ISSN 0025-7680

ORIGINAL ARTICLE

MEDICINA (Buenos Aires) 1998; 58: 262-264

COLONIC PROTEINASES: INCREASED ACTIVITY IN PATIENTS WITH ULCERATIVE COLITIS

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Abstract In order to study the colonic intraluminal proteinase-antiproteinase imbalance under inflammatory conditions, we determined proteolytic activity (PA), alpha-1-antitrypsin and the activities of trypsin, chymotrypsin and neutrophil elastase in feces from patients with ulcerative colitis (UC) comparing the results with a control group. A fecal sample was obtained from each of 25 patients with ulcerative colitis and 10 control subjects were studied. The severity of the disease was assessed by the Truelove index. Proteolytic activity was measured using azocasein as proteolytic substrate. The fecal concentration of alpha-1-antitrypsin was measured by radial immunodiffusion and the activities of the enzymes were measured using specific substrates. We found an increase in fecal PA, alpha-1-antitrypsin and neutrophil elastase in patients with UC and the correlation between the severity of the disease and the PA was statistically significant ($r = 0.62$, $P < 0.05$). We conclude that elevated colonic proteinase activity could contribute to the pathophysiology of ulcerative colitis.

Resumen ***Proteasas intracolónicas: actividad incrementada en pacientes con colitis ulcerosa.*** Con el objetivo de estudiar el posible desbalance del sistema proteasa-antiproteasa en el medio intraluminal colónico bajo condiciones inflamatorias, se determinó la actividad proteolítica (AP), la concentración de alfa-l-antitripsina y las actividades de tripsina, quimotripsina y elastasa de neutrófilos en heces de pacientes con colitis ulcerosa (CU), comparando los resultados con un grupo control. La severidad de la enfermedad en los pacientes con CU fue determinada por el índice de Truelove. La AP fue medida usando azocaseína como sustrato, la concentración fecal de alfa-1-antitripsina por inmunodifusión radial y las actividades enzimáticas usando sustratos específicos. Se observó un incremento en la AP, alfa-1-antitripsina y elastasa de neutrófilos en heces de pacientes con CU y la correlación entre la severidad de la enfermedad y la AP fue estadísticamente significativa ($r = 0.62$, $p < 0.05$). Concluimos que la elevación de la actividad de proteasas intracolónicas podría estar relacionada a la fisiopatología de la colitis ulcerosa.

Key words: ulcerative colitis, proteinases, neutrophil elastase

Under normal conditions intraluminal colonic proteolytic activity (PA) is the combination of PA from pancreatic enzymes that reach the large intestine and PA from colonic microflora[1-4]. In patients with ulcerative colitis (UC) proteinases from neutrophils may contribute to intraluminal proteolysis[5]. Proteinases in the gastrointestinal lumen can break the mucus barrier, digest the underlying epithelium and produce mucosal damage. Neutrophil elastase liberated into the intraluminal milieu can be neutralized by alpha-1-antitrypsin, thus protecting the colonic mucosa from proteolysis[5]. In the oxidative burst of neutrophils, myeloperoxidase (MPO) plays a major role by catalysing the reaction between hydrogen peroxide and chloride ions, to produce the highly cytotoxic hypochlorous acid[6]. Both MPO and reactive oxygen species may produce inactivation of alpha-1-antitrypsin[7], leading to an increase in the colonic PA. In orden to study the colonic intraluminal proteinase-antiproteinase imbalance under inflammatory conditions, we measured PA, alpha-1-antitrypsin, and the activities of trypsin, chymotrypsin and neutrophil elastase in feces from patients with ulcerative colitis comparing the results with a control group.

Received: 4-XI-1997 Accepted: 8-V-1998

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Materials and Methods

A fecal sample was obtained from each of 25 patients with ulcerative colitis, and 10 control subjects (members of the laboratory staff) were studied. Spontaneous feces were collected and the samples were kept at -20°C until analysed. The severity of the disease was assessed by the Truelove index[8]. Five hundred milligrams from each fecal sample were homogenized in 5 ml of 0.05 mol/L tris buffer, pH 7.4. After

centrifugation at 3 000 g for 10 min at 4 °C the supernatant was assayed for trypsin, chymotrypsin and neutrophil elastase activities.

Chymotrypsin activity was measured using 0.1 mM of succinyl-Ala-Ala-Pro-Phe 4 nitroanilide (Sigma) as substrate[9]. The assay were performed at 25 °C in 0.1 M tris-HCl and 0.01 M CaCl2, pH 7.8. For trypsin activity, we used a 0.01 M solution of benzoyl-DL-arginine P-nitroanilide (Sigma) as substrate[10]. The assays were performed at 25 °C in 0.05 M tris buffer, pH 8.2, containing 0.02 M CaCl2. For elastase activity a solution of Methoxysuccinyl-L-alanyl-L-prolyl-L-valine-p-nitroanilide was used[11]. The cuvette contained 0.32 mM of substrate,and 20 uL of elastase sample, in phosphate-buffered saline at pH 7,4.

For measurement of proteolytic activity and alpha-l-antitrypsin concentration 1 gr of feces was homogenized in 0.1 M sodium phosphate buffer, pH 7.0, to produce 10% (W/V) slurries. The proteolytic activity were carried out as described[1] using azocasein as proteolytic substrate. The fecal concentration of alpha-1-antitrypsin was measured by radial immunodiffusion[12].

The Wilcoxon signed rank test and the Kruskal-Wallis test were employed for paired samples and independent samples, respectively. Correlations were assessed with the Spearman rank correlation test.

Results

As table 1 shows, the PA in patients with ulcerative colitis was higher than in healthy subjects ($p < 0.01$). The Spearman correlation coefficient between PA and severity of the disease was statistically significant, 0.62 ($P < 0.05$). As written in table 2, we found and increased fecal activity of neutrophil elastase in patients with ulcerative colitis. The activity of this enzyme was undetectable in healthy subjects, but it was present in twenty-three out of 25 patients with ulcerative colitis. We did not find a significant correlation between the activity of this enzyme and the severity of the disease. Despicted in Table 3 is the concentration of alpha-l-antitrypsin in the supernatant of fecal homogenates from patients with ulcerative colitis and normal subjects. We found an increased fecal concentration of alpha-1-antitrypsin in patients with ulcerative colitis. However we found no significant correlation between the severity of the disease and the fecal concentration of alpha-1-antitrypsin.

TABLE 1.– *Proteolytic activity (mg Azocasein/h/gr of feces) in patients with ulcerative colitis and normal subjects*

	Control subjects (n = 10)			Ulcerative colitis (n = 25)
Median	2.88			10.80*
Range	(0.70-3.60)			(1.09-25.85)
	Quiescent (n = 6)	Mild (n = 8)	Moderate (n = 7)	Severe (n = 4)
Median	1.30	7.60	18.20	20.6
Range	(1.09-6.01)	(1.27-18.22)	(4.86-25.85)	(16.5-32.4)

* $p < 0.01$

TABLE 2.– *Trypsin, chymotrypsin and neutrophil elastase in patients with ulcerative colitis and control subjects*

	Trypsin	Chymotrypsin	Neutrophil elastase
Control (n = 10)	19.7 (16.9-81.7)	12.6 (2.2-44.8)	0 (0-0)
Ulcerative colitis (n = 25)	25.1 (3.8-55.3)	23.3 (5.50-118.4)	3.48* (0-27.88)

* $p < 0.01$. The results are expressed as median and range of U/L.

TABLE 3.– *Concentration of Alpha-1-antitrypsin (mg/dl) in patients with ulcerative colitis and healthy subjects*

	Controls (n = 10)			Ulcerative colitis (n = 25)
Median	1.7			48.0*
Range	(0-3.8)			(6.5-117.0)
	Quiescent (n = 6)	Mild (n = 8)	Moderate (n = 7)	Sever (n = 4)
Median	10.5	66.5	30.3	51.0
Range	(6.5-31.5)	(18.0-117.0)	(6.5-70.0)	(29.0-74.0)

* $p < 0.01$

Discussion

This study shows that fecal proteolytic activity was significantly higher in patients with ulcerative colitis than in healthy subjects and this PA had a significantly positive correlation with the severity of the disease.

The increased fecal neutrophil elastase in patients with UC suggests that this enzyme may have contributed to the increased fecal proteolytic activity in these patients. However, since there was no correlation between the level of the elastase and the severity of the disease, other proteolytic enzymes of white cell origin, such as collagenase and gelatinase, may have played a part[13].

Intracolonic neutrophil elastase could contribute to the pathophysiology of ulcerative colitis by a direct effect on colonic epithelial cells or by liberating a mediator of the inflammation. In this regard it has been shown that

neutrophil elastase stimulates platelet activating factor (PAF) synthesis by PMN and endothelial cells to levels comparable with those observed after the addition of tumor necrosis factor (TNF) or after phagocytosis[14]. PAF shows a wide range of biological effects inducing aggregation of platelets[15], promoting chemotaxis of neutrophils and monocytes[16, 17], increasing vascular permeability and altering the vascular tone[18]. We also found an increased fecal concentration of alpha-1-antitrypsin in patients with ulcerative colitis. Similar results have been shown by others[19, 20]. This fecal elevation probably reflects an increased permeability of the mucosal barrier, seen as a result of the inflammatory process in which plasma proteins diffuse into the intraluminal space of the intestinal tract. We found an increased activity of fecal neutrophil elastase in patients with ulcerative colitis, so that alpha-l-antitrypsin was unable to produce a total inhibition of this enzyme. Alpha-1-antitrypsin appears to be overcome by large amounts of free neutrophil elastase. The other possibility is that the catalytic activity of myeloperoxidase with hydrogen peroxide and Cl gives rise to highly reactive oxigen species, including O_2 and HOCl wich can oxidize the alpha-1-antitrypsin, especially to methionine in the active center of the molecule. After oxidation, alpha-1-antitrypsin has a much lower affinity to elastase[21]. In conclusion, we found an increased PA in feces from patients with ulcerative colitis and this PA had a significant correlation with the severity of the disease. It is probably due to a protease-antiprotease imbalance. Further studies are necessary to evaluate whether these patients profit from already available therapeutic options, such as infusion of active alpha-1-antitrypsin.

Acknowledgement: Ths work was supported in part by a grant from University of Buenos Aires.

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