Research Journal of Medical Sciences 7 (2): 50-53, 2013
ISSN: 1815-9346

Serum Gamma-Glutamyltransferase Activity: Inhibitory Study in Patient of Hepatitis

Namama S. Talabani
Department of Chemistry, Faculty of Science,
University of Sulaimania, Sulaimania, Iraq

Abstract: Elevation of Gamma-Glutamyltransferase (GGT) activity has been implicated in many pathologies and clinical inhibitors of GGT are under development for use in the treatment of liver, cancer, bone and other diseases. In this study, researchers try to find other new inhibitor of GGT researchers used new polar amino acid (threonine) as inhibitor, kinetic studies provide insight into the mechanism of inhibition. New uncompetitive inhibitors of physiological GGT reaction researchers found, development of new inhibitors is essential for exploiting GGT as a therapeutic target. L-threonine, in the presence study was detected as uncompetitive inhibitor, Km values were determined; researchers characterized the kinetic properties of GGT under a standard set of condition. Serum GGT was analyzed in healthy cases (normal 25: mean±SD) age 30±12 years (16.71±0.87), the mean values of GGT levels were statistically significantly higher in liver syndrome (70.79±1.24) and this values decreased in the presence of inhibitor to 60.82±0.87.

Key words: Gamma-glutamyltransferase, inhibitor, threonine, hepatitis, liver syndrome

INTRODUCTION

GGT (EC.2.3.2.2) is a cell surface enzyme that cleaves gamma-glutamyl bonds of extracellular subctances including Glutathione (GSH), GSH s-conjugates and leukotriene C (Elce and Broxeyer, 1976; Curthoys and Hughey, 1979; Wickham *et al*., 2011).

Elevation of serum γ-Glutamyltransferase (GGT) activity is frequently interpreted as an index of hepatobiliary dysfunction and as a nonspecific marker of excessive alcohol use (Whitifield, 2001). And elevation of GGT has been implicated in pulmonary disease, cardiovascular disease and cancer (Lowry *et al*., 2008; Lee *et al*., 2007; Hanigan *et al*., 1999).

GGT has been demonstrated in various human and animal organs including kidneys, liver and spleen. It is also present in various body fluids such as saliva, serum, bile and urine (Sener, 1997; Nemesanzky and Lott, 1985). GGT is present in serum as part of several molecular complexes with distinct physiochemical properties (Huseby, 1982; Wenham *et al*., 1984).

GGT measurement was introduced into clinical laboratories some 45 years ago and over that times a large amount of information on factors influencing its activity in serum has accumulated. Theories have been put forward about its normal function within the body and its role in numerous pathological conditions.

Threonine is an α-amino acid, this essential amino acid is classified as polar (Moss, 1984) while the highest growth and specific growth and specific growth rate were showed in diet containing 1.7% threonine. Dietary threonine imbalance is known to reduce the growth of the small intestine, liver and skeletal muscle in young animals, an excess or a deficiency of dietary threonine decreases protein synthesis in rapidly growing tissues (Wang *et al*., 2007; Benakappa and Varghese, 2002).

The present research, researchers have investigated the inhibitory effect of essential amino acid (L-threonine) on serum GGT activity in patient's with hepatitis, at the same time researchers study the effect of time on the activity, the results would help to select the new inhibitor which might useful for the treatment of some hepatitis.

MATERIALS AND METHODS

Collection of samples: Blood analysis is usually done on venous of capillary blood. Forty five samples of blood were throughout the investigation of GGT enzyme activity (age 20-50 years), these samples were diagnosed by consultants and proved by liver function test. They are no accompanied disease. Healthy normal (25) were used as controls.

The blood left at room temperature after clot formation the serum isolated by centrifugation. Serum obtained was use on the same day of experiment.

Chemicals: These were chosen for specific GGT activity determination and all other experiments related, they were of analytical grade and highly purified, purchased from BDH and FLUKA.

Gamma-Glutamyltransferase assay: The GGT activity was determined by the hydrolysis of g-glutamyl p-nitroanilide in the presence of the acceptor (Szaz, 1976).

Standard assay included final reagent concentration 2 mM of gamma-glutamyl p-nitroanilide, 62 mM of glycylglycine and 95 mM of Tris-HCl, pH 8.1. The rate of p-nitroanilide formation was measured at 405 nm by using spectrophotometer. The results were expressed as U/L, one unit of enzyme represents the amount of enzyme that catalyzes the release of 1 mmol of nitroaniline/min.

Inhibition studies: The GGT inhibition with L-threonine was studied by using Tris-HCl buffer containing various concentration of gamma-glutamyl p-nitroanilide (0.5, 1, 1.5, 2, 2.5 and 3 mmol L^{-1}) and 62 mmol of glycylglycine per liter. L-threonine (2.5 mM) were added to the reaction mixture. The GGT activities were assayed after 1 min incubation with the inhibitors.

Apparent Km and apparent Vmax values were determined from Michaelies-Menten and Lineweaver Burk plots for gamma-glutamyl p-nitroanilide.

RESULTS AND DISCUSSION

Investigating GGT activity data obtained shows an elevation of the activity in hepatitis (70.79±1.24 U L^{-1}) when compared with normal activity (16.71±0.87 U L^{-1}) (Table 1). In presence of L-threonine (2.5 mM L^{-1}) uncompetitively inhibited of the serum GGT activity at 2 mmol L^{-1} concentration of gamma-glutamyl p-nitroanilide (Fig. 1).

Determination of Vmax and Km values: These values of Vmax and Km were achieved using Michaelis-Menten analysis (Fig. 2) (Table 2) the data analysed were confirmed by plotting Lineweaver-Burk plot (Fig. 3). By these figures we can detect L-threonine as uncompetitive inhibitor.

The results showed that L-threonine could affect activity of GGT enzyme. Data from the study indicate that L-threonine amino acid inhibited the activity of GGT enzyme.

Elevation of GGT activity can be explained to be due to the affection of liver by damage of liver cells. the relationship between hepatic and serum GGT in human patients has been studied by several researchers.

Table 1: GGT-enzyme activity inhibition using L-threonine (2 mM) in normal and hepatitis sera

Cases	GGT activity (mean±SD) (U/L)
Normal	16.71±0.87
Hepatitis sera	70.79±1.24
Presence of L-threonine (2 mM)	60.82±0.87

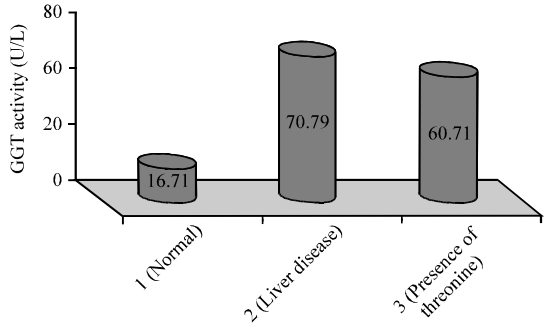

Fig. 1: Effect of (2 mM) of L-threonine on GGT activity in hepatitis sera

Table 2: Km and Vmax values for serum GGT in (hepatitis and presence of L-threonine 2 mM)

Parameters	GGT activity without L-threonine	GGT activity in presence of L-threonine
Km (mM)	1.25	1
Vmax (U/L)	12.5	10

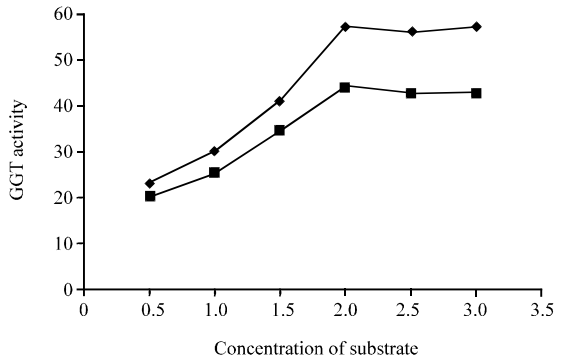

Fig. 2: Michaelis-Menten plot for serum GGT with gamma-glutamyl p-nitroanilide as substrate and L-threonine as inhibitor

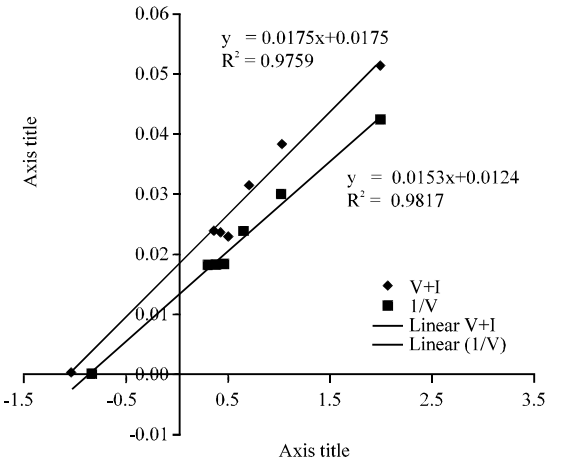

Fig. 3: Lineweaver-Burk plot for serum GGT with γ-glutamyl p-nitroanilide as substrate and L-threonine as inhibitor

Experimental work has mostly used rats who have substantially lower serum and hepatic GGT activity than humans (Teschke *et al*., 1983), found 20 fold higher values in normal humans than in control rats. As so much research is based on these animals, the assumption that the difference is purely quantitative is rather a crucial one.

A sensitive method for measurement of GGT activity in needle biopsy samples was developed by Satoh *et al*. (1980) found that in 57 patients with a variety of liver diseases either serum or hepatic GGT or both or neither could be elevated. Among those patients with both serum and liver GGT increased, higher values were found in alcoholic or drug-induced hepatitis than in viral hepatitis and in the former group serum GGT showed a greater increase above normal (approximately 6 fold) than liver GGT (around 3 fold).

In the study with S.GGT, it was reported that for gamma-glutamyl p-nitroanilide and glycyglycin, the Km values were 1.9 and 12.0 mM, respectively (Rosalki and Tarlow, 1974). The Km value of human liver GGT, when gamma-glutamyl p-nitroanilide was used as the substrate was reported as 1.4 mM and for kidney it was 1.2 mM (Shaw *et al*., 1978).

It was reported in the literature that L-serine (which is another essential amino acid) inhibits GGT in the presence of borate by interacting with the gamma-glutamyl binding site of the enzyme (Tate and Meister, 1978; Vesely *et al*., 1985). GGT activity is increased in a number of primary and metastatic tumers (Dominici *et al*., 2003). It has been shown that hepatocellular carcinoma can be expressed hepatoma specific GGT and secreted GGT in to circulating blood (Yao *et al*., 1998).

CONCLUSION

The experiment demonstrared that L-threonine amino acid or food that contain this type of amino acid might provide to be a valuable tool for the treatment of liver diseases.

REFERENCES

Benakappa, S. and T.J. Varghese, 2002. Dietary threonine requirement of indian major carp, *Cirrhinus mrigala* (Hamolton), juveniles. Israeli J. Aquaqult., 54: 183-188.

Curthoys, N.P. and R.P. Hughey, 1979. Characterization and physiological function of rat renal gamma-glutamyltranspeptidase. Enzyme, 24: 383-403.

Dominici, S., L. Pieri, M. Comporti and A. Pompella, 2003. Possible role of membrane gamma-glutamyltransferase activity in the facilitation of transiferrin-dependent and independent iron uptake by cancer cells. Cancer Cell Int., Vol. 3. 10.1186/1475-2867-3-7.

Elce, J.S. and B. Broxeyer, 1976. γ-glutamyl transferase of rat kidney. Simultaneous assayof the hydrolysis and transfer reaction with (glutamate-$_{14}$C) glutathione. Biochem. J., 153: 223-232.

Hanigan, M.H., B.C. Gallagher, D.M. Townsend and V. Gabarra, 1999. γ-glutamyltranspeptidase accelerates tumor growth and increase the resistance of tumor to cisplatin *in vivo*. Carcinogenesis, 20: 553-559.

Huseby, N.E., 1982. Multiple forms of serum γ-glutamyltransferase: Association of the enzyme with lipoproteins. Clin. Chem. Acta, 124: 103-112.

Lee, D.S., J.C. Evans, S.J. Robins, P.W. Wilson and I. Albano *et al*., 2007. Gamma glutamyltransferase and metabolic syndrome, cardiovascular disease, and mortality risk. Arteriosc., Thromb. Vasc. Thromb., 27: 127-133.

Lowry, M.H., B.P. McAllister, J.C. Jean, L.A. Brown and R.P. Hughey *et al*., 2008. Lung lining fluid glutathione attenuates IL-13-induced asthma. Am. J. Respir. Cell Mol. Biol., 38: 509-516.

Moss, G.P., 1984. Nomenccature and symbolism for amino acids and peptides. Pure Appl. Chem., 56: 595-624.

Nemesanzky, E. and J.A. Lott, 1985. γ-glutamyltransferase and its isoenzymes: Progress and problems. Clin. Chem., 31: 797-803.

Rosalki, S.B. and D. Tarlow, 1974. Optimaized determination of γ-glutamyltransferase by reaction rate analysis. Clin. Chem., 20: 1121-1124.

Satoh, T., M. Takenaga , H. Kitagawa and S. Itoh, 1980. Microassay of γ-glutamyltranspeptidase in needle biopsies of human liver. Res. Commun. Chem. Pathol. Pharmarmacol., 30: 151-161.

Sener, A., 1997. γ-glutamyltransferase. Biyokomya Dergisi, 22: 27-33.

Shaw, L.M., J.W. London and L.E. Petersen, 1978. Isolation of γ-glutamyltransferase from human liver and comparison with the enzyme from human kidney. Clin. Chem., 24: 905-915.

Szaz, G., 1976. A kinetic photometric method for serum gamma-glutamyl transpeptidase. Clin. Chem., 15: 124-136.

Tate, S.S. and A. Meister, 1978. Serine borate complex as a transition state inhibitor of γ-glutamyltranspeptidase. Proc. Natl. Acad. Sci. 75: 4806-4809.

Teschke, R., M. Neuefeind, M. Nishimurm and G. Strohmeyer, 1983. Hepatic γ-glutamyltransferase activity in alcoholic fatty liver: comparison with other liver enzymes in man and rats. Gut, 24: 625-630.

Vesely, J., V. Lisy and M. Cernoch, 1985. Partial purification of γ-glutamyltransferase from human brain microvessels. Neurochem. Res., 10: 1325-1334.

Wang, X., S. Qiao, Y. Yin, L. Yue, Z. Wang and G. Wu, 2007. Adeficiency or excess of dietary threonine reduces protein synthesis in jejunum and skeletal muscle of young pigs. J. Nutr., 137: 1442-1446.

Wenham, P.R., D.B. Horn and A.F. Smith, 1984. Physical properties of γ-glutamyltransferase in human serum. Clin. Chem. Acta, 141: 205-218.

Whitifield, J.B., 2001. γ-glutamyltransferase. Crit. Rev. Clin. Lab Sci., 38: 261-355.

Wickham, S., M.B. West, P.F. Cook and M.H. Hanigan, 2011. γ-glutamyl compounds: Substrate specificity of γ-glutamyltranspeptidase enzyme. Anal. Biochem., 414: 208-214.

Yao, D.F., Z.W. Huang, S.Z. Chen, J.F. Huang, J.X. Lu, M.B. Xiao and X.Y. Meng, 1998. Diagnosis of hepatocellular carcinoma by quantitative detection of hepatoma specific bonds of serum γ-glutamyltransferase. Am. J. Clin. Pathol., 110: 743-749.