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Systemic effects of local administration of vitamin C in experimental periodontitis and diabetic rats

Ayşe ToramanD.D.S.[1],Zeliha Aytekin D.D.S.[2], Ph.D.
[1] *Health Sciences University, Hamidiye Faculty of Dentistry, Department of Periodontology, 34668, İstanbul, Türkiye*
[2]*Department of Periodontology, Akdeniz University, Faculty of Dentistry, 07070, Antalya, Türkiye*

Correspondence: *Ayşe TORAMAN (ORCID ID: 0000-0001-7988-0765)*

HealthSciencesUniversity
Faculty of Dentistry
Department of Periodontology
34668 İstanbul / TÜRKİYE

ABSTRACT
Vitamin C is one of the antioxidants that are used topically or systemically as an adjunct in periodontal treatment and its benefits have been reported.The goal of this study is to evaluate the change in periodontal tissue, and serum levels of oxidative stress index (OSI), tumour necrosis factor (TNF)-a and interleukin-1 (IL-1)β after local vitamin C administration in rats with induced experimental diabetes and periodontitis.Totally, thirty (n=6, control, induced periodontitis 'EP', induced diabetes 'ED', ligature-inducedperiodontitis and alloxan-induceddiabetes 'EP-ED', and induced periodontitis+diabetes+locally Vitamin C 'ED-EP-LVitC') rats were included in this study.The locally vitamin C was applied three times at two-day intervals gingiva of rats in the treatment group, after induced diabetes and periodontitis. At the end of the experiment, the animals were sacrificed. Collected serum samples were analyzed biochemically and gingival samples were analyzedhistochemically.The level of serum OSI in the ED-EP-LVitC group was significantly lower than ED and ED-EP groups ($p<0.05$). Levels of serum TNF-a in the treatment group were significantly lower than EP groups ($p<0.05$). Levels of serum IL-1β in treatment groups were significantly lower than in groups of EP and ED-EP ($p<0.05$). Destruction in the periodontium was decreased by the local application of vitamin C ($p<0.05$).The present study suggested that the local application of vitamin C may be an effective adjuvant agent in the therapy of diabetes and periodontal diseases by reducing oxidative stress and inflammation. Further studies are needed to establish appropriate doses and intervals for local application of vitamin C in the clinic.
Keywords:*Vitamin C, Oxidative stress, Periodontitis, Diabetes, Rat*

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I. INTRODUCTION

Periodontal disease is a chronic inflammatory disease mainly caused by dental plaque, to which environmental, genetic, and systemic diseases (such as diabetes, and heart diseases vs.) may contribute[1]. Diabetes and periodontal disease are both chronic and common diseases that appear in the population, especially in individuals over the age of 65, and are related to each other[2].American diabetes association (ADA) reported that periodontal diseases are among the long-term complications of diabetes[3].Chronic local inflammation of the periodontium in periodontal disease may contribute more distantly to systemic inflammation [4]. Therefore, periodontal disease may aggravate the hyper-inflammatory process and hyperglycemiapresent in diabetes[5]. And, it may induce insulin resistance and then influence the metabolic process of diabetes. The persistence hyperglycemia, dysfunction of immune cells and advanced glycation end products (AGEs) in diabetes also exacerbate periodontal tissue inflammation and destruction [6]. Several pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin-1 (IL-1)β, andIL-6 areincreased in both diabetes and periodontitis[7]. The cytokines' overexpression and elevated cytokine levels inducethe formation of reactive oxygen species

(ROS) and increase oxidative stress [6]. The elevation of oxidative stress has an important place in the formation and progression of periodontal diseases [8, 9]. It has been reported that decreasing oxidative stress via antioxidant administration may be beneficial in reducing periodontal inflammation. Evidence revealed that the use of exogenous antioxidants in the treatment of periodontal diseases shows promising effects in reducing local inflammation[10, 11]. Vitamin C is one of the antioxidants that are used topically or systemically as an adjunct in periodontal treatment and its benefits have been reported[12-14].

The goal of this study is to examine the change in periodontal tissue, and serum levels of oxidative stress index (OSI), IL-1β, and TNF-α after local vitamin C administration in rats with induced experimental diabetes and periodontitis.

II. MATERIALS AND METHODS

2.1. Animals

Thirty male Sprague Dawley rats weighing ≈300 g were used in the study. The rats were randomly divided into five groups of six rats each: control, ligature-induced periodontitis (EP), alloxan-induced diabetes (ED),induced diabetes and periodontitis(ED-EP), and induced periodontitis+diabetes+locally Vitamin C (ED-EP-LVitC).This study was carried out after acceptance by the local ethics committee of Atatürk University for Animal Experiments (protocol 2021-4). All experiments were consistent with the National Institute of Health (NIH) Guide.The animals were maintained on a 12-h light/dark cycle at 23 °C to 25 °C. The animals had access to water and rat chow during the experiment.

2.2. Alloxan-induced diabetes

150 mg/kg alloxan monohydrate (Sigma Chemical Co., St. Louis, MO, USA) was injected intraperitoneally as a single dose to induce experimental diabetes in rats. Glucose values were recorded by a glucometer (Acon, Biotech, USA) from tail vein blood to check that diabetes was induced in rats three days and one week after injection.The rats used in the study had fasting blood glucose above 300 mg/dl [15].

2.3. Ligature-induced periodontitis

To establish experimental periodontitis, 3-0 silk sutures were placed around the teeth of the right mandibular first molars of rats and were removed on day 11 [16]. All procedures were performed under anesthesia with xylazine hydrochloride (Rompun, Bayer, Istanbul, Turkey) (0.1 ml/kg i.p.) and ketamine hydrochloride (Ketalar, Pfizer, Istanbul, Turkey) (1 ml/kg i.p.).

2.4. Locally vitamin C administration

After establishing ED and EP, locally 50 µL vitamin C (500 mg/5 mL Redoxon amp; Bayer Chemical Industry, Istanbul, Turkey) was applied to the subperiosteal at the buccal of right first molar gingiva in mandibles of rats with a 0.5 ml insulin needle (30 gauge; Becton Dickinson, Franklin Lakes, NJ) three times at intervals of 2 days[14, 17, 18].Locally 50 µL physiological saline was applied to create the same conditions in the EP, ED, and ED-EP groups.

2.5. Blood and tissue sampling

On day 20 of the experiment, cardiac blood samples (10 cc) collected under anesthesia were centrifuged at 1500 g for 10 minutes within 1 hour for biochemistry. Serum samples were kept at -80°C until the day of analysis. After the rats were sacrificed, the mandibles were dissected together with the surrounding tissues for histological analysis.

2.6. Histometricalanalysis

The loss of alveolar bone and attachment was evaluated by histological analysis. Mandibles fixed in 10% neutral buffered formaldehyde (48 hours) were decalcified with 5% nitric acid solution (7 days). Then the samples were embedded in paraffin. 5 µm thickness bucco-lingual sections were obtained from the mandible using a microtome, for hematoxylin–eosin staining. Systematically selected slices from among all sections in each mandibular first molar were used for immunohistochemical and histopathologic analysis.Alveolar bone level (ABL) (distance between the cemento-enamel junction 'CEJ'and the alveolar bone crest'ABC') and attachment loss (AL) (distance between the cemento-enamel junction and the periodontal attachment) were evaluated by a light microscope (KameramSLR;MikroSistem Ltd., Istanbul, Turkey) (Figure).

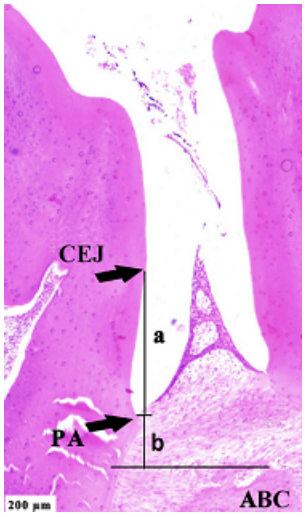

Figure. Photomicrographs of gingival mucosal tissues in the bucco-lingual sections of mandibular first molars (H&E staining).ABC, alveolar bone crest; CEJ, cemento-enamel junction; PA, junctional epithelium; a, distance between the CEJ and the coronal portion of junctional epithelium attachment; a+b, distance between the CEJ and the ABC.

2.7. Biochemical analysis

OSI (total oxidative stress -TOS-/total antioxidant capacity -TAOC-), is used to determine the level of oxidative stress with the balance of antioxidants. OSI values were calculated according to the formula: (OSI¼[(TOS, lmol/L)/ (TAS, lmol Trolox equivalent/L)100])[19].Levels of serum TOS, TAOC, IL-1β, and TNF-α were assessed by enzyme-linked immunoassay kit(Cusabio, Biotech Co.) according to the manufacturer's instructions.

2.8. Statistical analyses

Statistical analysis was done using IBM SPSS Statistics version 20. All data were presented as mean ± SD, and $p<0.05$ was considered significant. Kolmogorov-Smirnov was used for normality analysis and Levene's homogeneity test was used to evaluate for homogeneity of the data. Between-group differences were analyzed with one-way ANOVA and post-hoc Tukey test.

III. RESULTS

3.1. Biochemical results

Biochemical results were presented in the Table. Serum OSI levels of all experimental groups were significantly higher than the control group($p<0.05$).The level of serum OSI in the ED-EP group was significantly higher than in ED and EP ($p<0.05$).The level of serum OSI in the ED-EP-LVitC group was significantly lower than in ED and ED-EP($p<0.05$).

Levels of serum TNF-αin all experimental groups were significantly higher than the control group ($p<0.05$). Levels of serum TNF-αin ED-EP-LVitC group were significantly lower than in EP groups($p<0.05$).Serum TNF-αvaluein ED-EP-LVitC group was lower than ED and ED-EP groups, but it was not statistically significant ($p>0.05$).

Levels of serum IL-1β in EP, ED-EP and ED-EP-LVitC groups were significantly higher than in ED and control groups($p<0.05$). Levels of serum IL-1β in ED-EP-LVitC groups were significantly lower than in EP and ED-EP groups($p<0.05$).

3.2. Histometricalresults

Histometrical results were presented in the Table. There was a significantly higher AL in all experimental groups compared to the control group($p<0.05$). AL in EP, ED, and ED-EP-LVitC groups were significantly higher than the ED group ($p<0.05$). AL in the ED-EP group was significantly higher than the EP group ($p<0.05$).AL in ED-EP-LVitC group was significantly lower than ED-EP group ($p<0.05$). AL in ED-EP-LVitC group was lower than EP group, but it was not statistically significant ($p>0.05$).

There was a significantly higher ABL in groups of EP, ED-EP, and ED-EP-LVitCcompared to the ED and control ($p<0.05$). The ABL ofED-EP group was significantly higher than EP ($p<0.05$). ABL was significantly less in the ED-EP-LVitC group than in the ED-EP group ($p<0.05$).

Table: Comparison of Biochemical and Histometrical Data between the groups (mean ± SD).

Groups	C (n=6)	D (n=6)	P (n=6)	D-P (n=6)	D-P-LvitC (n=6)
OSI (n/µm)	0.91±0.07	1.51±0.09[a]	1.42±0.12[a]	1.82±0.12[a,b,,c]	1.24±0.19[a,b,d]
TNF-α (pg/ml)	40.60±2.32	54.18±4.78[a]	60.09±1.55[a]	56.07±4.69[a]	50.88±7.71[a,c]
IL-1β (pg/ml)	4.29±0.34	4.41±0.29	7.72±0.48[a,b]	8.3±0.4[a,b]	6.19±1.71[a,b,c,d]
AL (µm)	336.25±14.33	379.41±21.64[a]	1167.66±69.74[a,b]	1636±53.15[a,b,c]	1002±124.78[a,b,d]
ABL (%)	63.75±2.13	60.75±1.47	50.00±2.12[a,b]	37.50±2.82[a,b,c]	49.83±7.36[a,b,d]

[a] Statistically significant difference (P <0.05, Tukey HSD multiple comparison test) compared with C group.
[b] Statistically significant difference (P <0.05, Tukey HSD multiple comparison test) compared with D group.
[c] Statistically significant difference (P <0.05, Tukey HSD multiple comparison test) compared with P group.
[d] Statistically significant difference (P <0.05, Tukey HSD multiple comparison test) compared with D-P group.
Alveolar bone level: ABL; Attachment loss: AL

IV. DISCUSSION

As far as we know, no other study evaluating the systemic influence of local vitamin C application in periodontitis and diabetes has been conducted. The present study reveals that locally administrated vitamin C reduces levels of serum inflammation and oxidative stress markers and decreases attachment and bone loss in animals with diabetes and periodontitis.

Two factors, stage and grade, have been proposed as determinants in a new classification of periodontal disease. Stage indicates the severity of periodontal damage, while grade gives an idea of the patient's possible response to traditional periodontal treatment strategies.Diabetes is one of the risk factors that worsen the grade of periodontitis and affect the treatment [20].It has been reported that diabetes can modify the periodontal disease process as it increases the expression of pro-inflammatory cytokines (such as TNF-α, IL-1β, IL-6, IL-8), thereby increasing systemic inflammation [6]. Sun et al. [21]reported higher serum TNF-α values in patients with periodontitis and diabetes compared to healthy individuals. In addition, in the same patient group, they found that TNF-α levels at 3 months after periodontal treatment decreased compared with group without periodontal treatment [21]. A study [22]demonstrated that values of serum IL-1β and TNF-α in rats with periodontitis were significantly higher than control. In the present study, serum levels of IL-1β and TNF-α in the EP and ED-EP groups were significantly higher compared to the groups of control and ED. This result is consistent with previous studies [6, 21, 22].

Evidence suggests that oxidative stress increases in both local (in this case periodontal) and systemic inflammation, and that increased oxidative stress exacerbates inflammation [11, 23]. It is reported that oxidative stress increases significantly in both diabetes[24] and periodontitis [23].Studies revealed that periodontitis is associated with higher levels of serum OSI [25, 26]. In another study [27], the mean of TOS and OSI in gingival crevicular fluid were seen to be higher in subjects with only periodontitis, only diabetes, diabetes and periodontitis than in healthy individuals. In our study, serum OSI values of all experimental groups were significantly higher than the control group. We found that the presence of both diabetes and periodontitis caused the highest oxidative stress.

In recent years, it has been suggested that better results may be obtained with the combined use of antioxidants and periodontal therapy in patients with diabetes [10]. Vitamin C is one of the most powerful antioxidants that protects against harmful oxidants [28]. There are various studies in the literature regarding the systemic exogenous administration of Vitamin C in periodontitis and diabetes [13, 29-32]. However, the evidence for the local application of Vitamin C is very limited. One study reported that vitamin C injected locally into the gingiva reduced several degrees of chronic gingivitis [33]. Aytekin et al.[18] showed that locally vitamin C application to rats with experimental periodontitis decreased serum OSI values. In our previous study [14], locally vitamin C application reduced IL-6 and 8-hydroxy-2-deoxyguanosine in gingival tissues of rats with experimental periodontitis and diabetes.In the present study, serum TNF-α level in the ED-EP-LvitC group was significantly lower than in the ED-EP group.And, serum IL-1β level in the ED-EP-LvitC group was significantly lower than in the ED, EP, and ED-EP groups. Also, serum OSI level in the ED-EP-LvitC group was significantly lower than in the ED and ED-EP groups. The information is available in the literature that inflammation in the periodontium may contribute to systemic inflammation and that conversely, successful local periodontal treatment decreases levels of systemic inflammatory markers [4]. In light of this information, it may be concluded from our results that locally administration of vitamin C contributes to the attenuation of systemic inflammation and oxidative stress by reducing periodontal inflammation.

If IL-1β and TNF-α production exceeds the “critical level” in periodontal disease, the result is attachment and bone loss [34]. On the other hand, elevation of oxidative stress may play one of the most critical

roles in the destruction of periodontal tissue and loss of alveolar bone [10, 11]. In the current study, loss of attachment and alveolar bone were detected in all experimental groups in which oxidative stress and inflammation were increased. Periodontal destruction was mostly observed in the ED-EP group. The loss of attachment and bone were significantly less in the treatment group than in the ED-EP. These results showed the protective effect of local antioxidant application on periodontal tissues in the presence of diabetes and periodontitis.

This study has some limitations.Firstly,since the study was carried out under experimental conditions, it is not possible to apply the dose of vitamin C used and the results obtained directly to humans.Another limitation is the absence of micro-CT, that bone loss can be better evaluated.

V. CONCLUSIONS

The results of this study may hypothesize that local application of vitamin C may be an effective adjuvant agent in the treatment of diabetes and periodontal diseases by reducing oxidative stress and inflammation. Further studies are needed to establish appropriate doses and intervals for local application of vitamin C in the clinic.

DECLARATIONS

Authors' contributions

Ayşe Toraman: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Final approval of the version to be submitted

Zeliha Aytekin: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - Original Draft, Visualization, Final approval of the version to be submitted

Conflict of interest

The authors declare no conflicts of interest related to this study.

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Informed consent

This study was carried out after acceptance by the local ethics committee of Atatürk University for Animal Experiments (protocol 2021-4). All experiments were consistent with the National Institute of Health (NIH) Guide.

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