How reliable an indicator of inflammation is myeloperoxidase activity?

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A B S T R A C T

Background: Myeloperoxidase (MPO) and interleukin-6 (IL-6) are often used as markers of inflammation. The aim of this study was to ascertain whether MPO activity is as reliable as IL-6 as an indicator of inflammation.

Methods: Inflammation was induced in mice, using either turpentine or indomethacin. Duodenal tissue was removed from these animals at various time periods ranging from 6 h to 7 days later. Concentrations of IL-6 and MPO activity were estimated in the tissue. Histopathological examination was also carried out at some of the time periods to determine the presence of neutrophil infiltration in turpentine-treated mice.

Results: Concentrations of IL-6 and MPO activity were significantly higher in tissue that had been treated with the agents used, at all the time periods studied, when compared with corresponding control tissue. Fold-increases in MPO activity were higher than fold-increases in IL-6. Concentrations of the 2 parameters showed significant positive correlation. Histopathological examination did not show significantly higher numbers of neutrophils infiltrating the tissue in response to turpentine, at the time periods studied.

Conclusions: Estimation of MPO activity is a reliable indicator of inflammation, being more sensitive than histopathological examination of tissue and as good as measurement of IL-6 concentrations.

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1. Introduction

Myeloperoxidase (EC 1.11.1.7) (MPO), synthesized and secreted by neutrophils, catalyses the formation of hypochlorous acid and tyrosyl radicals during the process of respiratory burst [1,2]. Activation of leukocytes prompts secretion of myeloperoxidase and generation of these oxidants which play an important role in host defense [3]. Elevations in concentrations of MPO in both tissue and plasma have often been used as markers of polymorphonuclear leukocytosis in conditions of inflammation and sepsis [4]. By regulating the bioavailability of nitric oxide, MPO is also involved in modulation of vascular signaling and vasodilator functions by this molecule, during the processes involved in acute inflammation [5].

Interleukin-6 (IL-6) is a pro-inflammatory cytokine secreted by macrophages and T-cells in response to a variety of inflammatory stimuli. It is an important mediator of an acute phase reaction [6] and often used as a marker of inflammation [7,8]. Studies have shown that intestinal epithelial cells are an important source of IL-6 in inflammatory responses in the intestinal mucosa [9]. Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), both pro-inflammatory mediators, are known to act in synergy to enhance IL-6 secretion and may significantly contribute to overall systemic inflammatory responses by producing IL-6 [10].

The current work originated from our study to standardize a mouse model of inflammation in the duodenum using two agents, turpentine and indomethacin. Turpentine is an agent commonly used to produce inflammation in animal models [11,12]. Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) that is known to produce localized inflammation in the small intestine [13,14]. In order to confirm that administration of the agents concerned had induced inflammation, we decided to measure concentrations of MPO and IL-6 in the duodenum of the experimental animals, in response to the agents used.

2. Materials and methods

Male albino mice (weight 30–50 g) were used for all experiments, with age and weight-matched animals as controls. Approval for the use of animals was obtained from the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Government of India.

The mice were dosed with the following agents to produce inflammation:

1. Systemic inflammation: Turpentine oil (Sigma Aldrich, India) was given as a single subcutaneous injection into the intra-scapular fat pad of mice at a dose of 0.1 ml/20 g body weight [11]. This was done under inhalation anaesthesia, using halothane, The animals were sacrificed 6, 12, 24 h and 2, 3, 4, 5 and 7 days later. Control animals were injected with an equal volume of sterile saline (0.15 mol/l NaCl) and sacrificed at the time intervals detailed above.

2. Localized intestinal inflammation: Indomethacin (Sigma) was administered by gastric gavage, under inhalation anaesthesia with halothane, at a dose of 15 mg/kg body weight. The animals were sacrificed at the various time intervals described above. Control animals received the vehicle for the drug (0.6 mol/l NaHCO₃) and were sacrificed at time intervals corresponding to the treated groups.

When the mice were sacrificed, the small intestine was removed and opened along its anti-mesenteric border. The mucosa of the duodenum was scraped, using glass slides. The scrapings were then homogenized as described below.

For assays of IL-6, tissue samples were homogenized in phosphate-buffered saline (pH 7.0), to produce 0.05% homogenates. This homogenate was used for the assay carried out by

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an enzyme-linked immunosorbent assay (ELISA), using a commercially available kit (BD Cytokine Assay, BD Biosciences, San Jose, CA), as per manufacturer’s instructions.

For estimation of MPO activity[15], tissue samples were weighed and homogenized in 50 mmol/l potassium-phosphate buffer (pH 6.0), containing 5 mg/ml hexadecyltrimethylammonium bromide, to produce 0.05% homogenates. The homogenates were centrifuged at 12,000 rpm for 15 min. The reaction mixture, containing 16.7 mg of o-dianisidine, 90 ml of distilled water, 10 ml of potassium-phosphate buffer, and 50 µl of 0.29 M hydrogen peroxide, was prepared. 30 µl of homogenate was added to each well in a standard 96-well plate and the volume made up to 200 µl with reaction mixture. Using a microtiter plate scanner, three absorbance readings at 30-s intervals were recorded at 450 nm. MPO activity was measured in units/mg tissue, where one unit of MPO was defined as the amount needed to degrade 1 µmol of hydrogen peroxide per minute at room temperature.

Tissue from the animals treated with turpentine was also subjected to histopathological examination by light microscopy. The time periods of study used for this were 6, 12 and 24 h. Twenty high power fields were examined for 3 independent samples at each time period and the number of neutrophils in each of these fields counted. The mean number of neutrophils infiltrating the tissue per high power field was calculated for each of the periods studied.

3. Statistical analysis

Values for IL-6 and MPO were calculated as means±SD. Data from the test and control groups were compared by Student’s unpaired t-test. A p-value of less than 0.05 was considered statistically significant. Fold-increases in the two markers were calculated by dividing the value of each parameter in each treated group by its corresponding control. Spearman’s correlation coefficients for IL-6 and MPO were calculated using the Statistical Package for Social Sciences (SPSS), ver 11. Data from the histopathological studies were expressed as medians and compared by Mann–Whitney test.

4. Results

Values for IL-6 and MPO in the duodenal homogenates from turpentine-treated mice were significantly higher than corresponding controls at all the time periods studied. Fig. 1 shows fold-increases in concentrations of these parameters in the experimental animals. IL-6 concentrations showed elevations in concentrations that ranged from over 2.5-fold to nearly 8-fold. Increases in MPO activities ranged from 4-fold to more than 13-fold. Elevations in concentrations of both the parameters showed a significant positive correlation (Spearman’s correlation coefficient=0.475, p<0.01).

In the indomethacin-treated mice, values for IL-6 and MPO were significantly higher than corresponding controls at all the time periods studied. Fig. 2 shows fold-increases in concentrations of these parameters in the experimental animals. IL-6 concentrations showed elevations in concentrations that were ranged from nearly 2-fold to approximately 8-fold. Increases in MPO activities ranged from 2.5-fold to 17-fold. Elevations in concentrations of both the parameters showed a significant positive correlation (Spearman’s correlation coefficient=0.512, p<0.01).

Histopathological examination of duodenal tissue was done at 6, 12 and 24 h after injection with turpentine. At the 6 h time period, there were more neutrophils in the tissue from animals that had been treated with turpentine, when compared with corresponding control tissue (8.8 in turpentine-treated animals vs 1.4 in controls; n=3), with the difference being very close to statistical significance (p=0.05). There were no significant differences in the number of neutrophils in turpentine-treated tissue when compared with control tissue at the 12

Fig. 1. Effect of turpentine on IL-6 concentrations and MPO activity in mouse duodenum. Data represent means (±SD) of 3–6 separate experiments.

Fig. 2. Effect of indomethacin on IL-6 concentrations and MPO activity in mouse duodenum. Data represent means (±SD) of 3–6 separate experiments.
5. Discussion

IL-6 is used widely as a marker of inflammation in humans and in experimental animal models [7,8,16,17]. Estimation of MPO activity has also been used in numerous settings, as an indicator of influx of inflammatory cells into tissue [18,19]. In our study, we found that the activity of MPO and concentrations of IL-6 were significantly increased in duodenal tissue from animals that had been treated with turpentine or indomethacin. This was found to hold true for all the time periods studied and indicated the induction of inflammation in the tissue. Increased IL-6 concentrations were also seen in the serum of turpentine-treated animals (data not shown), confirming the presence of systemic inflammation.

Histopathological examination did not reveal significantly increased numbers of neutrophils in the duodenum in the first 24 h after turpentine injection. However, IL-6 and MPO concentrations were significantly increased at these time periods and up to the 7 days of the study. Therefore, our study showed that MPO and IL-6 concentrations were far more sensitive than histopathological studies in detecting the presence of inflammation. The latter require the services of a trained histopathologist while estimations of MPO activity and IL-6 concentrations have the advantage that they are simple to perform. The fold-changes seen in the activity of MPO were higher than those seen in concentrations of IL-6, indicating that MPO is a sensitive indicator of inflammation. Our results, thus, suggest that MPO is as reliable as IL-6 as an inflammatory marker in this model.

The advantages of using MPO as a marker of inflammation are manifold. The use of a microplate kinetic method for the assay of the enzyme, as we have done, involves use of small quantities of reagents (thus keeping costs low) and allows analysis of large numbers of samples. The method can also be adapted for use with a spectrophotometer. This enables the assay to be used by small laboratories which may not have access to an ELISA plate reader required for IL-6 estimations. An added advantage of the MPO assay over that of IL-6 is that the latter requires antibodies for use in an ELISA-based method. The use of antibodies is expensive, requires facilities for appropriate storage and entails dependence on limited shelf-lives of such products. In addition, the MPO assay can be carried out in a short period of time, unlike the IL-6 assay which requires overnight incubation of microtiter wells with the antibody.

In conclusion, our data show that in our experimental animal model, MPO performed as well, if not better than IL-6, as an indicator of inflammation in duodenal tissue. We conclude that this parameter is a sensitive and reliable marker of inflammation and is easier to carry out than estimation of IL-6.

List of abbreviations

MPO       myeloperoxidase
IL-6      interleukin-6
NSAID     non-steroidal anti-inflammatory drug
ELISA     enzyme-linked immunosorbent assay

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