

Functional activity of rabbit salivary glands in reduced and restored regional arterial blood supply conditions

Kaspars Stammers, Andrejs Skagers, Kalvis Pastars, Nonna Tomisheva, Maija Ratniece

SUMMARY

Background. Although the vascular pathology of carotid arteries is widespread, the function of salivary glands in reduced arterial flow conditions is not much investigated clinically and in experiments. At the same time blood supply is a keystone to normal functioning of every organ and especially of salivary secretion. The aim of this study was to estimate functional activity of salivary glands in reduced and restored blood supply conditions in experiment by sialoscintigraphy which is an approved method for functional assessment of salivary glands.

Methods. The ligation of *a. carotis communis dextra* was performed on 20 Californian rabbits. After 28 days sialoscintigraphy with Tc99 pertechnetate and revascularization through resection of the occluded part of *a. carotis communis* and reconstruction with venous autograft was performed. One month later sialoscintigraphy was done.

Results. The functional activity of rabbit salivary glands after the ligation of *a. carotis communis* is strongly depressed. The revascularized glands accumulated isotope slowly, but the level of accumulation was higher than on the control side.

Conclusion. The ligation and reconstruction of a common carotid artery on rabbits confirm the important role of the arterial blood supply in functional activity of salivary glands and may be an appropriate experimental model for investigation of ischemic disease of salivary glands.

Key words: sialoscintigraphy, carotid artery ligation.

INTRODUCTION

The occlusive diseases of carotid arteries are widespread and often accompanied by disturbances of cerebral circulation, like stroke and other vascular diseases which represent a significant public health problem. Stroke accounted for about one of every 16 deaths in the USA in 2004 and up to 25% are related to stenosis of the carotid arteries treated by carotid endarterectomy procedures on 98,000 inpatients in 2004 (1).

Regional hypoxia and ischemia cannot be without consequences also for tissue and organs of oro-maxillo-

facial region having blood supply from external carotid artery (2). Vascular pathology largely accounts for different diseases of salivary gland. In Sjogren's syndrome five stages for morphogenesis of arteritis are proposed: endothelial swelling, thrombosis, fibrinoid degeneration, necrotizing panarteritis and endarteritis obliterans which are closely connected with glandular tissue pathology (3). The experimental model of oro-maxillo-facial ischemic disease may be useful for evaluation of angiogenic pathology of different organs in this region and perhaps also for development of new treatment methods. Ductal obstruction is more often used in investigations on experimental pathology of salivary glands (4) and has vascular and secretory responses (5).

Functional activity of major salivary glands may be tested using scintigraphy with ^{99m}Tc – technetium pertechnetate (Tc-99) which has been used in clinical conditions during the three last decades for functional assessment in cases of Sjogren's syndrome (6,7,8,9,10), xerostomy after radiotherapy of thyroid and oro-facial cancer (11,12,13,14), sialolithiasis (15), and gastro-esophagal reflux disease (16). The use of Tc-99 scintigraphy is proposed for checking radioprotection of sali-

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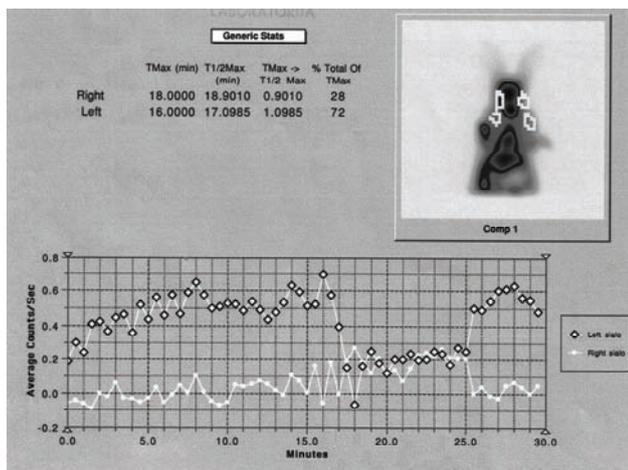


Fig. 1. Scintigramms of rabbit's salivary glands with Tc99: Right side – 28 days after ligation of common carotid artery; left side – control; stimulation at 15th minute

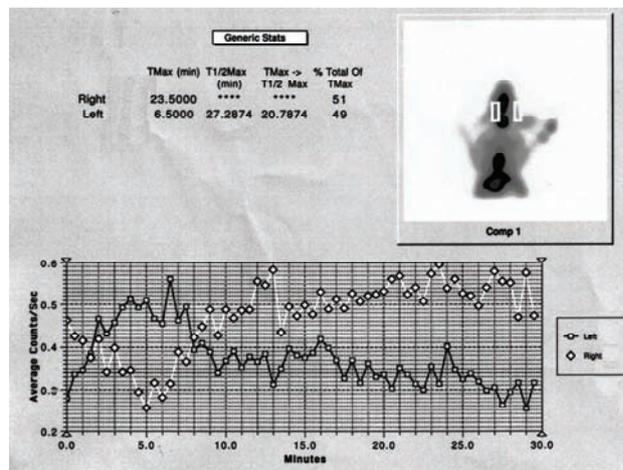


Fig. 2. Scintigramms of rabbit's salivary glands with Tc99: Right side – 28 days after reconstruction of common carotid artery; left side – control; stimulation at 15th minute

salivary glands (17). There is not yet a clear relationship between Tc-99 scintigraphic data and saliva secretion but there is a significant correlation between healthy and xerostomic patients (18), age and gender differences are also estimated (19). The quantitative analysis of time active scintigraphy curve usually includes the uptake rate and the washout fraction parameters enable objective testing of salivary gland function (20). The uptake parameters are only sensitive to differences exceeding 25% of gland mass and are not useful to detect the kind of disease causing xerostomia (10).

Our previous report (21) showed depressed functional activity of salivary glands after ligation of *a. carotis communis* on rabbits. The aim of this study was to estimate functional activity of salivary glands in reduced blood supply conditions in experiment by sialoscintigraphy.

MATERIALS AND METHODS

The experiments were authorized by the Animal Ethics Committee of the Latvian Food and Veterinary

Table 1. Maximal level of counts per second in accumulation phase (counts/sec) on side of *a. carotis communis* ligation (test) and control sides

No.	Control	Test (ligature of <i>a. carotis communis</i>)
1	0.35	0.15
2	0.40	0.10
3	0.70	0.10
4	0.45	0.15
5	0.60	0.20
6	0.60	0.10
7	0.50	0.15
8	0.35	0.10
9	0.45	0.15
10	0.40	0.10
11	0.35	0.20
12	0.55	0.15

Service. Twenty male Californian rabbits weighing approximately 3 kg were used. Under intravenous general anesthesia with Diazepam 2 mg/kg and 5% Ketamin hydrochloride 15 mg/kg and local infiltration with 0.5% Lidocain solution 5 ml, the ligation of *a. carotis communis dextra* was performed with 4-0 silk and the wound was closed. After 28 days under general anesthesia 6 MBq/kg Tc99 pertechnetate was injected intravenously and sialoscintigraphy using Siemens E.CAM equipment was performed on 12 rabbits (Fig. 1).

After 15 minutes salivary excretion was stimulated with five drops of lemon juice. Following the scintigraphy, 12 rabbits were sacrificed with over dosage of anesthesia and salivary glands were taken out for histological and immunohistochemical evaluation.

After general and local anesthesia, revascularisation was attempted on 8 rabbits: in two cases with extensive thrombosis resection of the occluded part of common carotid artery, reconstruction was performed with autovenous graft from *v. jugularis interna* using Monosof 8-0 sutures and 6X magnification; two

Table 2. The Bolton anterior ratio in different malocclusion

No.	Control	Test (ligature of <i>a. carotis communis</i>)
1	6	15
2	4	constant graphic representation
3	6	10
4	3	12
5	5	constant graphic representation
6	3	25
7	6	13
8	6	13
9	5	14
10	6	12
11	4	13
12	5	11

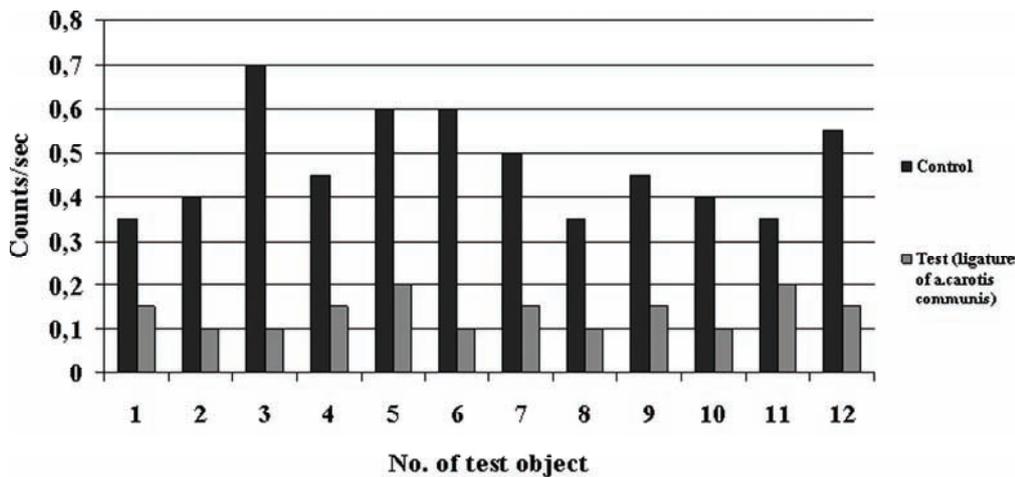


Fig. 3. Maximal counts per second in accumulation phase (counts/sec) in test (ligature of *a. carotis communis dxt*) and control sides

of those rabbits died from suppurative complications in early postoperative period. On 6 rabbits thrombosis of common carotid artery was not extensive and after resection the end-to-end anastomosis was possible; these rabbits survived and 28 days later sialoscintigraphy (Fig. 2) was performed on them, then the rabbits were sacrificed under general anesthesia.

RESULTS

After intravenous injection of Tc99 in salivary glands on the side of *a. carotis communis* ligature, small accumulation of radioactive isotope (mean 0.14 counts/sec, SD 0.04) was observed; while on the healthy side the glands accumulated isotope (mean 0.48 counts/sec SD 0.12) 2–3 times more (Table 1 and Fig 3). Comparing mean values with t test the result was $P < 0.01$

The mean difference in Tc99 accumulation between the test and the control side was 0.34 counts per sec with SD 0.12.

The duration of isotope accumulation was different for the test and the control side. On the control side

Table 3. Response of both side salivary glands after stimulation with lemon juice (counts/sec).

No.	Control side	Test side (ligature of <i>a. carotis communis</i>)
1	-0.25	0.05
2	-0.40	0.30
3	-0.20	0.10
4	-0.30	0.10
5	-0.35	0.20
6	-0.60	0.20
7	-0.40	0.15
8	-0.35	0.10
9	-0.20	0.10
10	-0.50	0.20
11	-0.55	0.10
12	-0.30	0.10

the maximum of accumulation was reached in 5 minutes on average (SD was 1.16), but on the test side it was reached only in 14 minutes on average (SD was 4.18); in two cases the graphic representation was constant (Table 2).

After stimulation with lemon juice, the response in salivary glands of both sides showed fast excretion, the mean being -0.42 counts per sec (SD was 0.17) on the

control side, and small secretion $+0.15$ counts per second (SD was 0.07) on the ligature side (Table 3).

After intravenous injection of Tc99, accumulation of isotope in salivary glands on the side of *a. carotis communis* ligature was on average 0.14 counts/min., SD 0.04. While salivary glands on the healthy side accumulated Tc 99 on average 0.48 counts/min., SD 0.12. The maximum of Tc99 accumulation on the healthy side was reached on average in 5 minutes, but on the test side – on average in 14 minutes.

On 6 rabbits 28 days after revascularization the sialoscintigraphy showed changes in functions as compared to the glands only with ligatures (Fig 4). During the accumulation phase, the control side showed the maximal accumulation in the 6th minute (SD 0.75), but the reconstructed (test) side reached the maximum mean accumulation in the 13th minute (SD 1.21) (Table 4). Statistical significance for revascularized values was $P < 0.001$.

Comparing the differences in accumulation between both sides, they were quite similar, even the test side reached a higher accumulation than the control side (Table 5).

The mean difference in accumulation between the test and the control side was 0.12 counts/per sec with SD 0.13. The revascularized glands can slowly accumulate isotope, but the accumulation remains spastic until the end of examination.

DISCUSSION

There is a close relationship between function of autonomous neural system and blood supply in salivary secretion. Salivary secretion is an active two-stage process. Primary secrete produced by acini has ionic concentration similar to blood plasma (22,23). Continuous stimulation of the parasympathetic *ramus com-*

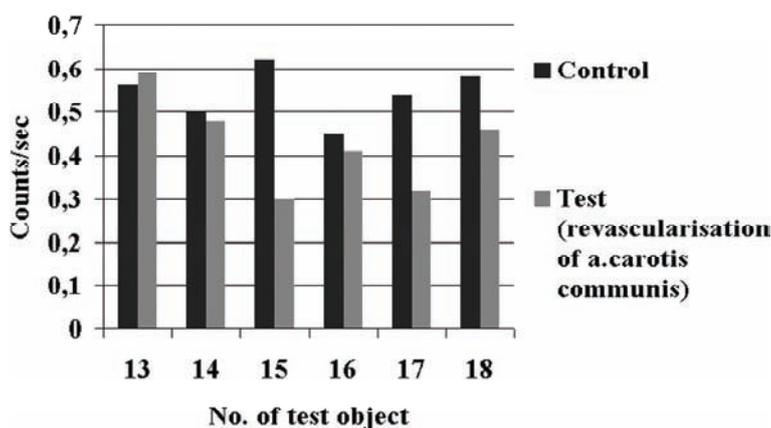


Fig. 4. Maximal counts per second in accumulation phase (counts/sec) in test (revascularization of *a. carotis communis dxt*) and control sides

municans of mandibular ganglion increased salivary gland arterial inflow and salivary secretion at low and moderate levels of parasympathetic stimulation not affected by reduction or cessation in arterial inflow, whereas the response to high level parasympathetic stimulation was significantly alleviated if blood flow to the gland was maintained at a level less than that of the resting arterial inflow (12). Clamping of carotid artery in rats reduced interstitial fluid pressure and glandular blood flow (-56.5±8.4% and -53.1±6.4 respectively, whereas clamping of jugular vein decreased glandular blood flow (21.6±14.3%) and increased interstitial fluid pressure (141.2±27.4%) (24).

Different methods in experiments are used to create carotid artery stenosis and occlusion. Tsuruta used rotation of balloon catheter/guidewire system inserted retrogradely from the external carotid artery into the common carotid artery and got homogenous stenoses due to intimal hyperplasia (25). Reduction of regional blood flow by intracarotid infusion of vasoconstrictor peptide endothelin-1 (ET-1) which acts directly on vascular smooth muscle cells diminished blood flow of submandibular salivary glands of anesthetized sheep by 48±4% and the flow of saliva to parasympathetic chorda – lingual stimulation by 50±1% due to diminution in the output of Na⁺, K⁺, and protein in saliva. Flow of saliva consistently amounted to 10% of the blood flow before, during and after the infusion of endothelin-

1 (26). Another group reported on reduction of blood flow through submandibular gland also in sheep by 56±5% and salivary secretion by 44±6% after intracarotid infusion of ET-1 (27). In anesthetized cat's intracarotid infusion of ET-1 reduced blood flow through submandibular gland by 64±7% (28). Withdrawal of blood also was used as a method to diminish blood flow in salivary glands (29). Submandibular secretor response to stimulation of parasympathetic chorda – lingual nerve in anaesthetized cats before, during and after withdrawal of about 50% blood resulted in significant reduction in submandibular blood flow and the secretion of both saliva and protein during stimulation. Under each set of conditions the flow of saliva was linearly related to the blood flow through the gland.

Experimental pathology of salivary glands more often was created through ligation of main salivary ducts. In some of these experiments the vascular response of salivary was also examined. On cat's submaxillary glands the increase in blood flow following a 10-sec stimulation of the chorda-lingual nerve was reduced to about 75% of control values (5).

The role of microcirculation is very important in all fields of surgery, especially in free flaps (30). Also the submandibular glands transferred for treatment of ceratoconjunctivitis sica show tracer uptake and excretion in scintigraphic findings. Consequently, the glandular tissue is able to function in compromised situations (31).

In our experiment we used ligation of common carotid artery that can create a condition close to the acute occlusive diseases and ligation of common carotid artery clinically and in human pathology. No neurological or general symptoms, or behavioral changes in rabbits were observed.

There was a large difference between the sialoscintigraphy data from the side with ligation of common carotid artery and the opposite control side regarding the uptake rate of Tc99 and the washout fraction as well. The reconstruction of closed common carotid artery 4 weeks after ligation resulted in increase of functional activity in salivary glands with slower yet higher accumulation of isotope.

It can be concluded that the functional activity of rabbit salivary glands tested by scintigraphy with Tc99 four weeks after ligation of common carotid artery is strongly depressed. Small activity shows that four weeks after decrease of arterial blood supply on the test side some salivary gland tissue is still functioning. Four weeks after restoration of blood flow in common carotid artery sali-

Table 4. Time of maximal accumulation of Tc99 (min) after revascularization

No.	Control side (min)	Side of revascularization (min)
13	6	13
14	5	12
15	6	14
16	7	14
17	6	12
18	5	15

Table 5. Maximal level of counts per second in accumulation phase (counts/sec) in test and control sides after

No.	Control	Test
13	0.56	0.59
14	0.50	0.48
15	0.62	0.30
16	0.45	0.41
17	0.54	0.32
18	0.58	0.46

vary glands on the experimental side accumulation of isotope is slow, but the level of accumulation is higher than on the control side.

CONCLUSION

The ligation and reconstruction of a common carotid artery on rabbits confirm the important role of

the arterial blood supply in functional activity of salivary glands and may be an appropriate experimental model for investigation of ischemic disease of salivary glands.

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