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Effects of LDD and CAPE administration on total antioxidant and total oxidant levels in experimental periodontitis model of rat brain

Sıçan beyninin deneysel periodontitis modelinde DDD ve KAFE uygulamasının toplam antioksidan ve toplam oksidan düzeyleri üzerine etkileri

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ABSTRACT

Aim: Observing the effects of caffeic acid phenethyl ester (CAPE) and/or low dose doxycycline (LDD) on total antioxidant and oxidant status of brain in experimental periodontitis is the purpose of the study.

Methods: 48 male Wistar albino rats were designed as the following: control group (C, n=8), periodontitis + CAPE group (PC, n=10), periodontitis + LDD (PD, n=10), periodontitis + LDD + CAPE group (PCD, n=10), and periodontitis group (P, n=10). The time period for the experiment was 14 days. 10 µmol/kg/day of CAPE was administered using an intraperitoneal injection (IP). 10 mg/kg/day of LDD was administered using an oral gavage method. Histopathological changes were evaluated.

Results: Beneficial results were seen in all of the groups after LDD and/or CAPE administration on decreasing the alveolar bone loss level and oxidative stress. All of the experimental groups showed signs of periodontitis with alveolar bone loss. The P group leads with the most alveolar bone loss compared to the other periodontitis groups and the lowest group was the PC group in the periodontitis groups. The evolution of alveolar bone loss from high to low was that group P, group PD, group PCD, group PC, and group C ($P < 0.05$). However, there is no statistical difference between brain total antioxidant status and brain total oxidant status average values according to brain groups ($p > 0.05$).

Conclusion: The combination of LDD and CAPE are not significantly different when applied alone or together on oxidative status. But both of the agents have beneficial effects on reducing the oxidative stress and tissue damages.

Key words: Brain, Oxidative stress, periodontitis, total antioxidant status

ÖZ

Amaç: Deneysel periodontitis modelinde kafeik asit fenetil ester (CAPE) ve/veya düşük doz doksisiklinin (LDD) beyin total antioksidan ve oksidanlar üzerine etkisinin araştırılmasıdır.

Yöntem: 48 adet erkek Wistar albino rat, kontrol grubu (C, n=8), periodontitis + CAPE grubu (PC, n=10), periodontitis + LDD (PD, n=10), periodontitis + LDD + CAPE (PCD, n=10) ve periodontitis grubu (P, n=10) şeklinde gruplara ayrıldı. Çalışma süresi 14 gün olarak belirlendi. 10µmol/kg/gün CAPE, bir intraperitoneal enjeksiyon (İP) kullanılarak,10 mg/kg/gün LDD, oral gavaj yöntemi kullanılarak uygulandı. Değişiklikler histopatolojik olarak değerlendirildi.

Bulgular: LDD ve/veya CAPE uygulamasının tüm gruplarda oksidatif stress ve alveolar kemik kaybı şiddetinin azaltması açısından faydalı sonuçlar gösterdiği belirlendi. Deney gruplarının tamamında periodontitisin kesin bulgusu olarak alveolar kemik kaybı tespit edildi. Sadece P grubunda, diğer periodontitis gruplarına kıyasla daha fazla alveoler kemik kaybı gözlenirken, PC grubunda ise en az alveolar kemik kaybı gözlendi. Gruplar arasında alveolar kemik kaybının yüksekten düşüğe sıralaması şu şekildeydi: grup P, grup PD, grup PCD, grup PC ve grup C ($p < 0.05$). Buna ragmen beyin gruplarına göre beyin total antioksidan durumu ve beyin total oksidan durumu ortalama değerleri arasında istatistiksel olarak anlamlı bir farklılık tespit edilmedi. ($p > 0.05$).

Sonuç: LDD ve CAPE kombinasyonu, tek başına veya birlikte uygulandığında oksidatif durum ve doku hasarı üzerine istatistiksel olarak herhangi bir anlamlı farklılık göstermedi. Ancak her iki ajanın da oksidatif stresi ve doku hasarlarını azaltmada faydalı etkileri vardır.

Anahtar kelimeler: Beyin, oksidatif stres, periodontitis, toplam antioksidan seviyesi

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INTRODUCTION

Periodontitis is a chronic inflammatory disease that originates from dental plaque. It is characterized by soft and hard tissue destruction around the teeth. Many studies have discussed the possible relationship between periodontitis and several systemic diseases such as diabetes, atherosclerotic cardiovascular diseases, low birth weight, metabolic syndrome, chronic renal failure, rheumatoid arthritis and neurodegenerative diseases [1]. This is emphasized in the new classification of periodontal diseases in 2017, as the main topic of this resource is the host immune response, associated periodontitis and relationship with systemic diseases [2].

The basic stimulant of inflammatory diseases is oxidative stress and periodontitis is a type of chronic inflammatory disease that is affected from the imbalance between the oxidative and antioxidative status [3]. In the activation of the disease, periodontopathogens in periodontitis stimulate neutrophils and cause to elevate oxidative stress status, reactive oxygen (ROS) and nitrogen species (RNS), load and by this way this is called like ‘‘respiratory burst’’. Hypochlorous acid, superoxide anion, and hydrogen peroxide are the most increased reactive specieses. The antioxidant barrier may be impaired after an attack of oxidative stress and then, oxidative/nitrosative modifications of cell components may be damaged [4]. The impaired antioxidant balance causes to decrease scavenging mechanisms of free radicals and the neutralization activity. Interestingly, periodontitis showed some differences with both increase/decrease in activity of scavenging [3]. The same ROS can severely damage the structure and function of cell membranes and even oxidative damage to tissues and organs. In a physiological state, ROS occurs as a natural byproduct of normal oxygen metabolism and has important roles in cell signaling and homeostasis. However, apart from environmental stress, increased ROS production along with a reduced antioxidant defense has been suggested to play a critical role in brain damage [5]. Brain tissue is more susceptible to oxidative damage due to high polyunsaturated fat content, high oxygen consumption and insufficient antioxidants. Astrocytes supply antioxidants and free radical scavengers that protect the brain from oxidative stress [6]. For all this, host modulation therapies (HMTs) deal to inhibit or stabilize the tissue breakdown, as well as increasing the levels of regenerative or preventive responses [7].

Caffeic acid is a biologic active content of honeybee propolis which is one of the well-known host modulator agent and scavenger of free radicals. It activates the antioxidant enzymes such as superoxide dismutase and catalase against free radicals and protects tissues against lipid peroxidation (LPO) which is produced by the oxidative stress and damages the cell integrity with its flavanoid content [8,9].

Caffeic acid and its derivatives have been extensively studied in the past, showing that these chemicals have functions such as acting as antioxidants, suppressing cerebral LPO, and reducing cerebral infraction in. It has also been suggested that caffeic acid and its derivatives have therapeutic potential in the treatment of neurodegenerative diseases [5].

Today, researchers suggest using CAPE as a therapeutic agent in periodontal treatment to increase host response by its known beneficial properties such as antioxidant, anticarcinogenic, immunomodulatory and anti-inflammatory [10]. The benefits of CAPE in vivo periodontal disease models have not been investigated completely. Only one study has reported in vivo experimental periodontitis [10]. However, in vitro studies, about the relation of periodontopathogens and cellular responses in culture media have been studied in a few studies [10].

Another immunomodulatory agent is doxycycline and it is a member of the tetracycline antibiotic family [7]. Besides its anti-microbial properties, at low (subantimicrobial) doses doxycycline (LDD) has anti-inflammatory and anti-oxidant effects by the way of decreasing nuclear factor-κappaB activity [7]. Yağan et al. has emphasized the beneficial effect of LDD on the treatment of periodontitis by its antioxidative performance and collagenolytic activity [7].

The multitude of enzymatic and non-enzymatic antioxidants as well as their synergistic effects cause an interest in the evaluation of the total oxidative and antioxidative capacity. The recent

research results indicate that the antioxidant/oxidant capacity of saliva is used in the diagnosis of such systemic diseases as chronic renal disease, hypertension, chronic heart disease, or psoriasis [3]. It is therefore necessary to evaluate several parameters characterizing the total antioxidant potential of the body [3].

To date, there have been no studies investigating the efficacy of LDD combined with CAPE in the management of periodontitis and its relationship with the brain. The aim of the present study was to evaluate the effects of LDD and/or CAPE on ABL and TAS-TOS levels, in an experimental periodontitis rat model.

MATERIALS AND METHODS

Animals, Care and Nutrition

A total of forty-eight female Wistar Albino rats weighing 200±20 g were kept under laboratory conditions with a 12-hour light/dark cycle and a room temperature of 21±3ºC. Standard pellet food was used for feeding and all animals had free access to water. The study was approved by the Mehmet Akif Ersoy University Experimental Medical Research Center's Experimental Animals Ethics Committee (17.02.2021-719).

Animals and Treatment

The forty-eight rats were randomly divided into five groups. The groups were group C (n:8); control, group P (n:10); periodontitis, group PC (n:10); periodontitis administered CAPE, group PD (n:10): periodontitis administered LDD, and group PCD (n:10); periodontitis administered CAPE and LDD.

Experimental periodontitis was induced in rats under general anesthesia by the intraperitoneal injection of ketamine, (90 mg/kg of body weight) and xylazine (10 mg/kg of body weight) by placement of sterile 3–0 silk ligatures in a subgingival position around the maxillary 2nd molars in all groups for 14 days, except Group C. All rats were periodontally healthy because of the exclusion of rats with 0.5 mm periodontal probing depths. CAPE3 10 µmol/kg/day, was intraperitoneally administered [11], with LDD,4 10 mg/kg/day, being administered by oral gavage [12], over the experimental 14-day period. The ligature-induced periodontitis model, which produces plaque accumulation and allows interaction between host and bacteria in the dentogingival area, is highly reproducible and reliable [12,13]. The ligature-induced experimental periodontitis model in rats is recommended for short observation periods (≤15 days) [13].

CAPE3 10 µmol/kg/day, was intraperitoneally administered [5], with LDD4, 10 mg/kg/day, being administered orally, over the experimental 14-day period [7]. There are three ways to administer the CAPE, namely topically, orally and intraperitoneally. All of these have shown beneficial effects, however the significant positive effects was seen in the intraperitoneal applied groups, therefore we chose the intraperitoneal administration, in accordance with the literature [14]. Novel "low doses" or non-antibacterial formulations of tetracyclines, such as subantimicrobial-dose doxycycline (SDD) or low-dose doxycycline (LDD) as an adjunctive treatment of periodontal disease, have been approved by the US Food and Drug Administration and other national regulatory agencies in Canada and Europe. According to previous studies, all of the application designs were focused on oral administration [12]. On the other hand, according to our reviews of the literature, the best absorption for CAPE should be administered as i.p. and the antibiotics are generally administered as oral gavage. We believe these two methods can be applied together.

Ketamine/xylazine (60/5 mg/kg) anesthesia was used for the sacrification. The tissues were removed and divided longitudinally into two sections after the sacrification, one part for biochemical analysis (stored under -70 °C) and the other for histologic evaluation (hidden kept in formaldehyde solution).

The total antioxidant status (TAS)

The method of Erel O. was applied for the measurement of TAS supernatant fractions [15,16]. Hydroxyl radicals and similar biologic radicals were produced using this method. Hydrogen peroxide was mixed with a ferrous ion solution and this application developed a potent radical the likes of dianisidinyl radical cations in the experiment. The measurement of the material antioxidative effect was done by the hydroxyl

radicals. The sensitivity values were lower than 3%. The total antioxidant response results were recorded as nmol Trolox equivalent/mg protein.

The total oxidant status (TOS)

The method of Erel O. was applied for the measurement of TOS supernatant fractions [15,16]. The oxidants in the sample oxidized ferrous ion-o-dianisidine complex to ferric ion. This ferric ion incubates a colored complex with xylenol orange in the acidic medium and the intensity of the color that is related to the total amount of oxidant molecules was able to be measured by spectrophotometric methods. The measurement was calibrated with hydrogen peroxide and the results were presented as nmol H2O2 equivalent/ng protein. Oxidation reaction was increased due to the glycerol molecules which were too abundant in the reaction medium.

Histological examination

Firstly, the tissues were kept in 10% neutral buffered formaldehyde and were then dehydrated in alcohol and embedded in paraffin. Tissues were divided to the 4 µm sections, deparaffinized and stained with hematoxylin and eosin. The samples were analyzed with light microscopy and blindly randomized. Inflammation, oedema, congestion, degeneration, necrosis and necrobiosis were evaluated. After the results were graded, all of the groups were compared with each other.

Statistical Analysis

Analyzed was performed with the IBM SPSS V23 and compliance with normal distribution was examined using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) was used in the comparison with normal distribution groups. The Tukey HSD multiple comparison test was preferred to find out from which group the significant difference originated in the analysis. Analysis results are presented as mean value and standard deviation. The significance level was taken as p <0.05.

RESULTS

All of the experimental groups showed periodontitis signs with alveolar bone loss. The P group leads the most alveolar bone loss, compared to the other periodontitis groups. The lowest group was the PC group in the periodontitis groups. The order from high to low was that group P, group PD, group PCD, group PC, and group C ($P < 0.05$) and the differences were significant. The alveolar bone loss was measured from the cementoenamel junction to the alveolar bone crest (Table 1 and Figure 1).

Table 1.Comparison of Brain TAS and TOS mean values according to groups

	Brain TAS (mmolTrolox Equıv/L)	Brain TOS (mmolTrolox Equıv/L)	ABL (mm)
Cape (n= 10)	0,64 ± 0,14	1,92 ± 0,70	0.42 ± 0.14
Dox (n= 10)	0,42 ± 0,17	2,49 ± 0,58	0.60 ± 0.12
Dox-Cape (n= 10)	0,58 ± 0,24	2,20 ± 0,72	0.56 ± 0.19
Control (n= 8)	0,58 ± 0,24	2,69 ± 0,56	0.22 ± 0.04
Perio (n= 10)	0,42 ± 0,14	2,09 ± 0,56	0.83 ± 0.24
Total (n= 48)	0,52 ± 0,21	2,27 ± 0,66	0,52± 0.14
p	0,05	0,1	

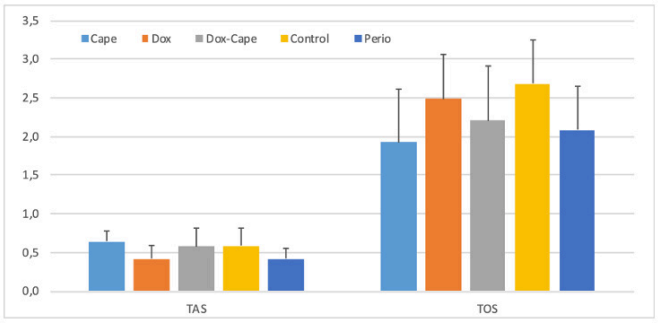

Figure 1. Mean and standard deviation graph of TAS and TOS values by groups

Biochemical Results

There was no periodontal inflammation and bone resorption in group C, where unligated animals and interdental papilla remained intact. All of the periodontitis-induced groups showed alveolar bone loss with increased oxidant activity. However, there were no statistical differences between brain TAS and brain TOS average values (p values 0.05 and 0.1, respectively). The brain TOS levels were precisely observed in the groups, as follows: the PC group was 1.92, in the PD group the level was 2,49, the level was 2.2 in the PCD group, the C group was 2.69 and the P group was 2.09 mmolTrolox Equiv/L. When the brain TAS mean values were examined by groups, the level of the group PC was 0.64 and, it was 0.52 in the

P group.

The results observed reveal that increased periodontal damage can affect periodontal tissues, alveolar bone levels and brain oxidative stress negatively.

DISCUSSION

The question of how the periodontal attachment apparatus is damaged in periodontal diseases has been discussed many times in previous studies. The most common answer is oxidative stress and its direct effect on extracellular matrix components degradation, such as collagen, elastin, proteoglycans and glycosaminoglycans (eg; hyaluronic acid) [3]. In fact, the periodontal infection can also be increased by oxidative stress and host response [3].The best evidence of this promotion is the increasing activity of nicotinamide adenine dinucleotide phosphate (NADPH) (NOX) oxidase and lysosomal enzymes, which are important in periodontal pathogens and tissue destruction because of its protection of redox stress accomplished by providing reducing equivalents to antioxidants, such as glutathione and thioredoxin [3].

There are various experimental periodontitis models In the literature [7,17]. The ligature-induced periodontitis model, which produces plaque accumulation and allows interaction between host and bacteria in the dentogingival area, is highly reproducible and reliable. The ligature-induced experimental periodontitis model in rats is recommended for short observation periods (≤15 days) [7]. Toker et al. reported that alveolar bone resorption reached a peak at 11 days and increased from 1 to 15 days in ligature-induced experimental periodontitis [17]. In the present study, experimental periodontitis was evaluated at day 14, with morphometric analysis revealing that periodontitis was successfully induced in the experiment groups.

There are a great number of studies that have shown the increased TOS levels in periodontitis. Wei et al. observed that LPO levels and TOS were higher in the periodontal region and also in serum, gingival crevicular fluid, blood and unstimulated saliva [9]. Baltacıoğlu et al. have reported similar results as the former: they showed that the increased TOS and decreased TAS, rather than LPO, had an important role in the periodontal pathology, and they also emphasized that TOS levels increase by the periodontitis levels [18]. Zhang et al. had reported the importance of saliva for diagnosis periodontal inflammation and they showed that oxidative stress and TOS are important for periodontitis progression, tissue damage and immune response [19].

CAPE is one of the effective regenerative solutions for bone loss, through its bone healing process and prevention capacity on RANKL-induced osteoclastogenesis. This property, as well as its antioxidant effects in periodontitis and bone healing, have been studied in various studies [20].

LDD is also an effective agent in periodontitis treatment, especially in preventing alveolar bone loss by way of decreasing NF-κB activity. Yağan et al. has emphasized the beneficial effect of low dose doxycycline (LDD) on the treatment of periodontitis by its antioxidative performance and collagenolytic activity [7]. Several studies have shown that LDD suppresses MMPs on the host in periodontal lesion, thereby inhibiting other components of periodontal tissues (fibronectin, proteoglycan ground substance, elastin and basement membranes) and bone resorption [12]. In addition to inhibiting MMPs, doxycycline has antioxidant effects that may be found against beneficial effects in recent studies [12]. Akamatsu et al. reported that doxycycline significantly reduced O2-, H2O2, and -OH levels in human neutrophils [21]. Recent research has focused on the role of ROS, LPO products and antioxidant systems in the periodontal pathology. These studies have reported that the level of ROS molecules with periodontitis increase and when this occurs, oxidative damage begins in the periodontal ligaments and cause osteoclastic bone resorption [12].

In this study, the CAPE induced groups (the PC and PCD group) were more successful than the other groups (the P and PD group) in reducing the alveolar bone loss. Many neuroinflammatory diseases have proven to be the underlying cause of oxidative stress associated with inflammation [6,22,23]. Researchers have reported different neurotoxic reactions and oxidative responses to

aldehydes in different regions of the brain such as the frontal and occipital lobes [6,24].

Uzar et al. reported that CAPE reduced the activities of antioxidant enzymes and malondialdehyde restriction in the cerebellum of rats exposed to methotrexate [25]. Ginis et al. reported that pretreatment with CAPE can protect brain tissue against central neurotoxicity due to ifosfamide [26]. Huang et al. reported that CAPE could provide a promising avenue for treating neurodegenerative diseases with oxidative stress acrolein, such as Alzheimer's disease [27].

MMP is a marker that arises in ischemic organs, such as heart and brain under any damage or injury, because MMPs regulate inflammation, epithelial-mesenchymal transition, cell proliferation, angiogenesis and apoptosis. Doxycycline inhibits MMPs and shows beneficial molecular effects in the brain and heart [28].

Although optimal doses of tetracyclines and drugs to the brain have been studied in experimental models for a wide variety of neurological diseases, tetracyclines, particularly minocycline, remain a concern [29]. However, inhibition of cerebral MMP-9 correlates well with drug levels measured in the brain, despite variability in drug distribution for tetracyclines. Previous studies have suggested several types of inhibition suggesting that it is linked to modulation such as gene transcription, scavenging reagent strains, or Zn2+ binding [29,30].

In this study, the increased TOS and decreased TAS levels were seen in the periodontitis group as well as brain tissues that induced periodontitis. The findings of this study demonstrate that CAPE is an effective additive agent in reducing oxidative stress, TOS versus increasing the TAS not only LDD, but also when given as a combination therapy with CAPE. But, the effects of CAPE and LDD combinations on ABL did not change so crazy in rats with ligature-induced periodontitis.

Many studies have showed that treatment with LDD or CAPE significantly reduced the inflammatory infiltration and bone resorption levels in rats [7]. In the present study, histomorphometric findings showed the lowest ABL in the PC group, when compared to the other experiment groups. ABL was not found to be different between the PD and PCD groups.

Exogenic radicals (radiation, pollution, smoking) and endogenic factors (inflammation, xenobiotic killing) may be a reason of increasing oxidative damage. [31]. Cells, cell membranes and extracellular fluids contain antioxidants and these antioxidants target and neutralize excessive and inappropriate ROS formation for improving the balance mechanism against the oxidants (e.g., chronic inflammation, cigarette smoking, and diets poor in antioxidants and/or rich in pro-oxidants [31]. The transporter in this balance mechanism is blood, through distribution of the related antioxidants to the related part of the body. The main problem is the reactions between catalytic metal ions and long-lived ROS, such as the superoxide anion or hydrogen peroxide and in this situation, plasma can break this chain and prevent more harmful effect of the ROS. Plasma can scavenge long-lived ROS, such as the superoxide anion or hydrogen peroxide, thus preventing reactions with catalytic metal ions to produce more harmful species [31]. For example, ascorbate can be turned back from oxidized ascorbic acid by plasma, so that many different compounds and systemic metabolic interactions present plasma antioxidant status [31].

Within the limitations of this study, we find that the different dose groups could not be included in the study, since there is no study in the literature on ideal dose adjustment for CAPE and LDD. Studies with different doses, durations and administration methods are needed within the limits of the study. Since our study is the first to investigate the antioxidant effect of CAPE and/or LDD on periodontal disease and its relation with the brain, there is a need for additional studies in which the effects on all periodontal tissues are examined and other host modulatory agents are compared, and dose-dependent/non-dependent and systemic effects are monitored.

CONCLUSION

The combination of drugs may be effective for anti-oxidative purposes and their different doses could be suitable for the treatment of neuronal diseases. In this study, the combination of LDD and CAPE are not significantly different when applied alone

or together on oxidative status. But both of the agents have beneficial effects on reducing the oxidative stress and tissue damages.

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