J. Clin. Chem. Clin. Biochem.
Vol. 18, 1980, pp. 237–239

Biochemical and Histochemical Estimations of Liver γ -Glutamyltransferase Activity in Ethanol-Fed Rats¹⁾

By G. Idéo, Silvana Tempini, A. Bellobuono, G. Bellati and G. Ronchi

Institute of Clinical Medicine (III); University of Milan, Milan, Italy

(Received May 31/December 17, 1979)

Summary: Ethanol-fed rats showed significantly elevated plasma and liver γ -glutamyltransferase activity, compared with rats fed carbohydrates isocalorically, or with untreated control animals. The activity of γ -glutamyltransferase in liver was detected by means of both biochemical and histochemical methods. These data suggest that increased plasma γ -glutamyltransferase activity commonly found in alcoholics can be related, at least in part, to a hepatic enhancement of the activity in the liver.

Biochemische und histochemische Untersuchung der Aktivität von γ -Glutamyltransferase in der Leber Ethanol-gefütterter Ratten

Zusammenfassung: Ethanol-gefütterte Ratten zeigten signifikant erhöhte katalytische Aktivitäten von γ -Glutamyltransferase in Plasma und Leber, verglichen mit isokalorisch Kohlenhydrat-ernährten oder unbehandelten Kontrolltieren. Die Aktivität der γ -Glutamyltransferase in der Leber wurde mit bio- und histochemischen Methoden nachgewiesen. Die Ergebnisse weisen darauf hin, daß die gewöhnlich im Plasma von Alkoholikern gefundene Erhöhung der Aktivität von γ -Glutamyltransferase mindestens teilweise auf deren Zunahme in der Leber bezogen werden kann.

Introduction

The activity of γ -glutamyltransferase in human plasma is elevated in alcoholics (2). The underlying mechanism was regarded as an induction of liver γ -glutamyltransferase, similar to that observed after the administration of enzyme inducing agents. However, experimental attempts to demonstrate this assumption by means of biochemical methods have given rise to conflicting results: in fact, rat liver γ -glutamyltransferase after feeding ethanol, was observed to be increased by Ishii et al. (3), Yasuroka et al. (4), Teschke et al. (5), but unchanged by Morland et al. (6) and even depressed by Kawaguchi (7) and Singer & Kaplan (8).

The values found for tissue enzyme activities could be influenced by a variation of K_m , by different methods of liver homogenization or subcellular fractionation, etc. In the present study rat liver γ -glutamyltransferase activity in ethanol-fed rats was detected by a histochemical method (which is less influenced by the above

variables), and compared to that obtained by the usual biochemical method.

Finally, γ -glutamyltransferase activity was measured in plasma of both intoxicated and control rats.

Material and Methods

Male Sprague-Dawley rats with a starting body weight of 230–250 g were pair fed a liquid diet according to De Carli & Lieber (9). The animals received 36% of their total calories, either as ethanol (experimental group), or as carbohydrate (control group).

The animals were sacrificed after 0 (pre-experimental group) and 24 days on their respective diets. They were fasted for 12 hours before exsanguination.

Heparinized blood was centrifuged and the plasma used for enzyme assay. Livers were homogenized in an Elvehjem-Potter apparatus (1 g + 9 ml distilled water). Homogenates were rapidly frozen, thawed and submitted to ultrasonic treatment (3 times 20 s), then centrifuged for 20 min at 10000 g. The supernatant fluid was employed for the enzyme assay. This method was shown to be suitable for enzymatic estimations in liver homogenates (10).

γ -glutamyltransferase (EC 2.3.2.2) was assayed by Szasz's method (11). Plasma enzyme activity is expressed as International Unit (IU) per litre (l) of plasma; tissue enzyme

¹⁾ A portion of this work was presented at the 11th Meeting of the European Association for the Study of the Liver, 1976, and has been published in abstract form (1).

activity as IU per g of liver protein. Total protein was determined by the method of Lowry et al. (12).

For the histochemical studies, small blocks of tissue from the right liver lobe were rapidly removed and frozen in isopentane which had been cooled to -170°C by liquid nitrogen. The staining reactions were performed on $9\text{ }\mu\text{m}$ cryostat sections, using the following procedure: sections were fixed for 1 hour in cold acetone and incubated at room temperature for 15 min in a medium prepared according to Rutemburg et al. (13), using γ -glutamyl-4-methoxy-2-naphthylamide as substrate.

The means ($\pm$ SEM) and differences were calculated, and their significances were assessed by the Student's t-test.

Results

The body weight of the animals at sacrifice was 280–310 g, without significant differences between the two groups of animals (ethanol and carbohydrate-fed respectively).

In control rats the histochemical pattern of γ -glutamyltransferase showed that the enzyme activity is localized at the apical border of biliary ductular and ductal cells, whereas parenchymal cells are negative, except for a very few strictly periportal canaliculi (fig. 1). A similar pattern was observed in the pre-experimental group (rats before any type of diet). The liver of ethanol-fed rats appeared to be richer in γ -glutamyltransferase activity than controls. The activity is present not only in ductular structures, but also on the canalicular membranes of periportal parenchymal cells (fig. 2). A weak cytosolic activity is often present as well. Centrolobular cells are always negative.

Biochemical determinations of γ -glutamyltransferase showed significantly higher activity in both liver and plasma in ethanol-fed rats, than in the controls, or in animals without treatment (pre-experimental group, tab. 1).

Fig. 1. Liver of carbohydrate-fed rats stained for γ -glutamyltransferase. Enzyme activity is present at the apical border of the biliary cells. Few periportal canaliculi are also delineated.

Fig. 2. Liver of ethanol-fed rats stained for γ -glutamyltransferase. Activity is located in ductular structures and in canalicular membranes of many periportal cells.

Tab. 1. Effect of chronic ethanol consumption on the γ -glutamyltransferase activity in plasma and liver.

Rats were given either a control or an ethanol diet for 24 days; activity in rats without treatment (pre-experimental group) is also shown. The number of animals is given in parentheses.

	γ -glutamyltransferase liver [U/g protein]	γ -glutamyltransferase plasma [U/l]
Carbohydrate-fed (8)	3.01 ± 0.35	1.32 ± 0.28
Ethanol-fed (8)	$5.25 \pm 0.65^*$	$3.87 \pm 0.45^*$
Pre-experimental group (6)	3.25 ± 0.26	1.60 ± 0.51

* $p < 0.001$

Discussion

By means of histochemical and biochemical methods the hepatic activity of γ -glutamyltransferase was shown to be increased in ethanol-fed rats.

Thus the behaviour of γ -glutamyltransferase is similar to that of other enzymes (particularly localized in microsomes), which were observed to be enhanced in the liver after ethanol administration (14–18).

As far as localization is concerned, the histochemical study showed that, while in carbohydrate-fed and control rats γ -glutamyltransferase is almost exclusively demonstrable in the ductular structures, after ethanol consumption this activity also became positive on the cytosol and on canalicular membranes of the liver cells in the periportal area. This finding was also observed after norethandrolone (19) and phenobarbitone administration (unpublished data).

The present study demonstrates, moreover, that alcohol consumption provokes an increase in plasma γ -glutamyltransferase, which cannot simply be attributed to enhanced activities in the liver. Under other experi-

mental conditions, such as after phenobarbitone administration, plasma activity was normal, despite the rise in liver γ -glutamyltransferase (20); only the addition of carbon tetrachloride significantly enhanced the plasma level of the enzyme (unpublished data). Thus the induced γ -glutamyltransferase must be liberated from the membranes and released into the bloodstream.

Alcohol itself not only seems able to increase γ -glutamyltransferase in the tissue, but also to "injure" the structure to which the enzyme is bound, thereby releasing it into the blood. It is interesting that addition of ethanol *in vitro* resulted in an increase of γ -glutamyltransferase activity in microsomes of alcohol-fed rats (5).

Therefore it is possible to hypothesize a similar mechanism in alcoholics who show a high level of plasmatic γ -glutamyltransferase. This assumption is in accordance with preliminary data of Seymour et al. (21), who observed that hepatic γ -glutamyltransferase localized in plasma membranes is elevated in patients affected by alcoholic liver disease.

Our results agree with those of Ishii et al. (3), Yasuraoka et al. (4), Teschke et al. (5). It is difficult to explain contrasting data of others (6–8), who did not observe any increase of γ -glutamyltransferase activity in such conditions. Morland et al. interpreted the difference between ethanol and carbohydrate-fed rats as a reduction of γ -glutamyltransferase activity in animals fed the control diet (6). We cannot confirm these observations, since the activity of carbohydrate-fed animals is equal to the pretreated group and this data was observed by means of both biochemical and histochemical methods. Conceivably, the above-mentioned diverse results might be related either to different strains of animals employed or to different types of diet (addition of antibiotics?). This interpretation could explain the various levels of enzymatic activity observed even in untreated animals – not only in the liver (in which γ -glutamyltransferase estimation could be influenced by different methods of homogenization, cell disruption, fractionation of subcellular components etc.) – but also in the plasma.

References

1. Ronchi, G. & Ideo, G. (1976) *Digestion* (abstract) 14, 544.
2. Rosalki, S. B. & Rau, D. (1972) *Clin. Chim. Acta* 39, 41–47.
3. Ishii, H., Kanno, I., Shigeta, Y., Tagagi, S., Yasuraoka, S., Kano, S., Takeshita, E. & Tsuchiya, M. (1972) *Gastroenterology* (abstract) 71, 913.
4. Yasuraoka, S., Takagi, S. T., Shigeta, Y., Kamiya, T., Ishii, H. & Tsuchiya, M. (1978) *Proceedings VI World Congress of Gastroenterology* (abstract), pag. 235.
5. Teschke, R., Brand, A. & Strohmeyer, G. (1977) *Biochem. Biophys. Res. Comm.* 75, 718–723.
6. Morland, J., Huseby, N., Sjoblom, M. & Stromme, J. H. (1977) *Biochem. Biophys. Res. Comm.* 77, 1060–1066.
7. Kawaguchi, Y. (1973) *Jap. J. Gastroent.* 70, 1157–1169.
8. Singer, J. S. & Kaplan, M. M. (1978) *Gastroenterology* (abstract) 74.
9. De Carli, L. M. & Lieber, C. S. (1967) *J. Nutr.* 91, 331–336.
10. Ideo, G., Del Ninno, E. & de Franchis, R. (1971) *Enzyme* 12, 242–254.
11. Szasz, G. (1969) *Clin. Chem.* 15, 124–136.
12. Lowry, O. H., Rosebrough, W. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275.
13. Rutemburg, A. M., Kim, H., Fischbein, J. W., Hanker, J. S., Wasserkrag, H. L. & Seligman, A. M. (1969) *J. Histochem. Cytochem.* 17, 517–525.
14. Lieber, C. S. & De Carli, L. M. (1970) *J. Biol. Chem.* 245, 2505–2512.
15. Rubin, E., Hutterer, F. & Lieber, C. S. (1968) *Science* 159, 1469–1470.
16. Teschke, R., Hasumura, Y. & Lieber, C. S. (1974) *Biochem. Biophys. Res. Comm.* 60, 851–857.
17. Teschke, R., Matsuraki, S., Ohnishi, K., Hasumura, Y. & Lieber, C. S. (1977) *Alcoholism* 1, 7–15.
18. Ideo, G., de Franchis, R., Del Ninno, E., Cocucci, C. & Dioguardi, N. (1971) *Enzyme* 12, 473–480.
19. Ronchi, G., Desmet, V. J. (1973) *Beitr. Pathol.* 150, 316–321.
20. Ideo, G., de Franchis, R., Del Ninno, E. & Dioguardi, N. (1971) *Lancet* II, 825–826.
21. Seymour, C. A., Neale, G. & Peters, T. J. (1975) *Gut* (abstract) 16, 839.

Prof. Gaetano Idéo
Via Pace 15
Milan (Italy)