Inhibitors of collagenolytic enzymes in the serum of patients with chronic liver disorders

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SUMMARY Inhibitory effect of serum on collagenolysis was studied in rat hepatic slices. Sera of patients with cirrhosis contained low concentrations of inhibitors. Higher concentrations were found in sera from patients with chronic active hepatitis and chronic persistent hepatitis, than in healthy individuals. It is suggested that changes of the serum inhibitory activity are responsible for the control of collagen degradation in the injured liver.

Chronic hepatic disorders are associated with accumulation of excess of collagen in the liver and hepatic fibrosis is important in the development of serious hepatic dysfunction.¹⁻³ Collagen degrading enzymes in fibrotic hepatic tissue have been studied by numerous investigators. It is generally accepted that collagen degradation is raised under conditions of fibrosis. Increased activity of collagenase,^{4 5} collagen peptidase^{6 7} and collagenolytic cathepsin^{8 9} has been described in the liver. It has been suggested that increased degradation of collagen is a defensive mechanism against fibrosis, but it is usually insufficient to successfully diminish the fibrotic process.^{8 10}

The mechanism of accelerated degradation of collagen fibres in the liver is unclear. Degradation of collagen needs the controlled action of a group of specific enzymes capable of selectively cleaving this fibrous protein. It was found that non-specific proteolytic enzymes were able to activate collagenase – the main enzyme responsible for cleavage of collagen. The nature of this phenomenon is controversial. Proteolytic removal of the inhibitor from enzyme-inhibitor complex, or proteolytic activation of procollagenase, have been suggested.^{11 12}

Recently increased activity of collagenase and changes of active to proteolytically activated enzyme ratio have been shown in hepatic tissue of rats chronically treated with carbon tetrachloride.¹⁰ In the present study an attempt was made to estimate the collagenolysis inhibitory activity of the blood serum from patients with chronic liver disorders.

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Methods

PATIENTS

The study was done on 25 patients with hepatic cirrhosis (16 men: aged 43–61 years). Seventeen had chronic active hepatitis (eight women, nine men: aged 39–56 years), 23 had chronic persistent hepatitis (13 women, 10 men: aged 42–55 years), and 30 healthy individuals (23 men: aged 32–53 years). Blood was collected at 7 am after 10 hours of fasting. The sera were diluted with physiological saline in order to normalise the total protein concentration to 50 g/l.

ANALYSIS

Wistar rat liver slices prepared as for tissue culture were used.¹³ It was found that these conditions were adequate for the investigations on living hepatic slices.¹⁴ Collagen degrading enzyme activity was measured as hydroxyproline concentration liberated from collagen by enzymes presented in hepatic slices. Collagen degradation was determined according to Sopata *et al.*¹⁵ In brief, rat hepatic slices (500 mg), reconstituted polymerised collagen fibres (5 mg), serum (0.5 ml) and 0.05M Tris-HCl buffer pH=7.5 containing 0.005M CaCl₂ and 0.2M NaCl were incubated for 18 h at 37°C. Control samples consisted of the same substances with potassium cyanate (0.05M) and collagenase inhibitor N-methyl maleimide (NMM), 1.25 µM. Control samples were used to allow for non-enzymatic liberation of hydroxyproline from collagen and to exclude the effect of the presence of hydroxyproline in the tested serum. Hydroxyproline was determined in the supernatant after acid hydrolysis with the

method of Drózd *et al*¹⁶ based on Stegemann's reaction.¹⁷ Similar estimations were carried out in the same mixture, though it did not contain serum.

The results were calculated as measured values minus control values (sample contained potassium cyanate). The following mean control values were obtained: normal subjects 0.012; cirrhosis 0.033; persistent hepatitis 0.018; active hepatitis 0.020 $\mu\text{mol/sample}$. The inhibiting activity was calculated as percentage of hydroxyproline enzymatically liberated from collagen substrate in the mixture with serum, compared with samples without serum. Each serum sample was measured in triplicate and the mean value was calculated.

Results are presented as mean $\pm$ SEM. Statistical analysis was performed with Student's *t* test.

Results

The measurements of inhibitory activity are in the Table. A significant decrease of inhibition was found in cirrhosis. By contrast, a significant rise was recorded in the patients suffering from chronic active or persistent hepatitis. Higher values were recorded in the patients with chronic active, than in those with chronic persistent hepatitis.

Discussion

Human plasma contains numerous inhibitors of proteolytic enzymes. Most of them are synthesised in the liver. It is believed that the inhibitors are produced as acute phase reaction to protect tissues against lysosomal enzyme-induced injury.¹⁸ Changes in the serum concentration of non-specific protease inhibitor have been described previously,

but the results were contradictory. Normal concentrations of alpha 1-antitrypsin in cirrhotic patients was reported by Sharp *et al*,¹⁹ and Aschauer *et al*.²⁰

Theodoropoulos *et al*²¹ and Bogdal *et al*²² described increased concentration of serum alpha 1-antitrypsin in chronic hepatic disorders. Alpha 1-antitrypsin does not inhibit collagenase. The specific inhibitor of collagenase was isolated from serum by Woolley *et al*²³ and described as beta 1-anticollagenase. The role of this protein in the control of collagen degradation is still unclear.

In the present study decreased inhibitory activity was found in sera from patients with cirrhosis. These changes were probably connected with hepatic cell failure. Increased inhibitory activity in sera from patients with chronic hepatitis can be explained as an acute-phase reaction to chronic inflammation.

It is difficult to determine the details of the described effect of sera upon collagen degradation, because substances originating from the liver samples and the sera acted simultaneously. The phenomena observed may depend on the action of the serum on proteolytic activation of collagenase, or on direct inhibition of the enzyme by serum proteins. Thus direct, or indirect mechanism can exist. Another limitation stems from the use of an exogenous substrate – the rat liver slices – for investigation of human sera. Substrate specificity of collagenase, as well as quantitative changes of the collagen fibres in the course of hepatic fibrosis, are still subjects of controversy. All these limitations have to be taken into consideration in the interpretation of the results.

The place of the present observations in the natural history of hepatic fibrosis is unclear. It is possible that the decreased serum inhibitory activity is connected with elevated collagen degradation in the liver.

This mechanism may act against collagen accumulation (hepatic self-defensive reaction) as was suggested previously.¹⁰ Further investigations, including studies with purified degrading enzyme preparations, are needed.

Table Inhibiting activity of serum on collagen degrading enzymes

	Hydroxyproline release* $\mu\text{mol/sample}$		inhibition %
	Without serum	With serum	
Healthy individuals n=30	6.21 $\pm$ 0.87	2.66 $\pm$ 0.63	57.17 $\pm$ 3.42
Cirrhosis n=25	6.28 $\pm$ 0.75	4.47 $\pm$ 0.94	28.82 $\pm$ 5.41†
Chronic persistent hepatitis n=23	6.15 $\pm$ 0.74	2.11 $\pm$ 1.05	65.69 $\pm$ 4.75†
Chronic active hepatitis n=17	6.15 $\pm$ 0.81	1.77 $\pm$ 1.12	71.22 $\pm$ 4.03†

* Obtained by calculating: determined values minus control containing sample potassium cyanate.

† $p < 0.001$ compared with controls.

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