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Alimentary Tract

Increased mucosal matrix metalloproteinase-1, -2, -3 and -9 activity in patients with inflammatory bowel disease and the relation with Crohn's disease phenotype

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Abstract

Background and objective. Matrix metalloproteinases are associated with matrix turnover in both physiological and pathological conditions. We postulate an association between aberrant matrix metalloproteinases proteolytic activity and the intestinal tissue destruction, seen in patients with Crohn's disease and/or ulcerative colitis.

Materials and methods. Surgically resected inflamed and non-inflamed ileum and colon with/without extensive fibrosis from 122 Crohn's disease, 20 ulcerative colitis and 62 control patients were homogenized. Protein levels of matrix metalloproteinases and tissue inhibitor of metalloproteinases were measured by enzyme-linked immunosorbent assays (ELISA), while matrix metalloproteinases and myeloperoxidase activity were measured by specific activity assays.

Results. Expression of total levels of matrix metalloproteinases-1, -2, -3 and -9 relative to tissue inhibitor of metalloproteinases-1 and -2 was increased in inflamed inflammatory bowel disease compared to non-inflamed inflammatory bowel disease and control intestinal mucosa. Also, net matrix metalloproteinases-1, -2, -3 and -9 activity in inflamed inflammatory bowel disease was increased, with similar expression profiles in Crohn's disease and ulcerative colitis. Within inflamed inflammatory bowel disease, a close correlation of matrix metalloproteinases with myeloperoxidase was observed. The expression of matrix metalloproteinases and tissue inhibitor of metalloproteinases was similar in inflamed Crohn's disease tissue with or without extensive fibrosis and not related to fistulizing disease.

Conclusions. We have shown increased net matrix metalloproteinases activity in intestinal inflammatory bowel disease tissue, likely to contribute to the tissue damage and remodelling seen in inflammatory bowel disease.

Keywords: Fibrosis; Fistulae; IBD; MMP; TIMP

1. Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are the major forms of chronic idiopathic inflammatory bowel disease (IBD). CD is characterised by periodic, segmental and often transmural infiltration of potentially the whole gastrointestinal tract, especially the ileocaecal area, by a variety of immune cells like T and B lymphocytes, histiocytes (developing into granulomas/giant cells) and granulocytes [1]. The inflammatory infiltrate and resident cells secrete pro-inflammatory chemokines and cytokines, proteinases, reactive oxygen radicals, etc., which cause extensive morphological damage manifested by ulcera, abcesses, fissures and fistulae. In a subset of patients the sustained intestinal inflammation activates (myo)fibroblasts and smooth muscle cells to deposit massive amounts of collagen III and V, resulting in fibrotic strictures, halting food passage and necessitating surgical removal of the affected bowel [2,3]. Idiopathic UC affects the superficial mucosal layers of the colon, often starting from the rectum and extending proximally over the years [4]. Although stenotic development is

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Table 1
Matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) analysed in this study, with their characteristics [5,38]

MMP–TIMP	Synonym(s)	Major substrate(s)	Molecular weight(s) (kDa[a])
MMP-1	Interstitial collagenase Collagenase-1	Fibrillar collagens, pro-MMP-2 and -9	52 and 41
MMP-2	Gelatinase A	Collagens, gelatins, elastins, laminin	72 and 66
MMP-3	Stromelysin-1	Extracellular glycoproteins, non-fibrillar collagens, pro-MMPs	57, 45 and 28
MMP-9	Gelatinase B	Collagens, gelatins, elastins, laminin	92 and 85
TIMP-1	Fibroblast collagenase inhibitor Collagenase inhibitor	(pro-) MMP-9	28
TIMP-2	CSC-21K	(pro-) MMP-2	21

[a] kDa = kilo Dalton.

not as common as with CD, in UC the inflammatory infiltrate may also result in extensive mucosal damage, with surgical removal of part or the whole colon as the final clinical outcome. The matrix metalloproteinases (MMP) are a family of calcium and zinc containing neutral endoproteinases implicated in matrix tissue turnover during normal growth, development and reproduction but are also involved in several pathological conditions, i.e. cancer metastasis, rheumatoid arthritis, atherosclerosis, psoriasis, etc [5]. They have been shown to degrade a considerable number of important structural matrix molecules (Table 1), and an altered production of MMPs might contribute to the tissue morphological changes seen in IBD patients. Actually, reports have demonstrated an increased level of several MMPs in inflamed intestine of IBD patients, which was accompanied by an insufficient upregulation of the endogenous MMP inhibitors, i.e., tissue inhibitor of metalloproteinases (TIMP) [6–14]. These studies do not report, however, about the actual activity of these MMPs in the IBD tissues. Also, in several models of IBD, e.g., DSS- and TNBS-induced colitis in rats and mice, immune infiltration was clearly associated with an upregulation of MMPs and the mucosal damage could be reversed by application of specific MMP inhibitors [15–17]. However, in fibrotic and stenotic areas, the increased synthesis of collagens by mesenchymal cells may actually lead to a thickening of the bowel wall probably because of dysregulated MMP matrix degradative capacity. For example, in fistulae with chronic inflammation and fibrosis only a moderate upregulation of MMP-3 and -9 versus TIMP was observed opposite to the massive upregulation of these MMP members in areas with acute inflammation without fibrosis [18]. Given the potential relevance in IBD pathogenesis, their interactions and substrate diversity (Table 1), we not only measured MMP-1, -2, -3, -9 and TIMP-1, -2 protein levels by ELISA but also their activity by specific immunocapture bioactivity assays. We show an upregulation of these MMPs relative to TIMP, associated with higher (patho)physiological MMP activity in inflamed IBD intestinal mucosa compared with non-inflamed tissue from both IBD and control patients. In CD patients, MMP levels were independent of the macroscopical/histological co-presence of fibrosis and were not related to the incidence of fistulae during follow-up.

2. Materials and methods

2.1. Tissue samples

Over the period 1983–2002 macroscopically inflamed/affected as well as non-inflamed/unaffected intestinal mucosa was prospectively collected immediately after surgical resection from 122 (49 male (M)/73 female (F)) CD and 20 (6 M/14 F) UC patients at the departments of Surgery, Pathology and Gastroenterology–Hepatology of our hospital and stored at −70 °C (Table 2). The IBD patients underwent operation because of stricturing processes, fistulae and/or luminal disease activity refractory to medical therapy (aminosalicylates, corticosteroids, azathioprine, methotrexate and/or anti–TNF-α antibody infliximab). Severe fibrosis in inflamed CD mucosa, often manifested by a thickened wall and narrowed lumen, was documented in the histopathology reports from the Pathology department. Incidence of perianal, entero–entero, entero–viscero and/or enterocutaneous fistulae in CD patients in their clinical history and during follow-up (evaluation period median 24.7, range 3.3–58.5 years) was recorded in patient files from the Gastroenterology department. Also, from 62 (26 M/36 F) colorectal carcinoma or adenoma patients, macroscopically normal control mucosa at least 10 cm away from the surgically resected neoplasia was collected. Reflecting the early onset, CD and UC compared to control patients were younger at time of surgery (Table 2). Intestinal mucosa was homogenised in 1 ml 0.1 M Tris–HCl, 0.1% Tween 80, pH 7.5 per 60 mg tissue using a Potter device (B Braun, Germany) as described previously [19]. Protein concentration was determined by the method of Lowry et al. [20] and myeloperoxidase (MPO) activity was measured as described elsewhere, based on the conversion of the ortho-dianisidine dihydrochloride MPO substrate in the presence of hydrogen peroxide [21].

2.2. Determination of MMP and TIMP by ELISA and BIA

Levels of MMP-2 and -9 protein were measured in appropriately diluted homogenates by our in-house ELISAs, as described previously [8]. TIMP-1 and -2 proteins were mea-