Myeloperoxidase Concentrations in the Stool as a New Parameter of Inflammatory Bowel Disease

TOMOHISA SAIKI

The Second Department of Medicine, Kurume University School of Medicine, Kurume 830-0011, Japan

Summary: Neutrophils play a predominant role in inflammatory and immune reactions in inflammatory bowel disease. It is well established that the level of myeloperoxidase, a constituent of neutrophil azurophil granules, reflects the number of neutrophil. We examined the usefulness of determining stool levels of myeloperoxidase in patients with inflammatory bowel disease. Myeloperoxidase levels in stool extracts were measured using a radioimmunoassay in 33 patients with ulcerative colitis, 32 with Crohn's disease, 9 inflammatory disease controls and 15 normal controls. Stool levels of myeloperoxidase in active inflammatory bowel disease patients increased significantly, and correlated with laboratory parameters and endoscopic grade of inflammation. A paired analysis showed a decrease in myeloperoxidase levels after the resolution of disease exacerbation. These results suggest that stool myeloperoxidase is a simple, noninvasive, and relevant marker of disease activity.

Key words inflammatory bowel disease, myeloperoxidase, stool

INTRODUCTION

Infiltration by neutrophils is a striking histologic feature in the lesions of active ulcerative colitis (UC), and is well documented in active Crohn's disease (CD) [1,2]. Myeloperoxidase (MPO) is an enzyme found in neutrophils [3] and, at much lower concentration, in monocytes and macrophages [4,5]. This enzyme catalyzes the reaction of hydrogen peroxide and halide ions to produce cytotoxic acids, such as hypochlorous acid [6]. The level of MPO in a suspension of neutrophils is directly proportional to the number of neutrophils present over a wide range of neutrophil concentrations. Previous studies applied the assessment of tissue MPO levels to the quantitation of intestinal inflammation in several animal models. In each model, MPO activity is directly proportional to the number of neutrophils present over a wide range of neutrophil concentrations. Although the MPO level in the intestine itself is important for the characterization of MPO profiles in human inflammatory bowel disease (IBD) [8], it is of course not suitable for practical use. Also, intestinal inflammation in both UC and CD usually exhibits a patchy and inhomogeneous distribution. Therefore, we asked whether the stools of IBD patients contain increased amounts of MPO and, if so, would measurement of stool MPO be a reliable and noninvasive means of understanding the disease status in the gut.

In the present study, we identified and quantitated MPO in stools of IBD patients using a specific and sensitive radioimmunoassay, and examined its relation to clinical parameters.

MATERIALS AND METHODS

Subjects

Thirty-three patients with UC and 32 with CD were investigated. The diagnoses were based on characteristic clinical, endoscopic, radiological, and histological features.

Patients with UC: There were 20 men and 13 women with a median age of 32 years (range 16-65). In
terms of disease distribution, 16 patients had pancolitis, 8 had left colon involvement, and 9 had disease limited to the rectum. Disease activity in each patient was analyzed according to the criteria of Truelove and Witts [9]. Eighteen patients suffered from active disease (4 mild, 12 moderate, 2 severe) and 15 had inactive disease. At the time of study, 12 patients had taken sulphasalazine alone, 5 both corticosteroid and sulphasalazine, 2 corticosteroid only, and 14 no specific treatment. Whenever possible, endoscopic findings were graded from 1 to 4 according to the severity of inflammation, using the criteria of Matts [10].

**Patients with CD:** There were 20 men and 12 women, with a median age of 34 years (range 17-67). In 14 patients the disease affected both the ileum and the colon, in 5 patients the colon alone, and in 13 patients the ileum alone. Disease activity was assessed by the score of the International Organization for the Study of Inflammatory Bowel Disease (IOIBD) [11]. A score of 1 or less was defined as corresponding to inactive disease and a score of 2 or more corresponded to more active disease. In this group, 18 patients had active and 14 inactive disease. Ten patients had taken sulphasalazine alone, 8 both corticosteroid and sulphasalazine, and 14 no specific treatment.

**Control subjects:** Fifteen healthy, age-matched subjects served as the normal controls. Nine patients with other colitides (7 infectious and 2 ischemic colitis) were examined as inflammatory controls.

This project was performed according to the Helsinki Declaration, and informed consent was obtained from every patient.

**Sampling**

Patients were instructed to defecate directly into a polystyrene container (diameter 11.5 cm; depth 7.5 cm). The stool extraction procedure for MPO measurement has been described previously. Briefly, the stool was weighed, diluted 1:2 in phosphate-buffered saline (pH 7.2) containing soy trypsin inhibitor (1 mg/mL, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and phenylmethylsulfonyl fluoride (1 mg/mL, Wako Pure Chemical Industries, Ltd.) and centrifuged at 10,000 G at 4 °C for 15 min. Supernatants were collected and kept frozen at −80 °C until use [12]. A blood sample was also obtained from every patient to determine various laboratory parameters.

**Determination of stool samples**

MPO levels in the stools were measured using a specific radio-immunoassay (detection limit, <8 μg/L; Pharmacia & Upjon Diagnostics AB, Uppsala, Sweden). Briefly, each stool sample (50 μL) was incubated with a fixed amount of [125I]-labeled MPO in the presence of a specific antibody against MPO. The antibody-bound and free MPO were separated by the addition of a second antibody immunosorbent, which was followed by centrifugation and decanting. The radioactivity in the pellet, which was then measured, was inversely proportional to the quantity of MPO in the sample [13].

As a stool marker for disease activity, polymorphonuclear cell elastase were determined by an enzyme-linked immunosorbent assay (<11.0 μg/mL, Sanwa Kagaku Kenkyusho CO., Ltd., Nagoya, Japan).

**Determination of blood samples**

Routine clinical laboratory methods were used for additional assessment of acute phase indicators, including the leukocyte count and erythrocyte sedimentation rate. Serum C-reactive protein concentrations (normal range < 350 ng/ml) were measured by laser nephelometry (NA latex CRP kit; Hoechst Japan, Tokyo, Japan).

**Statistical analysis**

The means of multiple groups were compared by Bonferoni's test after analysis of variance. Kruskal-Wallis and Spearman rank correlation were also used for statistical analyses. Differences were considered statistically significant for p<0.05.

**RESULTS**

Individual stool MPO levels are shown in Fig. 1. MPO was detected in every subject. Stool MPO levels were significantly higher in patients with active IBD compared to patients with inactive disease and normal controls. There was no significant difference in MPO levels between active UC and CD.

There was a significant correlation of MPO levels with laboratory parameters or stool polymorphonuclear cell elastase levels in UC and CD (Table 1). Further, MPO levels correlated significantly with the endoscopic grade of UC (p<0.05) (data not shown).

The level of MPO was compared between the active and following inactive phase in patients with UC and CD. As shown in Figs 2 and 3, MPO levels fell significantly after clinical resolution.

MPO levels were also analyzed in relation to the
Fig. 1. Stool concentrations of myeloperoxidase in normal subjects (A, n=15) and in patients with active (B, n=18) and inactive (C, n=15) ulcerative colitis, active (D, n=18) and inactive (E, n=14) Crohn’s disease, and inflammatory controls (F, n=9). Bars represent mean+SEM. n: number of patients.

Fig. 2. A paired analysis of myeloperoxidase levels in stools obtained from 9 patients with ulcerative colitis and 9 patients with Crohn’s disease.

Fig. 3. Time course study of C-reactive protein, polymorphonuclear cell elastase, and myeloperoxidase levels in a 16-year male patient with ulcerative colitis involving the entire colon (A) and in a 22-year male patient with Crohn’s disease involving both the small and large bowel (B). PSL: prednisolone; SASP: salazosulfapyridine; TPN: total parenteral nutrition; ED: elemental diet.
At present, it is not known whether inflammatory parameters in the circulation mirror the intestinal inflammation. However, the level in the intestine is not suitable for practical use. Our data suggest that stool MPO is a simple, noninvasive, and relevant marker of IBD activity, and merits further clinical investigation.

ACKNOWLEDGMENTS: The author wishes to thank Drs. Keiichi Mitsuyama, Atsushi Toyonaga and Kyuichi Tanikawa, the Second Department of Medicine, Kurume University, for their invaluable cooperation. Thanks are due also to Mr. Tetsuji Yamashita of Mitsubishi Kagaku Bioclinical Laboratories Inc.

REFERENCES

11. Raqib R, Wretlind B, Anderson J, and Lindberg A. Cytokine secretion in acute shigellosis is correlated to disease location. However, the MPO level was independent of this factor in both UC and CD (data not shown).

DISCUSSION

There are several reports concerning a stool marker of IBD activity, including polymorphonuclear cell elastase [14,15], α 1-antitrypsin [16], hemoglobin [17] or albumin [17]. In the present study, we focused on the MPO profile in stool samples of patients with IBD.

We found that MPO is present in low amounts in the stool of patients with inactive IBD and normal controls, and that it markedly increases in patients with active disease. The increase in MPO levels is not a specific phenomenon for IBD since the similar result was obtained in other colitides, such as ischemic or infectious colitis.

A paired analysis of the same individual also showed a decrease in MPO levels after the resolution of disease exacerbation. Daily variations of MPO levels during the course of the disease further support the reliability of this data. Therefore, MPO measurement may help determine disease prognosis and confirm treatment efficacy.

We further analyzed the linkage between MPO and various parameters of disease activity. We found a relationship between MPO levels and conventional markers for inflammatory activity, such as leukocyte counts, erythrocyte sedimentation rate or C-reactive protein, and endoscopic grade of inflammation. We also found a good correlation between stool levels of MPO and polymorphonuclear cell elastase, an established marker for disease activity [14,15]. Although the design of this study did not permit further comparison of these two stool markers, the present results suggest that stool MPO reflects the degree of disease activities.
disease activity and directed more to stool than to plasma. JID 1995; 171:376-384.