Effects of nonopsonized *Escherichia coli* on myeloperoxidase activity in medium used for incubation of leukocytes from patients with gingivitis and periodontitis

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Abstract : An attempt was made to explore the myeloperoxidase (MPO) activity in medium used for incubation of peripheral venous blood (PVB) leukocytes from patients with gingivitis and periodontitis and to compare it with that of periodontally healthy subjects. The study population included 54 gingivitis patients (G), 52 periodontitis patients (P) and 52 control subjects (C). All these groups were assessed by clinical, laboratory and statistical methods. The leukocytes were incubated with opsonized zymosan, *Escherichia coli* ATCC25922, nonopsonized *E.coli* or *Staphylococcus aureus* 256. The respective levels of MPO activity in incubation media of PVB leukocytes taken from group G patients were 598.0 ± 29.2 conventional units (c. u.), 640.0 ± 26.3 c. u., 662.0 ± 37.6 c. u. and 750.0 ± 40.8 c. u. (control incubation medium: 564.0 ± 25.1 c. u.); those for group P patients were 672.0 ± 34.3 c. u., 678.0 ± 43.1 c. u., 692.0 ± 47.9 c. u. and 762.0 ± 34.7 c. u. (control: 612.0 ± 35.2 c. u.); those for group C subjects were 556.0 ± 30.2 c. u., 714.0 ± 28.2 c. u., $1\ 276.0 \pm 69.0$ c. u. and 794.0 ± 47.1 c. u. (control: 534.0 ± 29.0 c. u.). MPO activity was increased most significantly when nonopsonized *E.coli* was added to the incubation medium of PVB leukocytes taken from subjects with intact periodontium. MPO activity was unchanged when the leukocytes were taken from periodontitis patients.

Key words: periodontal disease; neutrophils; enzymology; myeloperoxidase.

Introduction

Periodontal disease is considered to be inflammatory disorder whose patho-physiology is related to accumulated dental microbial plaque and the host response to this accumulation^[1, 2]. The host reaction to gingival microorganisms is characterized in part by an influx of polymorphonuclear leukocytes (PMN), which is one of the most important steps in host defense^[3]. PMN establish the first defense barrier against the microbial challenge^[3, 4, 5]. A decreased count of PMN and/or impairment of their function is detrimental to the maintenance of periodontal health^[3, 6, 7, 8, 9]. PMN contain the necessary material for killing pathogenic microorganisms, including MPO, which is considered to play an important role

in the host response, especially in oxygen-mediated defense mechanisms^[10]. The MPO-H₂O₂-halide system has been shown to be effective in killing periodontopathic microorganisms^[10, 11]. Interaction between bacteria and neutrophilic leukocytes activates a complex series of immunological and biochemical events, resulting in the uptake and subsequent killing of the microorganisms^[12]. MPO is central to these interrelated activities in the MPO-hydrogen peroxide-halide system^[13]. Studies on the relationship between periodontal disease and MPO activity of crevicular PMN have revealed decreased MPO activity in gingival crevicular neutrophils of subjects without inflammatory changes of the gingival crevice: the enzyme activity is not detected in approximately 50% of crevicular PMN. All PVB neutrophil MPO activity levels were found to be within the normal range, and the MPO activity of gingival neutrophils was also normal in all subjects with inflamed tissue^[14].

However, there seems to be little information about how the leukocytes of patients with periodontal disease release MPO into the surrounding environment in response to the presence of microbes.

The aim of the present study was to determine the MPO activity in medium used to incubate PVB leukocytes taken from patients with periodontitis and subjects with no inflammation of the periodontium.

Materials and Methods

Patient selection

Our study patients were selected from a large number of individuals with pathology of periodontal tissues who were examined clinically and radiographically and diagnosed as having gingivitis (gingival inflammation, bleeding on probing) or periodontitis (deep periodontal pockets, destruction of periodontal tissues surrounding the affected teeth and advanced vertical bone loss). They underwent periodontal treatment or routine check-ups at the Department of Stomatology, Kaunas Academic Clinic. We chose and included in our study only those patients with very marked signs of gingivitis and periodontitis using Russell's^[15] periodontal index (PI), which are given in Table 1. The study was performed on 158 systemically healthy subjects within an age range of 18 to 50 years: 54 patients (21 females and 33 males) with gingivitis (G), 52 patients (23 females and 29 males) with periodontitis (P) and 52 (29 females and 23 males) periodontally healthy controls (C), chosen from among donors, dental students and personnel.

Collection of blood samples

Ten milliliters of venous blood was collected from the subjects into a sterile syringe containing heparin (20 units/ml).

Laboratory studies

The test tubes with blood were positioned at an angle of 45 degrees and kept for 1 h at 37°C. Then the supernatant plasma rich in leukocytes was aspirated. The leukocyte count in the plasma was levelled to 1×10^9 cells / l, and 1.5 ml of the fluid was taken for each incubation medium. Then to the first specimen 0.1 ml of phosphate buffer was added (control); to the 2nd, 0.1 ml of opsonized zymosan; to the 3rd, 0.1 ml of opsonized *E.coli*; to the 4th, 0.1 ml of nonopsonized *E.coli*; and to the 5th, 0.1 ml of nonopsonized *S.aureus*.

Zymosan was opsonized by the method of Cates^[16]; its further preparation as well as the opsonization of *E.coli* and *S.aureus* were performed by the method of Peterson and Schmeling^[17].

The opsonized and nonopsonized *E.coli* and *S.aureus* were used at a concentration of 3×10^6 particles/ml. In the samples of leukocyte incubation media, prepared by the method of Talstad *et al.*^[18], MPO activity was determined spectro-photometrically in conventional units (the conventional unit is activity causing an increase in absorbance of 0.001 per minute at 20°C) by the method of Klebanoff^[19] using the modification of Yankovsky *et al.*^[20]. The reaction was followed for 10 min at 460 nm.

Statistical studies

Differences among the studied data were evaluated by Student's *t* test.

Results

Clinical data. The mean age of the group P patients was 40.5 ± 2.7 years; that of the group G patients was 32.9 ± 3.1 years, and that of the group C subjects, 32.5 ± 3.4 years. The difference in mean age between the group P, G and C subjects was not significant ($p > 0.05$). Russell's PI in the group C subjects equalled 0; that in the group G patients was 1.37 ± 0.13 , and that in the group P patients was 5.57 ± 0.36 (Table 1).

Laboratory data

It is evident from the data presented in Table 2 that there was no significant difference ($p > 0.05$) between any of the examined groups in their blood plasma percentages of phagocytes.

Data on MPO activity in the samples are given in Figure 1. They show that the most distinct rise of MPO activity occurred in the group C subjects' PVB leukocyte incubation media with nonopsonized *E.coli*: the activity was more than double that in the leukocyte control incubation media and in the incubation media of leukocytes with opsonized zymosan, and more than 1.5 times as high as that in the incubation media of leukocytes with opsonized *E.coli* and nonopsonized *S.aureus*. A significant rise ($p \leq 0.001$) of MPO activity was also observed in the PVB leukocyte incubation media with opsonized

E.coli and nonopsonized *S.aureus*, as compared with the MPO activity in the control leukocyte incubation media.

None of the studied groups (C, G and P) showed a significant ($p > 0.05$) elevation of MPO activity in the PVB leukocyte incubation media with opsonized zymosan in comparison with the enzyme activity in control leukocyte incubation media.

A significant rise ($p \leq 0.05$) in MPO activity was observed in the group G subjects' PVB leukocyte incubation media with opsonized and nonopsonized *E.coli*, but there was no significant difference in MPO activity ($p > 0.05$) between the latter media. A somewhat higher MPO activity was noted ($p \leq 0.01$) in the group G subjects' PVB leukocyte incubation media with nonopsonized *S.aureus*, significantly ($p \leq 0.05$) exceeding the analogous value in the PVB leukocyte incubation media with opsonized *E.coli*, but not differing ($p > 0.05$) from the MPO activity in the PVB leukocyte incubation media with nonopsonized *E.coli*. Interestingly, the latter activity was almost twice as low as that for the analogous group C subjects.

A significant rise in MPO activity was detected only in the PVB leukocyte incubation media of group P subjects with nonopsonized *S.aureus* ($p \leq 0.01$), but the activity did not differ significantly ($p > 0.05$) from that in the analogous leukocyte incubation media from group C and G subjects. In contrast, the activity of group P MPO assessed in the PVB leukocyte incubation media with nonopsonized *E.coli* was almost twice as low as the activity in the analogous C group PVB leukocyte incubation media.

Discussion

The difficulties encountered in determining the etiology of destructive periodontal diseases have been discussed at length^[21, 22]. Investigators have been handicapped by the dual problems of technical difficulty and inadequate understanding of the biology of destructive periodontal diseases^[23]. As has been shown by investigations in recent years^[24, 25], periodontal disease is initiated by subgingival infection with selective Gram-negative bacteria, but the presence of microorganisms alone is not the only factor responsible for periodontal destruction^[3, 26, 27]. The responses of the host to pathogenic bacteria are thought to be critically important^[28].

The determined clinical parameters (Table 1) showed significantly increased PI in both groups (G and P) of patients in comparison with the group C subjects. Bearing in mind that interactions between bacteria and the immune system play a central role in the etiology of periodontal disease^[29, 30], it was expected that our selection of subjects with severe periodontal lesions of inflammatory origin in the G and P groups would provide clearer data on the interaction between leukocytes and bacteria and their toxins, as compared with the analogous data for the group C subjects. It is also evident from the data that the mean age of the subjects in the three groups (G, P and C), and the leukocyte counts in PVB and the incubation media (Table 2) did not differ significantly ($p > 0.05$).

Table 1 : Data for study subjects and clinical evaluation

GROUPS OF STUDIED SUBJECTS	AGE (YR)	RUSSELL'S PI
C	29.6 $\pm$ 4.9	0.00
G	32.9 $\pm$ 3.9	1.37 $\pm$ 0.13
P	40.5 $\pm$ 3.1	5.57 $\pm$ 0.36

Table 2 : Leukocyte count and percentage distribution in incubation media

GROUPS	LEUKOCYTES IN INCUBATION MEDIA			
	LEUKOCYTE COUNT ($1 \times 10^9/l$)	DIFFERENTIAL LEUKOCYTE COUNT		
		GRANULOCYTES	LYMPHOCYTES	MONOCYTES
C	10.0 $\pm$ 0.14	42.8 $\pm$ 6.5	54.5 $\pm$ 7.5	2.7 $\pm$ 1.1
G	10.0 $\pm$ 0.15	46.5 $\pm$ 7.6	50.9 $\pm$ 8.1	2.6 $\pm$ 1.3
P	10.0 $\pm$ 0.12	50.5 $\pm$ 8.1	46.7 $\pm$ 8.6	2.8 $\pm$ 1.6
p		$P_{CG} > 0.05$ $P_{CP} > 0.05$		$P_{CG} > 0.05$ $P_{CP} > 0.05$

Fig. 1 : MPO activity in leukocyte incubation media (in conventional units)

C, MPO activity in incubation media of leukocytes taken from control group subjects;

G, MPO activity in incubation media of leukocytes taken from patients with gingivitis;

P, MPO activity in incubation media of leukocytes taken from patients with periodontitis.

Hence, the leukocyte count in the incubation media and the mean age of the subjects might not have had any substantial influence on the results for groups G and P.

Neutrophils are the principal cells of the host defense system^[31] and the primary protective cells against periodontal disease^[32]. Phagocytosis and the release of lysosomal factors are essential for the bactericidal activity of human polymorphonuclear leukocytes^[33]. According to Wilton^[34], extracellular release of lysosomal factors by neutrophilic leukocytes from gingival crevicular exudate is possibly more efficient than phagocytosis.

MPO is one of the enzymes considered to play a role in the host response, especially oxygen-mediated defense mechanisms^[14, 35]. From this view-point, the results of our study are of special interest: we revealed a very striking increase of MPO activity ($p \leq 0.001$) in the group C PVB leukocyte incubation media with nonopsonized *E.coli*, being almost double that in the analogous media for groups G and P. This may indicate that the leukocytes in groups G and P did not release enough MPO into their surroundings, and therefore periodontal tissue protection was insufficient. Our findings confirm indirectly the data obtained by Kowolik and Grant^[14] indicating that gingival neutrophil MPO levels were normal in all subjects with inflamed tissue, whereas no enzyme activity was detected in approximately 50% of those with no gingival inflammation. It appeared that some of the MPO had been released by gingival neutrophils of these subjects into the surroundings in response to oral bacteria.

The C group subjects' MPO activity in incubation media of leukocytes with opsonized *E.coli*, although significantly ($p \leq 0.001$) higher than the activity in the control leukocyte media, was, however, significantly lower ($p \leq 0.001$) than that in the PVB leukocyte incubation media with nonopsonized *E.coli*.

The G group subjects' MPO activity in media used for incubation of leukocytes with opsonized and nonopsonized *E.coli* was significantly higher ($p \leq 0.05$) than that in the PVB leukocyte control incubation media, showing no significant difference in the MPO activity ($p > 0.05$) between both of the media with *E.coli*. In contrast, the group P MPO activity in the leukocyte incubation media with opsonized and nonopsonized *E.coli* was not essentially different ($p > 0.05$) from that in the analogous PVB leukocyte control incubation media.

The C, G and P group MPO activity in the leukocyte incubation media with *S.aureus* was significantly higher than that in the analogous control leukocyte incubation media ($p \leq 0.001$, $p \leq 0.001$, and $p \leq 0.01$, respectively), without any significant difference ($p > 0.05$), but it was significantly ($p \leq 0.001$) lower than the activity of group C MPO in the leukocyte incubation media with nonopsonized *E.coli*. This indicates that MPO is released in various quantities by the leukocytes in response to different microbes. Various activities of MPO, especially of groups C and P, of leukocyte incubation media with nonopsonized *E.coli*, may give some idea of the disturbance of the bactericidal function of leukocytes by individual microbes in subjects with periodontal inflammatory pathology.

The present data confirm the importance of MPO for the normal function of periodontal tissues, although they are at variance with Över *et al.*^[36], who suggested that MPO activity may be related to the pattern and severity of periodontal breakdown.

Further studies are required for a final solution of this problem.

Conclusions

1. The activity of MPO increases when bacteria, especially nonopsonized bacteria, are added to human leukocyte incubation media.
2. The extent of the increase in MPO activity in the incubation media depends on the species of bacteria added.
3. The activity of MPO does not change significantly if nonopsonized *E.coli* is added to the incubation media of PVB leukocytes taken from the patients with periodontitis. On the other hand, its activity doubles in the analogous incubation media if the leukocytes are taken from subjects with an intact periodontium.

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