through a compensatory action on nondamaged cells as a response to signals from the radiation-induced damaged part of the parotid. Besides damaged cells, a sufficient number of remaining “healthy cells” must be present for the ameliorating effect of pilocarpine. Likely, this is not the case for glands irradiated with a dose above 30 Gy where no effect of pilocarpine was observed in the irradiated gland. For doses of 20 Gy and lower, the irradiated gland does contain a sufficient number of cells capable of responding to pilocarpine, which also could explain why we did find protective effects of pilocarpine after bilateral irradiation with 15 Gy^{11, this study}. Furthermore, the ability of pilocarpine to increase the function of the contralateral gland was independent on the dose given to the irradiated site.

The compensatory effects of pilocarpine were not seen before day 7 after irradiation. The time course of 7 days coincides with that observed for an increase in the fraction of proliferating cells within the salivary gland after X-irradiation²². It is tempting to speculate that pilocarpine enhances the extent of cell proliferation in response to irradiation, since we observed an increase in number of secretory cells per gland in the contralateral, nonirradiated gland (unpublished observations). As suggested previously¹¹, pilocarpine therefore may act by enhancing the replacement of damaged cells, thereby enhancing the functional recovery of the irradiated

gland. How the signals related to the prophylactic pilocarpine treatment and radiation-induced injury from the irradiated gland translates into the observed increase in saliva output of the nonirradiated, contralateral gland yet remains unclear. *Appos*, because pilocarpine had no effect on the flow rate of animals that were not irradiated at all, and because lower doses of pilocarpine had a reduced sparing effect on bilateral irradiated glands", it is likely that, in collaboration with a signal induced by irradiation, pilocarpine changes the status of the gland postirradiation.

In conclusion, irrespective of the exact mechanism, the ability of pilocarpine to ameliorate the early radiation-induced impairment of the parotid gland function seems to be dependent on the amount of damage induced. Therefore, the clinical implication of our data is that the type of fractionation scheme used and the volume of the gland that lies within the radiation portal will be crucial for the effectiveness of a prophylactic pilocarpine treatment to reduce the side effects associated with the radiotherapy treatment of head-and-neck tumors. A prospective, phase III clinical trial has recently been initiated in our institutes to test the ameliorating potential of prophylactic pilocarpine administration in relation to dose/volume parameters. This prospective, randomized, double-blind, placebo-controlled study evaluates whether oral pilocarpine given during radiation therapy may reduce salivary gland function in relation to dose/volume parameters. The patients are stratified into three groups: low volume, intermediate volume, and high volume of irradiation receiving at least a dose of 40 Gy.

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CHAPTER

A comparison of mean parotid gland dose with measures of parotid gland functions after radiotherapy for head-and-neck cancer: implications for future trials

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Abstract

Purpose

To determine the most adequate parameter to measure the consequences of reducing the parotid gland dose.

Methods

One hundred-eight patients treated with radiotherapy for various malignancies of the head-and-neck were prospectively evaluated using three methods. Parotid gland function was objectively determined by measuring stimulated parotid flow using Lashley cups and by scintigraphy. To assess xerostomia-related quality of life the head-and-neck cancer module EORTC QLQ-H&N35 was used. Measurements took place before radiotherapy and 6 weeks and 12 months after the completion of radiotherapy. Complication was defined for each method using cut-off values. The correlation between these complications and the mean parotid gland dose was investigated in order to find the best measure for parotid gland function.

Results

For both flow and scintigraphy data, the best definition for objective parotid gland toxicity appeared to be reduction of stimulated parotid flow to <25% of the preradiotherapy flow. Of all the subjective variables only the single item dry mouth 6 weeks after radiotherapy was found significant.

The best correlation with the mean parotid gland dose was found for the stimulated flow measurements. The predictive ability was the highest for the time point 1-year after radiotherapy. Subjective findings did not correlate with the mean parotid dose.

Conclusions

Stimulated flow measurements using Lashley cups, with a complication defined as flow <25% of the preradiotherapy output, correlated best with the mean parotid gland dose. When reduction of the mean dose to the parotid gland is intended, the stimulated flow measurement is the best method for evaluating parotid gland function.

Introduction

In patients with head-and-neck cancer, radiation treatment often involves the parotid glands, either because of the location of the primary tumor or of the lymph node metastases. Irradiation of the parotid glands is associated with loss in function, which leads to reduction in salivary flow and dryness of the mouth. Saliva facilitates speech and is essential for taste perception¹. A loss of stimulated saliva flow makes it difficult to soften a foodbolus and can cause difficulty in swallowing². A dry mouth (xerostomia) and sticky saliva are important complaints after radiotherapy³.

There have been several attempts to reduce the radiation damage to the major salivary glands. New radiation techniques have been introduced to spare parotid gland function. Using intensity-modulated radiotherapy (IMRT) it might be possible to preserve the function of the glands, while adequately treating tumors and regional lymph nodes⁴⁻⁶. In most IMRT studies a large effort has been performed to reduce the parotid gland dose. Numerous papers report the theoretical value of IMRT in reduction of parotid gland dose^{4,7-9}. However, only a limited number of papers report on measurements to assess the effect of reducing the gland dose. Furthermore, the effect of reducing the parotid gland dose has been evaluated in many different ways. In most studies, either objective method such as salivary flow measurements using sialometry and scintigraphy or subjective methods such as assessed by questionnaires have been used to quantify the function of the glands¹⁰⁻¹³. These different ways of recording the parotid gland toxicity complicates the comparison of the different IMRT studies. The question raises how to measure complications due to parotid gland irradiation and how to define complications.

Earlier, we described parotid gland function after radiotherapy in head-and-neck cancer in a large group of patients determined objectively by measuring stimulated parotid flow using Lashley cups and by scintigraphy^{14,15}. The corresponding quality of life outcome was assessed using the QLQ-H&N35 questionnaire. These three different measurements were used to quantify the complications due to damage of the parotid glands after radiotherapy for head-and-neck tumors. The aim of this study was to define complications and to determine which complication measurement correlates best with the mean parotid gland dose.

Methods

Patients

A total number of 108 patients treated with radiotherapy for various malignancies of the head-and-neck were studied. None of the patients received previous radiotherapy or surgery of the parotid glands, or suffered from malignancies or other diseases of the parotid glands. All patients were treated with radiation therapy without induction or concomitant chemotherapy. Details on radiation treatment

planning have been reported previously¹⁴. Fifty-one patients had surgical resections followed by postoperative radiotherapy. The primary site was located in the larynx in 45 patients, the floor of mouth/oral cavity in 19 patients, the oropharynx in 16 patients, the nose (nasal cavity) in 8 patients and other sites in 19 patients. One patient was treated for lymph node metastases with an unknown primary tumor. CT-based dose-volume histograms were generated for right and left salivary glands. Parotid gland function and quality of life were evaluated before radiation and 6 weeks and 12 months after the completion of radiotherapy. Informed consent was obtained from each patient.

Saliva collection

Stimulated parotid flow rates were determined by the collection of saliva from both parotid glands orifices (Stenson's duct) simultaneously with Lashley cups. No oral stimulus was permitted for 60 minutes before saliva collection. Applying three drops of 5% citric acid to the mobile part of the tongue every 30 seconds stimulated saliva production, and collection was carried out for 10 minutes. The volume of saliva was measured in tubes by weight, assuming the specific density of parotid saliva to be 1 g/mL. The flow rate for each gland was expressed in milliliters per minute (mL/min). Most samples were collected between noon and 5 PM. The measurements were performed by two of the authors in more than 90% of cases in order to reduce the effect of interobserver variability. Each subject's parotid flow rates were converted to percentages of the individual baseline flow rates (flow ratio).

Salivary scintigraphy

Radionuclide studies were performed with the patient in the supine position under a gamma camera with high-resolution collimators. After intravenous administration of 200 MBq ^{99m}Tc-pertechnetate fifteen sequential frames of 60 seconds (anterior view) were acquired. After 15 minutes, 5% citric acid was administered orally to induce the excretion of saliva. Time-activity curves were generated with regions of interest drawn around the right and left parotid gland. Background subtraction was performed using the temporal region. The response to citric acid was established using the salivary excretion factor (SEF). The SEF was defined as the uptake after secretion per gland as a fraction of the maximal uptake. Uptake and excretion was analyzed per patient and subsequently per individual gland. The SEF ratio was used to evaluate the percentage lost after radiotherapy (6 weeks and 1 year) with respect to the baseline SEF.

Xerostomia-related quality of life

To assess xerostomia and related symptoms the head-and-neck cancer module EORTC QLQ-H&N35 was used. This module contains seven symptom scales and six symptom items. All scales are rated on a four point Likert scale. All sub-scales

are linearly converted to a 0-100 scale. Higher scores represent a greater degree of symptoms. It has been validated in 622 patients with head-and-neck cancer from 12 countries¹⁶. In this analysis, only the single items regarded as most relevant for the purpose of the study was investigated, that is dry mouth, sticky saliva and an additional item on overall health.

Statistics

Models for flow, scintigraphy, and QLQ were built with the mean parotid gland radiation dose as explanatory variable. Due to multiple measurements on each patient for flow and scintigraphy, multilevel models were applied. Both flow and scintigraphy are continuous variables and therefore linear regression based on a normally distributed response variable might seem appropriate. However, this normality assumption is violated by a large number of zero response values at high dose of radiation. Therefore, we examined several cut off points by means of ROC (Receiver Operating Characteristic) curves in order to dichotomize flow and scintigraphy. We used both the area-under-the-ROC-curve and the spread in the predicted probabilities, based on the corresponding logistic model, to come to an optimal dichotomization. For the selected dichotomized variables, logistic models were estimated together with their predictive ability measure Nagelkerke's R^2 . For the QLQ variables, proportional odds models were used and Nagelkerke's R^2 was calculated as well.

Results

The best definition for objective parotid gland toxicity appeared to be reduction of stimulated parotid output to <25% of the preradiotherapy output. This was found for both flow and scintigraphy data. Based on the proportional odds models, of all the subjective variables only the early outcome (6 weeks after radiotherapy) of the single item dry mouth was found significant.

To find the best parameter for evaluating parotid gland function in relation to mean parotid gland dose, we correlated these definitions of complication for the three different methods, that is parotid flow ratio <25% at time points 6 weeks and 1 year after radiotherapy, the ratio of SEF <25% at time points 6 weeks and 1 year after radiotherapy and single item dry mouth 6 weeks after radiotherapy, with the mean parotid gland dose. To evaluate which of these complication measures is the best predictor for the mean parotid gland dose, Nagelkerke's R^2 was calculated. The higher the value of Nagelkerke's R^2 , the better the predictive ability of the corresponding model. The predictive ability was the highest for the parotid flow measurements, both for the early (6 weeks after radiotherapy) and the late (1-year after radiotherapy) time points (**Table 1**).

Table 1 Relationship between stimulated flow measurements using Lashley cups, scintigraphy (SEF), with complications defined as stimulated output <25% of preradiotherapy (RT) output, the subjective variable single item dry mouth and the mean parotid gland dose. Predictive ability was assessed using Nagelkerke's R^2 .

Variable	Value (Nagelkerke's R^2)
Flow 6 weeks after RT	0.785
Flow 1 year after RT	0.825
SEF 6 weeks after RT	0.379
SEF 1 year after RT	0.425
Dry mouth (QLQ) 6 weeks after RT	0.209

Discussion

Various methods have been used for many years in an attempt to evaluate the diminished salivary gland function after radiotherapy for head-and-neck carcinoma. Evaluation of the effects of radiation therapy on salivary gland function may comprise subjective rating of oral symptoms, i.e. the patient's sensation of dry mouth (xerostomia), or objective measures, such as flow rate and scintigraphic measurements.

The measurement of secretion from the parotid gland with suction cups allows the quantitative collection of the saliva and is reasonably easily performed, not invasive, and not expensive. In earlier studies the correlation between the parotid gland function using Lashley cups and the 3D-dose distribution in a large group of head-and-neck cancer patients was described^{14,17}. The stimulated salivary output depended on the mean parotid gland dose. Parotid scintigraphy also appeared to be a good indicator of gland function. A significant correlation between the ratio SEF and the mean parotid dose was shown, comparable with the flow results^{12,15}. Salivary gland scintigraphy seems to be a good indicator of gland function, but it is an expensive test and requires hospital equipment.

Xerostomia-specific questionnaires were found reliable and valid in measuring patient reported xerostomia¹⁸. However, different instruments for evaluating quality of life are available which makes the interpretation of the results more difficult. Furthermore, the correlation between the subjective quality of life scores and the objective flow measurements is not consistent.

Recent technical advances in radiotherapy have been obtained with the development of IMRT. These techniques can achieve dose distributions in the head-and-neck region that reduce the dose to the major salivary glands especially the parotid glands. Many authors suggested different planning goals for the parotid glands. Threshold doses of 22.5 Gy until 39 Gy have been published^{10,13,14,17,19,20}. These different results might have been caused by the different techniques to describe the prevention of xerostomia. The question is what is the best measure to evaluate

parotid gland function. To predict the outcome of parotid flow postirradiation and the subjective assessment of a dry mouth that is which measure should be used best is still a matter of debate.

In this study three different methods of measuring parotid gland function in the same large group of patients were compared in order to predict which complication measurement correlated best with the mean parotid gland dose. The parotid flow measurements one-year after radiotherapy using Lashley cups, with the complication definition parotid flow <25% of the preradiotherapy, correlated best with the mean dose to the parotid gland. Of the subjective scores, only the single item dry mouth as established early after radiotherapy could be defined as a complication. The subjective findings didn't correlate with the mean parotid gland dose. Using the subjective score of xerostomia and sticky saliva, it is argued that the role of the minor salivary glands for xerostomia should be taken into account. Alterations in submandibular and/or sublingual function may have the greatest impact on the sensation of oral dryness. The mucins are not found in the parotid secretions and may be an important component for imparting a sense of wetness and comfort to chewing and swallowing. Therefore to predict the parotid gland function in relation to the parotid radiation dose, the subjective score is not the best parameter to use. However, more recent studies showed a normal unstimulated salivary flow in patients after surgical resection of the submandibular glands or delivery of high radiation doses¹³. The subjective patient reports of oral dryness appeared not accurately or consistently reflect the actual salivary gland capabilities or performance. Therefore, in evaluating regimens to improve gland function subjective assessments alone are not adequate for therapeutic purposes²¹. Changes in quality of life and the relation with parotid output in the group of patients described in this paper will be reported elsewhere²².

In conclusion, parotid flow measurements using Lashley cups one-year after radiotherapy correlates best with the mean parotid gland dose. A complication was defined as stimulated parotid flow rate <25% of the preradiotherapy flow rate. Studies aiming at reduction of the parotid gland dose should preferably be validated using these parameters. Subjective findings didn't correlate with the mean parotid dose.

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CHAPTER

8 General discussion

Saliva is a most valuable oral fluid that is hardly recognized, until you lack it. Its fluid characteristics apply for cleansing and lubrication of the oral cavity and mucosa. It is responsible for facilitation of taste perception, mastication, swallowing and speech. Saliva is much more than water. It contains a number of proteins that serve to maintain oral health and function. These solutes perform an important role in maintaining oral health by the functions of protection, buffering action, antibacterial activities, digestion, and maintenance of tooth integrity^{1,2}. Any salivary gland dysfunction can result in reduced saliva secretion and inadequate composition as well as xerostomia. In the treatment of head-and-neck cancer by radiotherapy the salivary glands are often inevitably included in the treatment volume. The impairment of salivary gland function that can result is an important cause of late morbidity following treatment.

What is xerostomia?

Although the term ‘xerostomia’ is widely used, it remains difficult to define. Xerostomia is generally accepted as the subjective feeling of a dry mouth and is the most prominent long-term symptom in many survivors of head-and-neck cancer. It is a subjective experience and should be measured by the patient him- or herself. In his thesis, de Graeff³ showed that the EORTC Head-and-Neck cancer Quality of Life Questionnaire (QLQ-H&N35) (in conjunction with the EORTC QLQ-C30) is a reliable and valid instrument to measure quality of life in head-and-neck cancer patients. In this tumor specific module the symptom item ‘dry mouth’ is included. Long-term changes after treatment for head-and-neck tumors were described. For example at 36 months after treatment there was a significant deterioration of dry mouth, compared with baseline values. The SOMA (subjective, objective, management, analytic) criteria for salivary gland toxicity also reflect clinical symptoms⁴⁻⁶. The subjective part varies in degree from scant to complete, potentially debilitating, dryness. However, xerostomia, or oral dryness, may not reflect actual salivary gland capabilities or performance. It is a complex symptom unlikely to be solely determined by an irradiation-induced decrease in salivary flow. In fact, it is still not entirely clear what gives rise to the condition a patient describes as dry mouth. Certainly the correlation between the degree of xerostomia and the saliva flow rate is not random, but in many studies complaints of dryness are not significantly related to salivary output changes. Therefore, subjective assessments alone are not adequate for therapeutic purposes, especially when evaluating individual salivary glands. Individual gland secretions are quite different. Alterations in submandibular/sublingual function may have a great impact on the sensation of oral dryness. The volume and composition of secretions from the minor mucous glands also may be important⁷. Mucins are considered the main secretory products of the minor salivary glands⁸. These glycoproteins may be an important component for imparting a sense of wetness. The secretion of minor salivary glands in different regions

of the mouth may not be uniform and it is possible that localized areas of dryness could trigger the sensation of a dry mouth in the presence of a significant overall salivary flow rate. Especially when the soft palate is included in the radiation treatment volume, the affected minor palatine glands may give rise to dehydration of areas of the mucosa in that region with e.g. denture problems. However, if oral dryness is associated with eating, this is highly indicative for decreased (highly water containing) parotid output. Hence, it is essential that the hypofunction of the different individual salivary glands is substantiated by measuring the saliva secretion. Quantification of flow can help to adequately judge the effect or response of treatment. The analytic part in the SOMA criteria allows the quantification of salivary dysfunction. Grade 4 gland toxicity was defined as salivary flow rate reduced to <25% of the pre-treatment output (according to the RTOG/EORTC Late Effect Consensus Conference – ref. LENT SOMA tables 1995).

Methods of measurement of salivary gland function

Flow rate

The most direct quantitative study of salivary gland dysfunction measures the salivary flow rate at rest or after stimulation. Parotid saliva can be collected by using collecting cups (Lashley cups) placed over the orifice of Stenson's ducts. Salivary hypofunction is difficult to assess, because it is unclear how much saliva is required for normal oral function¹⁹. A wide range of variations is found to be accepted as normal. Therefore, the changes in salivary gland function should be evaluated prospectively over time with good monitoring of the baseline values. We prospectively evaluated parotid gland function by measuring selectively from each parotid gland before and periodically after radiotherapy, in conjunction with the corresponding gland dose-volume parameters. A strong correlation was found between the mean dose to the parotid gland and the parotid gland function after radiotherapy. In our study, the best definition for the objective parotid gland toxicity appeared to be reduction of stimulated output to <25 % of the preradiotherapy values. This is in agreement with the definition of a severe late complication as described earlier. Here, it is important to mention that parotid output can still recover long time after radiotherapy. At 5 years after radiotherapy a significant progression of flow rate was found compared to 12 months after radiotherapy²⁰.

Scintigraphy

Quantitative measurements of salivary dysfunction can also be performed with ^{99m}Tc-pertechnetate sialography. As in the flow data, the effects on parotid gland function could well be established. There was a significant correlation between the reduction in the excretion fraction and the mean parotid gland dose. Unfortunately, the scintigraphy measurements did not correlate better with the mean dose to

the parotid gland than the parotid flow measurements using the Lashley cups. The individual variations as seen in the cup measurements were also seen in the scintigraphy method. The scintigraphy is more expensive compared to measurements with Lashley cups. Furthermore, taking into account the fact that the patients experienced the technetium scans as more inconvenient, and the exposure of the patient to a radionuclide examination, we advise to use the scintigraphy measurements only when collecting saliva with the cups is not feasible.

Subjective score

In this thesis the single items dry mouth and sticky saliva and an additional item on overall health were selected from the EORTC QLQ-H&N35 to compare with the objective measurements. Mean scores of these items were comparable with the level of symptoms found in literature³.

Obviously, the patient's report on the symptom of dry mouth is the most important one¹¹. However, we experienced that it was not always very easy to achieve the patient's perception of a dry mouth. The individual opinion of the patient is sometimes difficult to score. Perhaps a subjective visual analog scale questionnaire will be a better way of evaluating xerostomia¹².

As already discussed the clinical symptoms may not correlate with the amount of available saliva. Of course, subjective measurements reflect all salivary glands, not one separate parotid gland. Therefore, it was difficult to correlate the subjective salivary symptoms with the separate parotid gland dose-volume parameters. Stimulated saliva as measured with the cups was collected from each parotid gland and for analysis of the scintigraphy data it was possible to create time-activity curves from the separate right and left parotid gland. But in the subjective reporting it is not easy to differentiate between left and right. It is questionable whether it is accurate to correlate the subjective scores with the sum of the left and right parotid gland dose divided by two. It is very likely that a unilateral radiation treatment with sparing of one gland may give a different subjective outcome when compared with a high dose treatment of both parotid glands for 50%. And, as already stated, the parotid flow measurements will not take into account the potential function of the submandibular/sublingual and minor salivary glands.

In our study, we analyzed the various parameters obtained with the three described methods to assess parotid gland function in relation to the parotid gland dose. The flow measured using the Lashley cups appeared to have the best correlation with the mean parotid gland dose.

Dose-volume effects

Eisbruch *et al.*¹³ reported the experience in Michigan on a group of patients in whom one of the two parotid glands was spared during head-and-neck irradiation.

The mean dose threshold for stimulated parotid saliva flow rates was 26 Gy. The glands receiving doses below the threshold demonstrated functional recovery in time, whereas most glands receiving higher doses did not recover. Chao *et al.*¹⁴ predicted a slightly higher threshold for stimulated whole saliva flow at six months of 32 Gy. Modelling resulted in an exponential relationship between saliva flow reduction and mean parotid dose at a rate of approximately 4% per Gy of mean parotid dose. Our study is best comparable to the study of Eisbruch *et al.*¹³, because both are based on CT data, dose-volume histogram analysis, and stimulated parotid gland flow measurements. In both studies the Lyman model was used as NTCP model, and the parameters of the model were found by the maximum likelihood estimation. The dose-response curve obtained from our data was less steep and the TD_{50} value at 1 year postradiotherapy was 10 Gy higher. Our flow measurements indicated partial recovery of parotid gland function over time. We were fortunate to have the possibility to combine the data from the two institutes. We assumed that a possible explanation for differences in dose-response results could be the difference in radiation techniques. In our study group, the majority of patients were treated with opposed lateral neck fields and the supraclavicular regions were treated with an anterior field using independent collimators with half-beam blocking. In Michigan the patients were irradiated with more advanced parotid-sparing conformal and multisegmental intensity-modulation techniques. The spatial distribution within the glands could be very different. The sets of DVHs from Michigan clearly showed the lack of data in the range 30-40 Gy mean parotid gland dose, which was near our TD_{50} value. This is probably due to the fact that the goal of the more advanced treatment techniques was to spare the contralateral gland. The number of patients in the study of Eisbruch in the mean dose group of 35 Gy and 45 Gy was very small: 3 respectively 8. However, below the mean parotid gland dose of 25 Gy at 1 year after radiotherapy the complication probability is only 4.7% (Michigan 2.9%, Utrecht 6.8%). So we can assume that, combining the data, there is no real threshold dose, it is rather save in terms of preservation of stimulated parotid gland function to have a mean parotid gland dose of less than 25 Gy. When a mean dose is reached above 50 Gy, nearly all patients will have a severe decrease in parotid flow rate.

In most studies the NTCP parameter TD_{50} is used to describe the data. This TD_{50} is the dose to the whole organ leading to a complication probability of 50%. This parameter is clinically less relevant. But it is difficult to define a complication probability that is acceptable for patients. The most practical guideline would be to try to reach a parotid gland dose As Low As Reasonable Achievable.

The complication probability curves gained after fitting the NTCP model to the clinical data can be very useful when parotid glands are partially irradiated. Remaining problem is that in the mean dose model, no information can be derived about the spatial dose distribution. Especially in more recently developed radiation techniques like intensity-modulated radiation therapy (IMRT) the dose distribution

within the gland is even more inhomogeneous comparing conventional or 3D conformal radiation therapy (3DCRT). It is assumed that the parotid glands behave as a parallel organ to the radiation damage, therefore the mean dose to the whole organ can be used as a predictor of the parotid gland dysfunction. However, as showed in animal models, differences in radiosensitivity between different parts of the parotid glands may be relevant¹⁵. Experiments, in which the parotid glands of the rats were partially and fully irradiated, have shown different degrees of radiation damage, depending on which part was irradiated. Reduction in flow rate was much more severe after irradiating the cranial part as compared to the caudal part of the gland. So, different structures or parts within one gland may respond to irradiation in different ways. It is very important to know whether there is a similar dependency on the human parotid gland, especially in order to steer the dose properly in IMRT treatments. It is possible that the upper-lower difference in rats is comparable with the inner-outer part of the human parotid gland related to the blood supply. Therefore, it is important to continue performing salivary gland function studies following radiotherapy using IMRT techniques. This may contribute to the development of more detailed NTCP models, including the area of the parotid glands irradiated, and eventually lead to a better understanding of the exact pathogenesis of radiation-induced deterioration of parotid gland function.

Preservation of function

IMRT

Currently, more conformal radiation treatment strategies are used, especially to prevent late normal tissue damage. CT-based 3DCRT have replaced standard beam portals based upon the bony anatomy. In conformal radiation therapy the individual radiation beams are shaped around the target using a planning CT scan resulting in a three-dimensional dose-distribution. IMRT is an advanced form of conformal radiotherapy and provides a sophisticated method of dose distribution. IMRT techniques use modifications in intensity of the beams across the beam portals as an additional degree of freedom to enhance the capability of conforming dose distributions in three dimensions. In head-and-neck cancer, preservation of salivary gland function is one of the most important goals of modern radiation treatment techniques such as 3DCRT and IMRT. Using these new tools we are able to create dose patterns covering the tumor while avoiding treating normal tissue.

IMRT can be delivered as the 'step and shoot' technique: the intensity-modulated fields are given as sequential static MLC (multi leaf collimator) shaped fields. A series of fields or segments from each beam direction are superimposed to achieve a modulation of intensity. The treatment planning can be performed as forward planning: the definition of the segment shapes is performed manually similar to conventional planning. IMRT plans can also be generated using the inverse treat-

ment planning: an optimization of fluence profiles for a fixed beam geometry takes place to obtain a dose distribution that best fits a series of dose constraints. As the mean dose to the parotid gland is a predictor for the salivary function of the glands after radiotherapy, this is often calculated in order to predict the NTCP value for the IMRT plans. Various studies showed reduction of the mean dose to the parotid glands using IMRT strategy¹⁶⁻²². IMRT plans also appeared to be better in reducing the mean parotid dose comparing to 3DCRT^{23,24}. The inverse planning strategies managed to improve the treatment plans due to a better sparing of the parotid glands compared to forward planning^{18,25}. However, in most studies a mean parotid gland dose beneath 25 Gy could not be achieved.

The reduction of the dose depends on the exact implementation of the IMRT technique, which determines the steepness of the dose gradient between the parotid glands and the planning target volumes (PTVs). In IMRT inverse treatment planning all dose constraints are related to a given contour. Therefore the choice of contours is important for dose painting. Especially the delineation of the elective node radiation can influence the mean dose to the parotid gland. For example lowering the cranial border of the level II lymph nodes from C1 to C2, in case of bilateral elective neck irradiation can reduce the mean dose to the contralateral parotid from 33 to 26 Gy when using IMRT plans. For the 3 DCRT plans only a reduction of 2 Gy (from 51 to 49 Gy) could be achieved²³. Another important point is the margins applied to the clinical target volume (CTV) in order to obtain the PTV. These margins can cause overlapping areas between the PTVs and the parotid glands, leading to high dose regions in these glands. The size of these margins depends on set-up uncertainties and organ motion. Van Asselen *et al.*²⁶ showed that, by improvement of the patient position accuracy, reduction of the CTV-PTV margin resulted in a decrease of the mean parotid dose by approximately 1.3 Gy per mm.

In most IMRT plans for patients with oropharyngeal or nasopharynx cancer, the parotid glands will receive a substantial dose, especially in the case of positive neck nodes. It probably is too optimistic to think that a low mean parotid gland dose can be achieved in all patients without risking a high incidence of tumor or neck node failure. So, IMRT can not offer a solution for parotid gland dysfunction in all cases. Consequently, we also should look for other strategies to further improve the possibilities of sparing parotid gland function. A better understanding of the mechanism of parotid gland radiosensitivity may be helpful.

Pilocarpine

Unfortunately, the underlying mechanism of radiation-induced injury to the salivary glands is still not exactly known. The differentiated salivary excretory cells have a slow turnover; nevertheless they are highly sensitive to radiation, as demonstrated by the functional changes shortly after the start of irradiation²⁷⁻²⁹. Information on the morphological changes that occur in human salivary glands following

radiation is limited. The largest study in humans examined salivary glands from surgical specimens removed 24 hours after exposure to single doses of 10-20 Gy³⁰. The glands exhibited acute inflammatory changes with infiltration by leukocytes and plasma cells. Marked degenerative changes in serous acinar cells were described along with pyknosis, cytoplasmic vacuolization, and loss of zymogen granules. Mucous glands showed little change. Microscopically the principal features of the chronic changes are atrophy and loss of serous acini, fibrosis, and chronic inflammation³¹. However, there is a great degree of variation in the extent of damage from patient to patient. Acinar cells are believed to be more radiosensitive than ductal cells. In humans it is not known to what extent the associated inflammation and eventual fibrosis are related to injury to the parenchymal cells and vascular/connective tissues. Most experimental studies on biological effects of radiation on salivary gland tissue have been conducted in animal models, generally in rodents and monkeys. In several of these animal studies it is concluded that the plasma membrane of the secretory cells is the likely target of the high radiosensitivity for the early effects. Late damage is mainly reproductive cell death linked to mitosis and damage by environmental changes³²⁻³⁵.

Understanding the exact pathogenesis of the radiosensitivity of the salivary glands can help us in finding strategies that may be beneficial for protecting the glands from irradiation-induced damage. Once chronic hyposalivation occurs, it cannot adequately be dealt with by the use of salivary replacements. The largest potential to manage oral complaints in humans comes from sialogogues studies. Attempts reported to improve radiation-induced salivary hypofunction include pharmacological stimulation with pilocarpine, a muscarinic-cholinergic receptor agonist with mild beta-adrenergic activity. Pilocarpine is well known for its largely parasympathetic stimulation. It has only slight side effects when used in a low dosage. It can increase secretions and diminish xerostomic complaints after the completion of radiotherapy in patients with sufficiently remaining exocrine tissue^{11,36-38}. Another attempt to improve radiation-induced salivary hypofunction and xerostomia is the concomitant use of pilocarpine with radiotherapy. In rodents, unilateral irradiation with graded doses of X-rays revealed that effects of a prophylactic pilocarpine administration were not restricted to the irradiated gland only, but also enhanced the flow rate in the contralateral, non-irradiated gland (chapter 6). This provides direct evidence that increasing the compensatory potential of the non-damaged gland, at least in part, underlies the radioprotective effect of pilocarpine in case of unilateral irradiation in rats. The ability of pilocarpine to ameliorate the early radiation-induced impairment of the parotid gland function appears to be dependent on the remaining number of functional cells, and thus the volume of the gland that lies within the radiation portal. However, long-term studies on the radioprotective effectiveness of a preirradiation treatment with pilocarpine in rats were unable to show substantial preservation of the gland function (*Coppes oral comm*).

In a collaborative study between Groningen and Utrecht, an analysis of pilocarpine-mediated protection in relation to radiation dose-volume criteria in humans has been performed. At the moment the results of this prospective randomized phase III trial are analyzed.

From our data so far, we propose that after radiotherapy for head-and-neck cancer, patients can be divided in three groups depending on the mean parotid gland doses:

1. below 25 Gy
2. between 25 and 50 Gy and
3. above 50 Gy.

When the parotid glands receive a mean dose of 25 Gy or less, it seems very likely that no reduction of parotid output is noticed. More advanced radiation techniques like IMRT can minimize the radiation dose to the parotid glands. A parotid gland mean dose below 25 Gy should be a planning goal if substantial sparing of the gland function is desired. When this can not be achieved, it is possible that patients with parotid glands receiving between 25 and 50 Gy may benefit from treatment with drugs, e.g. pilocarpine. Those patients with no sparing of the parotid glands and doses above 50 Gy on the parotid glands may have less chance to be among the favorable responders to pilocarpine.

Recommendations and future

MRI

Evaluation of parotid gland function should be done using measurements with Lashley cups, when looking at the separate glands. In addition, the excellent soft-tissue imaging capabilities of MRI may be helpful in assessing the salivary hypofunction after radiotherapy. Differences in gland volume or intensity and the visualization of the salivary duct architecture may provide useful information³⁹. We developed a special MRI protocol that includes T1 and T2 weighted MRI and MR sialography⁴⁰. MRI gives the opportunity to better delineate the glands. Using the MR sialography, the ducts could well be visualized. MRI might help to investigate radiation-induced changes in the parotid glands. These changes may correlate with the decreased parotid flow. Secondly, it can be studied whether the radiation-induced changes are correlated with the spatial dose distribution. Because MRI lacks the electron density information important for treatment planning, CT/MRI matching is necessary in order to evaluate MRI appearance and volume changes after radiotherapy with regard to the three-dimensional dose distribution. In our institute, it is possible to perform CT/MRI fusion: matching of images is performed using mu-

tual information matching. All images are obtained with the patient positioned on a flat table in the same plastic mould that is used for radiotherapy treatment. This ensures a very accurate co-registration of the CT and MRI.

Margins

The importance of reducing the position margins as stated previously, requires accurate patient position and therefore reliable treatment position verification. Implantation of gold markers, as done in prostate cancer, might be helpful to improve position accuracy during radiation treatment. The use of gold markers for position verification during radiotherapy seems safe and reliable⁴¹. Van Asselen *et al.*²⁶ stated that using gold markers it might be possible to reduce the margin from CTV to PTV to 3 mm and thus reduce the dose to the parotid glands. This may result in a NTCP reduction of approximately 20% (from 0.44 to 0.35). As margins shrink, there will be a need for high-resolution planning, delivery and verification systems.

Tomotherapy

Helical tomotherapy is a new dedicated image-guided IMRT system with on-board imaging capability and therefore differs from conventional treatment units^{42,43}. The ring gantry irradiation geometry of a helical CT scanner is combined with an intensity-modulated megavoltage fan beam. An on-board megavoltage CT scanner enables verification CT scans to be acquired before treatment. This information can be used to adjust the patient set-up or reconstruct the dose, if necessary. Helical tomotherapy allows also sharper dose gradients due to many beam directions. Eventually this may result in better sparing of the normal structures⁴⁴.

Drugs

From animal studies several other approaches have been presented to prevent or repair radiation damage to the salivary glands⁴⁵. For example the use of drugs before radiation treatment like radical scavengers, anti-oxidant enzymes, stimulators of the proliferation of acinar progenitor cells and, more recently, gene transfers technology and stem cell transplantation are studied⁴⁵. Of great concern is the difference between e.g. rodents and humans. It is not known whether the hypothesis on the underlying mechanism of irradiation-induced damage in animals, is exactly the same for humans. Furthermore, not all agents that proved to be beneficial in protecting the parotid glands in rodents are applicable in human conditions. For example adrenergic agonists like cycloctidine or isoproterenol are not likely to be useful because of systemic actions⁴⁶.

In humans, hyperbaric oxygen (HbO) therapy is used in the treatment of radiation-induced lesions in normal tissues. HbO therapy has been shown to durably increase vascular density in hypovascular irradiated tissue like skin, mucosa, and bone. As vascular depletion may also play a role in late salivary gland damage, this HbO therapy may have clinical implications.

Other considerations

In this thesis we concentrated on parotid gland function. The reason for this was that due to the conventional treatment plans and the great number of postoperative radiation treatments, in most of the patients' submandibular/sublingual glands received high doses or these glands were removed during operation. It is well known that the submandibular/sublingual glands are the main source of saliva in resting conditions, and the dose to these glands might have serious implications for the feeling of a dry mouth. Unstimulated whole salivary output may be a better indicator for assessment of xerostomia, since this represents the basal level of salivary output. By using more advanced radiation treatments like IMRT the relative contribution of the major and minor salivary glands becomes more important. Objective functions of all separate salivary glands should be measured to give a better definition of the dose constraints. The problem is that it is almost impossible to delineate and to measure the function of the minor salivary glands.

Conclusion

Preventing damage to the salivary glands that results from head-and-neck radiotherapy will require multiple approaches, probably each partially effective. It may include reduction of the exposed salivary gland treatment volume by highly conformal radiation dose distributions. It is possible that sparing certain parts within the gland is relevant. Preirradiation administration of agents like sialogogues or free radical scavengers can attribute to the prevention of damage. The results of new preventing strategies will continue to improve with the further development of the technologies and the increased expertise and involvement of radiation-oncologists, clinical physicists and radiobiologists.

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CHAPTER

Summary

Nederlandse samenvatting

Dankwoord

Curriculum Vitae

Radiotherapy is a common treatment for head-and-neck cancer patients. Unfortunately, it produces serious acute and long-term side effects to the oral cavity. One severe complication is the loss of salivary gland function, which can persist for many years. Saliva has multiple functions relating to speech, taste perception, mastication, and swallowing and bolus formation. Cleansing and dental and mucosal protection are also important functions. In this thesis, a detailed report is given on the effects of radiotherapy on changes in the parotid gland function. Different methods of measuring gland function are described. Moreover, a preventive measure to limit the gland toxicity was investigated in rodents.

The radiation field and, in particular, the volume of the parotid gland tissue exposed to radiation is of notable importance with regard to the development of gland damage and hypofunction. Therefore, it is important to achieve detailed information about the size and position of the parotid gland. In earlier studies bony structures were used to define the borders of the radiation fields. In **Chapter 2**, quantitative measurements of the volume of the parotid glands and of the relationship between the parotid glands and the surrounding bony structures were outlined. CT images and beams eye views of CT images projected on simulator films were used. Great differences in size and position of the parotid glands between the patients were seen. Therefore, it is essential to use CT-based simulation of radiation fields in order to exactly define the localization of the glands.

Parotid gland function of 108 patients treated with radiotherapy for various malignancies in the head-and-neck region were prospectively studied in detail. In **Chapter 3**, several parameters on parotid gland function before radiation treatment were evaluated. Stimulated parotid saliva was collected in ten minutes, simultaneously from both parotid glands with Lashley cups placed over the orifice of Stenson's duct. Stimulation of saliva flow was achieved by applying a 5% acid solution to the tongue. A considerable variability in stimulated parotid flow rate was found. No physiological or clinical variables including gender, age, tobacco and alcohol consumption, parotid volume, tumor location, T- and N-stage, or preradiotherapy surgery were correlated with parotid output. All these parameters apparently did not significantly change parotid gland function, in terms of stimulated flow rate, among patients with different head-and-neck malignancies.

The radiation tolerance of the parotid glands as a function of dose and volume irradiated was accurately described in **Chapter 4**. There are only a limited number of studies on dose-volume response relationships and earlier studies are difficult to compare because the volume of irradiated parotid tissue is either: not exactly defined, very variable or unknown. We used CT-based dose volume histogram analysis to quantitatively assess the radiation dose received by the parotid glands and the

volume of the glands irradiated. The stimulated parotid flow rate was used as a parameter for the assessment of parotid gland function and was measured before and 6 weeks, 6 months and 12 months after radiotherapy. The data were fitted using the NTCP model proposed by Lyman *et al.* The stimulated preradiotherapy flow rates of 174 parotid glands was 0.34 mL/min. The mean stimulated flow rate was reduced to 0.12 mL/min at 6 weeks postradiotherapy and slightly recovered to 0.17 and 0.20 mL/min at 6 months and 1-year postradiotherapy. Flow reduction depended on the mean parotid gland dose. For a posttreatment parotid flow ratio <25%, the TD_{50} (the dose to the whole organ leading to a complication probability of 50%) was found to be 31, 35 and 39 Gy at 6 weeks, 6 months and 1 year after radiotherapy respectively. A large volume dependency was found with no threshold effects for the mean parotid dose.

Chapter 5 evaluates the examination of the parotid glands by ^{99m}Tc -pertechnetate scintigraphy. The examination was performed with the patient under a gamma camera with high-resolution collimators. $^{99m}\text{TcO}_4$ is injected intravenously with computer acquisition of 1-minute frames for a total of 30 minutes. At the fifteenth minute the patient received citric acid, in order to provoke salivary secretion. For analysis of data regions of interest were selected over the parotid glands and corresponding time-activity curves were created. Uptake and the excretion response to citric acid were analyzed per patient and subsequently per individual gland before and 6 weeks and 12 months after radiotherapy. The mean maximal uptake of 192 parotid glands was 3329 ct/s before radiotherapy and decreased to 3084 ct/s and 3005 ct/s at 6 weeks and 1 year after radiotherapy. A significant correlation between the uptake 1-year postradiotherapy and the mean parotid dose was observed. The excretion, as assessed by the SEF (salivary excretion fraction) was 44.7% before radiotherapy. The SEF decreased to 18.7% at 6 weeks postradiotherapy, but recovered to a SEF of 32.4% at 1 year after treatment. When a complication was defined as a posttreatment SEF parotid ratio of <45%, the TD_{50} was found to be 29 and 43 Gy at 6 weeks and 1 year after radiotherapy. The reduction in excretion was dependent on the mean parotid gland dose, with some recovery of function, comparable with the flow results.

The consequences of parotid gland injury are still difficult to manage. Prophylactic treatment with sialogogues like the muscarinic receptor agonist pilocarpine has been shown to have radioprotective potential. It was suggested that the sparing effect of pretreatment with pilocarpine might be due to stimulation of salivary gland tissue outside the radiation portal. Therefore, the protective effect should decrease with increasing radiation dose and/or increasing irradiated gland volume. In **Chapter 6** the effects of preirradiation treatment of pilocarpine on rat parotid gland function were investigated in relation to radiation dose. Unilateral gland

radiation of male albino Wistar rats was performed with single doses of 10-30 Gy. Pilocarpine was administered intraperitoneally, 1 hour prior to irradiation. Separate left and right parotid gland saliva was collected using miniaturized Lashley cups 4 days before and 3, 7, 10 and 30 days after irradiation. Pretreatment with pilocarpine reduced the loss of function of the irradiated gland after single doses of 15 and 20 Gy. At 30 Gy this effect was lost. Yet, for all doses above 10 Gy, pilocarpine increased the flow rate in the nonirradiated gland. This latter result became apparent around 7 days after radiation treatment. Pilocarpine had no effect on the flow rate of animals that were not irradiated at all. So, pilocarpine induced compensation, which at least underlies the radioprotective effect of the drug. This effect seemed to be dependent on the amount of damage induced. Therefore, the type of fractionation scheme and the volume of the gland that lies within the radiation portal will be crucial for the effectiveness of a prophylactic pilocarpine treatment.

In **Chapter 7** we compared objective (as described in **Chapter 4** and **5**) and subjective methods to evaluate parotid gland function. The subjective assessment of a dry mouth was scored using the EORTC Head-and-Neck cancer Quality of Life Questionnaire (QLQ-H&N35). The single items dry mouth and sticky saliva and an additional item on overall health were used. The outcome of the different methods was correlated with the mean parotid gland dose. The best parameter for evaluation of the parotid gland function appeared to be the flow measurement using the Lashley cups. However, if direct flow measurements are not feasible, scintigraphy might be a good alternative.

With the more detailed knowledge on the dose/volume effects of radiation on parotid function, we can try to focus on sparing the parotid gland function. The prevention of radiation-induced loss of parotid gland function will depend on both optimal sparing radiation therapy techniques and on pharmacological agents, which can selectively interfere with the radiation-induced effects. These preventing strategies will need a joint effort of radiation-oncologists, clinical physicists and radiobiologists.

CHAPTER

Summary

Nederlandse samenvatting

Dankwoord

Curriculum Vitae

Wanneer patiënten met kanker in het hoofd-hals gebied bestraald worden liggen de grote speekselklieren, waaronder de oorspeekselklier (glandula parotis), vaak ten dele of geheel in het bestralingsveld. Deze speekselklieren zijn stralingsgevoelig. Daardoor kunnen patiënten na afloop van de bestraling last hebben van een permanent droge mond. Speeksel heeft tal van functies die voor ons dagelijks functioneren van groot belang zijn. Spreken, kauwen en slikken verlopen moeizamer wanneer de speekseluitscheiding sterk afneemt. Verder bevat speeksel een groot aantal componenten die betrokken zijn bij de bescherming van tanden, kiezen en zachte weefsels in de mond. In dit proefschrift wordt een gedetailleerde beschrijving gegeven van het effect van bestraling op de parotisFunctionie. Verschillende methoden om de speekselvloed te meten worden besproken. Bovendien is in een rattenstudie gekeken of een speciaal speekselsecretie inducerend farmacon de parotiden kan beschermen tegen de nadelige gevolgen van bestraling.

De verminderde speekselproductie na bestraling is afhankelijk van de bestralingsdosis en de hoeveelheid van het bestraalde speekselklierweefsel, oftewel het volume van de speekselklier gelegen binnen het bestralingsveld. Het is derhalve erg belangrijk om exact te weten waar de speekselklieren liggen. Tot voor kort werd voor het instellen van de bestralingsvelden gebruik gemaakt van botstructuren op doorlichtbeelden. Het bestraalde volume van de speekselklieren werd ingeschat aan de hand van deze botstructuren. In **Hoofdstuk 2** is gebruik gemaakt van CT beelden om de ligging van de parotiden en daarmee het bestraalde volume nauwkeuriger te kunnen vastleggen. Op CT opnames van de patiënt werden de parotiden ingetekend. Reconstructies van deze intekening werden over de röntgenopnamen van de bestralingsinstelling (simulator films) gelegd. De boven-, onder- en zijkanten van de parotiden werden ten opzichte van een vaste centrale botstructuur (tuberculum anterior van de atlas) bepaald. Tevens werd het volume van de parotiden gemeten. Grote verschillen in volumina en ligging van de speekselklieren werden gezien. Zo varieerde de afstand tussen de bovenzijde van de parotis en het tuberculum tussen de 0.7 en 4.8 cm. Het is gebleken dat het dus niet betrouwbaar is om gebruik te maken van de simulator films om de positie van de parotiden te bepalen.

In een groep van 108 patiënten met een grote variëteit aan hoofd-hals tumoren is prospectief de parotisFunctionie na radiotherapie bepaald. In **Hoofdstuk 3** zijn verschillende factoren welke invloed kunnen hebben op de speekselvloed voorafgaande aan de bestraling geëvalueerd. Het speeksel van linker en rechter parotis werd na prikkeling met citroenzuur geïsoleerd opgevangen met behulp van speciale (Lashley) cups. De speekselsecretie varieerde enorm. Géén van de onderzochte factoren (geslacht, leeftijd, alcoholgebruik, rookgedrag, parotismvolume, tumorlocatie, grootte en uitbreiding van de tumor, chirurgie voorafgaand aan de radiotherapie) hing samen met de hoeveelheid speeksel.

In **Hoofdstuk 4** zijn de resultaten van de parotisfunctie gerelateerd aan de dosis en volume berekeningen van de bestraalde parotiden. De bij aanvang van dit onderzoek uit de literatuur beschikbare data betreffende de speekselklierfunctie waren moeilijk te vergelijken omdat de hoeveelheid in het bestralingsveld gelegen parotisweefsel niet exact was beschreven. Reconstructie van bestralingsvelden vond merendeels plaats met behulp van simulatiefilms. In onze studie werden, gebruikmakend van dosis-volume histogrammen gemaakt aan de hand van CT-intekening, op een nauwkeurige manier de effecten van gegeven bestralingsdosis en bestraald parotivolume vastgelegd. De parotisfunctie werd bepaald door middel van speekselvloed metingen met Lashley cups. De metingen werden verricht voorafgaand aan en 6 weken, 6 maanden en 1 jaar na de radiotherapie. Data analyse vond plaats gebruikmakend van het NTCP model volgens Lyman. De speekselsecretie voorafgaand aan de radiotherapie bedroeg 0.34 mL/min en daalde naar 0.12 mL/min 6 weken na de bestraling. Herstel van functie trad op in de loop van de tijd met waarden van 0.17 en 0.20 mL/min 6 maanden respectievelijk 1 jaar na radiotherapie. De afname in secretie was afhankelijk van de gemiddelde dosis in de parotis. De NTCP model parameter TD_{50} (de dosis op de gehele parotis welke leidt tot een complicatie kans van 50%) bedroeg 31, 35 en 39 Gy voor 6 weken, 6 maanden en 1 jaar na bestraling, wanneer een complicatie werd gedefinieerd als speekselvloed <25% van de uitgangswaarde. Een grote volume afhankelijkheid werd gevonden zonder drempelwaarden voor de bestralingsdosis.

Middels speekselklierscintigrafie kan ook het functieverlies van de glandula parotis na bestraling worden beoordeeld. De patiënt krijgt intraveneus ^{99m}Tc -pertechnetaat toegediend. Dit wordt selectief opgenomen en uitgescheiden door de speekselklieren. De activiteit die gedurende het onderzoek zichtbaar wordt in het gebied van de mondholte, wordt veroorzaakt door radioactief speeksel, uitgescheiden door de speekselklieren. Om de maximale functionele capaciteit van de klieren te kunnen beoordelen wordt 15 minuten na de pertechnetaat injectie citroenzuur in de mond toegediend. De scintigrammen en curves worden beoordeeld op de mate van opname en gestimuleerde uitscheiding van pertechnetaat. In **Hoofdstuk 5** is de relatie tussen dosis/volume parameters en de parotisfunctie bepaald middels scintigrammen beschreven. Voorafgaand aan en 6 weken en 1 jaar na de bestraling werd gemeten. De gemiddelde opname was 3329 ct/s vóór radiotherapie en daalde naar 3084 en 3005 ct/s 6 weken en 1 jaar na radiotherapie. Een significante correlatie tussen de opname 1 jaar na radiotherapie en de gemiddelde dosis in de parotis werd gevonden. De uitscheiding vastgelegd middels de SEF (speeksel excretie fractie) was 44.7% voor bestraling. Deze daalde tot 18.7% 6 weken na radiotherapie, maar herstelde tot 32.4% 1 jaar na de behandeling. Ook hier werd gebruik gemaakt van de TD_{50} waarden. Wanneer een complicatie was gedefinieerd als een SEF ratio (t.o.v. voor de bestraling) <45%, was de TD_{50} 29 en 43 Gy 6 weken respectievelijk

1 jaar na radiotherapie. De afname in excretie en het herstel van functie in de loop van de tijd was, net als bij de metingen met de cups, afhankelijk van de gemiddelde dosis in de parotis.

De gevolgen van verminderde parotischfunctie zijn nog steeds erg moeilijk te behandelen. Uit eerdere studies is gebleken dat behandeling met secretie inducerende farmaca voorafgaand aan de bestraling, de speekselklier kon beschermen tegen de nadelige gevolgen van bestraling. Voor pilocarpine werd gevonden dat dit beschermend effect vooral werd veroorzaakt door zogenaamde compensatoire effecten. In ratten (**Hoofdstuk 6**) bleken deze effecten sterk afhankelijk van stralingsdosis en het volume bestraald weefsel. Wistar ratten ondergingen een éézijdige klierbestraling. Speekselproductie van de parotiden werd gemeten met behulp van mini Lashley cups. Pilocarpine gegeven voorafgaand aan de bestraling verminderde het verlies van functie in bestraalde klieren. Dit effect was dosis afhankelijk. Echter, pilocarpine zorgde ook voor een toename in speekselvloed van de niet bestraalde klier. Er was geen effect van het middel op klieren van niet bestraalde ratten.

In **Hoofdstuk 7** zijn we op zoek gegaan naar de beste parameter om de parotischfunctie te voorspellen. De metingen met de Lashley cups, de speekselklierscintigrammen en de subjectieve beleving zoals gemeten met de EORTC QLQ-H&N35 vragenlijst werden gecorreleerd met de gemiddelde dosis in de parotis. De meetmethode met de cups bleek de beste parameter om parotischfunctie te evalueren. Echter, wanneer de meting met de cups niet mogelijk is, is de scintigrafie een goed alternatief.

Nu we de exacte dosis/volume parameters weten die van invloed zijn op de parotischfunctie na bestraling zal verder gewerkt moeten worden aan het voorkómen van dit functieverlies. De preventie van stralings geïnduceerde schade zal gezocht moeten worden in nieuwe bestralingstechnieken en in de beschermende effecten van farmaca. Een goede samenwerking tussen radiotherapeuten, klinisch fysici en radiobiologen is hierbij onontbeerlijk.

CHAPTER

Summary

Nederlandse samenvatting

Dankwoord

Curriculum Vitae

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CHAPTER

Summary

Nederlandse samenvatting

Dankwoord

Curriculum Vitae

Judith Roesink werd geboren op 9 september 1966 in Almelo. In 1984 slaagde zij voor het VWO-diploma aan het Pius-X-College te Almelo. Aansluitend startte zij met de studie geneeskunde aan de Rijksuniversiteit Groningen. Na het artsexamen in 1991 werkte zij als arts-assistent radiotherapie in het Universitair Medisch Centrum Utrecht (UMCU). Van januari 1992 tot en met december 1993 volgde zij een KWF/NKB fellowship Radiobiologie. Daarna ving de opleiding tot radiotherapeut aan in het UMCU (hoofd en opleider Prof. Dr. J.J. Battermann). Sinds januari 1999 is zij als radiotherapeut verbonden aan het UMCU. Judith is getrouwd met Jeroen Smienk en samen hebben ze een zoon, Hugo en een dochter, Isabel.