

Serum cobalt-activated acylase and γ -glutamyl transpeptidase activities in toxic hepatitis

A. SZCZEKLIK, A. WIERNIKOWSKI, J. MUSIAŁ, AND E. WOŹNY

From the Institute of Internal Medicine, Copernicus School of Medicine, Kraków, Poland

SUMMARY Marked activity of cobalt-activated acylase was found in the sera of 33 of 37 patients with acute toxic hepatitis due to poisoning with either amanita mushrooms or chemicals. The activity of the enzyme showed a positive correlation with that of serum transaminases, reached the highest levels on the patient's admission to hospital and within a few days fell rapidly to undetectable levels. Slight acylase activity was observed in the majority of patients intoxicated with drugs or carbon monoxide but was not seen in sera of those poisoned with non-amanita mushrooms who showed no signs of liver injury. Unlike acylase, the serum activity of γ -glutamyl transpeptidase remained unchanged over the first days of acute toxic hepatitis. The determination of serum cobalt-activated acylase might be of value in the diagnosis of acute liver injury.

Recent studies have demonstrated the presence of cobalt-activated acylase in several animal and human tissues (Szewczuk and Szczeklik, 1971; Albert and Szewczuk, 1972). High activity of the enzyme was detected in human liver where it was localized in liver cell cytoplasm. Strong activation by cobalt ions is one of the features which differentiate this acylase from the known acylases I and II and from the arylamide acylase. The enzyme catalyzes deacylation of N-chloroacetyl- or N-butyryl- γ -L-glutamyl- β -naphthylamides but has only slight or no activity toward substrates with both a shorter or longer acyl moiety (Szewczuk, Szczeklik, Gładysz, Wellman-Bednawska, Nienartowicz, and Przygodzka, 1974).

Cobalt-activated acylase is absent from the sera of healthy subjects and from patients with liver cirrhosis, obstructive jaundice or chronic hepatitis. Its activity, however, appears invariably in serum during the early stage of acute viral hepatitis which helps in the diagnosis of this disease (Szewczuk and Szczeklik, 1971; Szczeklik, Sowa, and Pawlikowski, 1973). It seemed therefore interesting to study the activity in serum of this acylase in other cases of acute liver injury due not to viral but to toxic agents and to compare it with serum γ -glutamyl transpeptidase activity. The estimation of the latter enzyme has been found to be a valuable liver function test (Szczeklik, Orłowski, and Szewczuk, 1961; Gibiński, Szatoń, and Maraszek, 1963) but

information on its behaviour in toxic hepatitis is limited (Kokot and Śledziński, 1974).

Patients and Methods

Sixty-nine patients with acute intoxication in whom liver injury was suspected on admission to hospital because of a history and/or physical examination were studied. There were 38 men and 31 women. Their ages ranged from 15 to 74 years (average 35 years). Blood for enzymatic studies was drawn usually within the first hours after admission to hospital. Repeated estimations were performed over the following 10 days in several patients who showed abnormalities in the first results. Retrospective analysis based on the affecting toxic agent permitted us to divide the patients into the following groups.

1 Poisoning with cytotoxic amanita mushrooms: in 22 out of 26 patients of this group the diagnosis was confirmed by mycological analysis. *Amanita phalloides* was detected in 17 patients and *Amanita pantherina* in the other five. In the remaining four patients no mycological identification was performed and the diagnosis was based on a typical history and clinical findings. This was the only group with fatalities; seven patients died.

2 Eleven patients were intoxicated with hepatotoxic chemicals. These included carbon tetrachloride (four patients), pesticides composed of derivatives of phenoxyacetate or chlorinated hydrocarbons (four patients), chloroform (one patient),

arsenic (one patient), and trichloroethylene (one patient). All toxins were either drunk or inhaled by the patients. Two patients were under the influence of alcohol during exposure to hepatotoxic chemicals.

3 Four patients with acute alcohol intoxication had a serum alcohol level on admission which varied from 0.18 to 0.32%.

4 Ten patients swallowed drugs in an attempt at suicide. More than one drug was usually swallowed, most commonly barbiturates and benzodiazepines. Four subjects shortly before taking drugs drank alcohol. In none of them, however, did the alcohol level exceed 0.1%.

5 Four patients were intoxicated with carbon monoxide.

6 The last group consisted of 14 patients with acute gastroenteritis due to eating non-amanita mushrooms.

Serum Co^{++} -activated acylase was determined by the method described previously (Szewczuk and Szczeklik, 1971) using N-chloroacetyl- γ -L-glutamyl- β -naphthylamide as substrate; the results were expressed in mU/ml. In the majority of patients the activity was additionally measured by a simplified method, using commercial kits which have recently become available (Wytwórnia Surowic i Szczepionek, Kraków, Poland). The results obtained by both methods were found to be highly positively correlated ($r = 0.93$). All the determinations were performed less than four hours after drawing the blood.

Serum γ -glutamyl transpeptidase (GGTP) activity was determined by the method of Orłowski (1965) using γ -glutamyl-p-nitroanilide as substrate. Serum aspartate (AST) and alanine (ALT) transaminase activities were determined by the method of Reitman and Frankel (1957), while activities of alkaline phosphatase, cholinesterase, and prothrombin complex were determined by the standard methods (Kokot, 1969).

Results

In all patients poisoned with amanita mushrooms,

symptoms of liver injury were present. Their intensity varied greatly. Thus, in some patients the only expression of toxic hepatitis was subicterus and mild elevation of serum transaminases, while in others hepatic failure developed and led to death in seven patients within eight to 18 days following intoxication. In these cases necropsy revealed massive hepatic necrosis. This group was characterized by greatly increased transaminase activity and a fall in the cholinesterase concentration (table). Bilirubinaemia varied from 18.9 to 380.0 $\mu\text{Moles/l}$. Both GGTP and alkaline phosphatase activities remained within normal limits during the first days of illness. In 22 out of 26 patients of this group acylase activity was present in serum, ranging from 0.9 to 110.0 mU/ml. The mean activity recorded shortly after admission was higher in seven patients who died than in 19 who recovered. However, the subsequent course of acylase activity did not differ between these two subgroups. Thus, maximum acylase values were found in the first serum sample obtained. The activity of the enzyme then declined rapidly and often could not be detected in serum by the fifth day of the hospital stay. This rapid fall in activity contrasted with the behaviour of serum aminotransferases, the mean level of which was still distinctly raised on the tenth day of treatment.

The appearance of high acylase activity followed by a sharp fall was also typical for subjects poisoned with hepatotoxic chemicals. Thus, in a patient who swallowed about 50 ml of a solvent containing CCl_4 , extremely high values of acylase, up to 220 mU/ml, were noted within the first 12 hours after intoxication. Its activity declined to 2.0 mU/ml on the fourth day and could not be detected in serum on the sixth day. In this patient transaminases were elevated on admission, reached maximum values on the fourth day (AST exceeded by 15 times and ALT by 24 times the upper limits of normal) and then gradually began to decrease. Acylase activity was present in the serum of all patients of this group. Its values were high in subjects intoxicated with CCl_4 or chloroform, but only moderately elevated (1.0 to 2.2 mU/ml) in those poisoned with insecticides. In

Toxic Agent	No. of Patients	Acylase (mU/ml)	GGTP (mU/ml)	Bilirubin ($\mu\text{Moles/l}$)	AST (Reitman-Frankel Units)	ALT (Reitman-Frankel Units)	AP (Bodansky Units)
Cytotoxic							
Amanita mushrooms	26	10.8 \pm 22.2	3.2 \pm 1.9	95.7 \pm 53.0	323.2 \pm 180.2	596.1 \pm 349.7	4.4 \pm 2.2
Hepatotoxic chemicals	11	38.8 \pm 62.4	3.8 \pm 1.0	37.6 \pm 49.5	298.6 \pm 260.0	506.8 \pm 413.1	4.7 \pm 1.4
Alcohol	4	0.8 \pm 0.28	7.9 \pm 13.6	18.8 \pm 5.1	30.0 \pm 19.5	40.5 \pm 16.1	8.2 \pm 3.6
Drugs	10	1.2 \pm 1.1	2.1 \pm 1.1	13.6 \pm 6.8	16.6 \pm 7.6	30.1 \pm 16.5	3.9 \pm 1.1
Carbon monoxide	4	0.8 \pm 0.4	0.8 \pm 0.6	13.6 \pm 3.4	20.7 \pm 20.6	26.3 \pm 34.6	4.9 \pm 1.2
Non-amanita mushrooms	14	0	1.1 \pm 0.9	15.3 \pm 5.1	24.4 \pm 11.0	33.6 \pm 22.8	4.4 \pm 1.0

Table Mean serum activities of acylase, γ -glutamyl transpeptidase and other indices of hepatic function one to four days following the episode of acute poisoning

the latter patients serum bilirubin remained within normal limits during their hospital stay, while a definite transient decrease of cholinesterase and of prothrombin time was observed. The GGTP level as well as that of alkaline phosphatase were only slightly elevated in two patients and remained within normal limits in the rest.

In four patients with acute alcohol intoxication slight acylase activity (0.6 to 1.2 mU/ml) could be detected in serum. The GGTP level was within normal limits in two patients, but was markedly raised in two remaining patients in whom liver biopsy indicated chronic active hepatitis, most likely due to chronic alcohol consumption.

Out of 10 patients with drug intoxication, a three-fold increase in ALT was present in three persons only, while in others transaminases were within normal limits. Acylase activity was found in all patients of this group, but only in two did it exceed 1.0 mU/ml. No changes in GGTP activity were noticed. Acylase was also present in sera of all four patients intoxicated with CO; values ranged from 0.4 to 1.3 mU/ml.

In 14 patients poisoned with non-amanita mushrooms, symptoms of gastroenteritis dominated the clinical picture. They appeared within one to five hours after poisoning, thus differing from the amanita-poisoned group in which there was a delay of 10 to 24 hours between eating the mushrooms and the appearance of clinical symptoms. No liver injury could be detected on clinical grounds in this group. A transient rise of AST and ALT was observed in three patients. Acylase was absent from sera of all the patients; GGTP remained within normal limits. Analysis of all the patients studied revealed a highly significant correlation between serum acylase and AST ($r = 0.83$, $P < 0.01$) as well as between acylase and ALT ($r = 0.45$, $P < 0.01$). An inverse correlation between acylase and prothrombin time was found ($r = -0.35$, $P < 0.05$). There was no correlation between acylase and bilirubin, GGTP, alkaline phosphatase, prothrombin time, and cholinesterase.

Discussion

The results obtained indicate that cobalt-activated acylase appears in the serum of patients with toxic hepatitis. This is a common phenomenon and it occurred in 33 out of 37 patients (89%) in whom amanita mushroom or hepatotoxic chemicals led to liver injury. The high percentage of patients with toxic hepatitis showing acylase activity in serum suggest that estimation of this enzyme might be of value in the diagnosis of liver injury. Some of the patients with amanita mushroom poisoning were

admitted to the hospital late, ie, on the fourth or fifth days following poisoning. In these patients acylase was only moderately elevated or even absent from serum. In view of the rapid fall in serum acylase, it might be suspected that earlier determination of its activity would have increased the number of positive results.

A comparison of serum acylase activity with several other liver function tests points to its similarity in behaviour to serum transaminases in patients with toxic hepatitis. Thus, during the early phase of acute hepatitis high activities of both acylase and transaminases were recorded. At this time in the illness a positive correlation between the three enzymes was found. The highest values for acylase were usually recorded on the day of admission, while transaminases often reached their maximum one or two days later. This seems of interest in view of a recent observation pointing to acylase as the earliest enzyme to be released into the circulation in acute experimental CCl_4 intoxication in rats (Kokot, 1975). The data reported here do not permit the evaluation of the sequence of elevation of serum enzymes in the patients studied, since these were already raised in the majority of patients on admission to hospital. After reaching high values both acylase and transaminases declined subsequently, but they differed in the rapidity of decrease. Irrespective of the maximum values reached, the fall in acylase was sharp and led to the rapid disappearance of enzymic activity from blood. By contrast, aminotransferases decreased gradually. The rate of decrease of AST was usually more pronounced than that of ALT; however, both aminotransferases were on average still distinctly raised a few days after acylase fell to undetectable levels. This variation in the rate of clearance of both AST and ALT following toxic hepatitis seems similar to their removal from serum after viral hepatitis (Wilkinson, 1970). A longer period of observation, up to two to three weeks after intoxication, would be necessary to study the rates of clearance of aminotransferases in more detail.

Mild acylase activity was found in all the patients poisoned with either drugs or carbon monoxide, while transaminases increased only sporadically and other indices of liver function usually remained within normal limits. The average level of acylase in these patients was several times lower than that in subjects poisoned with amanita mushrooms or chemicals. Perhaps the presence of acylase in serum was a reflection of slight liver injury in these patients. Indirect evidence for such an explanation seems to be offered by the absence of acylase in serum among the patients with non-amanita mushroom poisoning, in whom liver injury could be safely excluded. Another

possibility which might explain the presence of acylase in serum is the induction of its biosynthesis in liver by drugs, a finding reported recently for several other liver enzymes.

The prognostic value of laboratory tests is believed to be relatively easy to ascertain in acute liver failure, because the patients either die in a short time or recover completely. It is therefore interesting to note that higher values for acylase were reported at the time of admission to hospital in those patients intoxicated with amanita mushrooms who died than in those who recovered. This raises the possibility that determining acylase levels might be of prognostic value in amanita-mushroom poisoning, a leading cause of toxic hepatitis in several European countries (Gaultier, Orcel, Fournier, Benhamou, Gervais, and Sicot, 1965; Larcán, Rauber, and Calami, 1968; Sicot, Bismuth, Pebay-Peyroula, Frejaville, and Rueff, 1970). No such index is at present available. However, prognostication of liver diseases from laboratory values is difficult and uncertain (Tygstrup, 1973) and therefore larger numbers of patients are necessary to evaluate statistically the validity of acylase determination in the prognosis of acute liver injury.

Unlike acylase, serum GGTP activity remained unchanged in the vast majority of patients with acute toxic hepatitis. This finding at first seems surprising, since the determination of activity of this enzyme is a very sensitive index of liver function in human pathology and its increase has been reported in experimental toxic liver injury (Kokot and Śledziński, 1974). The binding of GGTP to liver cell particles might explain, partly at least, the absence of changes in the serum activity at the time when cytoplasmic enzymes easily leak out into blood (Zimmerman, 1968). A similar situation probably occurs at the early stage of acute viral hepatitis when GGTP activity remains within normal limits or is only slightly raised (Szczeklik, Orłowski, and Szewczuk, 1961). Our observations are limited to the early stage of acute toxic hepatitis, and they do not rule out the possibility that an increase in GGTP might have occurred in our patients at a later stage of hepatitis. Such a late increase in GGTP has been described in other diseases, eg, viral hepatitis (Orłowski, 1963) or acute myocardial infarction (Agostoni, Ideo, and Stabilini, 1965; Szczeklik, Szewczuk, Nowosad and Kołaczowska, 1972).

Of the two enzymes studied, serum acylase, but not

GGTP, showed definite changes in activity in toxic hepatitis. The differential diagnosis of increased serum acylase should include acute viral hepatitis, another disease in which acute injury of liver cells leads to the appearance of high acylase activity in serum. High organ specificity of acylase and its absence from serum in chronic liver disease makes its estimation of clinical interest in acute hepatitis.

References

- Agostoni, A., Ideo, G., and Stabilini, R. (1965). Serum γ -glutamyl transpeptidase activity in myocardial infarction. *Brit. Heart J.*, **27**, 688-690.
- Albert, Z., and Szewczuk, A. (1972). Histochemical and biochemical studies on CO⁺⁺-activated acylase in rodent tissues. *Acta histochem.*, **42**, 68-76.
- Gaultier, M., Orcel, L., Fournier, E., Benhamou, J.-P., Gervais, P., and Sicot, C. (1965). L'hépatite phalloïdienne. *Presse Méd.*, **73**, 2349-2354.
- Gibiński, K., Szatoń, R., and Maraszek, J. (1963). Evaluation of gamma-glutamyltranspeptidase activity and leucyl-aminopeptidase determinations in internal diseases. *Gastroenterology, (Basel)*, **99**, 237-246.
- Kokot, F. (1969). Laboratory methods in clinical practice. (In Polish). Warsaw, PZWL.
- Kokot, F., and Śledziński, Z. (1974). Die γ -Glutamyltransferase (γ -GT). *Z. klin. Chem. klin. Biochem.*, **12**, 374-384.
- Kokot, F. (1975). Personal communication.
- Larcán, A., Rauber, G., and Calami, M. (1968). L'intoxication par l'amanite phalloïde. In *Les Hépatonephrites toxiques. Comptes rendus de la 8^{ème} Réunion Nationale des Centres de Lutte Contre les Poisons, Paris, 1968*. Masson, Paris.
- Orłowski, M. (1963). The role of gamma-glutamyl transpeptidase in the internal diseases clinic. *Arch. Immunol. Ther. exp.*, **11**, 1-61.
- Orłowski, M. (1965). A new method for the determination of serum gamma-glutamyl transpeptidase. *Arch. Immunol. Ther. exp.*, **13**, 538-541.
- Reitman, S., and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Amer. J. clin. Path.*, **28**, 56-63.
- Sicot, C., Bismuth, C., Pebay-Peyroula, F., Frejaville, J.-P., and Reuff, B. (1970). L'hépatite phalloïdienne. *Rev. Pract.*, **20**, 2485-2492.
- Szczeklik, A., Sowa, J., and Pawlikowski, T. (1973). The diagnostic value of determinations of cobalt-activated acylase in acute and chronic hepatitis. (In Polish). *Polish Arch. intern. Med.*, **50**, 1073-1077.
- Szczeklik, A., Szewczuk, A., Nowosad, H., and Kołaczowska, B. (1972). Serum peptidases in myocardial infarction. *Brit. Heart J.*, **34**, 232-237.
- Szczeklik, E., Orłowski, M., and Szewczuk, A. (1961). Serum γ -glutamyl transpeptidase activity in liver disease. *Gastroenterology*, **41**, 353-359.
- Szewczuk, A., and Szczeklik, A. (1971). Cobalt-activated acylase in human serum. Occurrence in hepatobiliary diseases. *Clin. chim. Acta.*, **33**, 309-315.
- Szewczuk, A., Szczeklik, A., Gładysz, A., Wellman-Bednawska, M., Nienartowicz, B., and Przygodzka, D. (1974). A simplified method of determination of serum acylase activity and its use in preclinical diagnosis of viral hepatitis. (In Polish). *Polish Arch. intern. Med.*, **51**, 159-166.
- Tygstrup, N. (1973). The prognostic value of laboratory tests in liver disease. *Scand. J. Gastroent.*, **8**, Suppl. 19, 47-50.
- Wilkinson, J. H. (1970). Clinical significance of enzyme activity measurements. *Clin. Chem.*, **16**, 882-890.
- Zimmerman, H. J. (1968). Toxic hepatopathy. *Amer. J. Gastroent.*, **49**, 39-56.