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Scintigraphic Assessment of Salivary Gland Function in a Rat Model

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Abstract. *Background: Salivary gland dysfunction with xerostomia is a major clinical problem without a causal therapy in most cases. The development of an animal model for scintigraphic assessment of salivary gland function has great clinical relevance for the investigation of promising new diagnostic and therapeutic strategies for chronic salivary gland diseases. This study reports the first experiences with scintigraphic analyses of salivary gland function in a rat model. Materials and Methods: Anatomical and scintigraphic studies were performed for topographic differentiation of major salivary glands of Wistar rats. ^{99m}technetium pertechnetate salivary gland scanning was performed, appropriate regions of interest were determined and the gland-to-background ratio was examined for the evaluation of salivary gland function. Results: The quantitative analysis of salivary gland scintigraphy revealed a reliable comparison of major salivary glands on both sides with the gland-to-background ratio ranging from 1.26 to 1.94 with an average of 1.51. Conclusion: This model seems to be appropriate for functional studies in an experimental setting.*

Chronic inflammatory diseases of the salivary glands are the most frequent cause of their dysfunction. Beside clinical examination, imaging procedures such as ultrasound, sialography and computed tomography are used for the indirect evaluation of salivary gland function. In some cases, standardised scoring schemes are used to assess the patients' impairment (4). Moreover, magnetic resonance imaging allows the estimation of gland function by signal intensity examinations (6, 28). However, the debate on the most reliable and suitable parameters for the diagnosis of loss of salivary gland function persists (29). Direct evaluation of salivary gland function may be performed by analysis of saliva after its collection (17, 19). The saliva can be collected directly from main excretory duct of the parotid and submandibular gland or by collection of saliva in the area of the main duct orifices. However, these methods are characterised by high inter-observer variability and low reproducibility.

Salivary gland scintigraphy, first described by Boerner *et al.* in 1965, is meanwhile a standard procedure in the clinical routine (3, 10). This method is well established for the evaluation of salivary gland function and shows significant correlation between scintigraphic findings and measured salivary flow rates (9, 26). The clinical impact of salivary gland scintigraphy has been reported especially for the diagnosis and clinical monitoring in chronic inflammatory diseases such as Sjögren's syndrome (5, 23, 28) and radiation-induced sialadenitis (14, 22). This standard procedure is non-invasive, can be repeated several times, is easy to perform and is well tolerated by the patients. The radiation exposure is about 1 mSv and can be considered as low compared to other radiological procedures with radiation exposures up to more than 30 mSv for a spiral CT (31).

For the technical improvement of salivary gland scintigraphy, evaluation of new radiotracers, and investigation of new treatment strategies, especially for chronic inflammatory diseases of salivary glands, an animal model for analyses of salivary gland function has high clinical relevance. In this study, a rat model for scintigraphic evaluation of salivary gland function is introduced.

Materials and Methods

Rats and anaesthesia. Eight adult male Wistar rats weighing 300-350 g were used. The animals were housed in the animal care centre under controlled light and environmental conditions. Food and water were supplied *ad libitum*. Subsequent experiments

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were approved by the University Animal Care Committee. All experiments were performed under anaesthesia using a mixed injection of 50 mg/kg ketamine (Ketavet; Alverta&Werfft AG, Neumünster, Germany) and 2.5 mg/kg Xylazin (Rompun; Bayer Health Care, Leverkusen, Germany). After sedation of the animals with CO_2, anaesthesia was conducted by intramuscular application in the rear femoral musculature. All animals awoke approximately after 45 minutes after anaesthesia and recovered well.

Topographic differentiation of the major salivary glands. The animals were anaesthetized and the cervical region was exposed by a horizontal neck incision. The topographic anatomy, the localisation of the major salivary glands and their relationship to each other were studied. To determine the exact position of the submandibular gland in relation to the parotid gland, a so-called dual isotope scintigraphy was performed (25). The submandibular gland was visualised by intraglandular injection of 1 MBq ^{99m}technetium (^{99m}Tc) pertechnetate while the parotid gland was labelled with the same activity of ^{111}indium-DTPA (diethylenetriaminepentaacetic acid). The cervical incision was closed with sutures. Subsequently static scintigraphy was performed, as described below.

Dynamic scintigraphy. The temporal accumulation of pertechnetate in the salivary glands was examined in all rats. After injection of 74 MBq ^{99m}Tc pertechnetate (in 0.3 ml), data was acquired for 35 minutes on a ECT-Gamma camera (13) (ECAM; Siemens, Erlangen, Germany). The animal was lying face down in a plastic box that was directly placed on the detector of the gamma-camera. The scan was processed using the ICON-Software (Siemens, Erlangen, Germany).

In analogy to functional scintigraphy in humans, 0.5 ml lemon juice was administered orally 10 minutes after the injection of ^{99m}Tc pertechnetate in 3 rats. In the remaining 5 rats, an intravenous injection of 1 mg/kg physostigmine followed 10 minutes after the injection of ^{99m}Tc pertechnetate (30). Scintigraphy was conducted for 25 minutes.

Static scintigraphy. Static scintigraphy was performed for the evaluation of salivary gland function in all rats. After injection of 74 MBq ^{99m}Tc pertechnetate (in 0.3 ml), scans were acquired by a digital gamma camera (Nucline TH/22-Gammakamera; Mediso Medical Imaging Systems, Laer, Germany) with a field of view of 180×180 mm and a matrix of 128×128 pixels, using a low energy high resolution collimator. Imaging started 10 minutes after injection and was conducted up to a quantity of 300,000 impulses, which took generally three to four minutes. The raw data were transferred to a workstation (Hermes Nuclear Diagnostics, Hermes Medical Solutions, Stockholm, Sweden). The processing software Gold (Hermes Nuclear Diagnostics) was used on SunOS Release 5.8 Generic operating system (Sun Microsystems, Inc, Santa Clara, CA, USA). The uptake of the salivary glands was determined by a region of interest (ROI) technique (1). All ROIs were defined manually for each single rat and gland region. The background activity was placed in the supraclavicular area. For the evaluation of glandular function, the ratio of the accumulation in the gland ROI to the accumulation in the background ROI, termed gland-to-background ration, was used.

Table I. *Average counts in the regions of interest (ROIs) in the right and the left salivary glands and the background. Gland-to-background ratio was calculated for both sides (n=8).*

	Analysis of the regions of interest (ROI)				
	Counts (average)			Gland-to-background ratio	
	Right	Left	Background	Right	Left
Rat 1	107.0	99.5	70.0	1.53	1.42
Rat 2	101.0	98.7	78.4	1.29	1.26
Rat 3	93.1	94.6	62.6	1.49	1.51
Rat 4	142.0	143.0	103.0	1.38	1.39
Rat 5	153.0	141.0	78.7	1.94	1.79
Rat 6	160.0	164.0	96.8	1.65	1.69
Rat 7	149.0	150.0	103.0	1.45	1.46
Rat 8	147.0	147.0	100.0	1.47	1.47

Results

After neck incision and exposure of the major salivary glands the submandibular gland impressed as the best definable salivary gland was surrounded by a well-marked capsule. The lateral margin was partially overlapped by the parotid gland, which layd diffuse and not well-bordered in the surrounding fat tissue. The sublingual gland appeared notably smaller and was found on the upper pole of the submandibular gland (Figure 1a).

In the dual isotope scintigraphy, the submandibular and the parotid glands on both sides were visualised *in vivo*. A small part of the activity of ^{99m}Tc pertechnetate was resorbed by the surrounding tissue and served as an anatomical landmark. The parotid gland partially overlapped the submandibular gland as shown in Figure 1b.

Dynamic scintigraphy revealed a fast accumulation of ^{99m}Tc pertechnetate in the first minutes. After approximately 10 minutes, a plateau was reached. Without a stimulus, activity decreased slowly in the following minutes (Figure 2).

The administration of lemon juice showed no significant decrease in the time–activity curve (data not shown). In contrast, the parasympathetic stimulation with physostigmine provoked a decrease of activity of about 20% (Figure 3).

In static scintigraphy, a clear accumulation in the area of the major salivary glands was seen in the Wistar rats. Due to their close anatomical proximity, the parotid gland could not be differentiated from the submandibular gland or the sublingual gland (Figure 4a). The right, left and background ROI were used for the calculation of the gland-to-background-ratio.

In investigations in human beings (Figure 4b), the differentiation between parotid gland and the other salivary

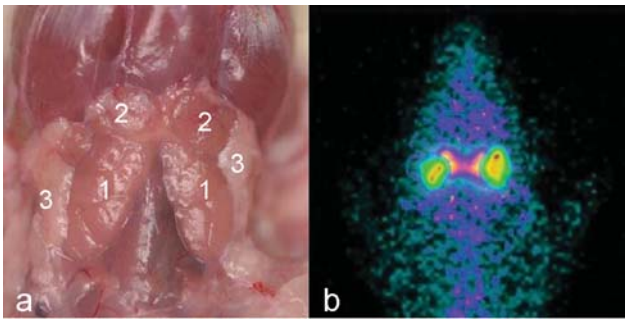
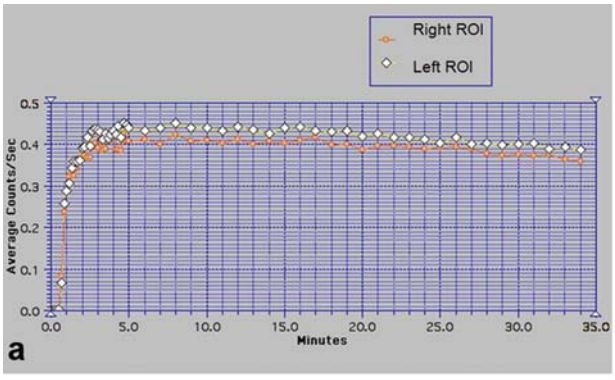


Figure 1. *Major salivary glands of the Wistar rat. a: Topographic anatomy, 1: submandibular gland, 2: sublingual gland, 3: parotid gland. b: Representation of the parotid gland (lateral) and the submandibular gland (medial) by dual isotope scintigraphy on both sides.*



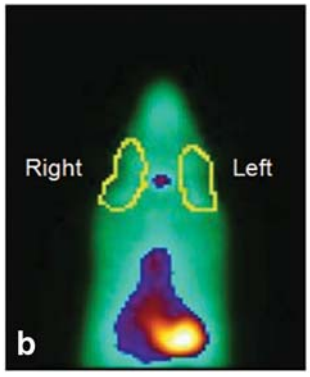


Figure 2. *Time–activity curve of ^{99m}Tc pertechnetate in the major salivary glands of an induvidual Wistar rat. a: Plot of the radioactivity within the right and left region of interest (ROI). b: Charting of the ROIs by the software drawing tool. Head, neck, chest and upper abdomen of the rat can be identified. The accumulation in the upper abdomen is caused by the stomach.*

glands is possible but there is no differentiation feasible for the submandibular gland or the sublingual gland.

The gland-to-background ratio varied from 1.26 to 1.94 with an average of 1.51. There was only a small intrasubject difference between the left and right side, not exceeding 8.5% (Table I).

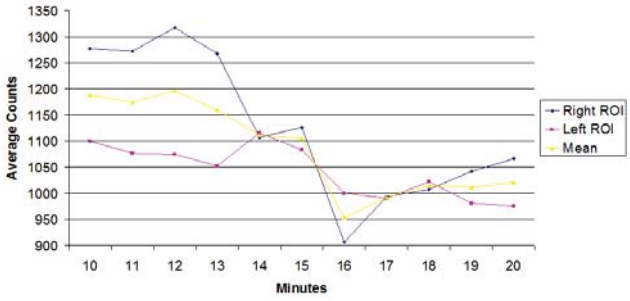


Figure 3. *Plot of the dynamic scintigraphy of another individual Wistar rat starting 10 minutes after injection of ^{99m}Tc pertechnetate and 0 minutes after injection of physostigmine. A decrease of activity about 5 minutes after physostigmine injection can be shown. Plot indicates the values of right and left ROI and their arithmetic mean. The charting of the ROIs is analogous to that shown in Figure 2b.*

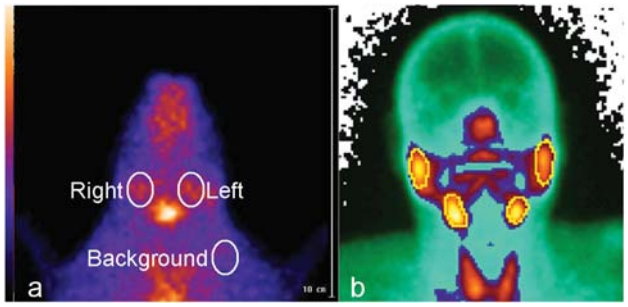


Figure 4. *Regions of interest (ROIs) in scintigraphic images of salivary glands in rat and man. a: ROIs for the calculation of the gland-to-background-ratio without differentiation between the major salivary glands. b: The upper ROI indicates the parotid gland while there is no differentiation between submandibular and sublingual gland (lower ROI) possible in humans.*

Discussion

Dry mouth with long-standing xerostomia is a particular problem in Sjögren's syndrome and after radiotherapy of the head and neck region. Uncontrolled diabetes mellitus, various chemotherapeutics and many commonly used drugs cause abnormal reduction of saliva. Xerostomia is accompanied by oral soreness and burning sensations, difficulty in mastication, swallowing, speech and altered or diminished taste acuity and leads to a considerably reduced quality of life (2). The treatment is frustrating both for the patients and the clinicians with no causal treatment for many cases of xerostomia. There is an urgent need for improved diagnostic and new treatment strategies for patients with salivary gland dysfunction (2).

Salivary gland scintigraphy with ^{99m}Tc pertechnetate is a standard and well-established procedure for the evaluation of human salivary gland function (7). Pertechnetate has a

comparable size and charge to iodide ions and is taken up therefore like iodine *via* the sodium/iodide symporter. The sodium/iodide symporter is mainly found in the thyroid gland and so the use of ^{99m}Tc pertechnetate is the gold standard in thyroid scintigraphy. However, it is also found in extrathyroidal tissues such as the salivary glands and the mucus-producing cells of the gastrointestinal tract (8, 11).

In the present study, a rat model was introduced for scintigraphic evaluation of salivary gland function. It is well-known that the salivary glands of rats are histologically and anatomically comparable to those of humans. In addition, the Wistar rat is the best studied animal model for salivary glands (12, 15, 16). For rats, a similar distribution of the sodium/iodide symporter is described (8), which suggests the suitability of these animals for scintigraphic studies of the salivary glands.

The results of the present study showed that this model seems to be appropriate for functional studies in an experimental setting. As a non-invasive procedure, scintigraphy of the salivary glands allows repeated analysis without impairment of the animals. In the present study, the determination of gland function using the gland-to-background ratio did not depend on the injected amount of activity or partial para-venous injection. The uptake of the salivary glands (percentage of injected dose) was not determined in the present study since there are many critical parameters that are either unknown or difficult to measure. Parameters that have deep impact on the accuracy of the determination of the uptake are the amount of the para-venous dose, the depth of anaesthesia (dose of ketamine, breathing) or the weight of the rat especially in follow-up studies (10, 20).

To the best of the Authors' knowledge, the only study of scintigraphic examinations in rats was published by Sagowski *et al*. in 2003 (21, 22). However, in that study, no scintigraphic images were shown and no description of the method was given. There are also published studies of salivary gland scintigraphy using other rodents such as rabbits (24); however these are more labour- and cost-intensive procedures compared to the use of Wistar rats in the present studies.

Ketamine is one of the most common anaesthetic agents used for small animals. As a side-effect, ketamine anaesthesia may lead to hypersalivation (30). Hypersalivation causes an early maximum of the time-activity curve in dynamic scintigraphy due to early excretion of salvia (18). Besides, the stimulation of salivary flow at a defined point is limited if there is a continuous stimulus caused by ketamine. Therefore, a relatively large amount of radioactivity was chosen in this study, namely 74 MBq (2 mCi). Other reasons for using such large amount of radioactivity were the favoured short time of acquisition and the sometimes inevitable partial para-venous injection (10, 20). The complete and fast intravenous injection of drugs in rats would require an intravenous port system (27).

Compared to salivary gland scintigraphy in humans, limitations result from the small anatomical dimensions and the close topographic relationship of the three major salivary glands and the thyroid gland in the rat. The scintigraphic differentiation of those glands is not possible in the rat because of their close topographic relationship. However, this model allowed the comparison of the left and right side and showed reliable results in the preliminary examination described in this study. Recently, this model was applied successfully in an animal study (27).

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