Time Course of Changes in Pancreatic Enzymes, Isoenzymes, and Isoforms in Serum after Endoscopic Retrograde Cholangiopancreatography

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Kinetics of the catalytic activities of total amylase (AMY; EC 3.2.1.1), pancreatic (P)-AMY isoenzyme, P₂ and P₃ isofoms, and pancreatic lipase (LPS; EC 3.1.1.3), and of the mass concentration of LPS in serum were studied in 10 patients who underwent endoscopic retrograde cholangiopancreatography (ERCP) and showed a distinct pancreatic injury. The temporal characteristics of enzyme changes described were (a) the maximal rate (Kₑ) at which enzymes are released into blood, (b) the time lag from ERCP until maximum concentration, (c) the peak value of each serum enzyme, and (d) the rate (Kₐ) at which each enzyme is cleared from serum. LPS activity and mass concentrations increased and decreased faster than AMY and isoamylases, and the time of the LPS peak tended to be earlier than that of the other enzymes, but not significantly. The average peak increase of LPS values was higher than that of total AMY, P-AMY, and P₂ isofom (P <0.001). The P-AMY time–activity curve was a composite of curves attributable to its isoforms; the isoforms increased and peaked sequentially, with P₂ returning to normal more slowly than did Pα. LPS mass and activity concentrations showed excellent parallelism, with no important differences. At 50 h after ERCP, only LPS values still exceeded the upper reference limit, returning to normal 70 h after the examination.

Additional Keyphrases: amylase • lipase • catalytic activity vs mass concentration • simulation of pancreatitis

The laboratory plays an important role in the evaluation of patients presenting with acute abdominal distress and possible pancreatitis. Recently, there have been several interesting developments in laboratory assays for detection and differentiation of pancreatic disease. A reagent of high stability, based on an ethyldene-protected 4-nitrophenylmaltotetraoside (EP 4NP-G7) substrate, has become available for quantifying amylase (AMY) (1). Moreover, a new procedure, based on a synergistic effect in which two monoclonal antibodies together inhibit the enzymatic activity of salivary (S) AMY, allows pancreatic (P) isoamylase activity to be determined directly (2). In addition, analyses for pancreatic lipase (LPS), involving supplementation with colipase, and P-AMY isoforms (P₂ and P₃, detected electrophoretically) have added new dimensions to the diagnosis of acute pancreatic disorders (3).

At present, little is known of the time course of these biochemical variables during the evolution of acute pancreatic damage. In the present study we investigated the serum kinetics of total AMY activity, P-AMY isoenzyme, P₂ and P₃ isoforms, and the activity and mass concentration of LPS in serum of patients undergoing endoscopic retrograde cholangiopancreatography (ERCP), which produces a pancreatic injury similar to acute pancreatitis (4). For comparison of the temporal sequence of different pancreatic enzymes released in pancreatic disease, ERCP represents a suitable model because the type of pancreatic injury can be assumed to be identical in most patients and the exact time of the pancreatic damage is known, allowing for collection of serum samples at defined time intervals thereafter.

Materials and Methods

Analytical Procedures

We measured total AMY activity at 37 °C, using EP 4NP-G7 as substrate [Boehringer Mannheim Diagnostica (BMD), Mannheim, F.R.G.] (1). To measure P-AMY activity, we used the Testcombination Pancreatic Amylase EPS kit (BMD), in which S-AMY is inhibited by two monoclonal antibodies (66C7 and 88E8), after which P-AMY is measured at 37 °C with EP 4NP-G7 as substrate (2). We determined LPS activity at 30 °C by colipase-supplemented turbidimetry according to Neumann et al. (5), using the commercially available test kit from BMD. The activity of the porcine LPS calibrator was determined titrimetrically. All three measurements were carried out with a Cobas-Bio centrifugal analyzer (Hoffmann-La Roche, Basel, Switzerland) (6). Immunoetric LPS was measured by using the Enzymnost Lipase assay purchased from Behringwerke AG, Marburg, F.R.G. (7). Pancreatic isoforms were separated by cellulose acetate electrophoresis and stained with the Phadebas dye–starch polymer (Pharmacia Diagnostics, Piscataway, NJ 08854), as previously described (3). For densitometric tracings of the isoamylase electrophoretic patterns, we used a Cliniscan densitometer (Helena Labs., Beaumont, TX 77704). Each isoform was expressed as its percentage of total AMY activity in the original serum sample; the absolute enzyme activity was calculated by multiplying measured total AMY by

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³Nonstandard abbreviations: EP 4NP-G7, ethyldene-protected 4-nitrophenyl-α-D-maltotetraoside; AMY, amylase (1,4-α-D-glucan glucohydrolase; EC 3.2.1.1); S-AMY and P-AMY, salivary and pancreatic isoamylases of AMY, respectively; LPS, pancreatic lipase (triacylglycerol acylhydrolase, EC 3.1.1.3); ERCP, endoscopic retrograde cholangiopancreatography; BMD, Boehringer Mannheim Diagnostica; URL, upper reference limit; Kₑ, maximum rate of enzymatic concentration increase; and Kₐ, rate of fractional disappearance of enzyme.

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the percentage of AMY attributable to each isofrom. The electrophoretic mobilities of serum isoamylase fractions were compared with those of specimens of saliva and pancreatic juice assayed on the same cellulose acetate membrane. Nomenclature of pancreatic isoforms followed accepted practice (3).

Upper reference limits (URLs; 97.5th percentiles), previously calculated by this laboratory from data on about 500 healthy persons with use of nonparametric statistics (3), were 100 U/L for total AMY activity, 60 U/L for P-AMY and P2 isofrom activity, 150 U/L for LPS activity, and 50 µg/L for LPS mass concentration. P3 isofrom was electrophoretically undetectable in serum from healthy people (9). During the study period, day-
to-day CVs calculated from monthly control periods never exceeded 2.3% for total AMY (mean 90 U/L), 2.6% for P-AMY (mean 28 U/L), 6.0% for LPS activity (mean 110 U/L), and 10.4% for LPS mass concentration (mean 40 µg/L). The between-run CV for P-isoform fraction-
ation was <12.7%.

Patients and Clinical Protocol

Enzyme studies were carried out in 41 consecutive patients who underwent ERCP, 11 because of suspected pancreatic or biliary disease (diagnostic ERCP), and 30 after endoscopic sphincterotomy because of choledocholithiasis (surgical ERCP). The patients were exam-
ined after an overnight fast. Before examination, they received diazepam, 5–10 mg, as a sedative and an anticholinergic agent to induce duodenal hypotony. The injection of the contrast medium into the pancreatic duct system was monitored carefully by fluoroscopy.

Before inclusion in the study, informed consent was obtained from each patient. Peripheral venous blood samples were obtained for determination of pancreatic enzymes immediately before ERCP and every 3 h for five days after examination. Patients' sera were ali-
quoted and stored at −20 °C until analyzed (within one week after collection). In particular, under these condi-
tions, there was no significant modification of migratory characteristics of isoamylases (10).

Data Analysis

Because values for the serum enzymes were not gaussianly distributed, we used nonparametric statisti-
cal tests for the statistical analyses; we also used non-
parametric group tests to compare two data groups (Wilcoxon rank sum test). The level of significance was set at 0.05. Serial results for total and P-AMY had to change by more than about 35% and 40%, respectively, before significance was claimed (11). Time–concentration curves, constructed for each patient with a significant enzyme increase, allowed us to derive the peak value and the time required to reach this maximum concentration value for all the enzymes evaluated (12).

The relationship of two continuous variables was tested by linear least-squares regression analysis. In particular, the rate of increase (Kv) and the fractional disappearance rate (Kd) for each enzyme were calculated by linear-regression analysis from the linear portions of the ascending and declining slopes of the time–concen-
tration curves, plotted semilogarithmically. The points used in calculations were the corrected enzyme concen-
trations, i.e., the measured concentrations minus indi-
vidual baseline values. However, P3 isofrom activity was not adjusted.

Results

The concentrations of the pancreatic enzymes in se-
rum were normal in 31 cases but pathologically in-
creased after the start of the examination in 10 cases (24.4%). In these 10 patients, we calculated the Kd, the time lag from ERCP until maximum concentration value, the peak value, and the Kd from serum for each pancreatic enzyme studied (Figure 1). LPS activity and mass concentrations increased (P <0.02) and decreased (P <0.05) significantly faster than AMY and isoamylases; their time of peak also tended to be earlier than that of the other enzymes, but not significantly. P3 isofrom increased faster than P2 (P <0.05), and P3 peaked significantly later (P <0.02) than did P-AMY. The delay of the serum change in P3 can be explained by the time required for P3 to be produced from P2. The average peak increase of LPS activity (13.3 × URL) and mass concentration (15.1 × URL) was higher than that of total AMY (3.5 × URL), P-AMY (3.9 × URL), and P3 isofrom (3.7 × URL), and the difference was statistically significant (P <0.001). Finally, P3 isofrom activity returned to normal significantly more slowly than did P2 isofrom (P <0.05).

Figure 2 shows as a semi-log plot the serial changes of the pancreatic enzymes (median values) in the serum of the 10 ERCP patients studied. The P-AMY time–activity curve is a composite of curves attributable to its isoforms: these are seen to increase and peak sequen-
tially, with P2 remaining detectable for about 10 h after the total AMY, P-AMY, and P3 isofrom had returned to normal values. There was an excellent parallelism be-
tween LPS mass and activity concentrations, with no important differences. At 50 h after ERCP, only LPS values still exceeded URL, returning to normal 70 h after the examination. With ERCP, only the main pancreatic duct was speci-
fied in 30 subjects, whereas the main duct and periph-
eral branches were viewed in 11. In four of the subjects (13.3%) whose main pancreatic duct alone was opacified, serum concentrations of pancreatic enzyme increased after ERCP. However, six (54.5%) of 11 subjects whose main pancreatic duct and peripheral branches were examined had significant increases in enzyme concentra-
tions after ERCP. Thus, the degree of opacification of the pancreatic ductal system in our patients signifi-
cantly correlated with the presence of abnormalities in serum pancreatic enzymes (chi-square test, P <0.01).

Discussion

The results of the present investigation show clearly that hyperamylasemia appearing after pancreatic duct visualization by ERCP should be regarded as indicative of some induced pancreatic alteration. ERCP resulted in
### Rate of Enzyme Increase (h⁻¹)

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<tr>
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<td>P₃ isoform</td>
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<tr>
<td>LPS mass</td>
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### Rate of Enzyme Disappearance (h⁻¹)

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Fig. 1. Data for kinetics of pancreatic enzymes, isoenzymes, and isoforms in serum in 10 patients with increased concentrations of enzymes after ERCP.

Fig. 2. Time course of changes in serum pancreatic enzymes, isoenzymes, and isoforms (median values) for 10 patients with increased concentrations of enzymes after ERCP (abscissa: time after cannulation).

Pancreatic hyperamylasemia in about 25% of cases, a frequency encountered also in previous reports (13). Typical acute pancreatitis as evidenced by clinical signs, chiefly acute pain in the upper part of the abdomen, is not frequent in these patients (14). However, the enzymatic alterations should not be disregarded, because they represent only quantitatively different manifestations of the same pathological process. Furthermore, the present study demonstrates a conspicuous correlation between the presence of above-normal serum concentra-

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tions of pancreatic enzymes and the degree of opacification of the pancreatic duct system, as also observed previously (16).

The results presented have led us to form the following general model of the kinetics of the pancreatic enzymes after acute pancreatic injury. In the beginning of the damage period, LPS and P-AMY gene product (the P₂ isoform) are discharged into the blood from the pancreas, with LFS tending to increase the most. The increase in total AMY activity is due to the P-type isoenzyme. Later, posttranslational modification of P₂, probably due to inflammatory intra-pancreatic processes, produces P₃ isoform, an AMY fraction with different temporal characteristics, i.e., a delay in serum kinetics. Total AMY rapidly returns to normal values. LPS, which appears to be cleared more rapidly from plasma than is P-AMY, returns to normal values somewhat later than this. P₃ remains detectable until 50 h after ERCP. All the elimination kinetics are first-order, as occurs in a short-lasting pathological event (16). The excellent parallelism between the catalytic activity and mass concentration curves for LFS—nearly identical results for Kₐ, the time at which the peak value is obtained, and Kₐ suggests that the LPS protein is cleared intact from the circulation.

Our findings agree with the report of Okuno et al. (15) that the concentration of LPS in serum tends to change earlier and more pronouncedly than does P-AMY after ERCP. The more rapid kinetics of enzymatic concentrations, compared with that seen in clinical pancreatitis (17), leads us to suggest that the principal determinant of plasma enzyme concentrations after ERCP injury is direct leakage of the pancreatic secretions from the damaged gland into the circulation. Conversely, the dominant route for the transfer of pancreatic enzymes in the course of the attack of acute pancreatitis is via the thoracic duct lymph (18). Thus, our results concerning the kinetics of LPS and P-type isoenzymes in ERCP patients are interesting, but they do not permit final conclusions. However, the results of Junge et al. (19), obtained in patients with uncomplicated acute pancreatitis, confirm our data on the disappearance rate of enzymes, showing that AMY activity disappears more slowly from the circulation than does LPS activity. The difference in the Kₐ of the two enzymes probably corresponds to the difference in the major metabolizing organs. LPS has a renal elimination route in humans (19), whereas renal elimination contributes little to the turnover of AMY, the liver probably playing an important role in the metabolism of this enzyme (20). Junge et al. (19) reported the apparent half-life in blood plasma after acute pancreatitis to be about 10 h for LPS activity and 15 h for AMY activity. Our Kₐ values agree with these data and are distinctly lower than those for most other clinically important enzymes (21–23). This finding is relevant to the diagnostic problem; repeated checks of LPS and P-AMY concentrations in serum make it possible to determine within hours whether the underlying pathological process involving the pancreas still persists and what course the disease will take (17).

References