MYELOPEROXIDASE AND ITS ACTIVITY PRODUCTS IN UNTREATED AND TREATED RHEUMATOID ARTHRITIS: SYNOVIAL FLUID ANALYSIS

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Abbreviations.

ACR, Acute Arthritis of Rheumatoid Arthritis;

Cl-tyr, 3-chlorotyrosine;

DAS, disease activity score;

DMARDs, disease-modifying anti-rheumatic drug

Hcit, homocitrulline;

IL-18, interleukin-18;

IL-8, interleukin-8;

LC-MS/MS, liquid chromatography coupled to tandem mass spectrometer;

Lys, lysine;

MPO, myeloperoxidase;

NSAIDs, non-steroidal anti-inflammatory drugs;

OA, osteoarthritis;

OS, oxidative stress;

RA, rheumatoid arthritis;

SF, synovial fluid;

SIEFED, Specific Immunological Extraction Followed by Enzymatic Detection;

TRA, treated rheumatoid arthritis;

Tyr, tyrosine;

URA, untreated rheumatoid arthritis.

ABSTRACT

Objective To study the relationship between myeloperoxidase (MPO) and its products, interleukin (IL)-8, and IL-18 in the synovial fluid (SF) of patients with rheumatoid arthritis (RA) or osteoarthritis (OA) and to determine the impact of RA treatment on these biological markers.

Methods Sixty-six patients with RA, 33 of whom were receiving specific treatment, and 39 patients with OA were studied. The DAS-28 was calculated and MPO activity and concentrations of C-reactive protein (CRP), MPO, chloro-tyrosine, homocitrulline, IL-8 and IL-18 were measured in SF.

Results The DAS-28 and CRP concentrations were not significantly different between groups. MPO activity, and MPO, chloro-tyrosine and homocitrulline concentrations were significantly higher in RA than in OA patients. MPO specific activity (MPO activity/antigen ratio) was significantly lower in treated than in untreated RA patients as were IL-8 concentrations. MPO activity and concentration were correlated with IL-8 and IL-18 in untreated but not in treated patients.

Conclusions MPO concentrations are related to IL-8 and IL-18 levels in untreated RA patients. Moreover, MPO specific activity, IL-8 and the homocitrulline/lysine ratio were lower in treated than in untreated RA patients. The causal role of MPO in SF inflammation and how treatment can affect MPO specific activity need further investigations.

KEYWORDS

Myeloperoxidase; synovial fluid; interleukine-8; chloro-tyrosine; homocitrulline

INTRODUCTION

There is increasing evidence of a relationship between oxidative stress and cartilage degradation in mouse and human models of arthritis.[1] Moreover, myeloperoxidase (MPO) and its activity products, including chlorinated and carbamylated peptides, are strongly associated with osteoarthritis (OA) and rheumatoid arthritis (RA).[2, 3] Fernandes et al observed significantly higher MPO plasma levels in RA patients compared to healthy controls, but no significant correlation between plasma MPO levels and the disease activity score (DAS-28).[4] More recently, Stamp et al described a statistically significant correlation between DAS-28 and plasma MPO levels but not with MPO levels in synovial fluid (SF) although they observed higher MPO concentrations in SF than in plasma.[5]

There is a relation between oxidative stress and cytokine levels. Recent review data indicate a critical role for interleukin-18 (IL-18) in the degradation of articular cartilage in RA,[6] and interleukin-8 (IL-8), a potent proinflammatory cytokine, has a key role in the recruitment and activation of neutrophils during inflammation.[7]

Our aim was to study the relationship and correlation between MPO and its activity products, IL-8 and IL-18, in the SF of RA and OA patients. Moreover, we assessed for the first time the impact of RA treatment on these biological markers.

PATIENTS AND METHODS

Patients

RA patients were selected according to the ACR 1987 criteria. Thirty-three patients were receiving only non-steroidal anti-inflammatory drugs (NSAIDs; untreated RA group [URA]), and 33 patients were being treated with disease-modifying anti-rheumatic drugs (DMARDs) and/or biological agents (treated RA group [TRA]). SF was obtained in treated and untreated RA patients and in 39 OA patients as a control group. DAS-28 and CRP were established according to the EULAR guidelines.[8]

Neutrophil count

SF was analysed for neutrophil count according to standard procedure. Briefly, manual counting was performed using a Brüker numerating cell followed by staining with May-Grünwald Giemsa to differentiate blood cells and finally neutrophils were counted according to their morphology.

Protein bound homocitrulline (Hcit) and chloro-tyrosine (Cl-Tyr) analysis

SF samples were centrifuged and 40 µL of supernatant were analysed by microwave-assisted acid hydrolysis and LC-MS/MS analysis according to the method described by Delporte et al.[9] (for further detail see online supplement).

Activity of MPO and total MPO assay

The activity of MPO in SF was measured using the "Specific Immunological Extraction Followed by Enzymatic Detection" (SIEFED) method as described by Franck et al.[10] Total MPO content in plasma was measured using a sandwich human MPO ELISA kit (ELIZEN MPO, Zentech SE, Belgium). These analyses enable the quantity of MPO to be distinguished from MPO activity and give its specific activity (activity/antigen ratio).

Cytokine quantification

IL-8 and IL-18 concentrations in SF were quantified using ELISA tests (Becton Dickinson®).

Statistics

Sigma Stat 3.0 Software was used for statistical analysis. Differences were considered statistically significant with a two-tailed $p<0.05$. Comparisons among URA, TRA and OA patients were made using an ANOVA on Rank and a Dunn's post-hoc test. Regression analyses were tested using a Spearman correlation.

RESULTS

Table 1 shows the comparison between the URA, TRA and OA groups. The median (min-max) DAS-28 in the URA group was similar to that in the TRA group [4.87 (3.24-5.69) vs. 4.94 (3.81-5.83), p= 0.47]. The median CRP concentrations were 1.3 (0.5-3.37) mg/dL and 1.05 (0.6-3.5) mg/dL, respectively, in URA and TRA patients. There were no differences in DAS-28 or CRP concentrations between the URA and TRA groups (**Table 1**). The neutrophil count was higher in URA and TRA patients than in OA patients. MPO antigen and MPO activity were greater in URA and TRA patients than in OA patients; however, there were no significant differences in MPO antigen or activity between the URA and TRA groups. In contrast, MPO specific activity (MPO activity/antigen ratio) was significantly lower in TRA than in URA patients [2.1 (1.4-4.9) vs 0.39 (0.15-1.01) $p< 0.05$]. Although IL-18 concentrations in URA patients were twice those in TRA patients [217 (94-532) vs 109 (39-397)], these differences were not significant and there were also no significant differences compared to OA patients (p= 0.34). Interestingly, the IL-8 level was lower in TRA than in URA patients [65 (9-1638) vs 1055 (72-2144) ($p<0.005$)] at the same level as that of OA patients [58 (26-102)]. MPO activity and concentration were correlated with IL-8 (**Fig 1**) and IL-18 (**Fig 2**) in untreated but not in treated patients.

Cl-tyr/Tyr and Hcit/Lys ratios were significantly higher in URA patients than in OA patients (both p= 0.03). There was no significant difference in Cl-tyr between TRA and URA patients, but the Hcit/Lys rate was higher in URA than in TRA patients ($p<0.005$). MPO activity and antigen were not correlated with Hcit orCl-tyr concentrations.

DISCUSSION

MPO concentration was higher in patients with URA and TRA than in those with OA but there was no significant difference in concentrations between URA and TRA patients. Treatment of RA, therefore, does not seem to affect the concentration of MPO in SF. Interestingly, MPO was present in OA patients although no neutrophils were detected in their SF. However, MPO specific activity was significantly higher in the URA group than in the TRA and OA groups. This observation highlights the importance of measuring the concentration and activity of MPO rather than just the concentration. Few data are available about the level of MPO activity when it is present in human fluids. Van Antwerpen et al showed that the glycobiology of MPO had an impact on its activity, because partially deglycosylated MPO has reduced activity.[11] When neutrophils are activated, they also release sialidases, which can target their environment and, potentially, deglycosylate MPO.[12] Nevertheless, global MPO activity in RA patients was higher than in OA patients, and this may play a role in advancement of disease by producing locally oxidative agents.

We further looked at two products of MPO activity, protein-bound Cl-tyr and Hcit. Both products were higher in URA patients than in TRA or OA patients, suggesting a greater impact of MPO on protein modification in URA patients. Mydel et al (2010) suggested that protein-bound Hcit triggers a primary immune response and enhances the pathogenesis of autoimmune arthritis.[3] Because MPO can catalyse Hcit formation in proteins,[13] we investigated the concentration of Hcit in SF. Our data suggest that treatment of RA significantly decreased Hcit concentrations although only a trend was observed for Cl-tyr concentrations. These decreases paralleled the lower specific activity of MPO and highlight a potential role of MPO in the pathogenesis of RA.

The correlations between IL-8, IL-18 and MPO antigen or activity in URA patients confirm the important role of IL-8 and IL-18 in the inflammatory process mediated by MPO in SF but do not establish a causal association. It is noteworthy that these correlations were not observed in TRA patients (data not shown), suggesting a key role of treatment on this inflammatory process. In

addition, the correlations between IL-18 and MPO antigen or activity in URA patients suggest that MPO may have a role in the degradation of articular cartilage in RA [6].

The DAS-28 and CRP concentrations were not statistically different in URA and TRA patients, and were moderate or high, suggesting that there was still some inflammatory activity in the joint. Moreover, there was no correlation between DAS-28 and MPO antigen/activity in SF whereas Stamp et al reported a correlation of DAS-28 with plasma MPO concentration. Although the DAS index is, in part, a subjective measure, non-paired analysis (heterogeneity between groups) could introduce a bias. Additional analyses or studies should be performed in paired RA patient groups (before and after treatment in the same patient) in order to investigate the impact of RA treatment on MPO antigen, activity, and products of activity.

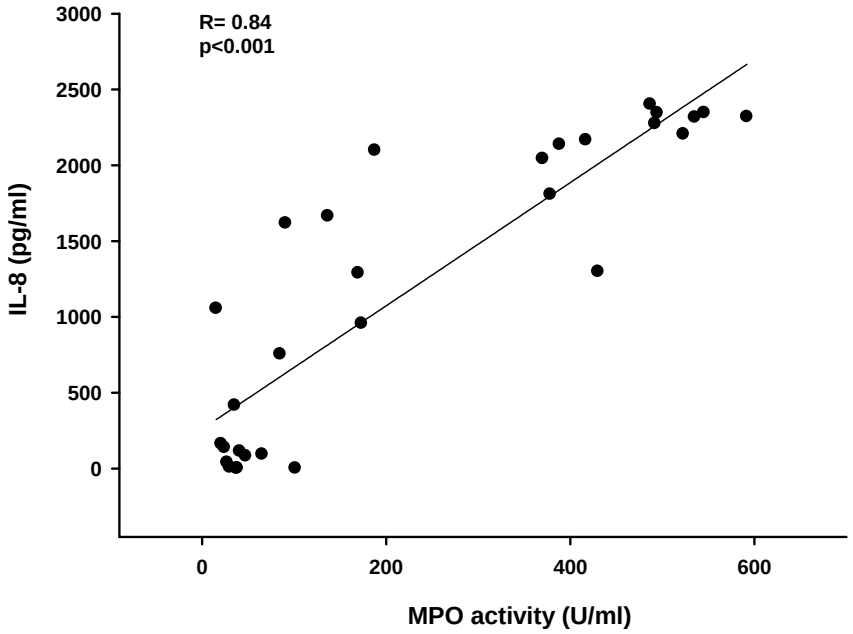
In summary, this study demonstrates a different pattern of MPO and IL-8/18 expression in the SF of RA and OA patients. Moreover, we have shown for the first time that treatment with DMARDs is associated with a lower MPO specific activity, Hcit/Lys ratio and IL-8 production in the SF of patients with RA. To further validate MPO as a biomarker for RA, a paired study should be performed in RA patients, including DAS-28 assessment and measurements of MPO expression, its products of activity and IL-8/18 in SF.

Table 1: Measured parameters in the untreated (URA) and treated (TRA) rheumatoid arthritis and osteoarthritis (OA) groups.

	URA group (n=33)	**TRA group (n=33)**	**OA group (n=39)**	**p**
DAS-28	4.87 (3.24-5.69)	4.94 (3.81-5.83)		0.47
CRP mg/100mL	1.3 (0.5-3.37)	1.05 (0.6-3.5)		0.82
Neutrophils (cells/µL)	0.5 (0-3) §	0.5 (0-2) §	0 (0-0)	<0.001
MPO antigen (ng/mL)	491 (60-823) §	124 (50-1006) §	28 (8.7-75.3)	<0.001
MPO activity (mU/mL)	153 (38-430) §	69 (32-223) §	12.19 (5.5-25.7)	<0.001
MPO act/antig (mU/ng)	2.1 (1.4-4.9) §	0.39 (0.15-1.01)*	0.48 (0.21-0.83)	<0.001
IL-18 (pg/mL)	217 (94-532)	109 (39-397)	180 (120-270)	0.34
IL-8 (pg/mL)	1055 (72-2144) §	65 (9.4-1638)*	58 (26-102)	0.003
Cl-tyr/Tyr ratio ($x10^{-5}$)	4.7 (2.4-5.3) §	2.9 (1.5-6.6)	2.7 (1.5-4.5)	0.03
Hcit/Lys ratio ($x10^{-4}$)	1.8 (1.7-2.1) §	1.2 (0.7-2.4)*	1.4 (1.1-2.5)	0.03

*Median (25-75%). ANOVA on Rank, Dunn's post-hoc test. *p<0.005 URA vs TRA ; § p<0.05 vs OA*

Fig 1: Correlation between IL-8 and (**A**) MPO activity or (**B**) MPO concentration in untreated RA patients.



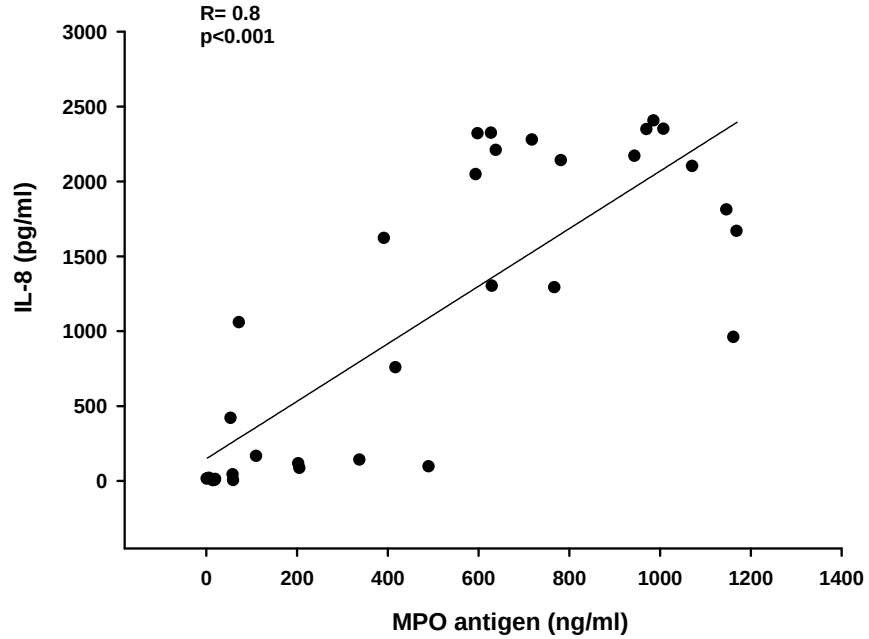
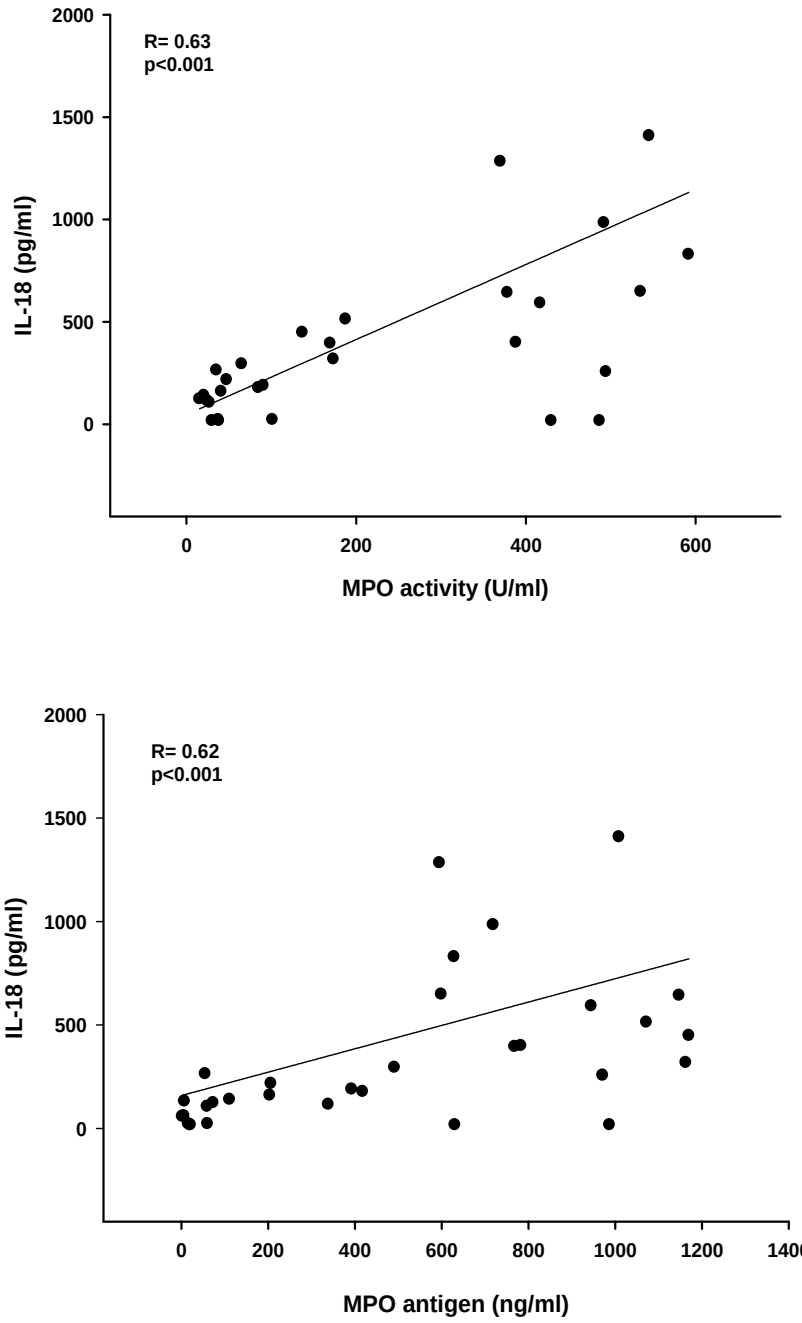


Fig 2: Correlation between **IL-18** and (**A**) MPO activity or (**B**) MPO concentration in untreated RA patients.



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