ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LXI, N 2, 122 SECTIO D 2006

Department of Dental Surgery, Medical University of Lublin

PAWEŁ ZAŁĘSKI, ANNA M. SZYSZKOWSKA,
URSZULA ORZĘDAŁA-KOSZEL

Total protein content in mixed saliva in patients treated with beta-blockers for hypertension disease

Saliva is a secretion which plays an important role in initial digestive processes and in maintaining homeostasis of the oral cavity environment. Constant presence of saliva in the oral cavity helps to keep its tissues in healthy condition. The chemical complexity of saliva, variety of protein compounds determines its properties and many of the functions it performs in the organism. These include protective, buffering, immunological and digestive functions.

The protective functions of saliva consist in its interaction with the teeth, soft tissues of the oral cavity and bacterial flora. Saliva protects the mucosa against mechanical, thermal and chemical irritating agents, by moistening it enables gentle passage of air through the oral cavity and facilitates speech articulation. Saliva is responsible for maintaining of neutral pH in the oral cavity, bacterial plaque and esophagus during deglutition.

In the rest phase the content of bicarbonates is low, and the buffering function is performed by histidine-rich peptides and phosphates. The rest of the buffering capacity falls to urea, from which ammonia is released during dental plaque metabolism.

The role of saliva in the homeostasis of hard dental tissues consists in maintaining the process of equilibrium between demineralization and remineralization on the enamel surface. Due to such proteins as staterin, which stabilizes the solution of saliva in respect of calcium and phophates content, saliva takes part in the maturation and remineralization of enamel. The immunological functions of saliva are performed by means of the immunoglobulins IgA, IgG, IgM, and glycoproteins, enzymes and peptides. Non-immunological antibacterial factors in the form of mucins, peptides and enzymes protect the organism against biological and physicochemical pathogenic factors.

The major immunological antibacterial factor is IgA, the immunoglobulin secreted by the cells of the salivary glands. IgA neutralizes viruses, can act as antibody for bacterial and digestive antigens. Salivary mucins protect against the virus of simplex herpes, and display activity against yeast-like fungi.

The oral cavity as the first section of the alimentary tract performs digestive and gustatory functions. Initial digestion is caused by salivary amylase. The level of gustatory abilities depends on the presence of certain proteins and gustins binding zinc, as well as saliva hypotonics which enables the taste buds to properly perceive different substances. Even little changes of composition and amount of secreted saliva that last for a long period of time can affect the health condition of the oral cavity by limiting one or more of the saliva functions mentioned above.

The precise regulation of saliva composition and secretion in physiological conditions can be disordered in salivary gland diseases and some other systemic diseases. Some medications can also have an effect on the secretion and composition of saliva.

The pharmacotherapy of chronic diseases such as arterial hypertension arouses special interest both due to the mechanism of medications action, and the pathomechanism of the very disease.

In the considerable percentage of cases of chronic hypertensive disease, especially in the 'disease prime form', the decisive role is played by the dysfunction of the autonomic nervous system related to the increased secretion of noradrenaline, as well as changes in the number and sensitivity of adrenergic receptors, which are responsible for noradrenaline's effect on the vessels.

In that type of arterial hypertension the drugs of choice are the blockers of beta-adrenergic receptors, which affect the receptors in the heart and arterial vessels, and simultaneously block appropriate receptors in other organs. Hence the hypothesis concerning the mechanism of their, frequently adverse, side-effects. The role of receptor mechanisms in saliva secretion and regulation of its composition allows to presume that therapeutic, systemic effect on the beta receptors of the sympathetic system may cause significant changes of protein content in that secretion.

The aim of this study is to evaluate the changes in the total protein content in the saliva of patients with arterial hypertension treated with beta-blocker, as compared to the saliva of healthy persons.

MATERIAL AND METHODS

A group of 76 people were included into the clinical examinations. The studied group comprised 44 patients, treated for arterial hypertension with the selective beta-blocker *Metocard* produced by the company POLPHARMA. The patients who were diagnosed with no other systemic diseases apart from arterial hypertension, were qualified for the study. The additional selection criterion was the monotherapy with the beta-blocker mentioned above, and taking no other medications. All the patients received *Metocard* for the period of at least 6 months. The patients' average age was 27.4 years, the age range was from 19 to 38 years. During the study the control blood pressure measurements showed values ranging from 120 to 140 mm Hg for systolic pressure, and from 80 to 90 mm Hg for diastolic pressure, which implies the effective treatment of chronic hypertensive disease.

The control group consisted of 32 healthy persons, aged from 20 to 36 years (on average 27.4), who were not on any form of medication, whose systolic pressure varied from 120 to 130 mm Hg, and diastolic pressure from 80 to 85 mm Hg, and these results were within the range of normal blood pressure values.

Saliva sampling for tests. Saliva was collected into sterile disposable plastic test tubes in the conditions without stimulation, always between 9:00 and 11:00 hrs, but not earlier than 2 hrs after the last meal. Before the test the patients rinsed their mouths with water. Saliva was collected into plastic graduated test tubes with the volume scale accuracy to 0.5 ml. The volumes of the collected saliva were recorded after 5 min, then collecting of saliva was continued to reach the volume of 10 ml. During saliva collection test tubes were placed in a container with ice. Immediately after saliva collection its pH was determined. Then the collected material was cetrifuged at 5,000 rpm for 15 min, frozen at a temperature of –25°C and stored in such conditions until biochemical tests were made.

Determining of total protein concentration. Total protein was determined using the Lowry method as modified by Markwell et al. (9). Four reagents were prepared to determine the protein: reagent A – 2% Na_2CO_3, 0,4%NaOH, 0.16% sodium potassium tartrate, 1% SDS, reagent B – 4% $CuSO_4$, reagent C – a mixture of reagents A and B in the volume to volume proportion of 100 : 1, and reagent D – Folin-Ciocaltau reagent diluted in the ratio of 1:1 with deionized H_2O. A saliva sample of 20 µl in volume was diluted with water to reach 1 ml in volume and 3 ml of

reagent C were added, and then it was incubated at a temperature of 25°C for 1 h. After that time 0.3 ml of reagent D were added and the mixture was incubated for another 45 min. To determine protein concentration in saliva a calibration curve was made using standard protein solutions of the following concentrations – 20, 40, 60 i 120 μg/dl, obtaining linear and proportional relationship between the absorbance and protein concentration. The absorbance was read at the wave length of 660 nm using the spectrophotometer Spectronic 20 Genesys and compared to identically prepared control sample, to which instead of protein solution the same amount of distilled water was added. On the basis of the calibration curve protein concentration in saliva was calculated using the formula: protein concentration in saliva (μg /dl) = absorbance $E_{660\,nm}$ x 30.

Statistical analysis of the results. Differences between variables were estimated by means of non-parametric Mann-Whitney test. To interpret the statistical tests results 5% inference error was assumed. Consequently, relationships between variables were considered as statistically significant at the level of significance $p < 0.05$.

DISCUSSION

Appropriate composition and secretion of saliva are controlled by the autonomic (sympathetic and parasympathetic) nervous system, which innervates the salivary glands. Many diseases associated with the hyperfunction or hypofunction of the system, as well as medications affecting it can have an effect on the composition and secretion of saliva. Salivary gland cells are equipped with beta-adrenergic receptors, mainly beta-1 subtype (5, 6, 12). These receptors are the place of action of beta-adrenergic blockers used in the treatment of chronic hypertensive disease. The most frequently described side-effects of beta-blockers therapy are the disorders of the alimentary tract, especially of its first section, i.e. oral cavity. The disorders mainly affect the amount and, as it is supposed, composition of saliva.

Our studies have revealed the differences in saliva protein parameters between the control and studied groups. Mean protein concentrations in the saliva of healthy persons and the patients with hypertension treated with *Metocard* have shown statistically significant differences. The mean protein concentration in the studied group was 1.9 g/l, in the control group, however, 2.15 g/l. Observations of other authors also confirm that protein concentration values in the saliva of the patients receiving beta-blockers tend to decrease (8, 10, 11, 13).

Table 1. Protein concentrations in saliva in the control group and studied group

Concentration g/l	N	Control group	N	Studied group	p (Mann-Whitney test)
		Mean ± SD		Mean± SD	
Protein	32	2.15 ± 0.317	44	1.90 ± 0.243	$p < 0.002$

Experimental studies on animals often make a good comparative model in relation to people. Similar autonomous innervation of the salivary glands in people and rats allows to compare both species in an approximate manner. Many authors have analyzed the influence of beta-adrenergic blockers on the protein composition of rat saliva. The study results of most of them are consistent and report a decrease of protein content in saliva in the groups receiving beta-blocker (1). The studies by Baum et al. (2) also confirm the significance of adrenergic receptors in the regulation of protein synthesis and secretion into saliva. They have found an increase in protein level in rat saliva after

administration of beta-adrenergic receptor agonists. Then Watson et al. (15) in their studies have revealed that protein concentration in saliva tends to decrease as the level of beta-blocker rises in rat blood serum. The authors have also observed qualitative changes in the electrophoretic picture of examined saliva, which demonstrated the lack of some of the bands in the studied group. The missing bands corresponded in their pattern with proline-rich proteins and some glycoproteins.

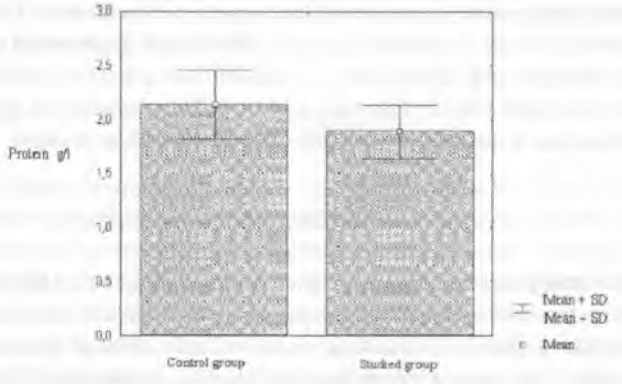

Fig.1. Protein concentration (g/l) in control group and studied group
Mean concentration of total protein in the saliva of the patients from the studied group was statistically lower than the value of the parameter registered in the saliva of the healthy persons

Similar study results have been presented by Johnson and Cortez (7). In rats which were given beta-blocker twice a day, a 40% fall in the volume of secreted saliva was observed, no statistically significant changes in protein concentration were found. However, such changes were revealed when selective beta-blocker was administered constantly by means of mini infusion pomp. A decrease in the content of proline-rich proteins in saliva was observed then. The differences resulting from the way of drug administration are explained with 'first passage' effect, which may occur after an oral or parenteral administration of too small a dose of medication.

Nederfors and Dahlóf (11) have presented similar relationships. In their studies they observed a marked increase in the secretion of protein into saliva after the cessation of beta-blocker administration in patients treated for arterial hypertension, and a considerable decrease in the protein secretion after the medication administration was again resumed during the therapy. In another study the same authors (10) describe a statistically significant decrease of total protein level in saliva during the administration of both selective and non-selective beta-blocker. Baum (3) reports that stimulation of beta-receptors of salivary gland cells activates the synthesis and secretion of protein into saliva. The studies of Laurikainen (8) performed on healthy volunteers imply that beta-blockers can cause qualitative changes in saliva protein composition by reducing the synthesis and/or secretion of proteins. A decrease in the content of proteins with antibacterial properties in saliva (peroxidase, lactoferrin) may weaken protective properties of saliva against micro-organisms (8).

Nederfors et al. (13) studied the influence of beta-adrenergic receptors antagonists on the secretion of the human salivary glands. The content in the secretion from the parotid gland as well as from the submandibular and sublingual glands was significantly smaller during the treatment with beta-blocker (13). In the parotid gland saliva the ratio of hexosamine to total protein decreased, while the ratio of sialic acid content to hexosamine increased. The reported facts may imply disorders in

the synthesis and secretion of various proteins, especially glycoproteins with short oligosaccharide lateral chains (13). The same authors (11) have discovered a simultaneous rise in the level of total protein, hexosamine, sialic acid and amylase activity in patients with arterial hypertension after the cessation of beta-blocker administration. After the medication administration was again resumed, they observed a marked decrease in the content of those ingredients in saliva.

In professional literature there are rather few reports on the functioning of the salivary glands in patients with chronic hypertensive disease before the beginning of the therapy. Ben-Aryeh et al. report that saliva secretion in basic conditions in patients with chronic hypertensive disease, before the beginning of the treatment with beta-blockers, is smaller than in healthy individuals (4, 14). These findings allow to presume that all disturbances of the balance between the sympathetic and parasympathetic systems lead to disorders in the composition and secretion of saliva.

CONCLUSION

The results of the study and the reports of other authors indicate that the hyperfunction of the sympathetic system and medications inhibiting its activity administered to patients with chronic hypertensive disease over a long period of time, change the content of total protein in mixed saliva. It appears interesting and sensible to analyze these changes in view of their possible effect on physiological processes which occur in the oral cavity.

REFERENCES

1. Abe K. et al.: The effects of 5-hydroxydopamine on salivary flow rates and protein secretion by the submandibular and parotid glands of rats. Experimental Physiology, 81, 645, 1996.
2. Baum B. et al.: Adrenergic regulation of protein synthesis in rat submandibular salivary gland cells. Arch. Oral. Biol., 29, 499, 1984.
3. Baum B. J.: Neurotransmittor control of secretion. J. Dent. Res., 66, 628, 1987.
4. Ben-Aryeh H. et al.: Salivary composition in patients with essential hypertension and the effect of pindolol. J. Oral. Med., 36 (3), 76, 1981.
5. Carlsoo B. et al.: Characterization of the rat parotid beta-adrenoreceptor. Br. J. Pharmac., 72, 271, 1981.
6. Edgar W.: Saliva and dental health. Clinical implication of saliva: Report of a consensus meeting. Br. Dent. J., 169, 96, 1990.
7. Johnson D., Cortez J.: Chronic treatment with beta Adrenergic-agonist and antagonist alters the composition of protein in rat parotid saliva. J. Dent. Res., August 67 (8), 1103, 1988.
8. Laurikainen K. et al.: Effects of a beta-blocking agent, timolol maleate, on saliva in healthy volunteers. Scand. J. Dent. Res., 96, 121, 1998.
9. Markwell M. et al.: Protein determination in membrane and lipoprotein samples : manual and automated procedures. In: Methods in Enzymology, 72, 296, 1981.
10. Nederfors T. et al.: Effects of the beta-adrenoreceptor antagonists atenolol and propranolol on human unstimulated whole saliva flow rate and protein composition. Scan. J. Dent. Res., 102, 235, 1994.
11. Nederfors T., Dahlöf C.: Effect on salivary flow rate and composition of withdrawal of and reexposure to the beta 1-selective antagonist metoprolol in a hypertensive patient population. Eur. J. Oral Sci., 104, 262, 1996.

12. Nederfors T., Dahlöf C.: Effects of the beta-adrenoreceptor antagonists atenolol and propranolol on human whole saliva flow rate and composition. Archs Oral Biol., 37, 579, 1992.
13. Nederfors T. et al.: Efects of the beta-adrenoreceptor antagonists atenolol and propranolol on human parotid and submandibular-sublingual salivary secretion. J. Dent. Res., 73, 5, 1994.
14. Streckfus C.: Salivary function and hypertension: A review of literature and case report. JADA, 126, 1012, 1995.
15. Watson G. et al.: The effect of propranolol treatment on salivary composition and caries in the rat. Archs Oral Biol., 35, 435, 1990.

SUMMARY

Pharmacological therapy is a frequent cause of side-effects affecting the physiology of other organs or systemic processes not directly associated with the main disease. Numerous groups of medications debilitate the functions of the salivary glands. This study comprises the analysis of the content of total protein in mixed saliva of the patients with chronic hypertensive disease who were treated with beta-blocker. Special attention was paid to the potential effects of changes in the content of protein substances in saliva. The study was conducted on a group of patients with hypertension disease, treated with the selective beta-blocker *Metocard* (the only medication used by the patients during the therapy). The study results were compared to the results of a control group consisting of healthy people, selected to match the group of patients in terms of age and gender. The people in the control group did not receive any medications. Total protein was determined using the Lowry method as modified by Markwell (9). The study revealed a statistically significant decrease of total protein content in the patients' saliva.

Zawartość białka całkowitego w ślinie mieszanej u pacjentów leczonych beta-blokerem z powodu nadciśnienia tętniczego

Terapia farmakologiczna jest często przyczyną działań ubocznych dotyczących fizjologii innych narządów lub procesów ustrojowych niezwiązanych bezpośrednio z chorobą zasadniczą. Wiele grup leków wpływa na osłabienie funkcji gruczołów ślinowych. W pracy dokonano analizy zawartości białka całkowitego w ślinie mieszanej osób chorych na nadciśnienie tętnicze, leczonych beta-blokerem. Zwrócono uwagę na potencjalne następstwa zmian w zawartości substancji białkowych w ślinie. Badaniom poddano grupę chorych na nadciśnienie tętnicze, leczonych beta-blokerem selektywnym *Metocard* (jedyny lek stosowany przez chorych w trakcie terapii). Wyniki badań porównywano z wynikami badań grupy kontrolnej, którą stanowili zdrowi ludzie dobrani pod względem płci i wieku do grupy badanej. Ludzie w grupie kontrolnej nie przyjmowali żadnych leków. Białko całkowite oznaczono metodą Lowry w modyfikacji Markwell. Stwierdzono istotny statystycznie spadek zawartości białka całkowitego w ślinie osób chorych.