EFFECT OF ANGIOTENSIN-CONVERTING ENZYME INHIBITORS ON VASCULAR ENDOTHELIAL FUNCTION IN HYPERTENSIVE PATIENTS AFTER INTENSIVE PERIODONTAL TREATMENT

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ABSTRACT

There is a relation between vascular endothelial function, atherosclerotic disease, and inflammation. Deterioration of endothelial function has been observed twenty-four hours after intensive periodontal treatment. This effect may be counteracted by the action of angiotensin-converting enzyme inhibitors, which improve endothelial function. The aim of the present study was to evaluate vascular endothelial function after intensive periodontal treatment, in hypertensive patients treated with angiotensin-converting enzyme inhibitors.

A prospective, longitudinal, comparative study involving repeated measurements was conducted. Fifty-two consecutive patients with severe periodontal disease were divided into two groups, one comprising hypertensive patients treated with converting enzyme inhibitors and the other comprising patients with no clinical signs of pathology and not receiving angiotensin-converting enzyme inhibitors. Endothelial function was assessed by measuring post-ischemic dilation of the humeral artery (baseline echocardiography Doppler), and intensive periodontal treatment was performed 24h later. Endothelial function was re-assessed 24h and 15 days after periodontal treatment. Statistical analysis: Results were analyzed using the SPSS 20 statistical software package. Student's t test and MANOVA were calculated and linear regression analysis with 95% confidence intervals and $\alpha < 0.05$ was performed. Arterial dilation at 24 hours was lower compared to baseline in both groups; values corresponding to the groups receiving angiotensin-converting enzyme inhibitors were 11.89 ± 4.87 vs. $7.30 \pm 2.90\%$ ($p < 0.01$) and those corresponding to the group not receiving ACE inhibitors were 12.72 ± 4.62 vs. 3.56 ± 2.39 ($p < 0.001$). The differences between groups were statistically significant ($p < 0.001$). Conclusion: The increase in endothelial dysfunction after intensive periodontal treatment was significantly lower in hypertensive patients treated with angiotensin-converting enzyme inhibitors. Endothelial function improved 15 days after periodontal treatment, reaching baseline values. These results support the protective effect of angiotensin converting enzyme inhibitors on the endothelial function after intensive periodontal treatment.

Key words: Hypertension, angiotensin-converting enzyme inhibitors, periodontal diseases.

EFECTO DE LOS INHIBIDORES DE LA ENZIMA CONVERTIDORA SOBRE LA FUNCIÓN DEL ENDOTELIO VASCULAR EN PACIENTES HIPERTENSOS QUE RECIBIERON TRATAMIENTO PERIODONTAL INTENSIVO

RESUMEN

Existe relación entre la disfunción del endotelio vascular, la enfermedad aterosclerótica y la inflamación. A las 24 h del tratamiento intensivo de la enfermedad periodontal se produce un deterioro de la función endotelial. Este efecto podría ser balanceado por la acción de los inhibidores de la enzima convertidora de la angiotensina que mejoran la función endotelial. El objetivo del presente estudio fue evaluar la función endotelial vascular después del tratamiento periodontal intensivo, en pacientes hipertensos tratados con inhibidores de la enzima convertidora de la angiotensina. Se realizó un estudio prospectivo, longitudinal, comparativo, con mediciones repetidas. Se incorporaron 52 pacientes consecutivos, con enfermedad periodontal severa divididos en dos grupos, uno con hipertensión arterial tratados con inhibidores de la enzima convertidora y el otro sin inhibidores ni patología clínicamente evidente. Se determinó la función endotelial cuantificando la dilatación de la arterial humeral post isquemia ecocardiografía Doppler basal. A las 24 h se efectuó el tratamiento periodontal intensivo; a 24 h y 15 días posteriores se reevaluó la función endotelial. Análisis estadístico: se empleó el paquete estadístico SPSS 20. Se realizaron: t-test de Student, MANOVA y análisis de regresión lineal con intervalos de confianza del 95% y $\alpha < 0.05$. Resultados: a las 24 h post tratamiento periodontal se observó una menor dilatación arterial en ambos grupos en relación a la dilatación arterial basal, siendo para el grupo con inhibidores 11.89 ± 4.87 vs. $7.30 \pm 2.90\%$, $p < 0.01$ y para el grupo sin inhibidores 12.72 ± 4.62 vs 3.56 ± 2.39, $p < 0.001$, con diferencias significativas entre ambos $p < 0.001$. En conclusión el aumento de la disfunción endotelial post tratamiento intensivo periodontal fue significativamente menor en hipertensos que recibieron inhibidores de la enzima convertidora de la angiotensina. La función endotelial mejoró a los 15 días de efectuado el tratamiento, alcanzando los valores inciales. Estos resultados permitirían relacionar a los inhibidores de la enzima convertidora con un efecto protector del endotelio posterior al tratamiento intensivo de la enfermedad periodontal.

Palabras Clave: Hipertensión arterial, inhibidores de la enzima convertidora, enfermedad periodontal.

INTRODUCTION

Vascular endothelial function (VEF) plays a fundamental role in the pathogenesis of atherosclerosis[1]. The endothelium plays an important part in maintaining vascular homeostasis, and is involved in hemodynamics and antithrombotic activity[2]. It consists of a single layer of cells that separates the circulating blood from the smooth vascular muscle, and produces signaling molecules that regulate vascular tone, monocyte and neutrophil adhesion, and platelet aggregation. The endothelium produces nitric oxide (NO)[3] from the amino acid L-arginine, through activation of NO synthase. NO plays a key role because of its potent vasodilator action. It inhibits leukocyte adhesion and platelet aggregation, and inhibits smooth muscle cell proliferation. Because NO acts as a free radical scavenger, it has antioxidant properties, and it is involved in the release of plasminogen activator, which has a fibrinolytic effect and antithrombotic properties. [4-6] There are certain substances that act on the vascular endothelium exerting a proinflammatory effect, and have the capacity to generate reactive oxygen species (ROS). The latter are responsible for the degradation of NO and modulate vascular tone. One of such substances with the strongest vasoconstrictor effect is angiotensin II. [2-7]

A disturbance in the balance between vasodilators and vasoconstrictors causes vascular endothelial dysfunction, with decreased production and increased degradation of NO. This alteration in VEF causes vasoconstriction, platelet aggregation, leukocyte adhesion, and proliferation of smooth muscle cells[8-11]. VEF is assessed by measuring brachial artery flow-mediated dilation following ischemia[12]. The latter is associated with NO released by the arterial endothelium in response to the shear stress in the blood flow. The decrease in NO release from dysfunctional endothelium generates a paradoxical vasoconstrictive response to acetylcholine[4].

Other works reported in the literature have shown vascular endothelial dysfunction to be associated with a variety of diseases and risk factors for atherosclerosis, ranging from hypertension (HTN), aging[13], dyslipidemia[14], diabetes[15] and smoking [16-17], to specific cardiovascular diseases (CVD), including coronary, cerebral, and peripheral arteriopathy[14-16] as well chronic infection and inflammation, such as periodontal disease.[18-22] Case-control-cohort studies have shown that periodontitis is associated with endothelial dysfunction[23], atherosclerosis[24-26], and increased risk of myocardial infarction and stroke[27-29]. Despite these findings, the statement of the American Heart Association indicates that there is no causative relationship between periodontal and atherosclerotic vascular disease. It is of note, however, that both pathologies share risk factors, such as diabetes and smoking[30]. Tonetti M et al.[31] found that intensive periodontal treatment caused endothelial dysfunction during the first 24 h, though dysfunction improved 60 days after treatment. Deterioration of VEF leads to an increase in pro-inflammatory and thrombogenic potential, an increase in the likelihood of ischemic episodes, and a higher incidence of cardiovascular events in periods immediately after invasive periodontal treatment.

Hypertensive patients have vascular endothelial dysfunction[32-34] and administration of angiotensin-converting enzyme inhibitors (ACEI) [35-38] is a therapeutic pillar in the treatment of HTN. In addition to lowering blood pressure, renin-angiotensin system blockade with ACEI provides a rational approach to reverse endothelial dysfunction.

The beneficial effects of ACEI on VEF in hypertensive patients may go beyond HTN. The aim of the present study was to evaluate vascular endothelial function after intensive periodontal treatment, in hypertensive patients treated with ACEI.

MATERIALS AND METHODS

A prospective longitudinal study involving repeated measures was conducted at the Departments of Periodontics and of Buccodental Pathology of the School of Dentistry, University of Buenos Aires, and the Department of Cardiology of the Spanish Hospital of Buenos Aires, between September 2010 and August 2014. The study design was similar to that used in controlled clinical trials. The study protocol was approved by the ethics committees of both institutions. Consecutive patients with severe periodontal disease (SPD) were divided into two groups: one group comprised patients with HTN treated with enalapril (ACEI) in doses according to blood pressure (cases), and the other group included non hypertensive patients (controls). The subjects were male and female outpatients over the age of 18 years. Patients receiving vasodilators, transplant patients, renal failure patients on dialysis, patients with concomitant infection or inflammation, pregnant and lactating patients, patients receiving

antibiotics within three months and/or periodontal treatment within 6 months prior to the study, patients with fewer
than 10 teeth, inadequately controlled hypertensive patients or with blood pressure values above 140/90 mmHg at the onset of the study, and patients who failed to sign the informed consent form, were excluded from the study.

The following covariables were recorded: sex, age, CVD, smoking, HTN, dyslipidemia and diabetes (DBT). The patients who met the inclusion criteria underwent VEF assessment (baseline), and received intensive periodontal treatment 24 h later. VEF assessment was repeated twenty-four hours and 15 days after periodontal treatment. The study design is shown in Fig. 1.
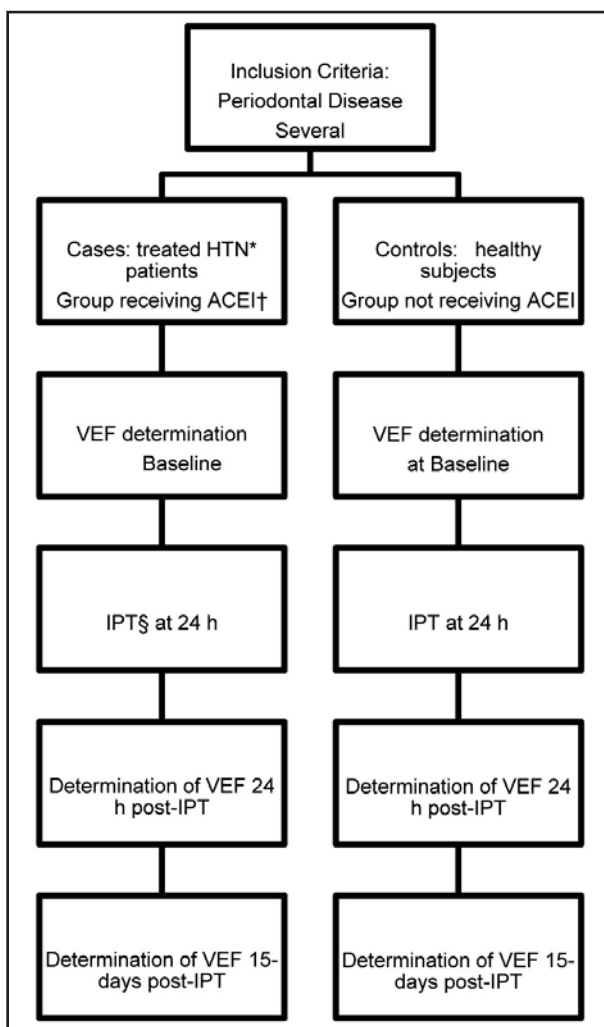

Fig. 1: Study Design. *HTN: Arterial hypertension; †ACEI: Angiotensin-converting enzyme inhibitors; ‡VEF: Endothelial Vascular Function; §IPT: Intensive Periodontal Treatment.

All enrolled patients underwent clinical and radiographic dental examination. The radiographic study included a standardized periapical radiographs using a CS2200 - intraoral x-ray system (Carestream Health, Inc). All permanent teeth, except for the third molars, were evaluated. Patients with fewer than 10 teeth were excluded from the study in order to ensure that periodontal diagnosis was representative of the clinical dental status of the patient and avoid overestimation of diagnosis of periodontal disease.

Clinical periodontal examination was performed by a single calibrated operator. Periodontal measurements were performed at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual/palatal, mid-lingual/palatal and disto-lingual/palatal) on all present teeth, using a manual Marquis Type periodontal probe (Hu-Friedy®). The studied periodontal parameters included Probing depth (PD), Clinical attachment level (CAL), Bleeding on probing (BOP), and radiographic alveolar bone loss (ABL), which were assessed following a standardized protocol.

Intra-examiner reproducibility of PD and CAL measurements was assessed before the study. The weighted K coefficients for PD and CAL were 0.96 and 0.91 respectively.

Diagnosis of periodontal disease was performed based on CAL values, in keeping with the 1999 International Workshop for a Classification of Periodontal Disease accepted by the American Academy of Periodontology (AAP)[39].

Severe generalized periodontal disease (SPD) was established when:

• Average CAL values were ≥ 5 mm. This value was obtained by averaging all six determinations performed on each of the teeth. This method allows avoiding overestimation of periodontal disease diagnosis, since many patients have a high CAL value at only one periodontal site as a result of trauma (for example, trauma associated with tooth brushing, ill-fitting removable dentures, or harmful oral habits, among other causes).

• Average CAL values were < 5 mm but with values ≥ 5 mm at more than 30% of studied sites, in addition to horizontal alveolar bone loss > 1/3 of the root length in at least 30% of teeth, as measured on the radiographic images.

Periodontal treatment

One hour before intensive periodontal treatment, all patients underwent a protocol of antibiotic prophylaxis, and intra and extra oral antisepsis was carried out. Intensive periodontal treatment was performed in a single session[40]. Under local infiltration anesthesia (lidocaine), root scaling and planing was performed using rigid Gracey-type curettes (Hu-Friedy®), numbers 5/6, 7/8, 11/12 and 13/14 according to the surface, and an ultrasound cavitation machine (cavitron plus scaler, Dentsply®).

Endothelial function assessment

Endothelial vascular function was assessed in the morning after 12 h fasting, before smoking and after 15 minutes rest under controlled temperature conditions (22 - 26º C). An ECG lead and a blood pressure cuff were placed on the patient's forearm. Endothelial-dependent dilation was assessed with the FMD (Flow mediated dilation) of the brachial artery using Doppler ultrasonography, according to the Guidelines of the International Brachial Artery Reactivity Task Force[41]. The forearm was occluded by cuff inflation to at least 50 mmHg above systolic pressure for 5 min, resulting in a reactive hyperemia after the release of the cuff, and the increased shear stress led to endothelial-mediated vasodilatation. FMD was measured with an ultrasound scanner with a 7.5 MHz linear transducer (ATL HDI 3000); five consecutive discrete measurements were obtained and averaged into the final value at each time point. The coefficient of variation of brachial artery hyperemic flow values was calculated by dividing peak hyperemic flow by peak baseline flow. After 15 minutes, smooth muscle response to an exogenous stimulus was measured. Brachial artery diameter was determined at baseline, and three times consecutively 3 minutes after sublingual administration of 0.4mg; the obtained values were averaged[41].

Statistical Analysis

The data were entered on an Excel spreadsheet and analyzed using the SPSS 20 software package. Frequency distribution and percentages of categorical variables were calculated. Number of cases, minimum value, maximum value, arithmetic mean, standard deviation of each variable measured on ordinal scale were calculated. Data were analyzed using Student's t test, MANOVA, and linear regression analysis, with $\alpha < 0.05$ and 95% confidence intervals (CI). Sample size calculation: Because the statistical analysis was based on regression procedures, given an effect size of 0.35, a power of 0.8, and an alpha value of 0.05, and considering a maximum of 5 predictors, minimum sample size was calculated to be 43 patients.

RESULTS

Out of a total of 86 patients studied initially, 52 patients with SPD were included in the study. The ACEI group comprised 51.9% (n=27) of the study population, and the remaining 48.14% (n=25) were included in the control group. The patients in the ACEI group received 14.20 ±4.25 mg of enalapril twice daily (IC 95% 12.51-15.88) for HTN treatment. Baseline values of both groups are shown in Table 1.

Severity of periodontal disease was similar in ACEI - treated patients and controls, as shown by the following results: present teeth 19.11 ±5.6 vs. 16.14 ±6.18 (p=0.258), CAL 3.49 ±1.49 vs. 4.39 ±1.72

CHARACTERISTICS	HTN* WITH ACEI (n27)	WITHOUT ACEI (n25)
Age in years	64.85 ± 9.7	62 ± 6.7
Female gender	11 (40.74%)	11 (44%)
Active smokers	1 (3.70%)	2 (8%)
Diabetes mellitus	2 (7.40%)	0
Hypercholesterolemia	8 (29.60%)	6 (24 %)
Systolic pressure in mmHg	121.36 ± 9.43	116.57 ± 11.14
Diastolic pressure in mmHg	79.48 ± 4,36	72. 51 ± 6.23
†HR Bpm	64.38 ± 10.68	62.72 ± 8.42

Table 1: Baseline characteristics of the population.

* HTN: Arterial hypertension ; † HR Bpm: Heart rate in beats per minute

mm (p=0.21), PD 3.45 ± 0.63 vs. 3.59 ±0.64 mm (p=0.61), sites with BOP 43.56 ±20.58 vs. 42.5 ±30.34 (p=0.92), and ABL 36.87 ±14.64 vs. 38.7 ±10.99 % (p=0.76).

Baseline values of end-diastolic humeral artery diameter and nitroglycerin-mediated humeral artery dilation were 4.3 ± 0.50 vs. 4.7± 0.3 mm and 19.32 ± 8.62 vs. 20.64 ±6.43% respectively.

A significantly lower percentage of humeral artery flow mediated dilation was observed in both groups 24 h after intensive periodontal treatment, as compared to baseline values, showing deterioration of VEF (ACEI p<0.01; Controls p<0.001) (Table 2).

Deterioration of VEF was significantly greater 24 after intensive periodontal treatment in patients not receiving ACEI (p<0.001). VEF improved 15 days after treatment, and was not significantly different from baseline values (Fig. 2). ACEI treatment was a significant variable in the differences between groups, having an average impact of 26.7% (95% CI 1-49.2).

DISCUSSION

A number of studies have demonstrated association between periodontal disease and CVD. The link between both pathologies remains to be elucidated in order to determine the causal, contributing or coincidental role of periodontitis in CVD, irrespective of shared risk factors. Periodontal disease is caused by an array of microorganisms, such as *Porphyromonas gingivalis,* which have the ability to invade endothelial cells[42]. In addition, periodontal pathogens have been identified in atherosclerotic plaque specimens obtained during carotid endarterectomy[43].

These microorganisms can act directly on the endothelium or via a systemic inflammatory response, thus contributing to arterial damage[44].

Atherosclerosis is a complex process involving a number of different cell types (monocytes, macrophages, T lymphocytes, smooth muscle cells, endothelial cells) and inflammatory molecules, such as C Reactive Protein (CRP), interleukin 1β, interleukin 6, and tumoral necrosis factor (TNF), among others[19, 45-49].

Its pathogenesis involves a variety of immune mechanisms which interact with multiple causal factors capable of triggering, developing, perpetuating, and activating arterial lesions[50].

The aforementioned factors include metabolic disorders such as DBT, hypercholesterolemia,
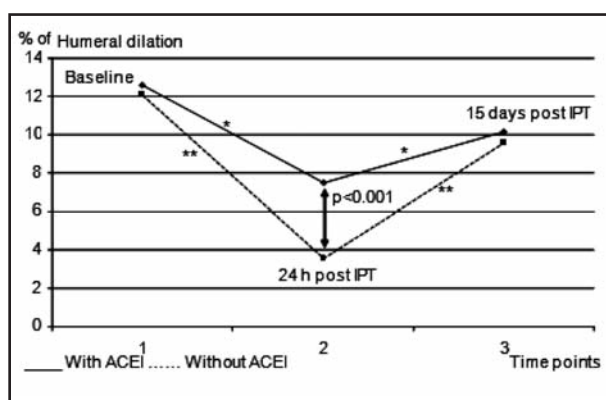
Fig. 2: Endothelial function at the different post-treatment time points. 1) Baseline determination: Humeral artery flow-mediated dilation; 2) Determination 24 h after intensive periodontal treatment; 3) Determination 15 days post-treatment.
* Level of significance p<0.01
** Level of significance p<0.001

Table 2: Endothelial function in the studied groups at the studied time points.

Time points	Group	VEF*	n	95 % IC
Baseline	Without ACEI	12.72 ± 4.62	25	10.81-14.62
	With ACEI	11.89 ± 4.87	27	9.96 -13.81
	Total	12.29 ± 4.72	52	10.97-13.60
24 hs post treatment	Without ACEI	3.56 ± 2.39	25	2.57-4.54
	With ACEI	7.30 ± 2.90	27	6.15 -8.44
	Total	5.50 ± 3.25	52	4.59 -6.40
15 days post treatment	Without ACEI	10.12 ± 3.38	25	8.72 -11.51
	With ACEI	12.19 ± 5.00	27	10.21-14.16
	Total	11.19 ± 4.38	52	9.97-12.40

* VEF: Vascular Endothelial Function, assessed by the percentage of humeral artery flow-mediated dilation

obesity, and other independent risk factors like hypertension, smoking, and infection[51-56].

Elevated inflammatory reactants have been found in patients with periodontitis and vascular endothelial dysfunction[57-58].

Underlying acute cardiovascular events, there is an inflammatory phenomenon that triggers plaque instability. Endothelial dysfunction is the initial pathophysiological step in a progression of vascular damage, and it is considered an early event in the development of atherosclerosis[59].

Periodontal disease treatment would lower the inflammatory component and improve endothelial function (6 months after intensive periodontal treatment), as shown by Tonietti et al [31], providing an additional benefit to VEF. It is well documented that HTN results in endothelial dysfunction[60]. Nevertheless, no significant differences in baseline VEF indices were observed between HTN patients and controls in the present study. This finding is most likely the result of treatment with ACEIs, which have been shown to improve endothelial dysfunction. [61-62]

The decrease in inflammatory reactants and improvement in VEF observed 6 months after intensive periodontal treatment[31] is preceded by a period of endothelial function deterioration and increase in inflammatory response immediately after treatment.

The increase in inflammatory reactants is shown by the increase in serum levels of RCP, IL-6, E-selectin, tissue plasminogen activator (tPA), plasminogen activator inhibitor type 1 (PAI-1), von Willebrand factor, and neutrophils.

As a result, during that period, the patient is at higher risk for a cardiovascular event. Moreover, a higher incidence of stroke and acute myocardial infarction has been observed in the first 4 weeks after periodontal disease treatment.[32]. It must be pointed out that around 40% of the patients studied here had coronary disease and an even higher percentage also had other risk factors for CVD. In agreement with findings reported by Tonetti et al, deterioration of VEF was observed immediately after intensive periodontal treatment (24h) in both groups, though the impact in the group receiving ACEI was lower.

Our results showed no significant differences between VEF determinations obtained at baseline and 15 days after intensive periodontal treatment.

No cardiovascular events occurred during the study, likely due to treatment with ACEI, in view of the beneficial effect of these drugs on the endothelium[63]. One of the limitations of the present study is the small number of patients. However, the differences in VEF observed after intensive periodontal treatment are so significant that they render the sample sufficient to demonstrate the protective effect of ACEI. Follow-up at 15 days does not allow establishing the incidence of cardiovascular events beyond that period. Further studies using longer follow-up periods are necessary to establish the incidence of events beyond the time point established in the protocol used herein.

CONCLUSIONS

Endothelial dysfunction after intensive treatment of severe periodontal disease was significantly lower in patients receiving angiotensin converting enzyme inhibitors.

Endothelial function improved 15 days after treatment, returning to baseline values.

There are data that would seem to indicate a protective effect of ACEI on vascular endothelium after intensive treatment for severe periodontal disease.

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