Plasma Glutathione S-Transferase and F Protein Are More Sensitive than Alanine Aminotransferase as Markers of Paracetamol (Acetaminophen)-Induced Liver Damage


Concentrations of glutathione S-transferase (GST; glutathione transferase; EC 2.5.1.18) B1, subunits, F protein, and the activity of alanine aminotransferase (ALT; EC 2.6.1.2) were measured in sequential plasma samples taken from nine patients with self-administered paracetamol (acetaminophen) poisoning. GST exceeded the reference interval in all patients at the time of admission, and F protein was increased in seven. In contrast, abnormal activities of ALT in plasma were found in only one of the nine on admission, a patient admitted 12 h after poisoning. Subsequent to admission nine, eight, and five patients, respectively, had abnormal concentrations of GST, F protein, and ALT. When expressed as multiples of the upper reference limit, the highest values for GST measured in each patient always far exceeded the greatest abnormalities in ALT; this was true for F protein in only five patients. Patients in whom the concentration of GST exceeded 10 μg/L on admission subsequently went on to develop moderate or severe liver damage, despite treatment with N-acetylcytystine. F protein and ALT measurements on admission were not as efficient as GST at predicting the clinical outcome of the patients. We conclude that GST and F protein offer clear advantages over ALT for detecting minor degrees of acute liver dysfunction, particularly when only centrilobular damage may be involved.

Biochemical assessment of liver damage usually includes measurement, in plasma or serum, of aspartate aminotransferase (AST; EC 2.6.1.1) or alanine aminotransferase (ALT; EC 2.6.1.2) activity. These enzymes are found in the cytoplasm of the cell and are released into the plasma space when the integrity of the hepatocyte is compromised. Although measurements of the aminotransferases have been used for decades, they are not entirely satisfactory, and values may often be normal in patients with chronic liver disease (1–3). In part, the inadequacies of the aminotransferases in detecting damage in certain types of liver disease lie in their lobular distribution. The pericellular hepatocytes contain the highest concentrations of the aminotransferases, with the centrilobular hepatocytes, which are relatively deficient in aminotransferases, being more susceptible to damage from hypoxia and toxins such as alcohol and paracetamol (acetaminophen) (4).

The advent of immunoassay has allowed an appraisal of other hepatic proteins as markers of liver damage. Two proteins that show the most promise in this respect are glutathione S-transferase (GST; EC 2.5.1.18) and F protein.

The GST are a family of dimeric, cytosolic enzymes found in high concentrations in many tissues. Several immunologically distinct isoenzymes of GST have been described most frequently classified as alpha, mu, and pi (5). The alpha class of GST is found in highest concentration in liver and kidney (5). These alpha-class GST can consist of immunologically similar or distinct subunits, B1 and B2 (6). In acute liver damage, measurement of the B1 subunit in plasma by radioimmunoassay provides a more sensitive index of hepatocellular integrity than does measurement of the aminotransferases (7).

F protein, a cytosolic protein of unknown function, is found predominantly in the liver; it can only be measured by immunoassay (8, 9). Measurement of F protein in serum appears to offer a more sensitive alternative to the aminotransferases for detecting hepatic dysfunction. Moreover, concentrations of F protein in serum apparently correlate well with histological assessment of liver damage (10).

Both GST and F protein are distributed throughout the liver lobule (9, 11) in high concentration. Thus their measurement in serum or plasma may provide a good index of centrilobular damage, which may not be detected by the more conventional measurement of the aminotransferases.

Here we have compared the sensitivity of GST, F protein, and ALT measurements for detection of liver damage in patients with paracetamol overdose.

Materials and Methods

Patients

We studied nine patients admitted with paracetamol overdose to the Regional Poisoning Treatment Centre of the Royal Infirmary of Edinburgh. Informed consent was obtained and the study was approved by the local Ethics Committee. The mean age was 25 years (range 13–36); seven were women. Six of the nine patients had taken ethanol with the paracetamol, one patient also took dihydromephine, and one took d-propoxyphene. Treatment of six of the patients with N-acetylcytystine began within 9 h of the overdose and of the other three 14 h or more after the overdose. Table 1 shows the paracetamol concentration in plasma, the ingestion-treatment interval, and a summary of clinical details for each patient.

The treatment regime involved administering N-acetylcytystine (150 μg per kilogram of body weight) in 200 mL of 50 g/L dextrose solution over 15 min, followed by a 50 mg/kg (body weight) dose in 500 mL of dextrose solution over 4 h, and finally 100 mg/kg in 1000 mL of dextrose during the next 16 h.

Blood was sampled on admission and at frequent intervals until discharge. The plasma samples were divided, with portions stored at −20 °C for later measurement of

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5 Nonstandard abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; and GST, glutathione S-transferase(s).

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GST and F protein, and a portion was stored at 4 °C and assayed for ALT activity within 24 h.

Assays

Bilirubin, alkaline phosphatase, and creatinine were measured in a SMAC II system (Technicon Instruments Corp., Basingstoke, U.K.).

ALT activity in plasma was measured in a Cobas-FARA centrifugal analyzer (Roche Diagnostics, Welwyn Garden City, Herts., U.K.) with a Boehringer Mannheim Diagnostics (Lewes, Sussex, U.K.) kit method.

The concentration of GST B2 was measured by a specific RIA method as previously described (12). The antisera used showed no cross-reactivity with GST B2 subunits nor with the mu and pi classes of GST.

The concentration of F protein was measured by an immunoradiometric assay on microtiter plates as described previously (13)

The between-assay coefficients of variation for ALT, GST, and F protein were 5%, 8%, and 14%, respectively; the upper limits of the reference intervals for these assays were 40 U/L, 4.0 μg/L, and 60 μg/L, respectively.

Results

All patients completed the course of treatment and all survived. All ALT activities were within normal limits 14 days after treatment. Normal renal function was maintained in all patients during the period of study. In four patients (KM, PF, TG, MC) no significant liver damage occurred; their ALT was <50 U/L. Severe damage occurred in four patients (MH, GB, HM, MW) and ALT was >1000 U/L. One patient had moderate liver damage with ALT activities ranging 380 U/L (EA).

Admission samples. The activity of ALT was within reference limits on admission in each of the eight patients who were admitted to hospital within 6 h of the overdose (Table 1, Figures 1 and 2). In one patient (MW, Figure 2) who was admitted 12 h after the overdose, the ALT was fourfold the upper reference limit.

Abnormal concentrations of GST were recorded in all nine patients on admission, and in six patients these abnormalities in GST exceeded twice the upper reference limits (Table 2, Figures 1 and 2). The concentration of F proteins exceeded twice the upper reference interval in seven of the nine patients at the time of admission.

Patients with no significant liver damage. In four pa-
tients, the activities of ALT in plasma did not exceed more than 1.2 times the upper reference limit at any time during the period of the study (Table 1, Figure 1). In each of these patients abnormal GST concentrations were recorded, on average maximal concentrations being 2.2 times the upper reference limit. In three patients, abnormal F protein concentrations were found and, on average, maximal concentrations were 9.8 times the upper reference limit.

Patients with moderate or severe liver damage. The remaining five patients suffered moderate or severe liver damage, with maximal activities of ALT ranging from 9.4 to 390 times the upper reference limit being recorded. In each case GST was relatively more increased than was ALT, with maximum values for individuals ranging from about 300 to 3000 times the upper reference limit (Table 1). F protein was increased relatively more than ALT in two patients when maximum values were compared.

The GST profiles showed discrete peaks and troughs of GST concentration in some patients (e.g., MH, GB, and HM, Figure 2). The discrete changes in hepatic protein release into plasma were less apparent with ALT or F protein. In each patient, concentrations of GST, ALT, and F protein continued to increase after N-acetylcysteine administration began.

Association between admission values for GST, F protein, and ALT and case outcome. The five patients who developed severe or moderate liver damage had GST concentrations on admission that were in excess of 10 μg/L. In the four patients who did not develop significant liver damage, GST concentrations were <10 μg/L. No such differentiation between values at the time of admission and outcome could be found with ALT or F protein, because these data for the two groups of patients overlapped considerably.

Discussion

Measurements of GST and F protein concentrations in plasma evidently are more sensitive than measurements of ALT activity for detection of acute hepatotoxicity after paracetamol overdose. All nine of our patients had abnormal GST concentrations and eight had abnormal F protein concentrations at some time after taking the paracetamol overdose, whereas values for ALT became abnormal in only six. The degree of the maximal abnormality recorded for GST far exceeded that for ALT in every patient studied, and this was also the case for F protein in six patients.

A prolonged half-life of paracetamol in plasma may be an
Fig. 1. Sequential concentrations of glutathione S-transferase (●), F protein (○), and alanine aminotransferase activities (△) in plasma of four patients who did not develop moderate or severe liver damage after a paracetamol overdose. Values are shown as multiples of the upper value for the reference interval. The arrows indicate the times at which treatment with N-acetylcysteine was begun.

Fig. 2. Sequential measurements of glutathione S-transferase (●), F protein (○), and alanine aminotransferase (△) in plasma from a patient (EA) who developed moderate liver damage and four patients who developed severe liver damage after paracetamol overdose. Values are shown as multiples of the upper value for the reference interval. The arrows indicate the times at which treatment with N-acetylcysteine was begun.
early indication of liver damage in patients with paracetamol poisoning (14). However, abnormalities in results of the conventional biochemical tests of liver function are not usually seen so soon after an overdose (15). The high frequency of abnormal results for GST and F protein in our patients on admission indicates that hepatocellular damage is measurable as early as 4 h after an overdose of paracetamol.

Abnormal GST and F protein concentrations found on admission in patients who have normal values for ALT is consistent with the view that paracetamol initially damages centrilobular hepatocytes that contain high concentrations of GST and F protein but relatively little ALT. Later, as the hepatic damage becomes more extensive, periportal hepatocytes that contain ALT, GST, and F protein become damaged and the concentrations of all three of these proteins in plasma increase. The histological findings of centrilobular necrosis in patients with paracetamol poisoning also lend weight to our suggestion that the liver damage is at first confined predominantly to centrilobular hepatocytes (16).

Although the number of patients we studied was small, our data suggest that measurement of plasma GST concentrations at the time of admission may provide a good prognostic indicator of which patients will subsequently develop moderate or severe liver damage, with or without treatment with N-acetylcysteine. Each patient who was admitted with a GST value exceeding 2.5 times the upper reference interval subsequently developed moderate or severe liver damage (Table 1, Figure 2). Such a clear discrimination could not be obtained by using F protein or ALT measurements. However, if GST measurements were to be used routinely as early predictors of liver damage, it would be necessary to develop an immunoradiometric assay that could provide a result within a few hours of admission. As yet, results for GST measurement by RIA cannot be available for at least 24 h.

The short half-life of GST (<1 h) in plasma makes it particularly suitable for assessing the mechanisms of drug-induced hepatotoxicity because, when cellular damage and GST release has ceased, its concentrations in plasma will decline rapidly. This is illustrated in Figure 2, where some patients showed distinct multiple peaks of plasma GST, which are less readily observable with F protein or ALT, both of which have much longer plasma half-lives.

In conclusion, it is apparent that GST and F protein measurements offer advantages over ALT for the detection of minor degrees of acute liver dysfunction, particularly when damage is limited to the centrilobular zone.

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References