Increased Activity of Lysosomal Enzymes in the Peritoneal Fluid of Bacterial Peritonitis

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ABSTRACT. Objective. The activity of lysosomal enzymes is increased in the cerebrospinal fluid of bacterial meningitis, suggesting that inflammation may cause leakage of lysosomal enzymes into the extracellular fluid. Our objective was to study the activity of 3 lysosomal enzymes in cell-free peritoneal fluid of patients with peritoneal inflammation.

Methods. The $\beta$-galactosidase, $\beta$-glucuronidase, and $\alpha$-mannosidase activity (nmol 4-methylumbelliferone/mL); the total, polymorphonuclear, and mononuclear cell number; and chemical parameters were determined in the peritoneal fluid of 26 patients with culture-positive acute bacterial peritonitis, 13 patients (under antibiotic treatment) with culture-negative bacterial peritonitis, 6 patients with acute mesenteric lymphadenitis, and 26 control subjects who were operated on for surgical conditions without peritoneal inflammation.

Results. The median $\beta$-galactosidase activity in the culture-positive bacterial peritonitis, mesenteric lymphadenitis, and controls was 175 (range: 63–2210), 50 (range: 37–56), and 16 (range: 8–52), respectively. The $\beta$-glucuronidase was 488 (range: 79–998), 53 (range: 27–98), and 15 (range: 3–22), respectively. The $\alpha$-mannosidase was 801 (range: 100–3172), 78 (range: 33–157), and 41 (range: 16–63), respectively. The differences of the enzyme activities among the groups of the subjects studied were significant, with the exception of the $\alpha$-mannosidase activity between mesenteric lymphadenitis and controls. There was no significant correlation between the enzyme activities and the cytologic or chemical parameters studied.

Conclusions. The elevation of the lysosomal enzymes' activity in the peritoneal fluid of patients with bacterial peritonitis seems to be a reliable index of peritoneal infection. Of the enzymes studied, the $\beta$-glucuronidase and $\beta$-galactosidase activities provide the best means for diagnosing bacterial inflammation of the peritoneal cavity. Pediatrics 2002;109(3). URL: http://www.pediatrics.org/cgi/content/full/109/3/e44; bacterial peritonitis, $\beta$-galactosidase, $\beta$-glucuronidase, lyosomal enzymes, $\alpha$-mannosidase, mesenteric lymphadenitis, peritoneal fluid.

ABBREVIATIONS. CSF, cerebrospinal fluid; MU, methylumbelliferone.

The precursors of the lysosomal enzymes are synthesized on ribosomes, and their subsequent maturation and targeting to the final destination in lysosomes are directed by a sequence of recognition signals localized on the enzymes' molecule. Approximately 5% to 20% of the mature lysosomal enzymes are secreted into the extracellular fluid before their delivery to lysosomes; subsequently, a portion of these enzymes bind to mannose 6-phosphate receptors on the surface of the cells, are internalized, and are delivered to lysosomes. This secretion-recapture mechanism functions as a salvage pathway. In cultured skin fibroblasts, the salvage pathway accounts for the delivery of 5% to 20% of the total lysosomal enzymes in the lysosomes.

Inflammation could affect the final packaging or the secretion-recapture mechanism of the lysosomal enzymes, thus resulting in a leakage of these enzymes into the extracellular fluid. Also, during inflammation, there is an increased rate of phagosome formation; close contact between the plasma membrane and phagosome membrane results in membrane fusion and the rapid opening of the cytoproct followed by the expulsion of the vacuoles' residual content, which includes the remaining lysosomal enzymes, into the extracellular space. Previously, our group found that the activity of 4 lysosomal enzymes—$\beta$-glucuronidase, $\beta$-hexosaminidase A and B, and $\alpha$-mannosidase—is increased in the cell-free cerebrospinal fluid (CSF) of patients with bacterial meningitis but not aseptic meningitis.

Acute infections of the peritoneum are characterized as primary or secondary. In primary peritonitis, the origin of the infection is outside the abdominal cavity; the infecting organism is derived from the blood or the lymphoid system. In secondary peritonitis, the infection initiates from extension or rupture of an intra-abdominal viscous or of an abscess. In both cases, the infected peritoneal fluid usually contains an elevated protein concentration and >300 leukocytes/µL, >25% of which are polymorphonuclear. However, the diagnosis of bacterial peritonitis, particularly primary, is not easy, and it may be difficult to recover organisms from cultures of peritoneal fluid, possibly because the load of organisms is low.

The purpose of this study was to determine whether measurements of the activity of lysosomal enzymes in the peritoneal fluid can be used for the diagnosis of bacterial peritonitis, as well as to investigate whether there is a correlation between the

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enzyme activities and the cytologic or chemical parameters in both normal and infected peritoneal fluid.

METHODS

Patients

Thirty-nine patients with acute bacterial peritonitis, 6 patients with acute mesenteric lymphadenitis, and 26 control subjects were studied. All patients included in the study were operated on, and peritoneal fluid was obtained during the operation directly from the peritoneal cavity without lavage. Also, from the 39 patients with bacterial peritonitis and the 6 patients with mesenteric lymphadenitis, the appendix was removed and was studied histologically.

Culture-Positive Bacterial Peritonitis

Twenty-six patients with acute bacterial peritonitis had positive peritoneal fluid cultures; in 25 of them, the peritonitis was secondary to an intra-abdominal process and in 1 primary. The median age of the patients in this group was 7.5 years (range: 3–14 years). Of the 25 culture-positive cases with secondary bacterial peritonitis, the peritoneum was contaminated as a result of spillage from appendix in 24 and through a necrotic defect of the intestine as a result of volvulus in 1. One patient, who was previously operated on for appendicitis, had multiple intra-abdominal abscesses. In 11 of these patients, Escherichia coli was isolated, in 2 Pseudomonas aeruginosa, in 2 β-hemolytic Streptococcus, in 1 Enterobacter, in 1 Salmonella typhi (from the case with intestinal necrosis as a result of volvulus), and mixed flora in various combinations of E coli, P aeruginosa, group D Streptococcus, S viridans, Klebsiella pneumoniae, and Acinetobacter in 8 cases. In the peritoneal fluid of the patient with primary peritonitis, S pneumoniae was cultured.

Of the 25 patients with secondary bacterial peritonitis and positive cultures, 11 had started treatment with 1 antibiotic—ceftazidime, cefamandole, or ceftriaxone—6 to 12 hours before the operation, and 1 (the case with intra-abdominal abscesses) with 3 antibiotics 24 hours earlier.

Culture-Negative Bacterial Peritonitis

Thirteen patients with acute secondary bacterial peritonitis associated with appendicitis but with negative cultures were studied. In these patients, the median age was 8.3 years (range: 2.5–13 years). All patients in this group had received a combination of antibiotics (ceftazidime, metronidazole, and tobramycin, or ceftriaxone, metronidazole, and tobramycin) 8 to 24 hours before the operation.

Mesenteric Lymphadenitis

Six patients with acute mesenteric lymphadenitis were studied. The median age of these patients was 8.8 years (range: 3.5–14 years). At surgery, the mesenteric lymph nodes of the terminal ileum were enlarged and the peritoneal fluid was xanthochromatic. Lymph node biopsies were obtained for histologic examination. No culturing of the lymph nodes was performed.

Control Subjects

Of the 26 cases used as controls, 22 had communicating hydrocele, 1 intussusception with a normal intestinal wall, 1 congenital megacolon, 1 meconium ileus, and 1 adhesive intestinal obstruction. The median age of the control subjects was 3 years (range: 1 day to 13 years). The study was approved by the Ethics Committee of the University Hospital. Informed consent was obtained from the parents of the patients.

Enzyme Assays

A portion of the peritoneal fluid obtained from each case was centrifuged within 20 minutes, and the supernatant cell-free fraction was stored at −70°C until assayed.

The activity of the lysosomal enzymes studied was measured by incubating cell-free peritoneal fluid with the appropriate substrate. The reactions were allowed to proceed for 30 minutes at 37°C and then were stopped with 5 mL of 85 mM glycine-carbonate buffer (pH 10.5). β-Galactosidase was determined by applying a modification of the method used for measurement of the enzyme activity in cultured skin fibroblasts. In short, 25 μL of peritoneal fluid was incubated with 75 μL of 0.5 mM 4-methylumbelliferyl (MU)-β-galactoside in 100 mM citrate-phosphate buffer (pH 4.35) with 0.4 M NaCl. The β-glucuronidase and α-mannosidase activities were determined by using techniques applied for measurement of the enzyme activities in CSF, adapted for peritoneal fluid assays. β-Glucuronidase was measured by incubating 50 μL of peritoneal fluid with 150 μL of 1 mM 4-MU-β-glucuronide in 100 mM acetate buffer (pH 4.0). α-Mannosidase was measured by incubating 20 μL of peritoneal fluid with 200 μL of 4 mM 4-MU-α-mannopyranoside in 100 mM citrate-phosphate buffer (pH 4.0). The substrates were purchased from Sigma Chemical Co (St. Louis, MO). The released 4-MU was measured against blank and standard 4-MU solutions. Fluorescence was determined with a Sequoia-Turner fluorometer (Model 450; Sequoia-Turner Corp, Mountain View, CA) at 450 nm after excitation at 360 nm. Assays were performed in duplicate, and the 2 values were averaged. The within-assay coefficient of variation for β-galactosidase, β-glucuronidase, and α-mannosidase was 2.5%, 2.8%, and 1.8%, respectively.

Statistical Analyses

For the comparisons, the 2-tailed t test or the Mann-Whitney U test was used, as appropriate. When multiple comparisons of nonparametric data were performed, the Bonferroni correction was applied. The enzyme activities were correlated to the findings of the cytologic and chemical analyses of the peritoneal fluid by applying the Spearman rank correlation. Parametric data are expressed as mean ± standard deviation; nonparametric data are expressed as median with range. Significance was set at .05. Enzyme activities are expressed in nmol 4-MU/mL peritoneal fluid per hour.

RESULTS

β-Galactosidase Activity

In the patients with bacterial peritonitis and positive cultures, the median β-galactosidase activity was 175 (range: 63–2210), whereas in the acute mesenteric lymphadenitis patients it was 50 (range: 37–56; U = 156; P = .002) and in the control subjects was 16 (range: 8–32; difference from bacterial peritonitis U = 676; P < .000001). The patient with bacterial peritonitis and the lowest enzyme activity was treated with ceftazidime for 8 hours before the peritoneal fluid was obtained. Similarly, the patient with the second lowest activity (108) had received cefamandole for 12 hours. Also, the difference was significant between patients with acute mesenteric lymphadenitis and control subjects (U = 156; P = .0002; Bonferroni correction, significance at P = .017). The median β-galactosidase activity in the patients with secondary peritonitis and negative cultures was 155 (range: 86–805); the difference is not significant from the peritonitis patients with positive cultures (Fig 1).

β-Glucuronidase Activity

The median β-glucuronidase activity in the bacterial peritonitis patients with positive cultures was 488 (range: 79–998); in the patients with acute mesenteric lymphadenitis was 53 (range: 27–98; U = 2; P = .0002), and in the control subjects was 15 (range: 5–22; difference from bacterial peritonitis: U = 0; P < .000001). In 1 of the patients with acute mesenteric lymphadenitis, the activity overlapped with that of 2 patients with bacterial peritonitis. Both of these patients were under treatment, 1 with cefamandole for
12 hours and the other with ceftazidime for 6 hours before the operation. Also, the enzyme activity was significantly greater in the patients with acute mesenteric lymphadenitis than in the control subjects \((U = 156; \ P = .0002;\) Bonferroni correction, significance at \(P < .017\)). The activity in the patients with secondary peritonitis and negative cultures (235; range: 47–940) tended to be lower than in the bacterial peritonitis patients with positive cultures, but the difference between them was not significant (Fig 2).

\(\alpha\)-Mannosidase Activity

The median activity of \(\alpha\)-mannosidase in the patients with culture-positive bacterial peritonitis was 801 (range: 100-3172), in the acute mesenteric lymphadenitis was 78 (range: 33–157; \(U = 150, \ P = .0005\)), and in the control subjects was 41 (range: 16–63; difference from bacterial peritonitis: \(U = 676; \ P < .000001\)). In 1 of the patients with acute mesenteric lymphadenitis, the enzyme activity overlapped with that of 4 patients with bacterial peritonitis, and in another patient with 2 such patients. The enzyme activity in the peritoneal fluid of the patients with acute mesenteric lymphadenitis just failed to reach the level of significance when compared with the control subjects (\(U = 126; \ P = .02;\) Bonferroni correction, significance at \(P < .017\)). There was no significant difference in the activity between the patients with secondary peritonitis and negative cultures (median: 728; range: 111-2249) and the patients with bacterial peritonitis and positive cultures (Fig 3).

Enzyme Activity in Primary Peritonitis and Predictive Values

In the patient with primary peritonitis caused by \(S\) pneumoniae, the \(\beta\)-galactosidase activity was 202, the \(\beta\)-glucuronidase was 714, and the \(\alpha\)-mannosidase was 3172. These activities are more than 6-fold, 32-fold, and 50-fold greater, respectively, than the corresponding highest activity measured in the control subjects.

The positive and negative predictive values of the 3 enzymes measured in bacterial peritonitis, acute mesenteric lymphadenitis, and control subjects are listed in Table 1.

Other Laboratory Parameters

The laboratory parameters of the peritoneal fluid from the patients with culture-positive bacterial peritonitis and with acute mesenteric lymphadenitis and the control subjects are listed in Table 1. The peritoneal fluid from the patients with bacterial peritonitis had a significantly higher leukocyte, polymorphonuclear, and mononuclear cell number; a greater concentration of total protein, albumin, and \(\gamma\)-globulin; and a lower glucose concentration than the fluid.
from the patients with acute mesenteric lymphadenitis and from the control subjects. Similar laboratory parameters were observed in the peritoneal fluid from the patients with acute mesenteric lymphadenitis and the control subjects.

Correlations of Enzyme Activities With Cytologic/Chemical Analyses

Spearman rank correlation failed to demonstrate a significant correlation between the activities of the 3 lysosomal enzymes measured in the peritoneal fluid of the patients with culture-positive bacterial peritonitis on the one hand and the cytologic/chemical analyses performed in the fluid of the same patients listed in Table 2 on the other hand. In addition, there was no significant correlation between the activities of the 3 enzymes and the age of the patients or the control subjects.

Histologic Examination

The histologic examination of the appendixes removed from the patients with mesenteric lymphadenitis, the patient with primary peritonitis, and the patient with intestinal necrosis as a result of volvulus. The lymph nodes obtained from the patients with mesenteric lymphadenitis showed reactive lymphoid hyperplasia.

DISCUSSION

The observation that the activity of 3 lysosomal enzymes is significantly greater in the peritoneal fluid of patients with bacterial peritonitis than in normal peritoneal fluid seems to provide the means for a rapid and reliable diagnosis of bacterial peritonitis. Actually, the median activity of β-galactosidase in the peritoneal fluid of the patients with culture-positive bacterial peritonitis was 5.5-fold greater than in the control subjects, the median activity of β-glucuronidase was 33-fold greater, and the median activity of α-mannosidase was 20-fold greater. Compared with the enzyme activities measured in the peritoneal fluid of patients with acute mesenteric lymphadenitis, the activities in the bacterial peritonitis patients were 3.5-fold, 9-fold, and 10-fold greater, respectively. The absence of overlapping values between bacterial peritonitis and control subjects indicates the capability of the approach to identify inflammatory processes of the peritoneum caused by bacterial contamination.
Diagnosis of bacterial peritonitis may be difficult. An acute onset of symptoms, abdominal pain, and peritoneal irritation can be helpful diagnostically, but peritonitis may be present in the absence of any of these symptoms. The findings of the study suggest that in suspected cases, sampling of the peritoneal fluid and measurement of the activity of any of the lysosomal enzymes studied—β-galactosidase, β-glucuronidase, or α-mannosidase—might be helpful in identifying patients with bacterial peritonitis.

On the basis of the observation that the β-glucuronidase showed the greatest increase in the activity of the enzymes studied, it seems that measurement of this enzyme's activity will provide the means for a sensitive diagnostic approach to bacterial peritonitis. In addition, the earlier report of this group, indicating that the β-glucuronidase activity is increased in the CSF of patients with bacterial meningitis very early in the disease, makes plausible the assumption that elevation of this enzyme's activity occurs early in the peritoneal fluid of bacterial peritonitis as well. Repeated sampling of peritoneal fluid during treatment may prove useful for early evaluation of the patients' response to treatment. This is supported by the finding that in patients with culture-negative bacterial peritonitis, all of whom were treated before peritoneal fluid sampling with a combination of 3 antibiotics for 8 to 24 hours, the median β-glucuronidase activity was 48% of that measured in patients with culture-positive bacterial peritonitis. Conversely, the median α-mannosidase and β-galactosidase activities were only 9% and 11% lower, respectively, than the median activities in the culture-positive patients. This observation may indicate that

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PPV indicates positive predictive value; NPV, negative predictive value; BP, bacterial peritonitis; ML, mesenteric lymphadenitis; C, control subjects; β-Gal, β-Galactosidase; β-Gluc, β-glucuronidase; α-Mann, α-mannosidase.
the activity of β-glucuronidase is the first to herald the improvement of the peritoneal inflammatory process during treatment. This aspect has clinical importance and warrants additional investigation. That the patients with bacterial peritonitis who had the lowest β-glucuronidase and β-galactosidase activities were previously treated with antibiotics may indicate that the reduction of the enzyme activities in these patients resulted from the suppression of the peritoneal inflammation by the antibiotic treatment. This is supported by the observed tendency for lower enzyme activities in the patients treated with antibiotics. However, it seems that the β-galactosidase activity provides the means for a better differentiation between bacterial peritonitis and acute mesenteric lymphadenitis because there was no overlapping of this enzyme’s activities between the 2 groups in the patients studied.

The maintenance of increased enzyme activity in the peritoneal fluid of the patients with bacterial peritonitis for at least 24 hours after the onset of antibiotic treatment supports the prospect that an important clinical application of the lysosomal enzymes measurement will be in bacterial peritonitis patients who have previously started treatment and whose peritoneal fluid cultures are negative.

The finding that the activity of the 3 enzymes measured in the patient with primary *S pneumoniae* peritonitis was greatly increased indicates that the lysosomal enzymes’ activity is elevated in the peritoneal fluid of bacterial peritonitis regardless of the origin of the inflammation. Actually, in all cases of bacterial peritonitis studied, there was an increased enzyme activity regardless of the cause of the peritoneal inflammation. Whether the presence of increased concentrations of lysosomal enzymes in the peritoneal fluid plays a role in the pathogenesis of inflammation remains unknown.

That patients and control subjects were unmatched for age does not seem to affect the findings of the study because no correlation was observed between the age and the activity of the enzymes measured in both patients and control subjects.

The findings of the chemical analyses show that although the mean concentrations of total protein, albumin, and γ-globulin as well as the mean specific gravity were greater, whereas the mean glucose concentration was lower in the peritoneal fluid of bacterial peritonitis, there was a broad overlapping of individual values among the 3 groups of patients studied. However, the cytopathic studies confirmed that an increased number of cells in the peritoneal fluid is a stronger diagnostic index for bacterial peritonitis but not for acute mesenteric lymphadenitis.

Important questions arise from the findings of this study: 1) Can the lysosomal enzymes activity diagnose pyogenic peritonitis from chemical peritonitis or sterile peritonitis, such as in systemic lupus erythematosus, porphyria, or familial Mediterranean fever? 2) Is there different enzyme activity in the peritoneal fluid of patients with tuberculous peritonitis and acute peritonitis caused by common bacterial agents? 3) How early in the disease process of bacterial peritonitis does the activity of the lysosomal enzymes increase in the peritoneal fluid? 4) Can follow-up measurements of enzyme activity in the peritoneal fluid of bacterial peritonitis provide the means for determination of the patient’s response to treatment? Additional studies are needed to answer these questions, which are important for the clinical application of the reported findings. However, if we infer from the findings in bacterial meningitis, we may assume that the activity of lysosomal enzymes in the peritoneal fluid will differentiate early in the disease between inflammatory processes of the peritoneum and other conditions that lead to fluid accumulation in the peritoneal cavity.

The most plausible explanation for increased lysosomal enzyme activity in the peritoneal fluid of patients with bacterial peritonitis is enzyme leakage through the cell membranes facilitated by the inflammatory process. A disturbance of the final packaging or of the salvage pathway of the lysosomal enzymes as well as increased phagocytosis, autolysis, or cellular death seem to be the most likely explanations of the increased enzyme leakage into the peritoneal fluid.
fluid of patients with peritonitis. Increased activity of lysosomal enzymes in extracellular fluids has been found in experimentally induced inflammation\(^\text{10–14}\) and in inflammatory disorders, such as in the synovial fluid of rheumatoid arthritis,\(^\text{15}\) pulpal inflammatory disease,\(^\text{16}\) gingival crevicular fluid,\(^\text{17}\) and CSF of bacterial meningitis\(^\text{18}\) and in the plasma of patients with acquired immunodeficiency syndrome.\(^\text{18}\) The absence of a correlation between enzyme activity and the number of leukocytes in the peritoneal fluid indicates that the enzyme leakage is not restricted to the freely floating leukocytes in the peritoneal fluid, but it results mainly from the inflamed tissues of the peritoneal cavity.

**CONCLUSION**

The activity of the lysosomal enzymes \(\beta\)-galactosidase, \(\beta\)-glucuronidase, and \(\alpha\)-mannosidase is significantly increased in the peritoneal fluid of patients with bacterial peritonitis. Thus, measurements of the activity of these enzymes in the peritoneal fluid can differentiate between bacterial peritonitis and non-inflammatory peritoneal fluid. Additional studies for the determination of the activity of lysosomal enzymes in chemical peritonitis, sterile peritonitis, and ascites of various origins are warranted. The levels of enzyme activity may vary in the peritoneal fluid of these conditions; thus, they may be useful in the differential diagnosis of disorders with accumulation of fluid in the peritoneal cavity.

**REFERENCES**


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