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Serum and Intestinal Dipeptidyl Peptidase IV (DPP IV/CD26) Activity in Children With Celiac Disease

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ABSTRACT

Objective: Dipeptidyl peptidase IV (DPP IV/CD26) is involved in the degradation of proline-rich proteins such as gliadin and in modulation of the immune response. The aim of this study was to examine the possible causal connection between DPP IV enzyme activities and celiac disease (CD) in children.

Patients and Methods: Intestinal mucosal biopsy specimens were obtained from 97 patients. The patients were divided into 3 groups: patients with active CD (n = 38), patients with malabsorption syndrome (MS) of other causes (n = 37), and control patients (n = 22). In addition, blood samples were collected from 48 patients with active CD and 50 control patients without gastrointestinal diseases. DPP IV enzyme activity was measured in the intestinal mucosal biopsy specimens and in the serum samples.

Results: DPP IV activity in the small intestine correlated inversely with the grade of mucosal damage in the CD ($r = -0.92$, $P < 0.001$) and MS groups ($r = -0.90$, $P < 0.001$). Intestinal DPP IV activity was statistically significantly lower in the CD and MS groups than in the control group ($P < 0.001$). By contrast, serum DPP IV activity was not significantly different between the CD and control groups.

Conclusions: Our results suggest that the decrease in intestinal DPP IV activity is not specific to CD because it correlates with the level of mucosal damage in both patients with CD and those with MS. In addition, it seems that serum DPP IV activity cannot be used as a specific noninvasive diagnostic or prognostic marker of CD. ***JPGN 45:65–70, 2007.*** **Key Words:** Celiac disease—Children—Dipeptidyl peptidase IV (DPP IV/CD26)—Malabsorption syndrome. © 2007 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

INTRODUCTION

Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten and/or related cereal proteins in genetically predisposed individuals (1). The disease is characterized by a lesion in the small intestine that shows villous atrophy, crypt cell hyperplasia, and infiltration by intraepithelial lymphocytes (2,3). CD commonly occurs in early childhood with severe symptoms, although in many patients the symptoms do not develop until later in life. CD is one of the most frequent lifelong disorders and has an approximate prevalence of about 1:300 to 1:80 in many populations (4,5). In 95% of patients CD is associated with human leukocyte antigen (HLA)-DQ2 (DQA∗05_DQB1∗02), and in the remainder, it is associated with HLA-DQ8 (DQA1∗03_DQB1∗0302) (6). The recognition of gluten epitopes after deamination by tissue transglutaminase and the activation of gluten-reactive $CD4^+$ T cells in a DQ2-restricted or DQ8-restricted manner seem to be critical events in the development of the disease (3). The gluten epitopes that bind to DQ2 or DQ8 peptides are rich in proline and glutamine, which makes them relatively resistant to proteolysis (7–9). Only a few types of proteolytic enzymes are capable of digesting such glutamine-rich and proline-rich proteins (8,10).

Dipeptidyl peptidase IV (DPP IV; EC 3.4.14.5; CD26) is a unique multifunctional type II transmembrane glycoprotein that acts as a receptor, binding protein, and proteolytic enzyme. This serine protease catalyzes the cleavage of X-Pro or, less frequently, X-Ala dipeptides on the *N* terminus of polypeptides. DPP IV is widely distributed in mammalian tissues, and a high level of DPP IV activity is found in intestinal brush border membranes. The proteolytic cleavage of membrane-bound DPP IV releases a soluble form that circulates in the plasma and other biological fluids (11). In addition to its localization and

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enzymatic activity, DPP IV may play an important role in the hydrolysis and assimilation of proline-rich proteins. Indeed, the importance of intestinal DPP IV has been verified by studies showing that rats deficient in DPP IV rats lose weight when fed gliadin (12).

Furthermore, DPP IV plays an essential role in the T cell–mediated immune response through the cleavage of some natural substrates that have immunoregulatory activity, which triggers a Th1 or Th2 immune response. DPP IV expression is higher on Th1 than Th2 cells and correlates with the production of Th1 cytokines (13,14). Therefore, because CD is a Th1 immune-mediated disease, it is possible that DPP IV regulates physiological immune processes, including the adaptive systemic immune response to local inflammation.

Because of the key role of DPP IV in the digestion of gliadin peptides and in the modulation of the immune response, we hypothesized that DPP IV is altered in children with CD. Therefore, the aim of this study was to determine whether there is a causal connection between the intestinal and serum DPP IV enzyme activity and CD in children.

PATIENTS AND METHODS

Intestinal mucosal biopsy specimens for histological analysis and for determination of DPP IV enzyme activity were obtained from 97 patients with malabsorption syndrome. Blood samples for analysis of DPP IV enzyme activity were collected from 48 patients with CD (22 girls and 26 boys; median age 3 years; age range, 1–9 years) and a control group of 50 children (23 girls and 27 boys; median age 3; age range, 1–6 years) without gastrointestinal diseases.

According to their clinical, histological, and serological data, the 97 patients with malabsorption syndrome were divided into 3 groups. The first group consisted of 38 patients (21 girls and 17 boys; median age, 3 years; age range, 1–5 years) with active CD. The second group was made up of 37 patients (16 girls and 21 boys; median age, 3 years; age range, 1–5 years) with malabsorption syndrome caused by other factors (MS), with mucosal lesions and negative antiendomysial (EMA) immunoglobulin A (IgA). Of the patients in the MS group, 16 had diarrhea, 15 had psorenteritis syndrome, and 4 had intestinal parasitosis. The third group included 22 control patients (10 girls and 12 boys; median age, 3 years; age range, 1–7 years) who had histologically normal intestinal mucosal architecture and were negative for EMA IgA. The control participants had undergone esophagogastroduodenoscopy as a routine evaluation for isolated signs of gastroesophageal reflux disease (n = 3), abdominal pain (n = 5), anemia (n = 4), or delayed growth (n = 10).

All of the patients included in the study were consuming a normal gluten-containing diet. CD was diagnosed according to the criteria recommended by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. In patients with diagnosed CD, a gluten-free diet was prescribed.

Sample Collection and Preparation

Intestinal mucosal specimens were obtained during esophagogastroduodenoscopy while the individuals were under deep sedation by intravenous propofol and fentanyl. Two biopsy samples were taken from the mucosa of the small intestine. One sample was fixed in 4% formalin and processed for routine histological analysis, and the other was weighed and stored in a closed test tube at −80°C for later analysis. The samples for histological analysis were evaluated by an expert pathologist. The ratio of the height of the small intestinal mucosal villous atrophy to the crypt depth, and the density of intraepithelial lymphocytes, were measured in well-oriented biopsy specimens as described previously (15). The degree of mucosal damage was graded according to the histological classification of Marsh (16).

Tissue samples (0.8–1.0 mg) for enzyme analysis were diluted in distilled water, homogenized in cold homogenization buffer (10 mmol/L Tris-HCl, pH 8.2), and analyzed immediately for enzyme activity. Blood samples were collected between 8:00 and 10:00 AM. The serum from blood samples was separated by centrifugation and stored at −80°C until analysis of enzyme activity.

Assay of Enzyme Activity and Serology

Determination of intestinal and serum DPP IV activities was performed as described by Kreisel et al (17). DPP IV activities were determined by measuring the release of 4-nitroaniline from an assay mixture containing 0.1 mol Tris-HCl (pH 8.0), 2 mmol Gly-Pro *p*-nitroanilide (Sigma Chemical, Steinheim, Germany) as the substrate and enzyme in a total volume of 0.20 mL. After 30 minutes of incubation at 37°C, the reaction was stopped by the addition of 800 μL of 1 mol sodium acetate buffer (pH 4.5). The absorbance at 405 nm was measured by use of a Varian Cary UV/VIS spectrophotometer (Cary, NC). All of the reactions were performed in duplicate.

Protein concentrations in homogenates were determined according to the method of Bradford (18). Enzyme activities in homogenates were expressed as international units per gram of protein, and in serum as international units per liter of serum. One unit corresponds to the hydrolysis of 1 μmol of substrate per minute under the assay conditions.

EMA IgA was measured in the serum of all of the patients by an indirect immunofluorescence method using a commercial kit (Eurospital, Trieste, Italy). Total IgA in serum was measured for all of the patients included in the study. All of the patients had normal serum levels of IgA.

Statistical Analyses

The collected data were statistically evaluated by use of the data analysis software system STATISTICA, version 7.1. StatSoft, Inc. (2005). The frequencies in contingency table (degrees of mucosal lesions in different groups: MS, CD, CG) were analyzed by Yates corrected χ^2 test. For the parametric statistical analysis, the DPP IV activity was logarithmically transformed to achieve the normal variable distribution (normality checked by Kolmogorov-Smirnov and Shapiro Wilks tests; in case of logarithmically transformed variables both tests showed $P > 0.20$). Inasmuch as the original DPP IV data were not normally

TABLE 1. *Intestinal and serum DPP IV activity in patients with CD, MS, and controls*

Sample	Group	Mucosal lesion	*n*	DPP IV activity		
				Median	Mean ± SD (95% CI)	Measurement
Intestine	Control		22	53.95	55.46 ± 12.04 (50.12–60.80)	(U/g protein)
	CD		38	13.92	16.40 ± 9.23 (13.36–19.44)	
	MS		37	29.03	28.01 ± 9.54 (24.83–31.19)	
	CD	I	10	27.07	28.66 ± 6.40 (24.08–33.24)	
		II	13	14.78	16.47 ± 4.48 (13.76–19.18)	
		III	15	8.10	8.17 ± 1.37 (7.41–8.93)	
	MS	I	15	35.34	36.63 ± 5.69 (33.48–39.78)	
		II	11	25.38	27.23 ± 5.85 (23.31–31.16)	
		III	11	16.59	17.03 ± 1.99 (15.69–18.37)	
Serum	Control		50	78.40	76.89 ± 23,13 (75.20–91.52)	U/L
	CD		48	87.00	83.36 ± 20.20 (66.89–86.90)	

95% CI, 95% confidence interval; CD = celiac disease; MS = malabsorption syndrome of other cause. Mucosal lesions were graded as follows: I, slight; II, partial; III, subtotal/total.

distributed, group values were presented by medians and by mean ± standard deviation (SD) with 95% confidence interval. Comparisons between groups were done by *t* test when 2 groups were compared, 1-way analysis of variance (ANOVA) in the case of between-groups effects (CD, MS, CG), and factorial ANOVA in the case of group comparisons (CD, MS, CG) adjusted for the grade of mucosal lesions. Correlation of variables was presented by Spearman rank correlation coefficient, with correspondent significance level. In all of the analyses the level of statistical significance was set at 0.05.

Ethical Considerations

All of the patients were admitted to the Department of Gastroenterology, Children's Hospital Kantrida, Clinical Hospital Centre Rijeka, Croatia. The study was performed according to the Declaration of Helsinki and was approved by the Ethics Committee for Medical Research, School of Medicine, University of Rijeka. Parents whose children were included in the study gave their written informed consent.

RESULTS

Histopathological analysis of small intestinal mucosal samples from 38 patients with CD indicated that 10 (26%) had grade I (slight), 13 (34%) had grade II (partial), and 15 (40%) had grade III (subtotal or total) mucosal lesions. All of the patients with CD had positive results for EMA IgA. Among the patients with MS, 15 (40%) had grade I, 11 (30%) had grade II, and 11 (30%) had grade III mucosal lesions. The Pearson χ^2 test showed no statistically significant difference in frequency distribution between the patients with CD and the patients with MS ($\chi^2 = 1.77$, $P = 0.413$). The 22 patients in the control group had histologically normal small intestinal mucosa.

All of the patients with CD for whom serum DPP IV activity was measured had villous atrophy with crypt hyperplasia, and all of them had positive results for EMA IgA. Of these patients, 13 (27%) had grade I, 18 (38%) had grade II, and 17 (35%) had grade III mucosal lesions.

DPP IV activity in the small intestine was determined in all of the patients (Table 1). Significantly lower intestinal DPP IV activity was observed both in the patients with CD and the patients with MS compared to the control individuals (comparison done between log values, ANOVA, $F = 129.97$, $P < 0.001$). The reduction of enzyme activity in the patients with CD on average was 70%, and in the MS group on average it was 49%. By contrast, a comparison between the patients with CD and the patients with MS showed that the intestinal DPP IV activity was significantly lower in the patients with CD (comparison between log values, *t* test, $t = 28.64$, $P < 0.001$).

The highest DPP IV activity was found in patients with CD and MS with slight (grade I) mucosal lesions, and the activity decreased as the grade of the mucosal lesion increased. As shown in Figure 1, decrease of DPP IV activity with the grade of mucosal lesion was significant in both groups (comparison of log transformed values, ANOVA, $F = 104.50$, $P < 0.001$ in CD; and $F = 72.87$, $P < 0.001$ in MS). Moreover, comparison of the values adjusted for the degree of mucosal lesion showed that enzyme activity reduction in the patients with CD was more pronounced than in the patients with MS (comparison of log transformed values, factorial ANOVA, $F = 10.11$, $P < 0.001$).

Linear regression analysis showed the correlations between the grades of mucosal lesions and the log transformed values of intestinal DPP IV activity in patients with CD and MS (Fig. 2). As already shown, intestinal DPP IV activity in patients with CD and MS correlated inversely with the grade of mucosal lesion in the brush border membrane. The correlation between the DPP IV activity and the grade of mucosal lesion in the patients with CD and MS was statistically significant (for CD, $r = -0.92$, $P < 0.001$; for MS, $r = -0.90$, $P < 0.001$).

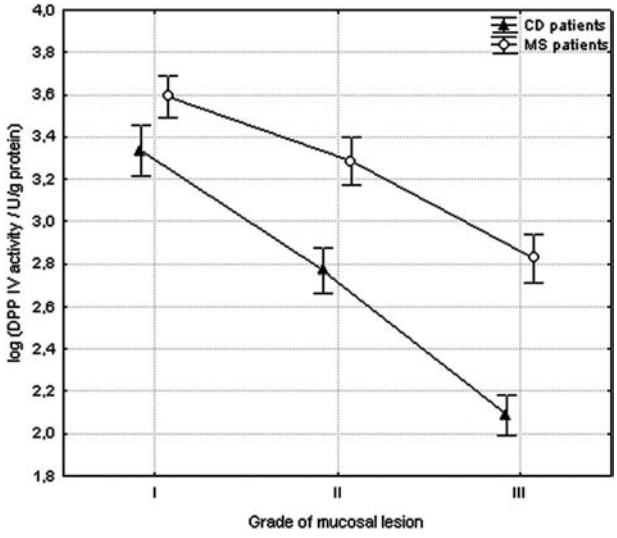

FIG. 1. The log transformed values of intestinal dipeptidyl peptidase IV (DPP IV) activity in CD and MS patients grouped by grade of mucosal lesion. CD = celiac disease (n = 38); MS = malabsorption syndrome of other cause (n = 37). The mucosal lesions were graded as follows: I, slight; II, partial; III, subtotal/total. Analysis of variance: CD, $F = 104.50$, $P < 0.001$; MS, $F = 72.87$, $P < 0.001$. Comparison between CD and MS groups, factorial analysis of variance adjusted for grade of mucosal lesion, $F = 10.11$, $P < 0.001$.

Serum DPP IV activities were determined in the patients with CD and in the control group (Table 1). There was no statistically significant difference in the serum DPP IV activity between these 2 groups (t test, $t = 1.04$, $P = 0.512$).

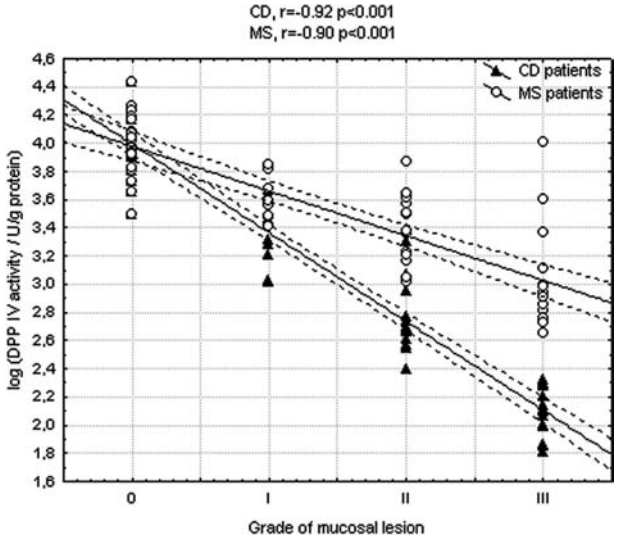

FIG. 2. Correlation between the log transformed values of intestinal dipeptidyl peptidase IV (DPP IV) activity and grade of mucosal lesion in CD and MS patients. CD = celiac disease (n = 38); MS = malabsorption syndrome of other cause (n = 37). Mucosal lesions were graded as follows: 0, normal; I, slight; II, partial; III, subtotal/total.

DISCUSSION

The pathogenesis and molecular mechanisms involved in the development and progression of damage to the small intestine in CD remains controversial. It is known that the recognition of ingested wheat gluten and related cereal proteins by mucosal T cells initiates an immunological cascade that ultimately leads to mucosal damage and other aspects of CD (19). The immunogenicity of gliadin peptides is influenced by glutamine residues, which become specifically deaminated by tissue transglutaminase, and by proline residues, which protect the peptides from proteolysis in the gastrointestinal tract (8). During digestion, a few brush border membrane proteases, including DPP IV, hydrolyze gluten proteins into smaller peptides (12).

In the small intestine the exopeptidase activity of DPP IV helps hydrolyze and assimilate proline-containing proteins. To evaluate the possible causal connection between DPP IV and CD, we determined the intestinal and serum DPP IV activity in patients with CD and with MS of other causes. We found that intestinal DPP IV activity was lower in patients with active CD than in control patients. These results agree with the findings of Smith and Phillips (20), who showed that the specific intestinal expression of DPP IV in microvilli is decreased in children with active CD. Furthermore, our results agree with a previous investigation showing a decrease in the level of several intestinal brush border membrane enzymes, including DPP IV, in adult patients with active CD (21). By contrast, this previous study also showed a persistent decrease in brush border aminopeptidase and DPP IV enzyme activity in patients in remission (21).

The present results also show that intestinal DPP IV activity in patients with MS was significantly decreased compared with control patients and that intestinal DPP IV activities were lower in patients with CD than in patients with MS. Mucosal lesions in CD can be differentiated from other mucosal lesions by the presence of a high number of intraepithelial lymphocytes with cytotoxic activity (22). This could explain the greater decrease of DPP IV activity in patients with CD than in patients with MS. Despite these findings, we cannot conclude that the decrease in DPP IV activity in patients with CD plays a role in the development of the disease.

A recent report by Hausch et al (8) showed that proline/glutamine-rich epitopes are exceptionally resistant to enzymatic processing. Moreover, estimation of the residual peptide structure and supplementation with exogenous peptidase indicate that DPP IV is the rate-limiting enzyme in the digestive breakdown of gliadin peptides (8,23). Shan et al (7) also demonstrated that a 33-amino acid stretch of α-gliadin is extremely resistant to digestion, leaving such regions available for T cell recognition and activation of pathogenic T cells. These findings and the results of the

present study agree with those of Clot et al (24), who did not find any evidence for a genetic defect in DPP IV in patients with CD.

Moreover, our results showed that the degree of the mucosal lesion correlates inversely with intestinal DPP IV activity in patients with CD and MS. Therefore, it seems that intestinal DPP IV cannot be used as a specific marker for differentiating CD and MS of other causes. Because we demonstrated a correlation between the degree of the mucosal lesion and the intestinal DPP IV activity in both patients with CD and patients with MS, we cannot exclude the possibility that the decrease in intestinal DPP IV activity is a result of the mucosal damage.

In addition to acting as an endopeptidase in the small intestine, DPP IV is an important modulator of the immune response (25). Previous reports showed that the proline-specific proteolytic activity of DPP IV is involved in regulation of the immune response because it cleaves biologically important peptides, cytokines, and chemokines. Chemokines processed by DPP IV have lower chemotactic potency, reduced ability to induce receptor signaling, and altered receptor specificity (26). Because of the key role of DPP IV in the T cell–mediated immune response and cytokine production, changes in DPP IV expression and serum activity have been analyzed in several autoimmune diseases such as rheumatoid arthritis (27) and inflammatory bowel diseases (Crohn disease and ulcerative colitis) (28).

As far as we are aware, the present study is the first to report serum DPP IV activity in children with CD. We did not find a statistically significant difference between the serum DPP IV activity in patients with CD and the control group. This indicates that the determination of serum DPP IV activity cannot be used as a specific noninvasive diagnostic or prognostic marker of the disease.

On the basis of previously published results and the data from the present study, it seems that the decrease in intestinal DPP IV activity is not specific for CD and is not necessary for initiation of the disease. Only patients harboring HLA molecules able to present undigested gliadin peptides to T cells are at risk for the development of CD, whereas individuals unable to react to these peptides are expected to remain healthy.

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