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Tramadol-induced oral dryness and pilocarpine treatment: Effects on total protein and IgA

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ABSTRACT

Pilocarpine induces a profuse flow of saliva, and it may re-establish saliva production in cases of drug-induced oral dryness. The aim of the study (a sub-study to the previous trial investigating the pilocarpine fluid effects in individuals suffering from drug-induced dry mouth) was to search for saliva quality changes induced by the treatments. Sixty-five individuals were enrolled in a randomized, double-blind, placebo-controlled trial. The subjects received tramadol to induce oral dryness. Secretion rate was measured before and after tramadol, and then after pilocarpine, placebo, or no treatment. All saliva was analyzed for its protein and IgA content in the pilocarpine ($n = 15$) and placebo groups ($n = 12$). At baseline, the flow of saliva was 0.47 ± 0.05 ml/min, the protein output 0.17 ± 0.2 mg/min and the IgA output 0.022 ± 0.002 mg/min. After tramadol treatment (50 mg 3×/day over two days), the flow was reduced by 64%, protein output by 52% and the IgA output by 38%. While placebo treatment did not affect any of the variables, the flow was 120%, the protein output 193% and the IgA output 83% of the baseline characteristics after pilocarpine treatment (5 mg). Thus, the pilocarpine-induced increase in the flow rate in the state of tramadol-induced oral dryness results in saliva with a well preserved protein concentration but with a decrease in IgA concentration. However, compared to baseline, there was neither a decrease in output nor in concentration of IgA.

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1. Introduction

Oral dryness, or xerostomia, is one of the most common adverse effects of pharmacotherapy.^{1,2} A decreased salivary secretion may be uncomfortable, may increase the susceptibility to caries, and may indirectly be the cause for mucosal infections.^{3,4} Several different treatments exist clinically which aim to increase the salivary secretion and thereby the oral health and comfort.⁵ One drug frequently employed for this purpose is pilocarpine, a parasympathomimetic agent

that stimulates sweat, lacrimal and salivary glands.⁶ Pilocarpine is currently used to treat xerostomia caused by Sjögren's syndrome or dryness emerged as a consequence of radiotherapy.^{7,8} However, in a recent study, pilocarpine was shown to also counteract the oral dryness and the xerostomia induced by drug treatment.⁹ In the study, pilocarpine did not only re-establish the flow of saliva but even increased it above a normal flow. The study thus further underlines the sialomimetic potency of pilocarpine and the importance of glandular muscarinic receptors as targets for drugs given for preserving

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the flow of saliva and maintaining oral health. Yet unknown is, however, whether or not pilocarpine changes the composition of saliva. Changes of the composition may *per se* have a deteriorating effect on oral comfort and health and the saliva content of IgA is inversely correlated to infections and may thus have an impact on oral health.¹⁰⁻¹³ The aim of the current study was to investigate the changes in the levels of the immunoglobulin IgA and in the total protein of the saliva following administration of pilocarpine to individuals suffering from drug-induced xerostomia. To address these questions, the present assessments were performed within a biochemical sub-study to the previously examination of oral pilocarpine fluid effects on opioid-induced oral dryness.

2. Materials and methods

In the randomized, double blind, and placebo-controlled trial 65 students were enrolled.⁹ Exclusion criteria were pregnancy, ongoing disease, previous radiotherapy to the head and neck region and medication. Six subjects were eliminated for one or other of these reasons before enrollment. After enrolment 5 subjects were excluded as a result of adverse effects of the tramadol treatment (dizziness, nausea, somnolence) and illness (viral infection). Subjects were also excluded if the tramadol treatment reduced the salivary secretion by less than 40%. Twelve subjects were eliminated for this reason (see Fig. 1). All subjects participated voluntarily. The Ethics Committee of the University of Lund approved the study. The trial was carried out according to the Helsinki Declaration.

Measurement of unstimulated saliva was made according to a standard method.^{14,15} At day 1, samples were collected for 5 min at 15 min intervals over 2 h to determine the basal secretory rate. After the volume had been measured, each sample was frozen in order to being analyzed for its protein and IgA antibody content. Subsequently, tramadol (50 mg 3×/day) was administered orally three times a day over two days in order to reach a steady state concentration of the drug. During the afternoon of day 3, unstimulated saliva was

collected for three consecutive 5 min periods to determine the effect of tramadol. After the examination of the tramadol effect, the subjects were randomly assigned to one of three groups. The first group was given pilocarpine 5 mg (pilocarpine-group), the second was given a placebo (placebo-group) and the third group was given no further treatment (control-group). Random assignment was computer-generated with a block size of six and beakers, which were number-connected to the coding, containing either a capsule or being empty were given to the participants consecutively. After 15 min saliva was collected by the same procedure for saliva collection as on day 1. With the exception of the volume measurements, no further examinations were made on the saliva from the control group. The drugs employed were tramadol hydrochloride (Tramadol GEA[®]), pilocarpine hydrochloride (Pilocarpine hydrochloridum Ph Eur 5 mg, Lactosum monohydricum Ph Eur 145 mg in Capsulae gelatinosae No. 4 ACL), placebo (Lactosum monohydricum Ph Eur 150 mg in Capsulae gelatinosae No. 4 ACL; *ex tempore*; Hospital Pharmacy, Malmö University Hospital). The dosages of tramadol (50 mg three times a day P.O.) and pilocarpine (5 mg P.O.) were the lowest recommended according to the Summary of Product Characteristics. For further details see Gotrick et al.⁹

The saliva samples were frozen at -18°C until analyzed. Before analysis, the samples were thawed, and then clarified by centrifugation at $1520 \times g$ for 10 min at 4°C . We were able to analyze a total amount of 27 individuals since seven individuals had to be excluded because of too little volume being secreted. The total number of analyzed individuals in each group was: pilocarpine group, $n = 15$ and placebo group, $n = 12$.

The protein concentration was measured with Bio-Rad Laboratories (Richmond, CA, USA) protein assay using bovine serum albumin as standard. For determination of IgA in saliva, an immunobead enzyme linked immunosorbent assay (ELISA) described by Sack et al.¹⁶ and modified by Bratthall and Ellen¹⁷ was used. Immunobeads were coated with alpha-chain specific rabbit-anti-human IgA (Irvine Scientific, Santa Ana, CA, USA). Goat-anti-human IgA (alpha-chain specific) conjugated with alkaline phosphatase (Chemicon International, Temecula, CA, USA) served as second antibody and human serum protein calibrator containing 2.27 mg IgA/ml (DAKO, Denmark) was used as standard.

2.1. Calculations

The design of the study was based on a pilot study including six subjects for power analysis of a three-group study design for examining effects on the flow of saliva. Forty-five participants had to be recruited to achieve a 90% power to detect an increase in unstimulated whole saliva from a dry mouth level (0.15 ml/min) to the level of average secretion for healthy individuals (0.35 ml/min), with a two-sided test and a type I error of 5%. In order to allow for screening failures and dropout during the study, the number of subjects needed in each treatment group was increased from 15 to 20. However, the number of individuals lost in the second part of the study, i.e., the analyses of the saliva content, was even larger than expected, since not only the compliance to inclusion criteria, but also the volume secreted had to be adequate. Therefore, the number of individuals included in the control and placebo groups is

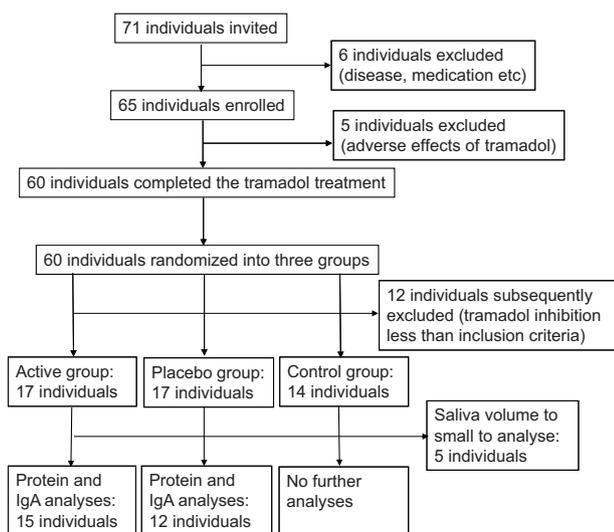


Fig. 1 – Trial profile.

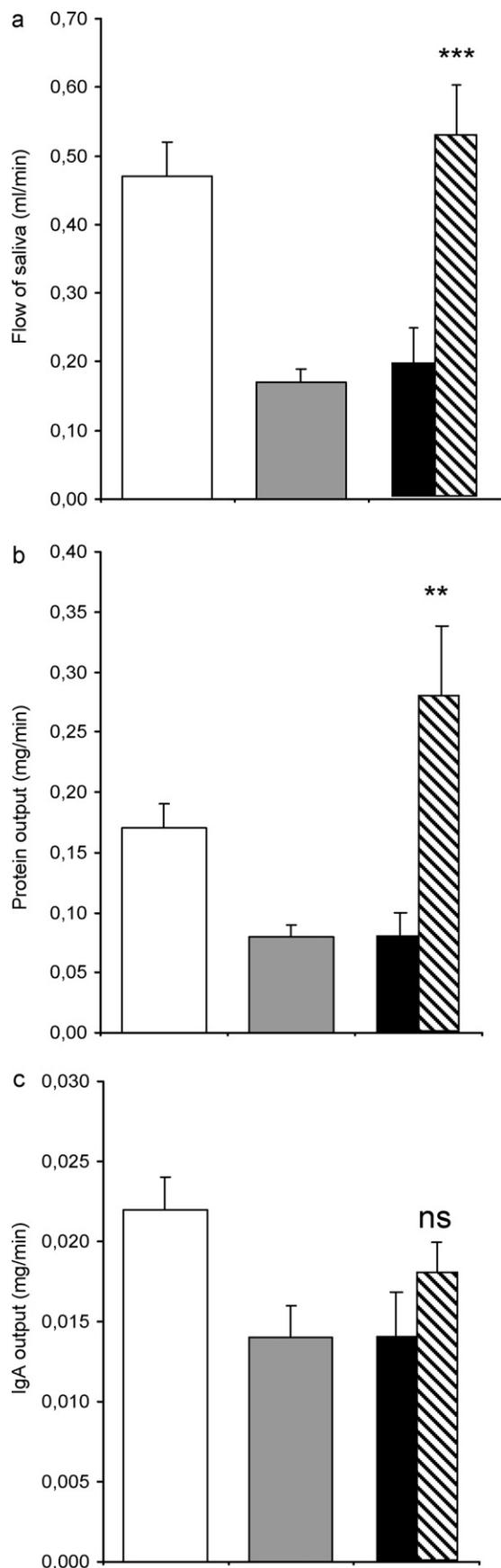


Fig. 2 – Mean values for unstimulated (a) flow of whole saliva, (b) protein and (c) IgA output at baseline, day 1 (□; $n = 27$), after treatment with tramadol, day 2–3 (■; $n = 27$)

somewhat less than 15. Assessment was based on per protocol analysis and statistical significance determined by one-way analysis of variance (ANOVA) followed by the Bonferroni multiple-comparison test. p values of 0.05 or less were regarded as statistically significant. Values are presented as mean \pm SEM.

3. Results

3.1. Basal observations and effects of tramadol

The mean basal flow of saliva during the 2 h collecting round at day 1 was 0.47 ± 0.05 ml/min ($n = 27$; mean value of all individuals) (Fig. 2) and the mean protein and IgA concentrations therein were 0.38 ± 0.04 and 0.06 ± 0.01 mg/ml, respectively (Fig. 3). While tramadol treatment (day 2–3) decreased the salivary flow rates by 64% (0.17 ± 0.02 ml/min; $p < 0.001$), it tended to increase the protein concentration (0.46 ± 0.04 mg/ml) and increased the IgA concentration by 50% (0.09 ± 0.009 mg/ml; $p < 0.05$). Despite the tramadol-induced increase in concentration of IgA and its tendency to increase protein concentration, the outputs were significantly reduced by tramadol. So decreased the protein output from 0.17 ± 0.02 mg/min to 0.08 ± 0.01 mg/min ($p < 0.01$), and the IgA output from 0.022 ± 0.002 mg/min to 0.014 ± 0.002 mg/min ($p < 0.05$) (Fig. 2). Thus, the tramadol administration had greater inhibitory effect on the total protein secretion than on the IgA secretion.

3.2. Effects of pilocarpine

The administration of pilocarpine restituted the flow of saliva above the baseline flow before administration of tramadol. The flow peaked 30–45 min after pilocarpine administration during which period the mean flow amounted to 0.53 ± 0.07 ml/min ($n = 15$; $p < 0.001$ vs placebo treatment). The placebo treatment caused no change of the tramadol-induced dryness. The flow was 0.17 ± 0.02 ml/min before placebo and 0.20 ± 0.05 ml/min after the treatment (Fig. 2a).

Placebo had no effect on the output of protein; 0.08 ± 0.01 mg/min before placebo and 0.08 ± 0.02 mg/min after the treatment (Fig. 2b). Pilocarpine, on the other hand, increased the output of protein to 0.28 ± 0.06 mg/min ($p < 0.01$ vs placebo). Since pilocarpine increased the protein output by 93% ($p < 0.001$) and the flow by 20% in comparison with baseline, the protein concentration increased; at maximal flow it was 147% of that at baseline ($p < 0.001$) (Fig. 3a).

Neither had placebo treatment any effect on the IgA output. It was 0.014 ± 0.002 mg/min before placebo and 0.014 ± 0.003 mg/min after the treatment (Fig. 2c) however

and after treatment (pilocarpine and placebo) day 3, in the pilocarpine group (■; $n = 15$) and the placebo group (▨; $n = 12$). Values are mean \pm SEM. ** $p < 0.01$, *** $p < 0.001$, ns $p > 0.05$; comparison between pilocarpine and placebo groups.

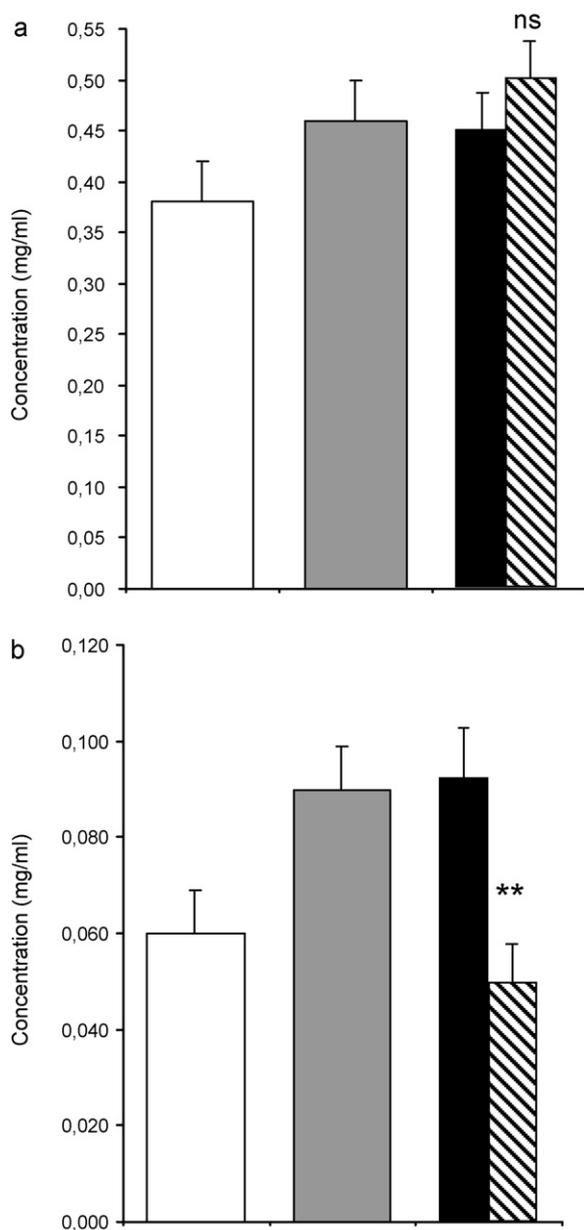


Fig. 3 – Mean values for unstimulated (a) protein and (b) IgA concentration at baseline, day 1. (□; n = 27), after treatment with tramadol, day 2–3 (■; n = 27) and after treatment (pilocarpine and placebo) day 3, in the pilocarpine group (■; n = 15) and the placebo group (▨; n = 12). Values are mean ± SEM. ** $p < 0.01$, ns $p > 0.05$; comparison between pilocarpine and placebo groups.

no effect was neither observed on the IgA output by the pilocarpine treatment (0.018 ± 0.02 mg/min; ns vs placebo). In fact the concentration of IgA was significantly lower than in the placebo group ($p < 0.01$, Fig. 3b), since the flow of saliva increased considerable more than the output of IgA after pilocarpine treatment. However, compared to baseline, there was neither a decrease in output of IgA (0.018 ± 0.002 mg/min vs 0.022 ± 0.002 mg/min; ns) nor in concentration of IgA (0.05 ± 0.008 mg/ml vs 0.06 ± 0.01 mg/ml; ns).

4. Discussion

This study provides novel and potentially important documentation of the impact of pilocarpine and opioid treatments on the quality of saliva. In a previous study regarding the sialogenic effects of pilocarpine, it was shown that oral pilocarpine re-establishes the flow of saliva in pharmacological states of oral dryness, and that low-dose regimes may be sufficient to restore secretion. It was also shown in the previous study that oral tramadol has pronounced xerogenic potency, since it induced a saliva flow reduction by 40% or more in 80% of the subjects.

In the current study, pilocarpine caused a conspicuous increase in the protein output in the saliva. The comparison of the effect on the fluid and the protein output revealed a larger effect on the latter variable, which thus resulted in a tendency of larger protein concentration after pilocarpine treatment. While pilocarpine seemed to increase the protein concentration in the saliva, it decreased the IgA concentration. When considering the total output of IgA after the pilocarpine treatment, it tended to be larger than in the saliva from the placebo group. But the pilocarpine stimulatory effect on the IgA output was conspicuously smaller than the corresponding effects on saliva flow rate and protein output. Consequently, parasympathetic activity via the muscarinic receptors had little effect on the IgA secretion in comparison with the effect on fluid secretion. This is consistent with the findings from studies on isolated salivary gland cells, in which β -adrenoceptor stimulation has been shown to far more potently induce IgA secretion, than both α -adrenoceptor and muscarinic receptor stimulation.¹⁸ In addition to the secretion of IgA being modulated by neurotransmitters,¹³ the flow rate of saliva has a great impact on the concentration. So has the IgA concentration been demonstrated to be negatively correlated to the flow rate.¹⁹

Secretory IgA is a polymeric molecule that consists of two IgA monomers, a joining chain and a secretory component.²⁰ The secretory component is present on the basolateral epithelial cell membrane and acts as a receptor for transepithelial transport. IgA exists in two different isotypes, IgA1 and IgA2, which have marked differences in their hinge regions. In saliva, IgA principally occurs as a dimer. In the literature the two forms are reported to occur in similar amounts in the salivary secretion.¹³ In the present study, the total IgA was examined (monomeric plus polymeric forms) and consequently we don't know the relative amounts of the different forms of IgA in our assessment. Nevertheless, salivary IgA is mainly produced by plasma cells located close to the ducts and acini.²¹ The secretion of S-IgA includes different steps and could theoretically occur by internalization followed by secretion or by paracellular secretion.¹³

The output of IgA is sometimes expressed in mg/100 mg total protein, since the concentration of the antibody in saliva often varies dramatically between individuals.²² However, in our examination the concentrations in the saliva showed only a moderate variation between the individuals. Consequently, the data show consistency irrespectively of the way of expressing it. Furthermore, the IgA concentrations agree well with those in other reports.²³ Nevertheless, the content of IgA has been shown to be correlated to oral health, both regarding

caries and periodontal disease.²² In this perspective it is important that saliva restitution strategies, such as pilocarpine treatment, do not hamper the defense systems towards infections. Our findings do not show such effects of pilocarpine, even though it stimulated the output of IgA less efficiently than fluid secretion.

Noradrenaline has been suggested to affect the IgA salivary response indirectly, by influencing the antigen sensitivity of B-cells and possibly to induce a paracellular secretion in salivary glands.¹³ Regarding the protein and IgA secretion, tramadol affected it to a lesser degree than the flow of saliva. This resulted in increases of the concentrations. In a study on the rat parotid gland, tramadol has been shown to reduce the salivary secretion by inhibiting the salivary reflex arch in the central nervous system and to cause the release of noradrenaline from glandular nerve terminals.²⁴ This resulted in a reduction of the flow of saliva, but with a higher concentration of protein. The current findings seem to support such tramadol mechanisms in humans also.

While the parasympathetic nervous system via acetylcholine acting on muscarinic receptors is the principal stimulation for fluid secretion in salivary glands, it also contributes via non-adrenergic, non-cholinergic transmitters to the secretion of protein.²⁵ The sympathetic nervous system, on the other hand, mainly contributes to the secretion of protein, although it may induce a sparse fluid secretion as well. This needs to be considered in view of the mechanisms by which pilocarpine induces secretion of saliva. Besides activation of the glandular muscarinic receptors, pilocarpine activates muscarinic receptors in the central nervous system.²⁶ The central activation could of course lead to stimulation of the salivary glands from both divisions of the autonomic nervous system. By this it follows that the composition of pilocarpine-induced saliva does not exclusively reflect a salivary response of the parasympathetic nervous system.

Secretion of whole resting saliva was assessed in our study. Therefore, the tramadol and pilocarpine effects could have affected one and not another type of salivary gland. It is well known that the parasympathetic nerves also affect salivary protein secretion, and that protein secretion of some glands (e.g., the sublingual and some of the minor glands) is only under parasympathetic control. In view of this, and that adrenergic and peptidergic stimuli, in contrast to cholinergic stimuli, potently stimulate S-IgA secretion^{13,27}, the differences between the pilocarpine effect on protein output and on IgA output may be explained.

In conclusion, the pilocarpine-induced increase in the flow rate in the state of tramadol-induced oral dryness results in saliva with a well preserved protein concentration but with a decrease in IgA concentration. Individuals undertaking pharmacological treatment often suffer from xerostomia, which causes both discomfort and pathological conditions. Finding a method of reestablishing salivary secretion in these individuals would be of great benefit for their life quality. Since our study not only shows that pilocarpine increases the salivary secretion, but also the output of protein and further, does not hamper that of IgA, it has to be considered a potent method of treating xerostomia.

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Ethnic approval: The Ethics Committee of the University of Lund approved the study. The trial was carried out according to the Helsinki Declaration.

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