Pharmacology Biochemistry & Behavior, Vol. 13, Suppl. 1, pp. 107–110. Printed in the U.S.A.

Plasma Glutamate Dehydrogenase: A Marker of Alcoholic Liver Injury

T. M. WORNER AND C. S. LIEBER

Alcohol Research and Treatment Center, Bronx VA Medical Center and Mount Sinai School of Medicine (CUNY), New York, NY 10468

WORNER, T. M. AND C. S. LIEBER. *Plasma glutamate dehydrogenase: A marker of alcoholic liver injury*. PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 107–110, 1980.—Forty-two male alcoholics admitted for alcohol detoxification or for the treatment of the medical complications of alcoholism, as well as two volunteers consuming 2 g/kg/day of ethanol under metabolic ward conditions, were evaluated to determine the time course of plasma GDH elevation. GDH values fell rapidly toward the normal range after cessation of the drinking episode. In patients who subsequently underwent a diagnostic liver biopsy, severity of histologic lesions correlated positively with early GDH elevations. In 32 patients followed up to 15 months, GDH values correlated well with clinical status.

Glutamic acid dehydrogenase Blood enzymes Liver enzymes Alcoholic liver disease

THE prognostic factors associated with the progression of alcoholic liver injury have not been elucidated. It is generally assumed that liver cell necrosis plays a significant role in the progression of this disease. Liver biopsies offer an accurate tool for diagnosing necrosis, but this is an impractical method for monitoring patients with alcoholic liver injury. "Liver enzyme" levels in serum, especially the transaminases, have been traditionally used to estimate the degree of liver cell damage, even though it has been shown that these blood enzymes correlate poorly with the degree of liver cell necrosis [7,10]. Indeed, it is possible that these tests may be only moderately elevated or even normal in patients with alcoholic hepatitis [1]. Additionally, the transaminases are not liver specific, and elevated values may reflect damage to organs other than the liver.

Glutamate dehydrogenase (GDH) has previously been reported to be a reliable marker of liver cell necrosis in the alcoholic [10]. This enzyme was chosen because of its high concentration in the liver, its predominant activity in the centrolobular portion of the liver (where alcohol produces its major effects) and its exclusively intramitochondrial location [6,11]. It has also been reported in another study that significant elevation of GDH (i.e., $>2^1/_2$ times normal) is not a common finding in alcoholics [8]. However, it is unclear in this latter study when GDH was measured in relationship to the alcoholic episode. Also, no histologic evaluation was performed.

On one hand, because cellular lesions may persist longer than elevation of serum enzymes, the time of sampling may be of importance. On the other hand, once the patient has been stabilized, frequent monitoring of blood enzymes may be of value in predicting the clinical outcome. Therefore, we wished to establish the time course of plasma GDH elevation after alcohol abuse and its relationship to alcoholic liver injury both during hospitalization and over a prolonged (fifteen months) outpatient clinic follow-up.

METHOD

Forty-one male and one female alcoholics admitted for alcohol detoxification or medical complications of alcoholism had sequential determinations of plasma GDH activity. In all patients, the first sample was obtained within 24 hours after cessation of alcohol consumption. In twenty-five of these subjects, samples were retaken on days 2–3, and in all patients after 5–10 days. None of these patients had overt clinical heart disease or symptomatic myopathy. Creatine phosphokinase activity (CPK) was not measured in these patients. In another study, however, we have assessed the relationship of GDH to CPK in 25 alcoholics without heart disease or symptomatic myopathy and found no correlation between elevated values of these enzymes.

A diagnostic liver biopsy was performed within 10 days after admission as part of the medical evaluation in thirty-seven patients. Prothrombin time was within 3 sec of control value at the time of admission and within 2.5 sec at the time of biopsy. Except for one subject with a cholestatic syndrome and a serum bilirubin of 13.7 mg/dl, no patient had a bilirubin value exceeding 3.2 mg/dl at the time of biopsy. This also pertained to the serum bilirubin at the time of admission, except for one patient with a transient increased value of 4.5 mg/dl.

Alcohol intake was assessed retrospectively through a detailed interview. Two male alcoholic volunteers admitted to the Medical Service of the Bronx VA Medical Center for detoxification from alcohol were studied. Liver biopsy showed minimal liver involvement. After obtaining informed consent, each volunteer was transferred to the metabolic ward. Abstinence from alcohol was assessed by clinical observation and measurement of blood alcohol levels three times per week. After a period of 2–3 weeks of documented abstinence, during which GDH remained constant, each subject was given alcohol in progressively increasing amounts over a period of 7 days, up to 50% of total calories (2

TABLE 1

CLINICAL PARAMETERS IN 13 ALCOHOLICS WHO DETERIORATED CLINICALLY DURING A 15 MONTH FOLLOW-UP PERIOD

Parameter	No. Improved	No. Unchanged	No. Deteriorated
Ascites/edema	0	8	5
Hepatomegaly	0	1	12
Weight	0	3	10
Bilirubin	3	4	6
Hematocrit	6	5	2
Prothrombin time	0	12	1

TABLE 2

CLINICAL PARAMETERS IN 19 ALCOHOLICS WHO IMPROVED CLINICALLY DURING A 15 MONTH FOLLOW-UP PERIOD

Parameter	No. Improved	No. Unchanged	No. Deteriorated
Ascites/edema	11	8	0
Hepatomegaly	19	0	0
Weight	15	4	0
Bilirubin	10	9	0
Hematocrit	7	10	2
Prothrombin time	3	16	0

g/kg/day), in addition to a nutritiously adequate diet as previously described [9]. Plasma GDH was measured three times per week as described below. After 28 days of alcohol consumption, each subject was withdrawn from alcohol over a seven day period.

Additionally, 32 alcoholics who had been discharged to a medical clinic after detoxification or treatment of the medical complications of alcoholism were evaluated. Patients were followed up to 15 months after discharge. Patients were seen at 1–3 month intervals, depending on their clinical status. Abstinence was encouraged. Subjects were evaluated for weight, hepatomegaly, ascites, edema, jaundice, anemia, and coagulopathies. Blood was obtained at each visit for determination of alcohol, hematocrit, prothrombin time, bilirubin, glutamic acid dehydrogenase (GDH), aspartate transaminase (SGOT), and alanine transaminase (SGPT).

Histology

Biopsy specimens were interpreted blindly without knowledge of the patients identity or blood enzyme levels. Sections were stained with hematoxylin eosin and trichrome and examined for the presence of fat, inflammation, fibrosis, necrosis, and Mallory's hyalin bodies. The degree of necrosis was graded as follows: 0—absence of necrosis and parenchymal inflammation; 1^+—occasional cell drop-out often shown by the inflammatory reaction, mononuclear in type; 2^+—scattered foci of necrotic cells in the parenchyma with polymorphonuclear infiltration predominantly in the centrolobular area ("mild alcoholic hepatitis"); 3^+—diffuse parenchymal necrosis with polymorphonuclear infiltrates ("frank alcoholic hepatitis"). The independent interpretation of the biopsy specimens by the hospital pathologists was consistent with our diagnoses in that none of the patients with grade 1 necrosis were classified as having alcoholic hepatitis, whereas most of those with grades 2 and 3 were given this diagnosis.

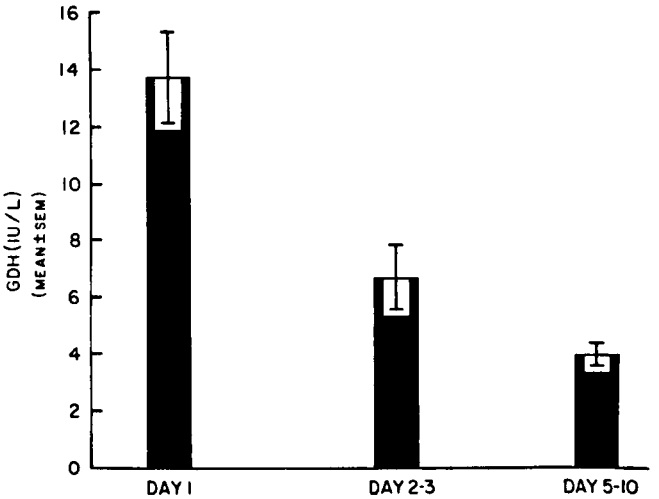

FIG. 1. Time course of GDH in 42 alcoholic men after alcohol withdrawal. GDH values were highest immediately after admission. Values fell rapidly, with a significant decrease by day 2–3. A still further reduction was apparent by day 5–10.

Blood Enzymes

Plasma was obtained immediately after venipuncture; hemolyzed samples were discarded. Samples were stored at −80° until they were analyzed. GDH activity was deter-

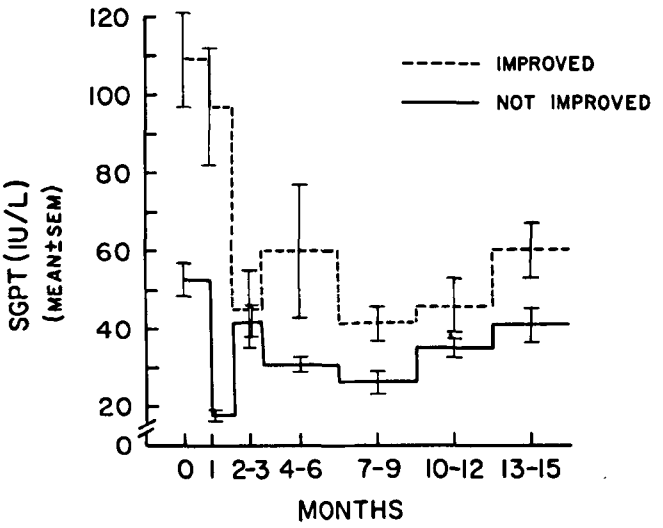

FIG. 2. Time course of SGPT in alcoholics with [19] and without [13] clinical improvement. Subjects were evaluated at 1–3 month intervals in an outpatient setting, for up to 15 months. No significant difference was noted between the groups.

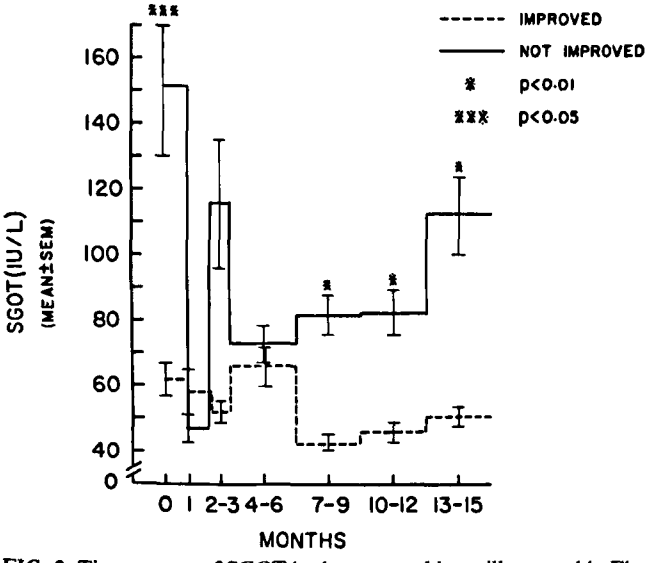

FIG. 3. Time course of SGOT in the same subjects illustrated in Fig. 2. Samples were obtained simultaneously. No significant difference was noted between the groups during the first 6 months. However, a persistent difference between the 2 groups is obvious after 6 months.

mined in duplicate at 37° by a standard laboratory technique [2]. Measurements of the activity of aspartate transaminase (SGOT), alanine transaminase (SGPT) and gamma-glutamyl transpeptidase (GGTP) were also carried out [3, 4, 5]. GDH and GGTP assays were performed manually in our laboratory.

SGOT and SGPT were measured by the hospital laboratory.

RESULTS

The time course of GDH elevation is illustrated in Fig. 1. GDH values were highest immediately after admission to the hospital. Of 42 patients, 27 had values above the normal range (>4 IU). After 2–3 days of abstention from alcohol, there was a marked decrease in GDH values. A further reduction was noted after 5–10 days, with the majority of patients returning to values below 12 IU/ml.

The correlation of GDH, GGTP, SGPT and SGOT values with alterations of liver histology has been reported [12]. As noted before, in samples obtained within 2 days after the drinking episode, there was marked overlap of values for all degrees of necrosis for both SGOT and SGPT. Although GGTP values correlated more closely with the degree of cell necrosis, there was an elevation of this enzyme in 30% of patients without necrosis. By contrast, a value of GDH>2½ times the upper limit of normal, differentiated between those patients without necrosis and those with 2–3+ necrosis.

For all the enzyme values obtained 5–10 days following abstention, there was no clear correlation with the severity of liver pathology.

In the subjects given alcohol (2 g/kg/day for one month) under metabolic ward conditions, GDH values never exceeded a 4.4 IU.

The self-reporting by patients of their alcohol intake revealed a wide spectrum; there was no apparent correlation with the initial GDH value. Three-fourths of these patients reported an alcohol consumption of 150–650 g alcohol/day prior to admission, with an average of 300 g/day. One-fourth of the patients reported no alcohol intake, but had blood

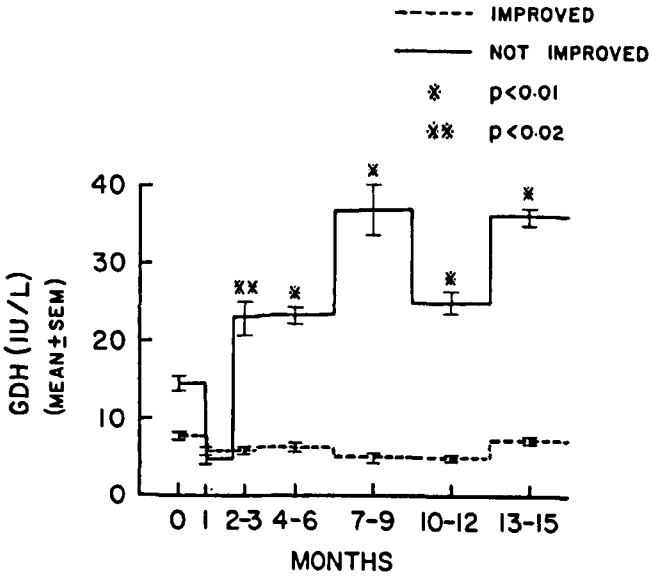

FIG. 4. Time course of GDH in these same subjects. Groups were similar at 1 month follow-up. However, thereafter a significant, persistent difference between the groups was observed.

ethanol levels ranging from 5–373 mg per 100 ml, with an average of 90 mg per 100 ml. In the latter group, when relatives could be contacted, they usually confirmed that these subjects had been drinking "heavily" prior to admission. However, in most cases they were unable to quantify the patient's intake.

Shown in Table 1 and 2 are the clinical parameters used to evaluate patients who were followed in a medical clinic for up to 15 months after discharge from the hospital. In each group, a substantial proportion of subjects did not have ascites. However, when ascites was present initially, its reso-

lution was associated with a favorable outcome. Hepatomegaly improved or resolved in all patients who did well clinically. The majority of patients who improved approached ideal body weight, while those who deteriorated clinically usually remained below ideal body weight. Bilirubin levels in general paralleled the clinical course. The "improvement" in those three patients who eventually deteriorated clinically can be accounted for by changes during the initial hospitalization. Hematocrit values improved in both groups in a large proportion of patients. A decreased value was observed in two subjects who improved clinically. In one of these, the decrease was secondary to post-operative blood loss after cholecystectomy. The other decreased hematocrit is not fully explained, but it is noteworthy that the patient had a chronic stable coagulopathy with occasional epistaxis and gingival bleeding. The prothrombin time was normal in the majority of patients in both groups.

Shown in Figs. 2, 3 and 4 are the time courses of SGPT, SGOT, and GDH in the patients described above. SGPT values did not allow for differentiation between these two groups. Actually, the highest values were recorded in the improved group. For SGOT, there was a wide variability in the elevated values of the non-improved group. Indeed, there was no significant difference in SGOT values between the two groups for the first six months of follow-up. During the last six months of follow-up, a significant difference was noted. For GDH, there was no significant difference between the two groups on admission or for the first month of follow-up. However, by 2–3 months, a significant persistent difference became obvious.

DISCUSSION

In the present study, we report the time course of plasma GDH elevation after alcohol abuse, the usefulness of initial serum GDH in the recognition of liver necrosis, and the value of GDH in predicting long term outcome. As previously reported, upon cessation of alcohol intake, elevated enzyme values rapidly return towards normal. We confirm here the importance of early sampling which has previously been stressed [12].

The usefulness of SGPT, SGOT and GDH in predicting long term outcome of patients was assessed in this study. SGPT was not found helpful in the prediction of outcome on a long term basis.

Although SGOT appears to have some delayed predictive value, its usefulness within the first six months of follow-up was negligible. In addition, SGOT elevation may result from injury to other tissues, such as muscle or heart [8]. By contrast, GDH correlated well with long-term treatment outcome. Significant differences between the groups were already noted within 2–3 months of follow-up. These differences persisted throughout the period of evaluation. Thus, chronically elevated GDH values seem to portend a poor clinical outcome.

ACKNOWLEDGEMENTS

We would like to thank Mr. M. Calka for his excellent technical assistance and the secretarial staff of the Alcohol Research and Treatment Center for their clerical assistance. This study was supported in part by the Medical Research Service, Veterans Administration and USPHS grants AA003508 and AA 00224.

REFERENCES

1. Beckett, A. G., A. V. Livingston and K. R. Hill. Acute alcoholic hepatitis without jaundice. *Br. Med. J.* **2**: 580–582, 1962.
2. Ellis, G. and D. M. Goldberg. Optimal conditions for the kinetic assay of serum glutamate dehydrogenase activity at 37°C. *Clin. Chem.* **18**: 523–527, 1972.
3. Gamma Glutamyl Transpeptidase (GGTP) in serum or plasma at 405 nm. Sigma Technical Bulletin No. 415.
4. Glutamic-Oxaloacetic Transaminase (GOT). Technicon Bulletin No. SG4-0010FH9.
5. Glutamic-Pyruvic Transaminase (GPT). Technicon Bulletin No. SG4-0022FH9.
6. Guder, W. G., A. Habicht, J. Kleibl, U. Schmidt and O. H. Wieland. The diagonistic significance of liver cell inhomogenicity: Serum enzymes in patients with central liver necrosis and the distribution of glutamate dehydrogenase in normal human liver. *Klin. Chem. Klin. Biochem.* **13**: 311–318, 1975.
7. Kallai, L., A. Hahn, V. Roder and V. Zupanic. Correlation between histologic findings and serum transaminase values in chronic diseases of the liver. *Acta Med. Scand.* **175**: 49–56, 1964.
8. Konttinen, A., H. Gottfried and L. Antti. Multiple serum enzyme analyses in chronic alcoholics. *Acta Med. Scand.* **188**: 257–264, 1970.
9. Shaw, S. and C. S. Lieber. Plasma amino acid abnormalities in the alcoholic: respective role of alcohol, nutrition and liver injury. *Gastroenterology* **74**: 677–682, 1978.
10. VanWaes, L. and C. S. Lieber. Glutamate dehydrogenase: a reliable marker of liver cell necrosis in the alcoholic. *Br. Med. J.* **2**: 1508–1510, 1977.
11. Wieme, R. J. and L. Demeulanaere. Enzyme assays in liver disease. *J. clin. Path. Suppl.* **4**: 15–19, 1971.
12. Worner, T. M. and C. S. Lieber. Glutamate dehydrogenase: Clinical application in alcoholic liver disease. *Alcoholism: Clin. exp. Res.* **4**: 431–434, 1980.