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# Prevention of vascular dysfunction following periodontal therapy: A single blind, randomized controlled trial

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## SUMMARY

**AIM:** Periodontitis has been associated with the development and progression of cardiovascular disease. Our group reported the beneficial effect of periodontal treatment on the vascular homeostasis. However, the therapy resulted in acute, short-term systemic inflammation and endothelial
dysfunction. We have designed a single blind, randomized clinical trial exploring mechanisms to prevent the vascular dysfunction following intensive periodontal treatment (IPT) using the remote ischemic preconditioning (RIPC).

**METHODS:** 49 otherwise healthy participants affected by periodontitis were
randomly allocated to receive either IPT preceded by RIPC or a sham RIPC. Endothelial function as assessed by flow-mediated dilatation (FMD), biomarkers of inflammation and endothelial activation were evaluated at baseline, 1, 7 days after IPT.

**RESULTS:** Twenty-four hours after treatment, flow-mediated dilatation was significantly lower in the control group showing a protective effect of the RIPC towards the endothelial dysfunction ($P<0.001$). TNF-α, CRP, s-ICAM3, s-Eselectin and s-Thrombomodulin levels were significantly reduced in the test group.

**CONCLUSION:** RIPC is an effective procedure for the reduction of the endothelial dysfunction following IPT. The potential mechanisms underlying
Its effect could be related to the modulation of the host transient inflammatory response following periodontal treatment.

## INTRODUCTION

Periodontitis (PD) is a chronic inflammatory disease of the tissues supporting the dentition led by the accumulation of a bacteria biofilm on the tooth surface[1]. The transient translocation of oral bacteria associated with PD in the blood stream triggers a host immune-inflammatory response that has been associated with systemic inflammation[2]. An increased inflammatory burden has been linked to higher future risk of cardiovascular diseases (CVD)[3]. Although the exact mechanisms linking PD and CVD remain unknown, endothelial cells function could represent an important mediator of this association. Endothelial cells line the inner surface of the blood vessels and have a crucial role in controlling vascular homeostasis. Their function is impaired early in the development of CVD as a result of exposure to traditional cardiovascular risk factors (i.e smoking, obesity, hypertension) and to inflammation[4].

PD treatment has been shown to modulate the individuals inflammatory burden[2]. Non surgical periodontal therapy consists of scaling and root planning (SRP) of the diseased dentition. The mechanical removal of bacterial deposits combined with an effective dental hygiene routine has been consistently associated with reduction of gingival inflammation. The same treatment has also been associated with a medium term reduction of systemic inflammation and this is more evident in patients with established co-morbidities[5]. However our group previously documented that an intensive session of periodontal treatment (IPT) caused an acute inflammatory response within 24hrs and resolving within 7 days of the treatment[2]. These changes correlated with an acute impairment of the vascular function and improvement over 6 months. Changes in endothelial function therefore support a possible role of systemic inflammation in the evolution of CVD in patients with PD. The exact impact of acute vascular dysfunction following IPT is still unknown.

Data from the U.S. Medicaid claims database confirmed an increased rate of vascular events following the 4 weeks after an invasive dental procedure (i.e. simple tooth extraction)[6]. This evidence is consistent with an increased vascular risk following acute inflammation already reported following simple respiratory and urinary infections. Remote ischemic preconditioning (RIPC) of the endothelium is a procedure associated with a reduction of endothelial dysfunction following systemic inflammation[7]. The exact mechanisms underlying the vascular benefit of RIPC are still under investigation but likely to involve modulation of inflammatory pathways. The aim of this study was therefore to ascertain whether RIPC performed prior to IPT would prevent acute vascular dysfunction.

## MATERIALS AND METHODS

**Study Design -** We designed a single blind, parallel group, randomized controlled trial to evaluate the effect of RIPC on endothelial function assessed by FMD within 7 days of IPT (Fig 1).

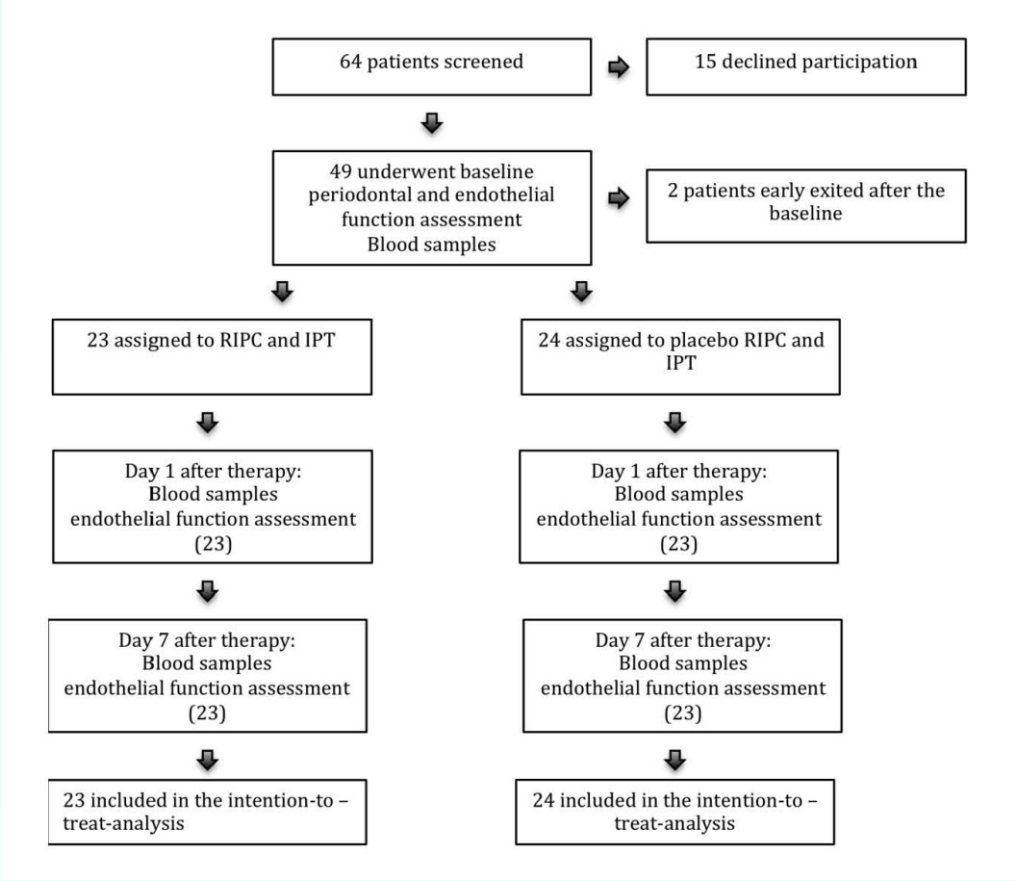


***Figure 1*** *Flowchart of the study design*

Consecutive patients referred to the Eastman Dental Hospital in London for periodontal therapy were invited to participate in this study if they had at least 30 periodontal pockets with probing pocket depth > 4mm and confirmed radiographic alveolar bone loss. Patient were excluded if they were: a) pregnant, lactating or of childbearing, b) on chronic treatment (i.e., two weeks or more) with specific medications known to affect periodontal status (phenytoin or cyclosporine) within one month of baseline visit, c) suffering from any systemic disease (assessed by medical history questionnaire), d) with limited mental capacity or language skills such that simple instructions cannot be followed or information regarding adverse events could not be provided, e) on any chronic medications or requiring antibiotic coverage for dental/periodontal procedures and f) had received a course of periodontal therapy in the preceding 6 months. All patients gave written informed consent. The study was approved by the London Queen Square Ethics Committee (06/Q0512/107).

A baseline periodontal examination was performed, and full medical and dental histories were collected by a single trained examiner (MO). Arterial blood pressure was measured in triplicate with (OMRON M10 -IT), and the average of the readings was recorded. The study participants were randomly assigned with the use of a computer-generated table to receive intensive periodontal treatment preceded by RIPC (test group) or placebo RIPC (control) in a 1:1 ratio. To prevent an imbalance between the two groups with respect to smoking status, sex, age, and severity of periodontitis, restricted randomization (minimization) was performed by the study registrar. Treatment assignments were concealed in opaque envelopes and revealed to the research staff performing the RIPC on the day the treatment was administered. Patients underwent dental examinations, blood samples and endothelial function assessment at Baseline, 1 and 7 days following periodontal treatment.

**Periodontal examination and therapy -** A single trained dental examiner at baseline recorded periodontal data. The data included, number of teeth, periodontal pocket depth (PPD) in mm, gingival recession and clinical attachment levels as previously described. The presence or absence of supragingival dental plaque and gingival bleeding on probing on whole mouth was also recorded.

All study participants received standard oral hygiene instructions and underwent a single-sitting full-mouth session of scaling and root planing (IPT) by means of hand and ultrasonic instruments under local anesthesia within one month from the baseline. A single experienced clinician (MO) completed all treatments in a blind fashion (unaware of to the preconditioning assignment).

**Remote ischemic preconditioning (RIPC) -** RIPC consisted of three 5 minutes cycles of lower arm ischemia alternated by 5 minutes of reperfusion using a 9cm blood pressure cuff inflated to a pressure of 200mmHg. Sham procedure consisted of three 5 minutes cycles alternated by 5 minutes of reperfusion with the blood pressure cuff being placed around the lower arm with 10mmHg. IPT commenced after 30 minutes of the completion of the RIPC protocol.

**Vascular function -** For this study, endothelium-dependent vasodilatation of the brachial artery was assessed using high-resolution ultrasound imaging (Acuson XP128 with a 7-MHz linear probe) in a quiet, temperature controlled room. All patients were fasting for a minimum of 6 hours prior to their assessment. Images of the right brachial artery were measured for 1 minute at baseline, 5 minutes of ischemia (using a blood pressure cuff inflated to 300mmHg) and during deflation.

Flow measurements were derived by using a pulsed wave Doppler signal. Vessel diameter measurements were analyzed using automated brachial software (Medical Imaging Applications, vascular research tools, version 5.6.7) and dilation was calculated as a percentage change from BL to the peak diameter. Endothelium-independent vasodilatation was also assessed following a 25µg dose of GTN administered sublingually. A single examiner who was blinded to the RIPC or sham procedure assessed all patients.

**Inflammatory and vascular biomarkers -** Serial blood samples were collected in fasting condition at baseline, 24 hours and 7 days after IPT. Samples were immediately processed and aliquots of serum and EDTA plasma were stored at -70 degrees as previously reported. At the end of the study all samples were processed in a blind fashion for a broad panel of inflammatory biomarkers using multiplex high sensitivity assays including interleukin-1β (IL-1β), IL-6, IL-8, IL-10, IL-12, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) according to manufacturer's instructions (Meso Scale Discovery, Maryland, USA). Serum C-reactive protein (CRP) was measured by immunoturbidometry (Cobas, Roche Diagnostic, Mannheim, Germany). sE-selectin, sP-selectin, intercellular adhesion molecule-3 (ICAM-3) and sThrombomodulin (sTM) were assayed with a multiplex assay (Meso Scale Discovery, Maryland, USA)

**Statistical analysis -** All data is presented as mean and standard error of the mean unless specified. Demographic data at baseline was compared between groups (RIPC versus placebo) at baseline by paired *t*-Test for numerical variables and Fisher's exact for categorical variables.

Changes in FMD 24 hours after IPT were the primary outcome. Changes in all biomarkers were analysed as secondary outcomes using ANOVA for repeated measures (with post-hoc Bonferroni corrections when between groups comparisons were performed). Covariates included in the model were, age, gender, smoking, temperature and body composition differences. For those variables with a statistical significant difference between groups at day 1, a relative increase was calculated as follows: day 1 serum concentrations minus baseline, divided by baseline and multiplied by 100. Comparison of relative increases at day 1 between groups was performed by t-test or equivalent non-parametric method. Data were graphically tested for normality and logarithmic or square root transformations were made as needed before applying the adequate non-parametric tests. All analysis was performed with the statistical software package SPSS 22 (SPSS Inc. Chicago, IL, USA).

A minimum of 22 patients per group were needed to demonstrate a 2% difference in FMD between groups after 24 hrs (90% power, a 0.05, standard deviation of $1.6^2$). A final sample of 24 participants per group was planned including a 10% drop-out rate.

## RESULTS

From April 2013 to December 2013, 64 patients met the inclusion criteria for the study. 49 accepted to be enrolled into the study and underwent randomization of whom 47 completed the trial. An intention to treat analysis was performed including 24 patients in the Placebo Group and 23 patients in the RIPC Group (Figure 1). The patients' baseline characteristics were similar in both groups, recruited individuals were middle aged, 60% Caucasians, equally distributed between genders, slightly overweight and with 25-30% of current smokers (Table 1, 2, 3).

| | Placebo (N=24) | RIPC (N=23) |
|---|---|---|
| Age | 47±9 | 45±9 |
| BMI | 26.5±3.8 | 26.1±3.7 |
| Gender, Male | 14(56.0%) | 11(45.8%) |
| Ethnicity, Caucasian | 15(60.0%) | 15(62.5%) |
| Smoking, Never | 7 (28.0%) | 8 (33.3%) |
| Systolic BP, mmHg | 120±16 | 118±10 |
| Diastolic BP, mmHg | 77.58±8.51 | 76.43±8.08 |
| FMD, % | 6.28±2.56 | 6.28±3.68 |
| GTN, % | 17.40±7.14 | 19.52±7.64 |
| Wcirc | 92±8 | 93±8 |
| Hcirc | 106±8 | 105±6 |
| Fat_perc | 29.2±9 | 28.5±8.3 |

***Table 1*** *Patients characteristics at baseline*

| | Placebo (N=24) | RIPC (N=23) |
|---|---|---|
| Tx_Time | 144±25 | 128±24 |
| PPD | 4.16±.82 | 3.84±.56 |
| REC | .85±.86 | .86±.82 |
| NPKTS | 69.33±28.96 | 61.00±21.81 |
| FMPS | 63.97±16.23 | 58.90±15.6 |
| FMBS | 49.86±21.97 | 50.05±16.04 |
| NTEETH | 28.46±2.72 | 28.57±2.84 |

***Table 2*** *Periodontal data at baseline and treatment (tx) time*

| | Placebo (N=24) | RIPC (N=23) |
|---|---|---|
| IL-6, pg/ml | 1.15 (1.20) | 0.99 (1.54) |
| CRP, mg/l | 1.30 (1.90) | 1.10 (1.85) |
| TNF-alpha pg/ml | 4.00±1.92 | 3.52±1.85 |
| E-Selectin pg/ml | 21.26±16.43 | 20.75±15.20 |
| P-Selectin pg/ml | 122.89±62.12 | 114.92±43.91 |
| s-ICAM3 pg/ml | 1.92±3.86 | 2.02±3.39 |
| Thrombomodulin pg/ml | 5.30±3.98 | 5.47±6.06 |

***Table 3*** *Biomarkers of inflammation and endothelial activation at baseline*

No serious adverse events were reported during the study. All study participants did not report any major changes in lifestyle for the entire duration of the trial. FMD was lower ($p<0.001$) in the Placebo group at 24 hours after IPT when compared to RIPC. This difference disappeared at 7 days after IPT (Figure 2 A). A 30% reduction of FMD was noted in the placebo group 24 hours after IPT when compared to RIPC (Figure 2 B). A greater reduction in endothelium independent vasodilatation (GTN) was further noted 24 hours after IPT in the placebo group (Figure 2 C) when compared to RIPC ($p<0.05$). Among all inflammatory biomarkers, repeated ANOVA analysis was statistically significant only for changes in CRP and TNF-α (Figure 3 A, B) whilst all endothelial markers were different between groups.

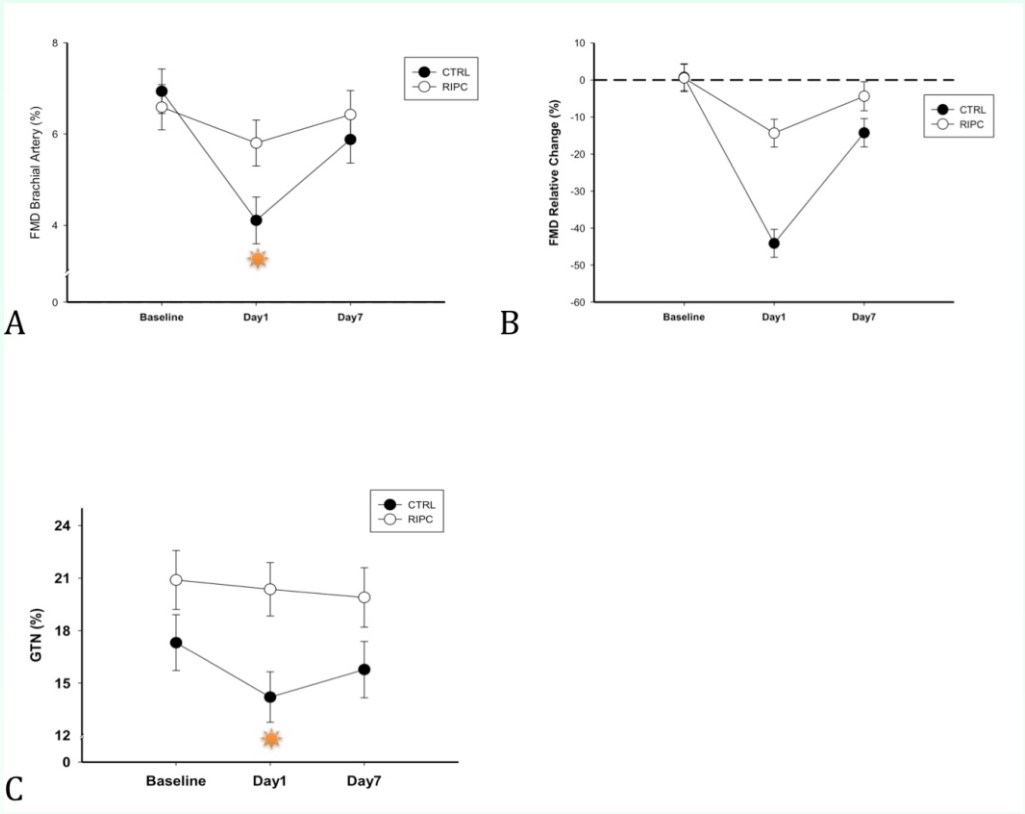


***Figure 2*** *Flow mediated dilatation during the study duration*

I bars represent SE. Data are for the 23 patients in the test group and the 24 patients in the control-treatment group. A FMD % changes, asterisks indicate significant between-group differences (P<0.001). B FMD relative % changes C GTN % changes (P<0.05)

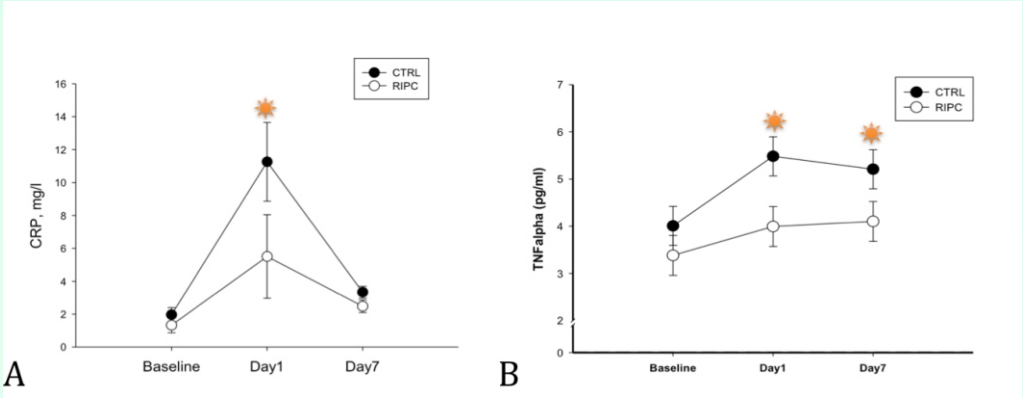


**Figure 3** Inflammatory biomarkers changes during the study duration

I bars represent SE. Data are for the 23 patients in the test (RIPC) group and the 24 patients in the control (CTRL) group. Asterisks indicate significant between-group differences (P<0.05). A C-reactive protein, B Tumor necrosis factor-α profiles at baseline, 1 and 7 days after the IPT.

In particular patients in the RIPC group exhibited lower CRP release at day 1, and lower TNF-α at day 1 and day 7 after IPT when compared to placebo group patients ($p<0.05$ for all comparisons). An inverse trend of acute release of endothelial markers was noted following IPT between groups. Indeed patients in the RIPC group presented with lower s-Eselectin at 24 hrs, sP-selectin at 7 days, lower s-ICAM3 both at 24 hours and 7 days after IPT when compared to placebo group patients (Figure 4 A, B, C). sTM levels between study groups although different at baseline, changed following an opposite trend. Patients in the RIPC group showed statistically significant lower levels of sTM at 7 days after IPT when compared to placebo patients (Figure 4 D).

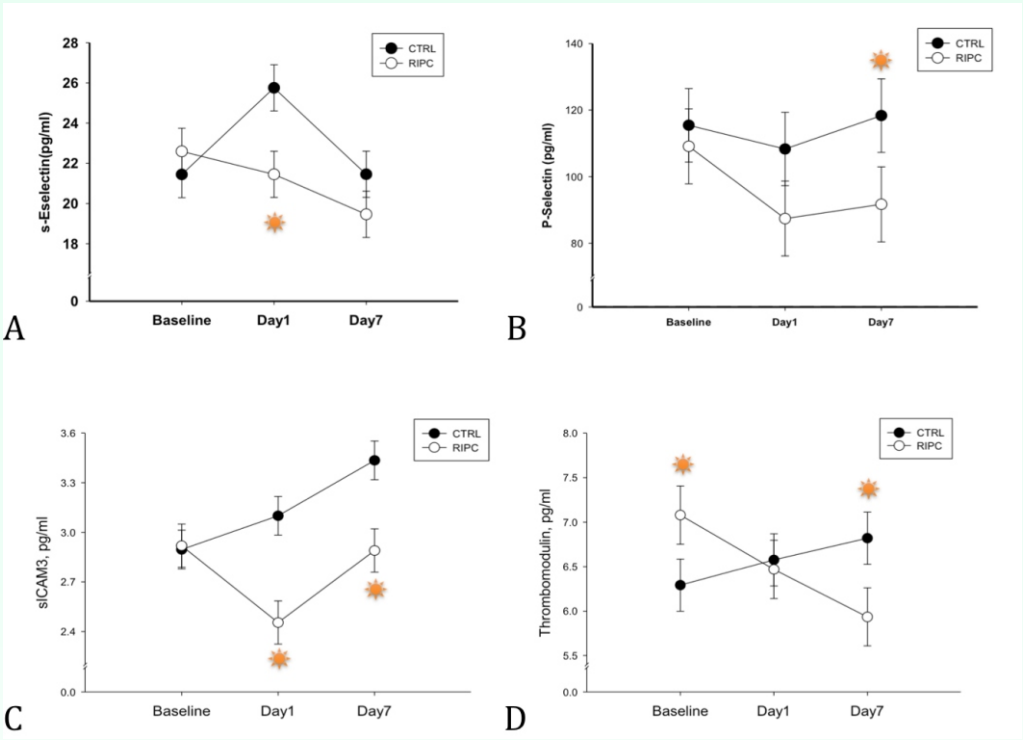


**Figure 4** Biomarkers of endothelial activation during the study duration

I bars represent SE. Data are for the 23 patients in the test (RIPC) group and the 24 patients in the control (CTRL) group. Asterisks indicate significant between-group differences (P<0.05). A s-ESelectin, B s-PSelectin, C sICAM3, D Thrombomodulin profiles at baseline, 1 and 7 days after the IPT.

## CONCLUSION

Our group showed that 24 hours following intensive periodontal treatment, endothelial function was mildly impaired when preceded by RIPC and if compared to placebo. The benefit on the endothelium was associated with a reduction of all soluble markers of endothelial cell activation and some inflammatory markers. Some of these differences were still present 1 week after IPT, although vascular function had returned to normal.

Full mouth scaling and root planing (IPT) induced a transient inflammatory response and endothelial impairment as previously reported[2]. Acute impairment of vascular function was associated with increase in systemic inflammation and endothelial activation.

Previous evidence suggested that RIPC is protective for the vasculature trough two different windows. The earliest one is active within 6 hours following RIPC, the second and longer protective effect has been identified between 24 and 72 hours[8]. As the IPT was initiated within 30 minutes from preconditioning, it is plausible to consider that the effects related to the first window of protection. RIPC has been previously used in human models in order to prevent endothelial dysfunction following different inflammatory stimuli (typhoid vaccination, strenuous exercise[9]) and it has shown a protective effect towards the endothelium. This is the first trial that explores its effects on markers of inflammation and endothelial activation following IPT. The changes in some serum inflammatory markers observed in this study suggest that the protective effect of RIPC on the endothelium might be partially mediated by the modulation of systemic inflammation. Similarly we could infer that the inflammatory mediators profile could be responsible for the endothelial impairment observed 24 hours following the dental treatment. Substantial changes in endothelial activation markers between the RIPC and placebo groups were noted. Macromolecular adhesive associations between cells are important for transmitting spatial and temporal information that is critical for immune system function. Among such group of proteins, the intercellular adhesion molecules (ICAMs) have multiple functions including intracellular signaling events[10]. The normal expression pattern of ICAM3 is high on all leukocytes but lacking on endothelium, it is likely that it plays an important role in early events during leukocyte-leukocyte contact, for example B cell activation mediated by T cell help or T cell activation by antigen-presenting cells (APCs).

Monoclonal antibodies (MAbs) to ICAM-3 have been shown to inhibit peripheral blood lymphocyte proliferation in response to phytohemagglutinin, allogeneic stimulator-cells, and specific antigen[11]. Endothelial activation often precedes endothelial dysfunction. Differential cell surface molecule expression between quiescent and activated endothelial cells influences not only the relative balance between pro- and anti-coagulant activity, but also the degree of adhesion of circulating blood cells. E-selectin is expressed on activated endothelial cells, where, in combination with P-selectin, it facilitates rolling of leukocytes along the endothelial layer as a prelude to leukocyte adhesion (facilitated by the upregulation of ICAM-1 and VCAM-1 [vascular cell adhesion molecule-1]) to activated endothelial cells and subsequent transmigration across the endothelial barrier to a site of injury or inflammation.

Given their specificity for endothelial cells in the activated state, soluble forms of these cell-surface molecules, shed from endothelial cells after activation, have been widely studied as diagnostic and prognostic markers in a variety of infectious diseases. Thrombomodulin (TM) is present in large quantities on the surface of the endothelium, particularly in the microcirculation, where it acts as an anticoagulant. The TM-thrombin complex catalyzes the generation of the anticoagulant molecule activated protein C, and prevents thrombin from converting fibrinogen to fibrin and from exerting other pro-coagulant effects. Consistent with the pro-coagulant properties of the activated endothelium, cell-surface thrombomodulin expression is reduced during sepsis, likely secondary to shedding of the molecule. Soluble TM (sTM) has therefore been proposed as both a diagnostic and prognostic marker of endothelial activation/dysfunction[12]. Lower serum levels of s-ICAM3, sE-Selectin and sTM, together with the reduction of TNF and CRP, reported 24 hours after RIPC and IPT suggest that RIPC could act as a modulator of the immune system and subsequently of endothelial activation. These properties could explain the differences observed in vascular function assessed by FMD in our study. An alternative mechanism could have been mediated on the immune response to the acute bacterial burden which the invasive dental treatment could have produced. There is increasingly interest on the impact of invasive dental procedure both with regards to the long term risk of vascular diseases as well as on the myocardial tissues (risk of endocarditis).

Although this study is the first one to investigate possible mechanisms underlying the transient effect of IPT on the vasculature there are some limitations to consider. This study included a small group of individuals and the results might not be valid for all patients suffering from periodontitis as well as following other dental invasive procedures (i.e. tooth extraction or surgical procedures). Although we included healthy individuals with no other systemic conditions known to impact on the endothelium such as hypertension, heart failure, atherosclerosis, hypercholesterolemia, diabetes mellitus, smoking, aging known to affect endothelial function, we cannot rule out an alternative mechanism of protection of RIPC on vascular dysfunction. Strengths of the study though include the adoption of a validated flow mediated dilatation protocol and using a single trained vascular examiner (DB) masked to the group allocation, who performed and analyzed all the scans with a standardized protocol. In addition, a single clinician (MO) performed all the IPT blind to the patients' randomization.

In conclusions intensive periodontal therapy is associated with acute impairment of vascular function associated with systemic inflammation and endothelial cell activation. RIPC performed 30 minutes before IPT can prevent both acute inflammation and endothelial dysfunction. Further research is needed to ascertain whether RIPC could be performed when IPT or other invasive dental procedures are performed in individuals with known comorbidities and produce a similar benefit.

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