Superoxide dismutase and myeloperoxidase activities in polymorphonuclear leukocytes in acne vulgaris

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ABSTRACT

Background and design: Acne vulgaris frequently occurs in the second decade of life. The pathogenesis of the disease is multifactorial and in the present study, we aimed to investigate the role of reactive oxygen species in the inflammation of acne by determining the activities of myeloperoxidase (MPO) and superoxide dismutase (SOD) in polymorphonuclear leukocytes (PMN).
Materials and methods: Forty-three patients with acne vulgaris and 24 healthy controls were enrolled. The severity of the acne was categorized from mild (subjects with only comedonic lesions) to severe (subjects with nodulocystic lesions). SOD and MPO activities in PMN were measured spectrophotometrically.
Results: There was no significant difference in the activity of MPO between the patients and controls. However, SOD activity in PMN was significantly lower in the patients than in the controls ($p<0.001$). No correlation was detected between the activities of enzymes and the severity of the disease.
Conclusion: *Propionibacterium acnes* may not play a primary role in the pathogenesis of acne as a bacterium. However, the low activity of SOD in PMN may be responsible for the increased levels of superoxide anion radicals in the epidermis. New anti-acne drugs should include substances with lymphocyte stimulating and anti-oxidative properties.

KEY WORDS

acne vulgaris, myeloperoxidase, superoxide dismutase, polymorphonuclear leukocytes

Introduction

Despite much research, the etiology and pathogenesis of acne are not completely understood. *Propionibacterium acnes* appears to play an important role in the pathogenesis of acne, initiating the inflammation by producing low-molecular-weight chemotactic factors which result in the accumulation of neutrophils at the site of acne lesions (1). The attracted neutrophils, after phagocytosis, are thought to release lysosomal enzymes and produce reactive oxygen species (ROS), with resultant damage to the follicular epithelium (2).

The role of polymorphonuclear leukocytes (PMN) in host defense is to destroy invading microorganisms, and several antimicrobial systems have been described in normal human PMN (3). Two cytoplasmic enzymes, superoxide dismutase (SOD) and myeloperoxidase (MPO) protect the cell contents against oxidizing activ-

ity by destroying superoxide anions ($^{-}O_2$) and hydrogen peroxide (H_2O_2) respectively (4). One powerful antimicrobial is composed of MPO, H_2O_2, and a halide cofactor (5). The release of the enzyme MPO from granules is considered to act by killing the bacteria (6,7). SOD reduces both the oxidative stress and the activation of mediators of inflammatory response (8).

Although acne vulgaris is the most frequent skin disease of the second decade in both sexes, only a few studies on MPO and SOD in acne pathophysiology have been performed to date (9,10). For this purpose, the present study was designed to determine the activities of MPO and SOD in patients with acne vulgaris.

Materials and methods

Subjects

The study included 43 patients (30 female, 13 male) with acne vulgaris and a control group of 24 healthy subjects (13 female, 11 male). The ages ranged from 13 to 35 (mean ± SD: 19.6±4.3) in the patients group and 14 to 31 (mean ± SD: 21.9±8.2) in the control group. These subjects were randomly selected from persons who had not received any treatment in the 6 month period prior to the study. Those subjects that smoked, took alcohol or that had any systemic or cutaneous diseases were excluded. Acne vulgaris was diagnosed on the basis of history and physical findings alone. The severity of acne was categorized from mild (subjects with only comedonic lesions) to severe (subjects with nodulocystic lesions). The patients with papulopustular acne were described as having moderate acne. Demographic and clinic characteristics of all subjects were recorded. Of all patients 28 (28/43) had only facial acne and 15 (15/43) had both facial and truncal acne. Seven patients had mild, 31 patients had moderate, and 5 patients had severe acne. Prior to initiation of the study, each person was informed about the aim of the study and signed an informed consent form.

Biochemical Analysis

Blood was taken from the cubital vein with a heparinized disposable syringe, and mixed with 6% dextran in saline at a ratio of 4:1 and allowed to stand for 60 minutes at room temperature. The supernatant was removed and centrifuged at 275 g for 10 minutes. The resulting cell pellet was resuspended in 8 ml of 0.1 M phosphate buffer (pH 7.4), layered over 3 ml of Ficoll-Hypaque and spun at 450 g for 30 minutes at 4 °C. The cell pellet containing the PMN was then resuspended in a phosphate buffer and 3 ml of erythrocyte lysing solution (0.87% NH_4CI) was added. The cell suspension was centrifuged at 275 g for 5 minutes, the supernatant was discarded, the pellet was washed three times with Hank's balanced solution (HBSS) and resuspended in 1 ml of HBSS. Cell numbers in the final suspension were counted and the suspension stored at -20 °C.

Preparation of PMN homogenates

The PMN suspension was frozen at -20 °C and thawed six times, then homogenized using a motor driven Teflon-glass homogenizer (9000 rpm for 5 min at 0 °C). The protein content of the homogenate was measured using Lowry's method (11).

Measurement of MPO activity

MPO activity was determined by a modification of the O-dianisidine method (12). The assay mixture, in a cuvette with a path length of 1 cm, contained a 0.3 ml 0.1 M phosphate buffer (pH 6.0), 0.3 ml 0.01 M H_2O_2, 0.5 ml 0.02 M O-dianisidine (freshly prepared) in deionized water and 10µl PMN homogenate in a final volume of 3 ml. The PMN homogenate was added last and the change in absorbance at 460 nm was followed for 10 min. All measurements were carried out in duplicate. One unit of MPO is defined as that giving an increase in absorbance of 0.001 per min and specific activity is given as IU/mg protein.

Measurement of SOD activity

SOD activity was measured according to the method described by Fridovich (13). This method employs xanthine and xanthine oxidase to generate superoxide radi-

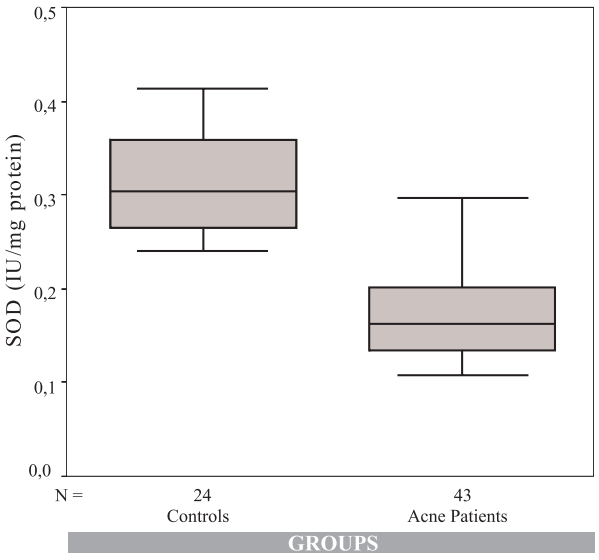

Figure 1. Superoxyde dismutase (SOD) activities polymorohonuclears of acne patients and control group; box graphics.

Table 1. Activities of myeloperoxydase (MPO) and superoxyde dismutase (SOD) in polymorphonuclears of acne patients and control group.

Groups	MPO (IU/mg protein)	SOD (IU/mg protein)
Patients (*n=43*)	0.22±0.004 (0.154-0.297)	0.17±0.005 (0.118-0.413)
Controls (*n=24*)	0.21±0.005 (0.118-0.298)	0.31±0.007 (0.107-0.297)
p value	***0.56***	***<0.001***

MPO: Myeloperoxidase, SOD: Superoxide dismutase, mean ± standard deviation, and minimum-maximum levels.

cals that react with p-iodonitrotetrazolium violet (INT) to form a red formazan dye that was measured at 505 nm. The assay medium consisted of a 0.01 M phosphate buffer, 3-cyclohexilamino-1-propanesulfonicacid (CAPS) buffer solution (50 mM CAPS, 0.94 mM EDTA, saturated NaOH) with pH 10.2, PMN homogenate, a solution of substrate (0.05 mM xanthine, 0.025 mM INT) and 80 IU/L xanthine oxidase. SOD activity was expressed as IU/mg protein.

Statistical analysis was performed by using the SPSS 10.0 version for Windows Operating System. The results were analyzed using the Mann-Whitney-U test, the Chi-Square test and linear regression analysis. The significance level was set at $p<0.05$.

Results

While the mean SOD activity was significantly lower in the acne patients than in the controls ($p<0.001$), there was no significant difference in MPO activity between the acne patients and controls ($p=0.56$). The results are given in Table 1. SOD activities in the two groups are shown in Figure 1.

No significant correlation was found between the severity and distribution of acne, and the mean values of MPO activities (respectively, $r=0.10$, $p=0.95$; $r=0.15$, $p=0.33$) and SOD activities (respectively, $r=0.05$, $p=0.97$; $r=0.05$, $p=0.78$). Regression analysis was carried out to test a correlation between MPO and SOD activities. Any statistically significant correlation was not found between MPO and SOD activities ($r=0.08$, $p=0.49$).

Discussion

The control of ROS production is necessary for physiologic cell function. The increased reactive oxygene species (ROS) are scavenged by SOD and subsequently produce H_20_2, which is partly used for bactericidal action in PMN (8). Previous studies have supported the effect of ROS in the etiopathogenesis of acne inflammation. In the present study, we found decreased SOD activity in acne vulgaris which is primarily responsible for antioxidant defense reaction. Our results are in accordance with earlier studies. Other authors have published similar results (9). This finding is consistent with the suspected role of ROS in acne. The function of SOD is to convert superoxide anion free radicals (O_2^-) that are detrimental to all living cells, to H_20_2 and molecular oxygen (14). Some authors suggested that O_2^- radicals could be released and cause damage to surrounding healthy epidermal cells (15). Increased levels of O_2^- radicals have been reported in mediums containing low SOD activity. It seems unlikely that PMN in acne patients, in which biochemical reactions are taking place that are known to produce O_2^-, would survive without protection against these free radicals. The low activity of SOD in PMN deposited in the epidermis may increase the level of O_2^- radicals. Our findings show that drugs with antioxidant effects may be valuable in acne treatment. However, whether impaired antioxidative defense results in papulopustular acne formation, or activation and consumption of the SOD enzyme after the beginning of inflammation still requires further investigation.

The bactericidal activity of MPO in the presence of H_20_2 and a halide such as Cl^-, Br^- or I^- has been demonstrated by various authors (16,17). The results of our study showed that the specific MPO activity in circulating PMNs in patients with acne vulgaris does not differ statistically from the values determined in healthy subjects. There is no causal relationship between altered MPO activity and the pathologic alteration of lesions in acne. However, we only examined circulating cells, and our findings on MPO activity cannot be transferred with certainty to PMNs that have migrated to the skin. The various influences on enzyme activity in acne lesion, such as mediators and activating or inhibiting substances, must also be considered. Webster et al. reported that *Propionibacterium acnes* was resistant to killing by PMNs (10) and we showed that MPO activities in PMN in acne patients were normal. We think that inflammation in acne is an indirect result of the proliferation of *Propionibacterium acnes* and it may not play a primary role in the pathogenesis of acne as a bacterium. The accumulation of PMNs may be secondary changes

in acne lesions and cellular immunity may play an important role against *Propionibacterium acnes* (18).

In conclusion, we showed that there were no significant differences in the activities of MPO in PMNs between the patients and the control group. However, SOD activity in PMNs was significantly lower in the patients than in the control group. We believe that new anti-acne drugs are well advised to incorporate substances with lymphocyte stimulating and anti-oxidative properties.

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