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Effectiveness of scaling and root planing versus modified Widman flap on nitric oxide synthase and arginase activity in patients with chronic periodontitis

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Background: Nitric oxide (NO) is synthesized from the conversion of L-arginine to L-citrulline by NO synthase (NOS). Arginase, which is an arginine-depleting enzyme, can compete with NOS for the common substrate L-arginine and thus inhibit NO production.

Objectives: In the present study, we aimed to examine the correlation between the arginase and NOS activity in patients with chronic periodontitis and to compare the effects of scaling and root planing and modified Widman flap procedures on enzyme activity.

Material and methods: The study included 13 patients diagnosed with chronic periodontitis. Using a split-mouth design, the defects showing ≥ 7 mm of attachment loss were treated either with scaling and root planing or with modified Widman flap. Gingival biopsies from both sites were obtained at baseline and 2 months after periodontal treatment. Immunohistochemical staining was performed for evaluating NOS expression and specific arginase activity was determined spectrophotometrically.

Results: Although inflamed periodontal tissues demonstrated a strong inducible NOS (iNOS) expression at baseline, immunostaining decreased after periodontal treatment. iNOS expression intensity and the number of inflammatory cells showing iNOS expression were found to be higher in the scaling and root planing group compared to the modified Widman flap group. The specific activity of arginase was measured as 0.18 ± 0.07 IU/mg protein in the modified Widman flap group and 0.25 ± 0.11 IU/mg protein in the scaling and root planing group at baseline. After periodontal therapy, the enzyme level was increased to 0.68 ± 0.14 IU/mg protein in the modified Widman flap and to 1.10 ± 0.23 IU/mg protein in the scaling and root planing group.

Conclusion: This study was the first report of evaluating the involvement of the arginine–NO pathway in chronic periodontitis and this might be considered to be of value in understanding the periodontal disease mechanisms.

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Periodontitis is a disease characterized by loss of connective tissue attachment and bone around the teeth in conjunction with the formation of periodontal pockets due to the apical migration of the junctional epithelium. Although bacteria are obviously the initiating agent in periodontitis, the complexity of the associated microflora and the critical role of the host in determining the outcome of the bacterial challenge cause difficulties in clarifying the immunopathogenic mechanisms involved in the disease process.

Both host-derived and microbial-derived factors, including several enzymes such as arginase, have been reported to be related to the destruction of the periodontium (1–3).

Recently, we investigated the salivary arginase activity in patients with periodontitis in order to elucidate the mechanism of its possible contribution to the disease process (3). We detected higher salivary arginase activity in periodontitis patients compared to healthy controls. The increased salivary arginase activity in periodontitis, perhaps causing a decrease in nitric oxide (NO) synthesis, also leads to a decrease in the antibacterial property of saliva and causes periodontal tissues to become more susceptible to existing pathogens. NO is a free radical with important immune functions and is produced from L-arginine by NO synthase (NOS). Arginase can compete with NOS for the common substrate L-arginine and thus inhibit NO production (Fig. 1). NO in periodontal tissue may be part of the non-specific natural defence mechanisms of the oral cavity against pathogenic bacteria or, alternatively, excessive amounts of NO may contribute to tissue destruction in periodontitis. Therefore, arginase activity in periodontitis, along with the arginine–NO pathway, may be involved in the disease process by using the common substrate.

NOS exists in three distinct isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). eNOS and nNOS release small amounts of NO for a short period of time following receptor stimulation, whereas iNOS is expressed in response to proinflammatory stimuli and produces large amounts of NO (4, 5). iNOS activity has been reported to be increased in inflamed periodontal tissues (6, 7). Kendall *et al*. (8) showed that iNOS was strongly expressed by inflammatory cells, basal keratinocytes and fibroblasts in the periodontal sites showing clinical attachment loss of greater than 6 mm.

In light of these findings we have hypothesized that the production of NO in periodontal tissues may be down-regulated by arginase, which is competing with iNOS for arginine as a common substrate.

The aims of this study were: (i) to demonstrate iNOS and arginase presence in gingival tissue from patients with chronic periodontal disease, (ii) to examine the correlation between arginase and iNOS activity in gingival tissue from patients with periodontitis, and (iii) to investigate the effect of periodontal treatment on NOS and arginase activity in patients with chronic periodontitis.

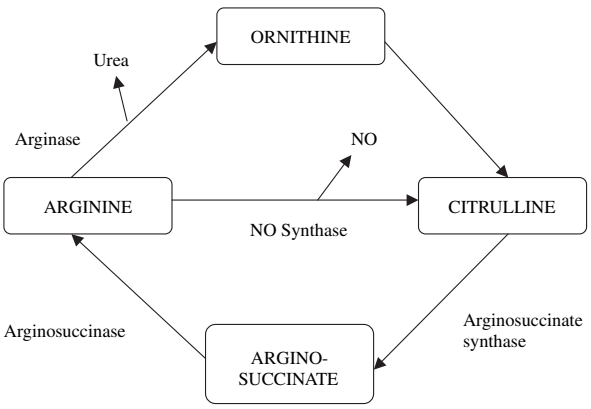

Fig. 1. Simplified scheme of production of ornithine and nitric oxide (NO) via the NO synthase (NOS)–arginase pathway.

Material and methods

Patient selection

The study included 13 patients (seven female and six male, aged 35–55 years) diagnosed with chronic periodontitis. Patients were chosen who had at least 18 teeth, including two single-rooted premolars and two incisors in contralateral quadrants of the same arch and five to six teeth with probing depth ≥7 mm. Chronic periodontitis patients were systemically healthy and had not taken anti-inflammatory agents, antibiotics, immunosuppressant or systemic contraceptives in the past 6 months and had never smoked. The study protocol was approved by Institutional Review Board at Gazi University, School of Medicine and all subjects were asked to give an informed consent to participate after a detailed explanation of the procedures and objectives of the study.

After periodontal examination, all patients were subjected to basic periodontal therapy including oral hygiene instructions and plaque control evaluation, supra- and subgingival scaling and root planing. Prior to any treatment, first biopsies were obtained from gingiva and clinical indices were obtained before biopsies. Periodontal disease status was determined by clinical periodontal assessments, including plaque index (9), gingival index (10), probing pocket depth, clinical attachment level, gingival bleeding index (11) and radiographic examination. Each single rooted tooth showing ≥7 mm of attachment loss was randomly assigned to treatment with either scaling and root planing or modified Widman flap procedures. The study schedule is shown in Table 1.

Biopsy technique

Gingival biopsies of patients were taken under local anesthesia with 2% xylocaine adrenaline from internal part of interdental papillae regions at baseline and 2 months postoperatively from the same site. An inverse bevel incision

Table 1. Study schedule

Baseline	Treatment	Postoperative
Index	Either scaling and root planing or modified Widman flap	Index
First biopsy obtained prior to any treatment Oral hygiene instructions Scaling and root planing		Second biopsy (2 months later)

was used to get tissue from the underside of the papilla. The gingival samples were divided into two parts and approximately 1-mm tissue samples were obtained. Biopsies included part of the pocket epithelium, connective, and granulation tissues. Care was taken to have good cicatrization without any esthetic consequences. After washing in sterile 0.15 M saline solution, one sample was placed in an eppendorf tube filled with 0.15 M saline for biochemical analysis and the other sample from the same pocket was placed in 10% neutral buffered formalin for immunohistochemical analysis. They were sent to the laboratory immediately.

Immunohistochemical staining

Four-micrometer thick sections of formalin-fixed and paraffin-embedded biopsy samples were processed by the avidin–biotin–peroxidase complex (ABC) method. Deparaffinization and rehydration of the sections were followed by the blocking of endogenous peroxidase activity by incubating the sections in 3% H_2O_2 for 10 min. After rinsing with phosphate-buffered saline, the sections were treated in a microwave by antigen retrieval solution (HK087-5K, Biogenex, San Ramon, CA, USA) for 15 min and then slides were left to cool at room temperature for 30 min. Non-specific binding was reduced with protein blocking serum (HK112-9K, Biogenex) for 20 min. Sections were incubated with iNOS primary polyclonal antibody (rabbit anti-iNOS polyclonal, Zymed, South San Francisco, CA, USA) at room temperature (25°C) for 120 min. After rinsing thoroughly with phosphate-buffered saline, the slides were incubated with biotinylated secondary antibody [murine (mouse), Signet, Dedham, MA, USA] for 30 min. The sections were washed with phosphate-buffered saline followed by treatment with the ABC (HK330-9K, Biogenex USA) for 30 min. The diaminobenzidine tetrachloride DAB (Lipshow Immunon, Pittsburgh, PA, USA) was used as a chromogen for visualization of the antibody binding. Finally, the sections were counterstained with hematoxylin, cleared and mounted.

Evaluation of sections and statistical analyses

Immunohistochemical slides were examined for positive staining by light microscopy. The immunoreactive cells were semiquantitatively estimated. The sections were graded according to the scale shown in Table 2.

The interpretation of the immunohistochemistry slides were performed according to a common methodology used in pathology (12).

Morphological identification of neutrophils, lymphocytes and macrophages was made in immunohistochemical slides. The morphology and the type of cells were confirmed in a slide of exactly the same area stained with hematoxylin and eosin. Ten fields at ×400 magnification (five fields of basal layers of epithelium and subepithelial area and five fields of deeper connective tissue) were examined microscopically so as to provide standardization.

Statistical analysis

The average of the clinical parameters (plaque index, gingival index, gingival bleeding index, probing pocket depth and clinical attachment level), the specific enzyme activity, and the scale of immunoreactive cells were calculated for each subject in the chronic periodontitis group. The Friedman test for iNOS parameters, Wilcoxon Signed Rank test for clinical parameters and for specific enzyme activity were used to evaluate the differences before and after treatment. The correlation between the specific enzyme activity, iNOS and clinical parameters was analyzed separately using the Spearman's correlation coefficient. Mann–Whitney *U*-test were used for comparative evaluations between scaling and root planing and modified Widman flap groups.

Arginase activity

Arginase activity was measured spectrophotometrically according to Chinard's method (13). The enzyme activity was determined by measuring the amount of ornithine produced from the hydrolysis of arginine by arginase and was expressed as IU/ml. The specific enzyme activity was referred to the protein amount and calculated by dividing the mean of enzyme activity

Table 2. Evaluation of sections from gingival biopsy samples

Inflammation intensity (II)		Rate of iNOS (+) inflammatory cells (RIC)		iNOS expression intensity (iNEI)	
Absent	0	Absent	0	Absent	0
Minimal	1	< 20%	1	Weak	1
Slight	2	20–50%	2	Strong	2
Moderate	3	> 50%	3		
Diffuse	4				

II is inflammation intensity and shows the intensity of inflammatory cell infiltration. iNEI is the intensity of iNOS expression in iNOS expressed cells. The intensity of iNOS expression can be absent. If it is present, it can be scored weak or strong. iNEI is scored per cell. RIC is the percentage of cells expressing iNOS. All these parameters are different from each other and relatively objective criteria for evaluating the immunohistochemical slides. iNOS, inducible nitric oxide synthase.

by the mean of the protein amount. The specific enzyme activity was expressed as IU/mg protein.

Briefly, tissue specimens were extracted, homogenized and diluted fivefold. The reaction mixture (0.5 ml) contained 0.5 μmol of $MnCl_2$, 10 μmol of arginine and 30 μmol of carbonate buffer, all adjusted to pH 9.8. Then 0.05 ml of supernatant was added and after incubation at 37°C for 15 min, the reaction was stopped with 1.5 ml of concentrated acetic acid. Afterwards, 0.5 ml of ninhydrin solution was added (2.5 g of ninhydrin in a mixture of 40 ml of 6 mol/l H_3PO_4 and 60 ml of glacial acetic acid) and heated in a boiling water bath for 1 h. After the mixture was cooled, the absorbance was measured at 515 nm. One unit of arginase was defined as the amount of enzyme that produces 1 μmol of ornithine per minute at 37°C. Each sample was analysed in duplicate. A 10 μM solution of ornithine was used for standard assay procedure. The analytical imprecision (i.e. the coefficient of variation, expressed as a percentage, CV%) of arginase assay in gingival tissue was determined. Within-run and day-to-day analyses yielded 4.8% and 5.6%, respectively. The assay was performed on the same day and in one and the same run by the same operator. Protein contents of the samples were measured by the method of Lowry *et al.* (14).

Results

iNOS expression was observed in both sites treated either with scaling and root planing or modified Widman flap. The level of iNOS expression was greater in biopsies where abundant inflammatory cells were present. Neutrophils, lymphocytes and macrophages in connective tissue displayed strong immunostaining for iNOS (Fig. 2A). Immunostaining decreased in iNOS positive cells that were located in vessel walls. Based on the location of the staining in the blood vessels, the positive cells were considered to be predominantly endothelial cells. Similarly, iNOS expression in fibroblasts and basal keratinocytes was weak (Fig. 2B).

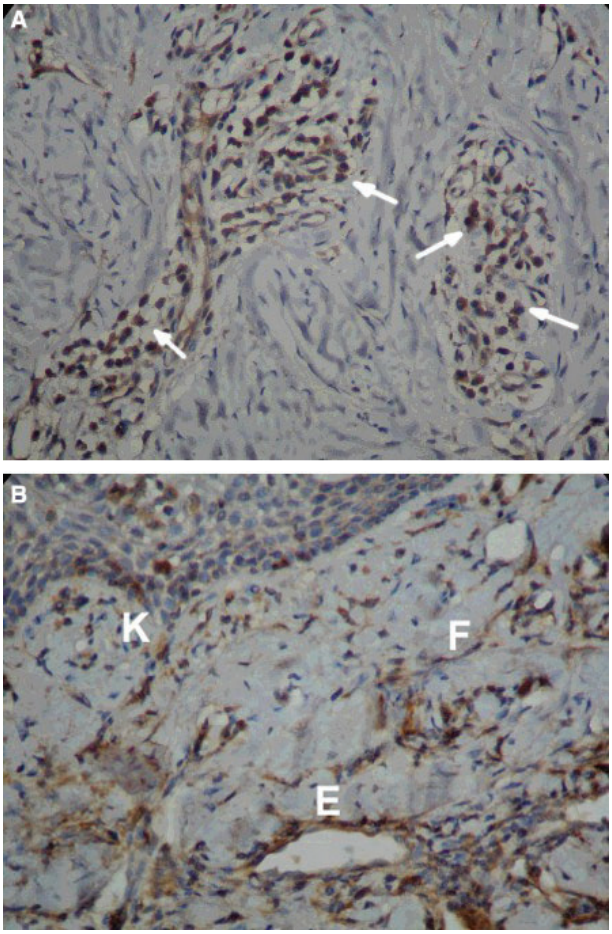

Fig. 2. (A) Formalin-fixed paraffin-embedded section of gingiva from chronic periodontitis patient. Inflamed gingival tissues showing expression of iNOS by inflammatory cells (arrows) (400 × magnification). (B) Note the iNOS expression in basal keratinocytes (K), fibroblasts (F) and endothelial cells (E)(400 ×magnification). iNOS, inducible nitric oxide synthase.

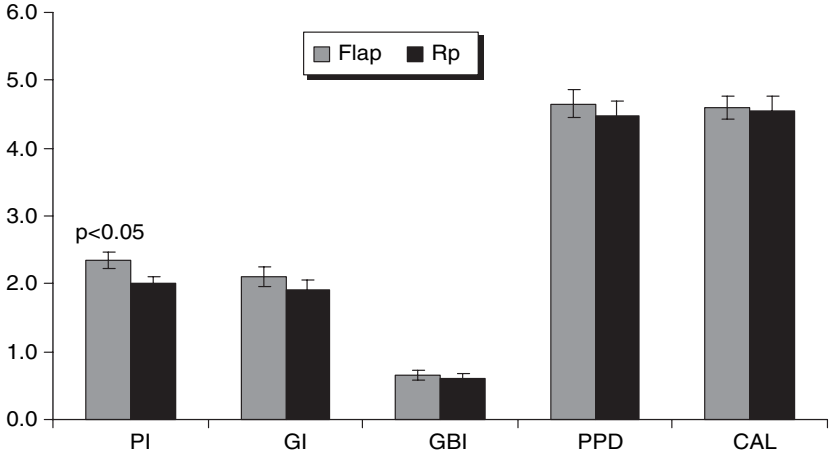

Fig. 3. Summary statistics for clinical parameters in sites treated with either flap or scaling and root planing (Rp) at baseline. PI, plaque index; GI, gingival index; GBI, gingival bleeding index; PPD, probing pocket depth; CAL, clinical attachment level.

The clinical indices for the sampled teeth at baseline and 2 months after modified Widman flap and scaling and root planing are shown in Fig. 3 and Fig. 4, respectively. Significant differences in plaque index were

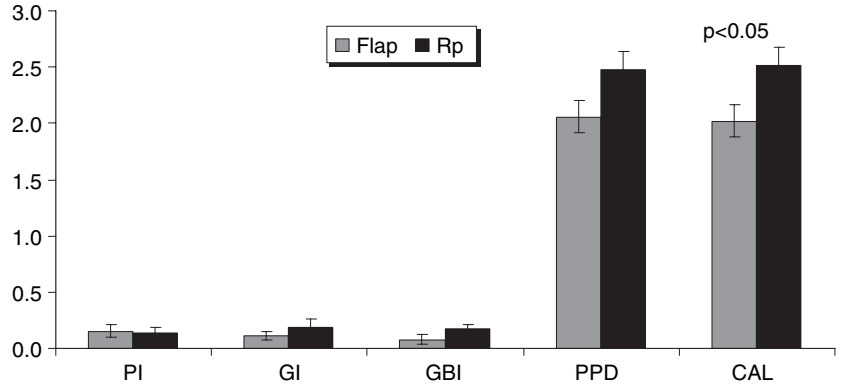

Fig. 4. Summary statistics for clinical parameters in sites treated with either flap or scaling and root planing (Rp) 2 months after therapy. PI, plaque index; GI, gingival index; GBI, gingival bleeding index; PPD, probing pocket depth; CAL, clinical attachment level.

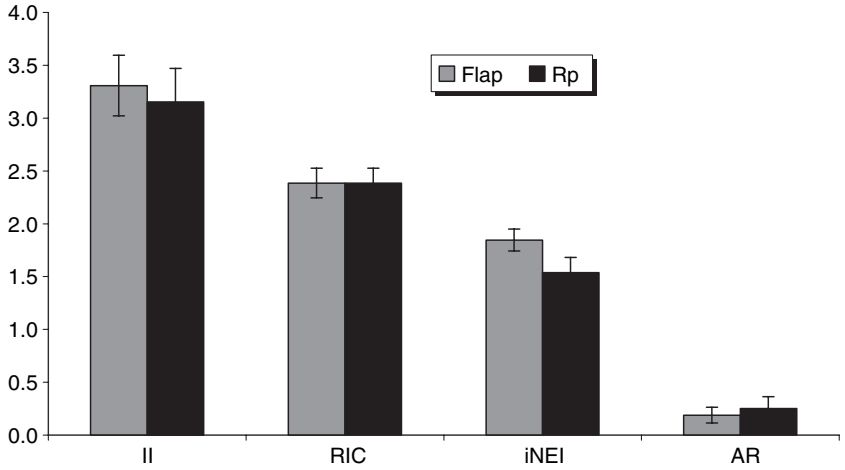

Fig. 5. Summary statistics for immunohistochemical and biochemical parameters in sites treated with either flap or scaling and root planing (Rp) at baseline. Ar, arginase; II, inflammation intensity; RIC, rate of inflammatory cells; iNEI, inducible nitric oxide synthase expression intensity.

Table 3. Comparison of activity of arginase in sites treated with either flap or scaling & root planing at baseline and 2 months after therapy ($n = 13$, mean ± standard deviation)

	Baseline	After therapy	Significance
Modified Widman flap	0.18 ± 0.07	0.68 ± 0.14*	$p < 0.05$
Scaling and root planing	0.25 ± 0.11	1.10 ± 0.23*	$p < 0.05$

*Significant difference, $p < 0.05$.

found between the statistical mean values of the sites treated with either modified Widman flap or scaling and root planing at baseline ($p < 0.05$). The means of clinical attachment level after periodontal therapy in the scaling and root planing group were measured higher than in the modified Widman flap group ($p < 0.05$). Although the gingival index and gingival bleeding index of sampled sites were found to be non-significantly higher in the modified Widman flap group at baseline compared to that in scaling and root planing ($p > 0.05$), these parameters were decreased after therapy.

No statistical differences were observed in immunohistochemical and biochemical parameters in both groups at baseline (Fig. 5). The specific activity of arginase was measured as 0.18 ± 0.07 IU/mg protein in the modified Widman flap group and 0.25 ± 0.11 IU/mg protein in the scaling and root planing group at baseline (Table 3). The difference was found to be non-significant between the two groups. After periodontal therapy, the enzyme level was increased to 0.68 ± 0.14 IU/mg protein in modified Widman flap, and 1.10 ± 0.23 IU/mg protein in scaling and root planing. The differences before and after treatment were statistically significant in both groups ($p < 0.05$); the specific enzyme activity was significantly greater in the scaling and root planing group than in the modified Widman flap group after treatment ($p < 0.05$). Similarly, iNOS expression intensity and the number of inflammatory cells showing iNOS expression was found to be higher in the scaling and root planing group compared to that in the modified Widman flap group after treatment ($p < 0.05$) (Fig. 6).

In both post therapeutic groups, iNOS positivity was observed in scant mononuclear inflammatory cells and the number of iNOS-positive fibroblasts, endothelial cells and basal keratinocytes was reduced after therapy (Fig. 7). iNOS decreased in both groups after therapy; however, the decrease in the modified Widman flap group was significantly different than the decrease in the scaling and root planing group ($p < 0.05$).The correlation between specific arginase activity, clinical and immunohistochemical variables in the modified Widman flap and scaling and root planing groups was evaluated (Tables 4 and 5). In sites treated with modified Widman flap, a significant correlation existed between plaque index and iNOS expression intensity and between probing pocket depth and inflammation intensity at baseline ($p < 0.05$). However, when the sites treated with scaling and root planing are considered, there was not any correlation between clinical, immunohistochemical and biochemical variables at baseline. Two months after treatment, only gingival bleeding index demonstrated a negative correlation with specific activity of

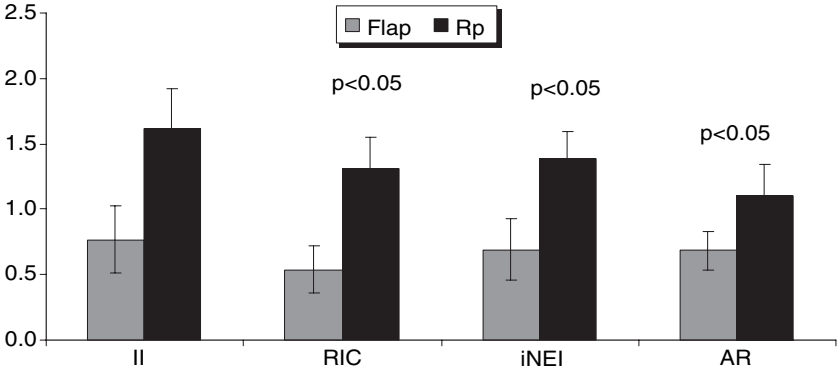

Fig. 6. Summary statistics for immunohistochemical and biochemical parameters in sites treated with either flap or scaling and root planing (Rp) 2 months after therapy. Ar, arginase; II, inflammation intensity; RIC, rate of inflammatory cells; iNEI, inducible nitric oxide synthase expression intensity.

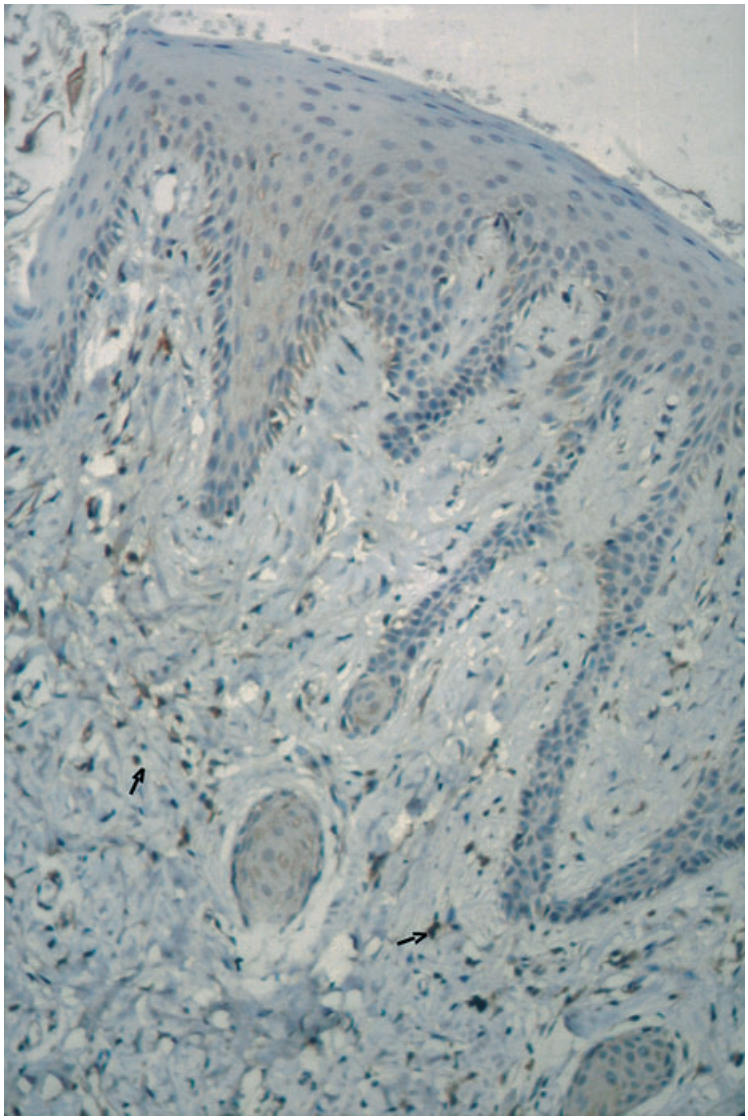

Fig. 7. In both post therapeutic groups, inducible nitric oxide synthase (iNOS) positivity was observed in scant mononuclear inflammatory cells (arrows) and the number of iNOS positive fibroblasts, endothelial cells and basal keratinocytes were reduced after therapy (400 × magnification).

arginase in the scaling and root planing group ($p < 0.05$) (Table 5). No significant correlation was found among the iNOS expression intensity, inflammation intensity, rate of inflammatory cells, specific arginase activity and clinical variables in modified Widman flap group after treatment.

Discussion

The present study comprised of both immunohistochemical and biochemical procedures for the localization of iNOS and arginase activity in gingival tissue from patients. The localization of iNOS in gingival tissue from patients with chronic periodontitis was demonstrated at different stages of periodontal therapy and iNOS was evaluated as to whether it correlated with arginase, which can compete with iNOS for the common substrate L-arginine and thus inhibit NO production.

Previously, Lohinai *et al.* (6) evaluated iNOS activity in rats with ligature-induced periodontitis and demonstrated that iNOS was expressed in periodontitis, with localization mainly in inflammatory cells and epithelial cells. Similar to the experimental study of Lohinai *et al.* (6), iNOS expression in gingival tissue obtained from chronic periodontitis patients has been reported to be higher than in clinically healthy tissue samples (7, 15, 16). However, so far there has been no evidence of the effect of the periodontal treatment on the profile of iNOS. Our results were in accordance with those previous reports. The level of iNOS expression was greater in biopsies where abundant inflammatory cells were present. The intensity of iNOS expression was decreased after periodontal therapy, and flap procedures seemed to be more effective in reducing the number of iNOS expressed cells compared to scaling and root planing alone. Similar to our results, Lappin *et al.* (7) have reported that iNOS presence was dependent on the extent of inflammation in the tissue and iNOS positive cells in connective tissue were observed predominantly in macrophages. The important sources of iNOS production were suggested to be

Table 4. Correlation between clinical, immunohistochemical and biochemical parameters for sampled sites at baseline ($n = 13$, r)

	Modified Widman flap				Scaling and root planing			
Baseline	Ar	II	RIC	iNEI	Ar	II	RIC	iNEI
Ar		0.14	0.04	0		0.11	−0.19	−0.08
II			0.38	−0.03			0.26	0.22
RIC				−0.17				0.33
PI	0.21	0.25	0.34	**0.50***	−0.08	−0.11	−0.19	−0.08
GI	−0.06	−0.06	−0.00	0.20	0.39	−0.06	−0.25	−0.10
GBI	−0.22	0.09	0.23	0.26	0.45	−0.01	−0.43	−0.45
PPD	−0.25	**0.42***	0.10	−0.00	0.06	0.35	−0.12	−0.18
CAL	−0.33	0.34	0.09	0.07	−0.02	0.35	−0.06	−0.06

*Significant correlation, $p < 0.05$.
Ar, arginase; II, inflammation intensity; RIC, rate of inflammatory cells; iNEI, inducible nitric oxide synthase expression intensity; PI, plaque index; GI, gingival index; GBI, gingival bleeding index; PPD, probing pocket depth; CAL, clinical attachment level.

Table 5. Correlation between clinical, immunohistochemical and biochemical parameters for sampled sites at 2 months postoperatively ($n = 13$, r)

	Modified Widman flap				Scaling and root planing			
After therapy	Ar	II	RIC	iNEI	Ar	II	RIC	iNEI
Ar		−0.38	−0.49		−0.24	−0.07	−0.18	
II		−0.23	**0.85*****	**0.76****			**0.95*****	**0.91*****
RIC				**0.88*****				**0.95*****
PI	0.20	0.19	0.36	0.30	−0.07	0.15	0.38	−0.16
GI	0.08	0.05	0.22	0.18	0.00	0.41	0.25	0.19
GBI	−0.13	0.26	0.15	0.14	**−0.59***	0.18	−0.16	0.04
PPD	0.08	0.09	−0.09	−0.10	0.00	0.31	0.27	0.47
CAL	0.06	0.03	−0.15	−0.16	−0.04	0.31	0.28	0.44

Significant correlation *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
Ar, arginase; II, inflammation intensity; RIC, rate of inflammatory cells; iNEI, inducible nitric oxide synthase expression intensity; PI, plaque index; GI, gingival index; GBI, gingival bleeding index; PPD, probing pocket depth; CAL, clinical attachment level.

macrophages and endothelial cells (7). Hirose *et al*. (16) have found that NO production by macrophages and polymorphonuclear leukocytes via the iNOS pathway was enhanced in periodontal lesions and resulted in the progress of periodontitis. This might also be valid for our patients, as macrophage infiltration and activation are known to be characteristics of chronic inflammation such as periodontitis provoked by pathogenic bacteria.

We found a weak staining in the epithelium, fibroblasts and endothelial cells. Daghigh *et al*. (17) have shown in an *in vitro* study that fibroblasts are a source for iNOS and overproduction of NO in human gingival tissues in the presence of proinflammatory cytokines. Our results supported this *in vitro* study, as in the present study fibroblasts demonstrated iNOS expression as well. The presence of iNOS and conceivably NO release at inflammatory sites was thought to have a role in selecting the type of T-cell response, and the reason for not observing the epithelial cell staining was suggested to be due to a down-regulation by T helper cytokines such as interleukin-4 and -10 (7). However, interleukin-1beta, tumor necrosis factor alpha and interferon gamma stimulated the production of iNOS in inflammatory cells and a combination of the three cytokines had a synergistic effect on induction of iNOS (17). As these cytokines are known to be increased in periodontal diseases (18), the increased iNOS expression in inflammatory cells in the periodontal tissues is not so surprising.

NO is relatively unstable in the presence of oxygen and quickly auto-oxidizes to produce nitrogen oxides. Moreover, because of NO's reactivity and short-life, direct measurements of NO in cells and tissues are very difficult. Therefore we prefer to detect iNOS in tissues, assuming that large amounts of NO are produced by iNOS expressing cells in periodontal tissues and excessive levels of NO production can lead to tissue destruction in periodontitis.

Elevated NO production is a reflection of an immune-activated state in which inflammatory cytokines and other mediators have up-regulated iNOS (19). Matejka *et al*. (15) have demonstrated that inflammed gingival tissue showed a higher content of L-arginine and L-citrulline when compared to healthy samples. As L-citrulline correlates with NO formation, elevated levels of L-citrulline indicate an increase in NO levels in periodontal tissues. In that way, during direct enzymatic conversion of arginine to citrulline, iNOS most likely also produced an elevation in NO (20).

NO has been implicated in modulating expression of matrix metalloproteinases, and it is also thought to down-regulate the synthesis of tissue inhibitors of matrix metalloproteinases (21). Matrix metalloproteinases including neutrophil collagenase and serine proteinases seem to be related to tissue destruction in periodontitis and matrix metalloproteinases degrade most components of the extracellular matrix, as well as many non-extracellular matrix molecules. Increased levels of NO production via iNOS enzyme in periodontal tissues may lead to activation of matrix metalloproteinases, a decrease in the level of their inhibitors and tissue destruction in periodontitis.

Mercaptoethylguanidine, which is a selective iNOS inhibitor, has been shown to inhibit the cytokine induced production of NO, suggesting that only the iNOS isoform is involved in inflammatory periodontal diseases, and not the other constitutive NOS isoforms; i.e. eNOS or nNOS (17).

In the present study, a significant correlation was found between plaque index and iNOS expression in periodontitis sites. Effects of lipopolysaccharides extracted from

periodontopathogenic bacteria on NO synthesis in murine macrophages were also recently examined (22). As high score of plaque index indicates a dense microbial stimuli in periodontal disease, the positive correlation between plaque index and iNOS expression suggested that periodontal bacteria induced a significant production of NO.

Although there is one report about arginase activity in periodontitis (3), to our knowledge, the possible association of the arginase–nitric oxide pathway to the mechanism of periodontal diseases has not been discussed and the presence of arginase in gingival tissues has not been shown before. In our previous work, we demonstrated a higher arginase activity in saliva of periodontitis patients (3).

It is stated that the arginase might originate from oral bacteria or host cells or from both sources (23, 24). Activated macrophages were also shown to produce arginase (25, 26). Arginase can down-regulate NO production by decreasing intracellular arginine concentrations. Therefore, the availability of intracellular arginine is a rate-limiting factor in NO production. In the present study, iNOS expression was decreased after periodontal treatment, whereas arginase was increased significantly in both scaling and root planing and modified Widman flap groups. As both iNOS and arginase use arginine as a common substrate, it seems logical to observe an inverse relationship between these enzymes. As large amounts of NO is toxic to many cells and cause tissue destruction, arginase may prevent sustained overproduction of NO. So, we speculated that the role of arginase in periodontal diseases may be protective. On the other hand, arginase may provide ornithine for polyamine and/or proline (and thus collagen) synthesis. Thus, arginine metabolic enzymes may be pertinent therapeutic targets to control NO production in various diseases associated with disordered NO production (27).

This study was the first report of evaluating the involvement of the arginine–NO pathway in chronic periodontitis and this might be considered to be of value in understanding the periodontal disease mechanisms. Besides, the use of specific iNOS antagonist drugs may also play a role in the future management of patients with aggressive forms of periodontitis.

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