SERUM PROLYL HYDROXYLASE ACTIVIY IN THE CLINICAL COURSE OF VIRAL HEPATITIS

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SUMMARY: Prolyl hydroxylase activity was measured in the serum of patients with viral hepatitis and healthy individuals. Serum prolyl hydroxylase levels were maximally raised in patients with viral hepatitis during the active stage of the disease. Themcan 4396 ± 1658 cpm/min/ml compared to the values of a comparable control group (995 ± 591 cpm/min/ml). The difference between the two was statistically important ($p<0.05$). 8-12 weeks later this difference was no longer significant ($p>0.05$). It is important to note that prolyl hydroxylase activity during the course of the disease revealed a similar trend to those of transaminases and serum bilirubin levels.

Key Words: Viral hepatitis, Prolyl hydroxlase, alanine transaminase, serum bilirubin.

INTRODUCTION

The enzyme prolyl hydroxylase catalyses the hydroxylation of prolyl residues to hydroxyprolyl residues during collagen synthesis (1-4). The liver tissue contains considerable amount of the enzyme and the significant increase in enzyme activity has been reported in many experimental clinical liver disorders associated with injury - induced damage (5-11). It would seem that the liver is the most likely source of prolyl hydroxylase activity in serum (12,13). Serum activity of this enzyme was found elevated in certain conditions affecting the liver, such as hepatocellular carcinoma, experimental liver fibrosis and experimental acute liver injury induced by dimethylnitrosamine and carbon tetrachloride (12-15).

The changes of serum activity of prolyl hydroxylase in viral hepatitis has been studied in only a few instances (12,13). Therefore we studied the serum activity of this enzyme with the clinical and laboratory follow up of viral hepatitis.

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MATERIALS AND METHODS

Twelve patients with viral hepatitis (5 males and 7 females) were included in the study. The age of the patients ranged from 18 to 50 years. Those who had any other disease and abuse of alcohol were excluded from the study. The serum samples were taken with regular intervals during the illness period. HB_sAg was found to be positive in four patients. HB_sAg was found to be positive in four patients. The control sera were obtained from 20 healthy volunteers of either sexes aged from 20 to 60 years.

To assay the serum prolyl hydroxylase activity, a nonradioactive substrate and α-ketoglutarate -1-C^{14} were used. In this method for each equivalent of proline, one equivalent of $14CO_2$ is liberated. 0.25 ml of serum was incubated with agitation at 30°C for 1 hour in a final volume of 1 ml containing 100.000 cpm

of 1-^{14}C-α-ketoglutarate (New England Nuclear), 250 μg of the synthetic substrate poly (L-prolyl-glycyl-L-proline)$_n$, (Milesyeda - Israel), 30 μg of catalase, 10^{-6} M L - ascorbic acid, 10^{-6} M Fe ammonium sulphate, $5x10^{-5}$ M Tris-HCI (pH 7.2) and 1 mg of denaturated bovine serum albumin. The incubation was carried out in an erlen with sidearm (Contes Co., USA). For the transferring and trapping of $^{14}CO_2$ sidearm of incubation erlen and the counting vial was connected with a short glass' tube and filter paper impregnated 10% KOH were placed in the counting vial. At the end of incubation, 10 ml of Bray's solution was immediately added into counting vial and kept in dark for 1 hour. Then $^{14}CO_2$ radioactivity was counted by a liquid scintillation spectrometer (16,17).

Table 1: Serum alanine transaminase and bilirubine levels (Mean ± SD) during the course of the disease.

Serum sampling time in the illness period	Alain Transaminase activity Mean ± SD	Serum bilirubin mg/dl Mean ± SD
1 - 2 weeks	771 ± 411 n=12	7.8 ± 5.7 n=10
3 - 4 weeks	375 ± 172 n=12	6.0 ± 5.0 n=12
5 - 6 weeks	208 ± 127 n=10	5.1 ± 5.7 n=10
8 - 12 weeks	54 ± 52 n=10	1.4 ± 1.2 n=12

Table 2: Serum prolyl hydroxylase activity in the control group and the patients with viral hepatitis during the course of the disease.

		ILLNESS PERIOD			
Prolylhydroxylase activity cpm/min/ml	Control group	1-2 weeks	3-4 weeks	5-6 weeks	8-12 weeks
Mean ± SD	995±591	4326±1658	2976±1426	1538±713	986±542
Range	0-1918 n=20	2104-6682 n=12	1025-4807 n=12	903-3155 n=10	375-1886 n=12

RESULTS

Table 2 shows the serum enzyme levels expressed as cpm/min/ml in the patients and the controls. The activity of serum prolyl hydroxylase was found very high in the first two weeks. The enzyme began to decrease in the 3-4 weeks and returned to normal values in the 7-8 weeks. The changes of serum prolyl hydroylase activity were similar to that of transaminases (Table 1 and Table 2). In statistically, prolyl hydroxylase activity in each sample compared to consecutive sampling and control group was significantly different ($P<0.05$).

DISCUSSION

Prolyl hydroxylase enzyme is present in many tissues and its level is related to the rate of collagen synthesis in those tissues (1,7,10,18,19). The large increases in enzyme activity have been found in a number of experimental and clinical disorders of the liver which are accompanied by the accelerated collagen formation (7,9,11,14, 18-21). These studies indicated that increase in prolyl hydroxylase activity occurred more earlier and to a greater extent than the increase in collagen hydroxyprolyl (7,21).

The presence of prolyl hydroxylase enzyme has been reported in rat and human serum, although its level is relatively low and has failed to be detected in some studies (12,13,15). Elevated serum levels of the enzyme were found in patients with hepatocellular carcinoma, tumors metastatic to the liver and receiving methoxyflurane or halothane anesthesia (12,13). Increase in serum prolyl hydroxlase activity has also been determined in patients with viral hepatitis (12,13). But the changes of the enzyme levels in serum had not been followed previously in the course of the disease.

The results of this investigation indicate that prolyl hydroxylase levels in serum were maximally raised in active stage of viral hepatitis, the changes of the enzyme during the course of the disease were similar to the transaminases and bilirubin.

The mechanisms, involved increase in prolyl hydroxylase activity in serum during the acute liver injury are not known in detail. Some studies indicated that prolyl hydroxylase enzyme in tissues is present partly as an inactive proenzyme (20). It was also concluded that the leakage of intracellular enzymes into serum due to liver cell injury may cause the elevated of serum enzyme levels (13, 15).

Since the high levels of serum prolyl hydroxylase in patients with viral hepatitis, determination of prolyl hydroxylase activity during the course of the disease may provide a useful biochemical indication of disease activity.

REFERENCES

1. Bornstein P: The biosynthesis of collagen. Annu Rev Biochem 43:567, 1974.

2. Grant ME, Prockop DJ : The biosynthesis of collagen (part I). N Eng J Med 286:194, 1972.

3. Grant ME, Prockop DJ : The biosynthesis of collagen (second of three parts), N Eng J Med 286 (5) : 242, 1972.

4. Takeuchi T, Kivirikko KI, Prockop DJ : Increased protocologen hydroxylase activity in the livers of rats with hepatic fibrosis. Biochem Biophys Res Commun 28(6): 940, 1967.

5. Grand ME, Prockop DJ : The biosynthesis of collagen (part III), N Eng J Med 286:250, 1972.

6. Guzelian PS, Diegelmann RF: Localization of collagen prolyl hydroxylase to the hepatocyte. Exp Cell Res 123:269, 1969.

7. Takeuchi T, Prockop DJ: Protocollagen proline hydroxylase in normal liver and in hepatic fibrosis. Gastroenterology 56(4):744, 1969.

8. Mc Gee JO'D, Patrick RS, Rodger MC, Lity CM: Collagen proline hydroxylase activity and ^{35}S sulphate uptake in human liver biopsies. Gut 15:260, 1974.

9. Activities of prolyl hydroxylase, lysyl hydroxylase, collagen galactosyltransferase and collagen glucosyltransferase in the liver of rats with hepatic injury. Biochem J 144:115, 1974.

10. Fuller GC, Nolan JC, Fink S, Rodil JV : Prolyl hydroxylase activity in normal diseased human liver. Proc Soc Exp Biol Med 151:61, 1976.

11. Mann SW, Fuller GC, Rodil JV, Vidins EI: Hepatic prolyl hydroxylase and collagen synthesis in patients with alcoholic liver disease. Gut 20:825, 1979.

12. Stein HD, Keiser HR, Sjoerdsma A : Proline hydroxylase activity in human blood. Lancet I:106, 1970.

13. Keiser HR, Vogel CI, Sadikali F: Protocollagen proline hydroxylase in sera of Ugandans with hepatocolluler carcinoma. J Nat Can Ins. 49(5):1251, 1972.

14. Mc Gee JO'D, Hare RPO, Patrick RS : Stimulation of the collagen biosynthetic pathway by factors isolated from experimentally injured liver. Nature (New Biol) 243:121, 1973.

15. Risteli J, Tuderman L, Tryggvason K, Kivirikko KI: Effect of hepatic injury on prolyl 3-hydroxylase and 4-hydroxylase activities in rat liver and on immunoreactive prolyl 4-hydroxylase concentration in the liver and serum. Biochem J 170:129, 1978.

16. Robert RE, Roberts EN, Udenfriend S: Collagen proline hydroxylase (rat skin) Methods in Enzyme Ed Tabor H, Tabor CW, Acad Press. New York London 27 (B): 306, 1971.

17. Tietz NW : Fundementals of Clinical Chemistry, WB Saunders Co Philadelphia London Toronto 184, 1976.

18. Mak KM, Leo MA, Lieder CS: Alcoholic liver injury in baboons. Transformation of lipocytes to transitional cells. Gastroenterology, 87:188, 1984.

19. Nakano M, Lieter CS : Ultrastructure of initial stage of perivenular fibrosis in alcohol-fed baboons. Am J Pathol 106:145, 1982.

20. Risteli J, Tuderman L, Kivirikko KI: Intracellular enzymes of collagen biosynthesis in rat liver as a function of age and in hepatic injury induced by dimethylnitrosamine. Biochem J 158:369, 1976.

21. Risteli J, Kivirikko KI : Intracellular enzyme of collagen biosynthesis in rat as a function age and in hepatic injury induced by dimethylnitrosamine. Biochem J 158:361, 1976.

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