BALKAN JOURNAL OF STOMATOLOGY

BaSS

ISSN 1107 - 1141

Effects of Local Application of Ascorbic Acid and Glutathione by Iontophoresis on Gingival Inflammation

SUMMARY

Management of gingival inflammation is always a priority in dental practice, regardless the possible involvement of other periodontal tissues. Besides the use of systemic medical therapy, such as antibiotics, vitamins are also recommended as a complementary therapy. Nutritional vitamin intake has been proven effective on gingival inflammation. The aim of this study was to evaluate the effects of local application of glutathione and ascorbic acid by iontophoresis on gingival tissue inflammation.

60 patients with periodontal disease were divided into 2 groups: the control group was treated by conservative treatment of periodontal disease only, and the study group was treated by ascorbic and glutathione, applied with iontophoresis in 10 sessions, besides conservative treatment. Values for gingival inflammation and gingival bleeding on probing were noted.

The obtained results showed significant differences for both gingival inflammation and gingival bleeding between the examined groups, with significant decrease in index values for gingival bleeding after 3 months in the study group.

Keywords: Gingival Inflammation; Iontophoresis; Ascorbic Acid; Glutathione

Maja Pandilova[1], Ana Ugrinska[2], Silvana Georgieva[1], Mirjana Popovska[1], Lidija Kanurkova[1], Katerina Smilevska[1]

[1]University Dental Clinical Centre "St. Pantelejmon"
[2]Faculty of Medicine,
Institute of Pathophysiology and Nuclear Medicine "Akademik Isak Tadzer"
Skopje, FYROM

ORIGINAL PAPER (OP)
Balk J Stom, 2012, 16:74-78

Introduction

Managing gingival inflammation is one of the first challenges that every peridontologist faces, no matter if the process is located only in the gingival tissue, or severe periodontal destruction had taken place. Furthermore, control of infection and gingival inflammation is necessary regardless the following course of periodontal therapy, conservative or surgical. Keeping inflammation under control is even more important in the maintenance period.

The methods used for dealing with gingival inflammation are various, such as: removal of local irritations, training the patients to maintain optimal plaque control, and applying different kinds of medication. Besides the antibiotic therapy, which in certain cases is applied, for control of inflammation and creating better conditions for managing microorganism challenge, vitamins are often used as a supplemental therapy. The effects of vitamins on the gingival tissue are confirmed when taken in natural forms, like fruits and vegetables[14]. Receiving vitamins as supplements in a chemical way (pills, tablets), according to our previous examinations[15] did not show measurable results.

Therefore the **aim** of this study was to verify the effects, if any, of topical appliance of ascorbic acid and glutathione delivered by iontophoresis in the gingival tissue, on gingival inflammation.

Material and Methods

For realization of our goal 60 patients with periodontal disease, randomly selected, participated in the study. The selected patients met the following criteria:

- None of the patients had attachment lost on probing greater then 5 mm;
- None of the patients had any systematic disease, or was receiving any drugs;

- All patients had forefeel the conditions for iontophoresis (patients with heart disease and pregnant women were excluded from the study);
- All patients were between 35 and 45 years of age;
- All patients, according to their history, brushed their teeth twice a day and occasionally used additional products for inter-dental cleaning.
- Patients were separated in 2 different groups, 30 patients each:
- The control group, treated only with conservative treatment;
- The study group, treated by iontophoresis with ascorbic acid and glutathione beside the conservative treatment.

Ascorbic acid was applied by all standard procedures, depending on the individual patients' sensitivity of current strength and the transportability of molecules of the applied solution. After a 30 min break, to each patient of the study group, glutathione was applied as solution on the electrodes covered with sterile gaze soaked in solution of distilled water and 0.9% NaCl. The iontophoresis of glutathione was performed from the active electrode with negative charge for a period of 15 minutes. Both glutathione and ascorbic acid were applied every day during period of 10 days.

All patients were trained to maintain oral hygiene and were called 3 months later for a control visit.

Gingival inflammation and gingival bleeding were noted according to the criteria proposed by Silness and Loe[22], for each patient on his/her first visit, after the treatment, and on the recall visit after 3 months. Data were statistically processed by the computer programme *Statistics 5*.

Results

After processing the data we gained the following results: both groups expressed excellent results during therapy according to the given criteria. The control group had a significant decrease of gingival inflammation index and gingival bleeding after initial therapy: inflammation went from 2.46 to 0.83 and gingival bleeding from 2.76 to 1.56. However, on the control visit after 3 months, gingival inflammation was 1.40, while gingival bleeding was almost at the start point (Tabs. 1 and 2).

Table 1. Average index values for gingival inflammation for the control group

	X	Sd	Se	t	p
Before therapy	2.46				
After therapy	0.83	0.80	0.11	11.06	<0.001
3 months after therapy	1.40	0.86	0.09	6.71	<0.001

Table 2. Average index values for gingival bleeding for the control group

	X	Sd	Se	t	p
Before therapy	2.76				
After therapy	1.56	0.55	0.11	11.39	<0.001
3 months after therapy	2.20	0.72	0.09	4.26	<0.001

The study group also had a significant decrease of gingival inflammation and gingival bleeding after the performed therapy (inflammation went from 2.30 to 0.93 and gingival bleeding went from 2.66 to 0.77). Nevertheless, the index values after the 3-month period showed bigger stability for the study group compared with the control group (Tabs. 3 and 4).

Table 3. Average index values for gingival inflammation for the study group

	X	Sd	Se	t	p
Before therapy	2.30				
After therapy	0.93	0.61	0.11	12.17	<0.001
3 months after therapy	1.01	0.70	0.09	10.30	<0.001

Table 4. Average index values for gingival bleeding for the examined group

	X	Sd	Se	t	p
Before therapy	2.66				
After therapy	0.70	0.66	0.11	16.10	<0.001
3 months after therapy	0.86	0.61	0.09	16.15	<0.001

Discussion

The general idea of our study was to administrate ascorbic acid and glutathione directly in gingival tissue in order to accomplish favourable ratio between over-generated reactive oxygen species (ROS) and antioxidants. ROS are highly reactive molecules which in their last electron circle have the ability to gain or lose 1 electron. This kind of structure makes them highly unstable, so they persist only a very short period of time (a nanosecond or less). Even so, they can cause serious damages on the cellular level. They are taught to be responsible for autoimmune diseases, atherosclerotic changes and cancerous growth.

Once created, ROS can interact among themselves, which is most preferred outcome, yet less likely to happen considering their brief life time. As long as the production of ROS is in physiological range, the organism ties them up in order to make them stable. But when their production is increased or when the antioxidant system is exhausted, then the increased presence of ROS

start a chain reaction of creating more free radicals, even more reactive and damageable from the initial one. The chain reaction will run until the one of the antioxidant mechanisms stops it.

ROS production is inevitable in all aerobic organisms, including humans, who necessarily posses a complex system of antioxidant defence[8,21]. If homeostasis is interrupted in favour of ROS, an oxidative stress situation is created[21]. Oxidative stress processes and alterations in the immune system are closely related and have been described in different diseases, thus both the aspects also seem to be linked to the pathogenesis of periodontal disease, and can also be detected in plasma of patients with periodontal disease[8,16,21]. However, the extent to which ROS over-generation influences the initiation and progression of periodontal diseases is still unknown.

The strong evidence linking ROS to the pathological destruction of the connective tissue during periodontal disease rests on the presence of neutrophil infiltration as the main event in the host's response to bacterial invasion[1,2,10,19]. The hydroxyl radical is able to initiate a classical chain reaction, known as lipid peroxidation, leading to vasodilatation and rat bone reabsorption[8]. An example of the damage caused by hydrogen peroxide is stimulation of phosphorylation of the NFkB-kB complex, activating the NK-kB and facilitating nuclear translocation and downstream of pro-inflammatory cytokines, including IL-2, IL-6, IL-8, β-interferon and TNF-α, that are very important in the pathogenesis of periodontal disease[9].

Experiments concerning the effects of ROS are generally focused on the effects of gingival inflammation. Such experiments, conducted on lab animals treated with pesticides, and given food with lowered elementary antioxidant, showed desquamation of the epithelium, swelling, elasticity loose and breaking of the collagen, bone resorption and lowered speed of C prolin incorporation, thickening of the blood vessels and thrombosis with all it's consequences[5]. On the contrary, in another study, the lab animals were treated with ascorbic acid, tocopherol and biophlavonides. In this experiment, less inflammatory-destructive process was noticed[12].

Mentioned data raises the questions on developing pharmaco-prophylactic measures with bio antioxidants and other bio-regulatives as an addition to the primary and preventive treatment of periodontal disease.

The chain of inhibition of ROS: glutathione - ascorbic acid - tocopherol, with transportation of electrons from pironucleotides (NAD NADF) towards ROS, guarantees permanent low level of free radicals in the cells. Applying glutathione at periodontal disease has proven to be effective in the early stages of the disease. Beside glutathione, other antioxidants can be used with good results such as: tocopherol or ascorbic acid[12,13].

Glutathione (GSH) is a tripeptide of glutamine, glycine, and cysteine. It is not an essential nutrient, since it can be synthesized in the body from the amino acids L-cysteine, L-glutamic acid, and glycine, and acts directly as a generic ROS scavenger of the so-called Phase II reactions[17]. GSH has multiple functions: it is the major endogenous antioxidant produced by cells, participating directly in neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants, such as vitamins C and E, in their reduced active forms[20]. It is used in metabolic and biochemical reactions, such as DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport, and enzyme activation.

Some periodonto-pathogenic bacteria deplete GSH, and this may explain why the amount of this antioxidant was not elevated in the gingival tissue of patients with periodontal disease[1,2,10]. A similar result was obtained in gingival tissue and blood, and lower levels of GSH were detected in the crevicular gingival fluid of patients with chronic periodontal disease, when compared to normal subjects[1,2]. Systemic depletion of antioxidants clearly indicates that in chronic periodontal disease the antioxidant system is affected by a relatively strong oxidation insult, which can deplete nutritional antioxidants, such as vitamin E and C in plasma, and also vitamin E in red cell membrane[16].

According to the literature data on the positive effect of this kind of additional therapy with vitamins, and in our previous studies, we confirmed that individuals which on regular daily base use citrus and citrus like fruits have less gingival inflammation and gingival bleeding from those who never use it, or use it occasionally[14]. But in our further studies we got to the conclusion that if the vitamins are received chemically (as pills) the significant results were not achieved[15].

In order to be able to get better topic apply of antioxidants, directly in the gingival tissue, we decided to use permeability of the oral mucosa and enhance the drug appliance by electric current using iontophoresis. The permeability barrier in oral mucosa is believed to be the result of intercellular material derived from the so-called 'membrane coating granules' (MCG)[6]. MCG start forming through cell differentiation and at the apical cell surfaces they fuse with plasma membrane. This barrier is present in the outermost 200 μm of the superficial layer. Permeation studies have been performed using a number of very large molecular weight tracers, such as horseradish peroxidase and lanthanum nitrate[3]. After being applied to the outer surface of the epithelium, these tracers penetrate only through outermost layer of cells. When applied to the submucosal surface, they permeate up to the top cell layers of the epithelium. While the basement membrane may present some resistance to permeation, the outer epithelium is considered to be the rate limiting step to mucosal penetration[11].

There are 2 permeation pathways for passive drug transport across the oral mucosa: paracellular

and transcellular routes[4]. These 2 routes can be used simultaneously, but 1 route is usually preferred over the other, depending on the physicochemical properties of the drug. Since the intercellular spaces and cytoplasm are hydrophilic in character, lipophilic compounds would have low solubility in this environment. The cell membrane is lipophilic in nature and hydrophilic solutions will have difficulty permeating[24].

Iontophoresis is a procedure based on the use of galvanic electricity and provides easy, fast and effective absorption of different kinds of substances in the tissue. Iontophoresis accomplishes faster metabolism of cells by movement of ions in the bodily fluids and opening on canals in the cell's membrane which create possibilities for easer absorption of different liquid substances in the tissue. Iontophoresis enhances drug delivery by 3 mechanisms: ion-electric field interaction provides an additional force that drives ions through the tissue, the flow of electric current increases the permeability of the mucosa, and electro-osmosis produces bulk motion of solvent that carries ions or neutral species with the solvent stream. Electro-osmotic flow occurs in a variety of membranes and is in the same direction as the flow of counter-ions. It may assist or hinder drug transport[18,23].

Iontophoresis can also enhance mucosa delivery by a possibly dependant pore formation in the upper stratum cell layer, attributed to a "flip-flop" gating mechanism that occurs due to restructuring of the polypeptide helices on application of electric current. Iontophoretic transport is capable of producing a 100 fold enhancement relative to passive diffusion[7,23].

The achieved effects from our study were expected. Inflammation and gingival bleeding showed significant decrease of the average index values for both groups after the therapy without noticeable difference between the groups. Noticeable data were achieved for the average index value for gingival bleeding after 3 months had passed. Since our study design didn't include methods of tracing the given medicaments into the tissue, the concentration they achieve and length of their permeability, the prolonged improvement of clinical parameters may convince us that we had created better host environment.

It is worth mentioning that during our study same problems occurred. Convincing the patients from the study group to undertake the procedure was an issue, since applied at the way we did the procedure, it was rather time consuming for both patient and doctor. So further work might be focused towards developing a hydro-gel containing these or some other drugs and, using iontophoretic enhancers, the time of appliance could be shortened.

Discoloration of the mucosa did not appear in any of the patients, since both agents are used in dermatology as blanching agents.

The achieved results of this study speak in favour of applying ascorbic acid and glutathione by iontophoresis in everyday clinical practice, especially for a long term maintenance on once achieved results of periodontal therapy.

References

1. *Chapple ILC, Brock G, Eftimiadi C, Matthews JB*. Glutathione in gingival crevicular fluid and its relation to local antioxidant capacity in periodontal health and disease. *J Clin Pathol*, 2002; 55(6):367-373.
2. *Chapple ILC*. Role of free radicals and antioxidants in the pathogenesis of the inflammatory periodontal diseases. *J Clin Pathol*, 1996; 49:247-255.
3. *Collins LMC*. The Surface Area of the Adult Human Mouth and Thickness of the Salivary Film Covering the Teeth and Oral Mucosa. *J Dent Res*, 1987; 66:1300.
4. *DeVries ME, Verhoef JC, Junginger HE*. Developments in buccal drug delivery. *Crit Rev Ther Drug Carr Sys*, 1991; 8:271.
5. *Enwonwu C*. Cellular and molecular effects of malnutrition and their relevance to periodontal diseases. *J Clin Periodontol*, 1994; 21:643-657.
6. *Gandhi RE*. Indirect atomic absorption determination of atropine, diphenhydramine, tolazoline, and levamisole based on formation of ion-associates with potassium tetraiodometrcurate. *Ind J Pharm Sci*, 1988; 50:142.
7. *Gangarosa LP, Sr*. Iontophoresis in dental practice. Chicago: Quintessence Publ Co Inc, 1983; p 40-52.
8. *Halliwell B, Gutteridge JMC*. Free Radicals in Biology and Medicine. Oxford: Clarendon Press, 1998; pp 617-783.
9. *Honda T, Domon H, Okui T, Kajita K, Amanuma R, Yamazaki K*. Balance of inflammatory response in stable gingivitis and progressive periodontitis lesions. *Clin Exp Immunol*, 2006; 144(1):35-40.
10. *Katsuragi H, Ohtake M, Kurasawa I, Saito K*. Intracellular production and extracellular release of oxygen radicals by PMNs and oxidative stress on PMNs during phagocytosis of periodontopathic bacteria. *Odontology*, 2003; 91(1):13-18.
11. *Lee JW, JHP, Robinson JR*. Bioadhesive-based dosage forms: The next generation. *J Pharm Sci*, 2000; 89:850.
12. *Meydani SN, Meydani M, Blumberg JB, Leka LS, Siber G, Loszewski R, Thompson C, Pedrosa MC, Diamond RD, Stollar BD*. Vitamin E supplementation and in vivo immune response in healthy elderly subjects. A randomized controlled trial. *JAMA*, 1997; 277(17):1380-1386.
13. *Nishida M, Grossi SG, Dunford RG, Ho AW, Trevisan M, Genco RJ*. Dietary vitamin C and the risk for periodontal disease. *J Periodontol*, 2000; 71(8):1215-1223.
14. *Pandilova M, Ivanovski K, Ugrinska A, Minovska A, Radojkova V, Ristoska S*. The effect of nutritional vitamin intake on periodontal health. Abstract Book of the 9th Congress of the Balkan Stomatological Society, Ohrid 2004; p 97.
15. *Pandilova M, Ugrinska A, Ivanovski K*. Effects of nutritional tocopherol intake on periodontal health. *Mak Stom Pregl*, 2009; 33(1-2):95-101.

16. *Panjamurthy K, Manoharan S, Ramachandran CR*. Lipid peroxidation and antioxidant status in patients with periodontitis. *Cell Molec Biol Letters*, 2005; 10(2):255-264.
17. *Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF*. The changing faces of glutathione, a cellular protagonist. *Biochemical Pharmacology*, 2003; 66(8):1499-1503.
18. *Rai R, Srinivas CR*. Iontophoresis in dermatology. *Ind J Dermatol Vener Leprol*, 2005; 71:236-241.
19. *Sakallioğlu U, Aliyev E, Eren Z, Akşimşek G, Keskiner I, Yavuz Ü*. Reactive oxygen species scavenging activity during periodontal mucoperiosteal healing: an experimental study in dogs. *Arch Oral Biol*, 2005; 50(12):1040-1046.
20. *Scholz RW. Graham KS, Gumpricht E, Reddy CC*. Mechanism of interaction of vitamin E and glutathione in the protection against membrane lipid peroxidation. *Ann NY Acad Sci*, 1989; 570:514-517.
21. *Sculley DV, Langley-Evans SC*. Salivary antioxidants and periodontal disease status. *Proc Nutr Soc*, 2002; 61(1):137-143.
22. *Silness P, Loe H*. Periodontal disease in pregnancy. *Acta Odontol Scand*, 1964; 22:121.
23. *Semalty A, Semalty M, Singh R, Saraf SK, Saraf S*. Iontophoretic drug delivery system: A review. *Technology and Health Care*, 2007; 15(4):237-245.
24. *Wertz PW*. The physical, chemical and functional properties of lipids in the skin. *Chemistry and Physics of Lipids*, 1998; 91:85.

Correspondence and request for offprins to:

Pandilova Maja
University Dental Clinical Centre „St. Panteleimon”
Department of Oral Pathology and Periodontology
Skopje
FYR Macedonia
E-mail: mpandilova@yahoo.com