

The effect of Captopril on electrogustometry thresholds, tongue tip vascularization, density and form of the fungiform papillae in humans

Pavlos Pavlidis^{1*}, Georgios Kekes², Haralampos Gouveris³

¹ ENT-Clinic, Ppanikolaou Hospital, Thessaloniki, Greece

² Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

³ ENT Clinic, University Hospital of Mainz, Mainz, Germany

* **Corresponding Author:** Pavlos Pavlidis **E-mail:** papavlid@googlemail.com

ABSTRACT

Objective: Aim of the study was to study in parallel changes in electrogustometric thresholds (EGM), in morphology and density of the fungiform papillae (fPap) and in vessels' shape and density at the tip of the human tongue of patients receiving Captopril.

Material and Methods: In 18 female subjects receiving Captopril (50mg, once per day) as Monotherapy against Hypertension EGM thresholds at the chorda tympani, at the soft palate were recorded bilaterally. Morphology and density of the fungiform papillae (fPap) and blood vessels' density and morphology at the tip of the tongue were examined using contact endoscopy (CE). As control-group 18 healthy non-smokers female subjects of the same age group were also examined.

Results: The evaluation of subjects treated with Captopril showed a higher EGM-thresholds as the subjects of the control-group. The difference between the density of patients' fPap and that of the control-group was statistically significant. It is obvious that Captopril affects the shape and the vascularization of fPap. Captopril can affect seriously taste acuity.

Conclusion: The present study provides data concerning the simultaneous changes in both EGM-Thresholds and shape and vascularization of fPap. The use of captopril seems to have a negative effect on them.

Keywords: Captopril, contact endoscopy, electrogustometry, fungiform papillae, vascularization

INTRODUCTION

Taste perception plays a key role in systemic health, nutritional status and quality of life. Increasing age, smoking and a number of drugs are associated with a decline in the sense of taste and many gustatory disorders are secondary to a wide variety of diseases. Taste disturbance is caused by several endogenous and exogenous factors, such as drugs (1).

Antihypertensive drugs have been identified as potential causes of taste disturbance (1,2). One of these drugs is an angiotensin-converting enzyme, captopril. These drugs have been identified as potential causes of taste disturbance. The captopril molecule contains a thiol-group (-SH) and has been shown to form chelates with zinc (1). However, other ACE inhibitors without the thiol radical have been reported to cause taste disturbance.

Though the wide use of captopril there is a lack of data concerning the effects of captopril on tongue papillae. The majority of the references in the literature presents case-reports of patients who manifested severe dysgeusia and impaired quality of life attributed to the angiotensin-converting enzyme (ACE) inhibitor (3).

The present study had the following aims: first to determine the variations of electrogustometric thresholds in relationship to treatment with Captopril and second to examine the possible differences in densities, shape and vascularization of fPap.

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MATERIAL AND METHODS

The study was conducted according to the guidelines of the Declaration of Helsinki on biomedical research involving human subjects and was approved by the local ethics committee. All subjects provided spoken and written consent after they had been extensively informed about the study. Volunteers participated in the study only after they were informed of its background and purpose and after their written consent was obtained. To minimize variability in technique and interpretation of the findings, all examinations were carried out by the same examiner (PP).

Eighteen female subjects (age $51,2 \pm 2,5$) receiving Captopril (50 mg, once per day) as Monotherapy against Hypertension participated in the study (long term treatment, mean \pm SE 2.6 ± 0.2 yr). Another 18 healthy female (age $50,6 \pm 2,54$) subjects participated in the study as control-group. Patient medical histories solicited information regarding current medical care, recent hospitalizations, significant medical histories such as hepatitis or epilepsy), allergies, cardiac murmurs and/or valve disease, prosthetic hip replacements, and pregnancy. No-one of the patients suffered from retinopathy, nephropathy, polyneuropathy or macroangiopathy. Exclusion criterias were determined as treatment with medications such as antirheumatic, antidepressant, or antiepileptic drugs. Subsequently, a complete otorhinolaryngological examination was performed. Two of the subjects receiving Captopril reported a reduced acuity to sweet substances and another two a metallic taste.

Electrogustometry testing: Taste acuity was evaluated with EGM. Electrical stimuli were delivered with an electrogustometer (TR-06, Rion Co, Tokio, Japan) with a single, flat, circular stainless steel stimulus probe (5mm in diameter). The device produces low-amplitude stimuli of pre-determined duration (1 second). A feedback circuit controls the output current with an error of $< 1\%$ (1).

All subjects were instructed not to drink an hour before the beginning of the testing session. First, a 30dB-stimulus was administered to test whether the subject was in a position to recognize electrogustometric stimuli. Stimulation started at the lowest stimulus amplitude (-6 dB) and increasingly stronger stimuli were presented until the subject recognized the stimulus. If the threshold for stimulus perception was not clearly determined, the next higher- and lower-strength stimuli were presented to the individual.

The electric threshold scores were measured at six locations, namely para-medially on both sides of the tongue apex (each 2 cm away from the tip), an area innervated by the chorda tympani, at the area of the vallate papillae on both sides of the tongue (innervated by the glossopharyngeal nerve) and at the soft palate (area innervated by the major petrosal nerve) bilaterally.

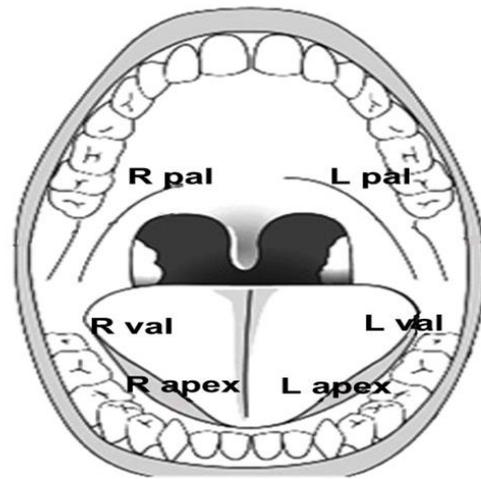


Figure 1. The figure depicts the locations of threshold measurements. The sites have been chosen to evaluate the function of the chorda tympani, glossopharyngeal and greter petrosal nerves.

In healthy subjects, electric gustatory thresholds for the tongue apex, vallate papillae and soft palate are generally set at levels up to 8, 14 and 22 dB respectively. A 500-ms electric stimulus was applied, beginning at -6 dB and increasing up to +34 dB (3-400 μ A) in 2dB-steps. Thresholds were first measured on the right side beginning at the soft palate (Rpal), then proceeding to the area of the vallate papillae (Rval) and finally at the tongue apex (Rapex). Electrogustometry thresholds were then recorded at the left side of the tongue first at the tongue apex (Lapex), then at the area of the vallate papillae (Lval) and finally at the soft palate area (Lpal).

The relationship between the logarithmic control settings and the output currents is shown on Table 1. All six areas were tested with the same stimulus duration before proceeding using stimulus duration. This procedure resulted in a 3-4 minutes' stimulus interval, therefore decreasing the possibility of the emergence of stimulus adaptation. The subjects had been instructed to discriminate between the perception of a sour/metallic taste (suggesting gustatory function - taste threshold) and the perception of an electrical sensation (suggesting trigeminal stimulation). We have measured the EGM-Thresholds as in previous studies (4). 2006). The subject was kept unaware of whether or not the current was applied (blind test) and no feedback was ever given to the subject. The test included successive applications of current in a staircase procedure 10- μ A steps, then a 2- μ A Up-and Down steps from the first "no" response. The EGM detection was taken as the lowest current intensity perceived by the subject for repeated trials (4).

Table 1: The relationship between the logarithmic control settings and the output current. The output current can be adjusted in 2-dB steps from -6 dB to 34 dB

Output Current dB Readings and Output Current																				
dB	-6	-4	-2	0	2	4	6	8	10	12	14	18	20	22	24	26	28	30	32	34
(μ A)	4	5	6.4	8	10	13	16	20	25	32	50	64	80	100	130	160	200	250	320	400

Contact endoscopy: Imaging was performed using a 30° contact endoscope (magnification $\times 60$ and $\times 150$; Karl Storz, Tuttlingen, Germany). Identification of fPap was first performed using a non-contact technique. Subjects were instructed to rinse their mouth with water before contact endoscopy. A contact technique was used first without staining for imaging of subepithelial vessels. After careful suctioning of the saliva, methylene-blue 1% solution was used to stain epithelia and taste pores. A filter paper strip delineating an area of 1 cm² was placed in a paramedian position on the tongue tip as proposed in a previous study (5). To address the problem of instability of the tongue during endoscopy the subjects were advised to hold gently the tip of their tongue between their upper and lower teeth, to avoid venous congestion and hyperemia which could eventually confound contact endoscopic findings. The subjects were asked to seat in the examination chair with their head and neck supported by a pillow. The patients were asked to keep the tongue in a fixed position as much as possible. Examination time by CE was about 30 s. Anesthesia was not necessary. A cold light source was used to minimize any heat produced at the tip of the endoscope. No change (increase or decrease) in vascularization has been observed during examination by CE.

The form of the fungiform papillae was classified to one of four types according to a previously introduced classification paradigm as following: Type 1, (egg-shaped or long ellipse type – without surface thickness), Type 2 (slight thicker surface compared to type 1), Type 3 (thick and irregular surface) and Type 4 (remarkably flat and atrophic surface). Type 1 corresponds to the healthier state, while Type 4 shows a remarkably flat surface. It should be stressed that the mushroom-shaped papillae with horny tips were counted as filliform (and not as fungiform) papillae (5). For estimation of the density of fPap (number of papillae per cm²), the contact endoscopic image with the highest fPap density taken from each individual was used. Due to their very light staining, fungiform papillae could be readily distinguished from filiform papillae, which stained dark. The classification of the blood vessels' morphology at the tongue apex was also performed according to the previous classification (Negoro et al. 2004). Five types of vessels' morphology Type A (clear loop and wooden branch shape), Type B (unclear loop and wooden branch shape), Type C (elongated blood vessels), Type D (granular shape or dotted shape) and Type E (unclear blood vessels). Type A represents the "healthiest" morphology and Type E corresponds to the "unhealthiest" morphology.

All participants completed the study. The findings, and particularly the CE images and the classification of the fPap, have been checked by two persons (P.P and G.K) in order to achieve consensus and avoid any possible mistakes.

Statistical analysis: The null hypothesis was that there was no statistical difference in EGM-thresholds between age groups and between sexes. We used a quantile-quantile test (QQplot) to examine the distribution of our findings. A quantile-quantile plot (QQ plot), a graphical tool for assessing normality, is a plot of the sorted values from the data set against the expected values of the corresponding quantiles from the standard normal distribution. The QQ plot of the data did not show any normal distribution.

As a result, non-parametric tests were applied. The level of statistical significance was set at $p < 0.05$. On each occasion, the EGM-thresholds between the two groups were compared using Kruskal-Wallis and Mann-Whitney tests. The Bonferroni correction was used when necessary. Tukey's multiple comparison test was used to detect differences significant at the 0.05-level in mean thresholds for the various age categories.

For analysis of the regression between age, form, and vascularisation of fPap, the Kendall rank correlation coefficient was applied. The null hypothesis was that the two variables examined on each occasion were independent. The results were analyzed using SPSS software (Version 12 for Windows, SPSS Inc. Chicago, IL, USA)

RESULTS

EGM-thresholds

The EGM-Thresholds on the right and left side of the tongue of the patients after 1-second stimulation are depicted in **Table 2**. As it can be seen the EGM-Thresholds of the patients were higher than these of the healthy subjects. We have also found a statistic significant difference between the thresholds of the two groups, those of the patients were obviously higher (Threshold A: $p=0.04$, Threshold B: $p=0.04$, Threshold C: $p=0.03$, Threshold D: $p=0.04$, Threshold E: $p=0.04$, Threshold F: $p=0.04$).

Table 2: The mean EGM-Thresholds on the right and left side of the tongue after 1-second stimulation in patients receiving Captopril and healthy subjects of the control group. It is obvious that patients receiving Captopril produce a diminished taste acuity.

	Patients treated with Captopril (n=18)			Healthy Subjects (n=18)		
	Mean	Min.	Max	Mean	Min.	Max
Rpal	28.56	24	34	21.43	18	28
Rval	23.43	20	28	18.12	14	22
Rapex	14.46	12	18	8.34	8	12
Lapex	15.65	16	18	9.12	8	14
Lval	22.34	20	26	19.34	12	26
Lpal	27.65	24	34	23.23	22	28

Fungiform papillae structure

Changes in shape and density of fPap as well as in vascularization of the tip of the tongue were also detected by means of CE. It should be mentioned that no change in vascularization or shape has been observed during the examination due to pressure on the tongue's surface. The shape of fPap and vascularization of the tongue tip worsen significantly, as it can be observed with the use of Negoro's classification, as shown on **Table 3**.

We have also reported a difference in densities of fPap in patients compared to the healthy participants (Patients: Right Side: 20.4 ± 2.2 , Left Side: 19 ± 4.6 ./Healthy Participants: Right Side: 24.4 ± 3.7 , Left Side: 25.3 ± 4.3). There was also a high regression-analysis by application of Kendall's tau (τ) between taste thresholds and vascularization ($\tau=0.73$), taste

thresholds and shape ($\tau=0.81$) and taste thresholds and density of fPap of women under treatment ($\tau=0.67$).

Table 3: The table depicts the classification according to shape and vascularization of fungiform papillae on both sides at the tip of the tongue in patients and healthy subjects.

Types of form and vascularization in Patients (n=18)	3A	3C	4A	4D
	5	7	4	2
Types of form and vascularization In Healthy subjects (n=18)	2C	2D	3A	3B
	6	4	5	3

DISCUSSION

The results of the present study show a significant deterioration, both functional (EGM-Thresholds), and morphological (vascularization and shape of the fPap in patients receiving captopril).

Many gustatory disorders are induced by drugs. Frequently, patients are aware of this relationship and report on the close temporal relationship between the occurrence of the taste disorder and drug-intake. Numerous mechanisms of drug-induced gustatory dysfunction have been identified, including disposition of silver sulfate, altered influx of calcium and other ions, chelation or depletion of zinc, disturbed bradykinin catabolism, alteration of second messenger synthesis and altered prostaglandin synthesis (6).

Their taste disturbance disappeared within a few weeks after discontinuation of the drug. A variety of drugs are reported to cause taste disturbance, including thiamazol, D-penicillamine and captopril (7). Taste disturbance induced by captopril has been attributed to the adverse effect by chelation of zinc (8). Recent advances in molecular biology have identified receptors and ion channels on taste cells. Sweet and bitter taste receptors are the proteins that couple with G-proteins (1,9). Coupling and uncoupling to G-protein causes 'taste-on' and 'taste-off' (1). Angiotensin II receptor, which is the target molecule of ARB, also belongs to the same category of the receptor (10). Zinc is an essential trace element playing an important role in many functions such as vision, taste and smell (11, 12). Previous studies suggested that taste impairment belongs to the symptoms of zinc deficiency (2,11). Takeda et al. (11) suggest that zinc deficiency is a predominant factor underlying hypogeusia even when zinc concentrations are within normal ranges in serum (1).

Because of the accessibility of the human tongue tip to examination by contact endoscopy (CE) and because of the established association between the fPap and the gustatory stimuli's thresholds we have chosen the fPap among the four different tongue papillae types to study structural variables that may be important for taste sensation as well as structure-function correlates (13). Similarly to the work of other investigators, we concentrated on the study of the fungiform papillae. In this way the results we obtained can be easily compared to those of previous studies and avoid any other measurements or observations coming from other areas of the tongue's surface. Seen from the patient's perspective, the CE procedure was simple because the gag-reflex or other complaints that could lead to the interruption of the evaluation were avoided.

CE has been used to assess the vascularization, shape and density of fPap as in previous studies (13, 14, 15, 16). The use of CE has the advantages that the procedure is not time-consuming (it lasts 10-15 minutes for each examined person) and that it is non-invasive. Of note, CE cannot detect taste pores. Even after methylene blue staining, reliably detecting of taste pores probably cannot be reached, as suggested by other authors (13). The previous findings concerning the effects of Captopril on the lingual mucosa were experimental. Chou et al. investigated the zinc-deficiency-induced morphologic changes in the vallate taste buds of weanling and young adult male Wistar rats. The authors concluded that the main effects of zinc deficiency were changes in the number and size of taste buds, and fine structure changes in the taste bud cells (17).

The use of CE provided the advantage to study the vascularization of the tongue tip and the shape of the fPap. The results of this study suggest that these two parameters are significantly associated with taste acuity as assessed by EGM. Nonetheless, it should be stressed again that not all fPap contain taste buds and therefore not all fPap produce taste sensation (18, 19) and that there are variations in sensitivity of fPap to chemical stimuli (13). It is obvious that patients receiving Captopril present worse vascularization and form of fPap than healthy patients. The above finding alone does not give the final explanation of taste disturbances caused by ABRs. Further study is needed to solve this issue.

CONCLUSION

In conclusion, the present study offers new data concerning the changes in fPap shape and density as well as the changes in vascularization of the tongue tip in correlation to EGM-thresholds in patients treated with Captopril. As far as we know this is the first time in which combined data such as the above are presented in the literature.

Author contributions: PP, GK, HG; Literature search and study design, patient examinations, data collection and analyzes PP; Writing article and revisions

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Ethical issues: All authors declare originality of research.

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