Desmosine and isodesmosine contents and elastase activity in normal and cirrhotic rat liver

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The contents of desmosine and isodesmosine, the cross-linking amino acids of elastin, were increased 4-fold in rat liver with carbon tetrachloride-induced cirrhosis, which suggests that insoluble elastin accumulates in cirrhosis. Elastase activity in the cirrhotic liver, as determined with 3-carboxypropionyl-L-alanyl-L-alanyl-L-alanine *p*-nitroanilide, was 17% less than in the normal liver; no change was found when Congo Red–elastin was used as a substrate.

The amount of elastic fibres was found by morphological methods to be greatly increased in human livers with various fibrotic and cirrhotic processes. The increase was observed in the liver capsule, the portal spaces, fibrous bands and the walls of portal and systemic veins when stains specific for elastic tissue were used (Liban & Ungar, 1959). Thung & Gerber (1982) detected elastic fibres in livers with massive hepatic necrosis. There was a good correlation between the amount of elastic fibres and the duration of the illness.

Popper & Lieber (1980) noted formation of elastic fibres in the liver of baboons given an ethanol-containing diet for 6 years. Elastic fibres appeared in thick connective-tissue septa in late phases of alcoholic disease. Shikata & Sakai (1974) isolated elastin from human livers by boiling the tissue in 0.1 M-NaOH and found that the ratio elastin/collagen increased in cirrhosis.

The content of hepatic hydroxyproline insoluble in 0.1 M-NaOH was increased in carbon tetrachloride-treated rats. Elastic fibres were observed in the septa surrounding liver pseudolobules (Kanta & Bartoš, 1978). Carbon tetrachloride-induced liver cirrhosis thus seemed to be a good model to study this feature of human liver cirrhosis.

Desmosine and isodesmosine are amino acid cross-links found only in elastin (Starcher, 1982). The determination of desmosine and isodesmosine contents in tissues can thus reveal elastin accumulation. Elastin is extremely resistant to a proteolytic attack and is degraded only by elastases (Baici, 1980). The determination of elastase activity in the liver is therefore pertinent when elastin accumulation is studied.

Materials and methods

Female Wistar rats weighed 120–130 g at the beginning of the experiment. They received 0.5 ml of carbon tetrachloride in 1 ml of olive oil/kg body wt. intragastrically twice weekly. Control rats were given olive oil only. The rats were treated with carbon tetrachloride for 17 weeks and then left without treatment for 2 weeks.

Hydroxyproline content of the liver was determined as described by Stegemann (1958), to evaluate the extent of fibrosis. The mean value was 3.6 μ mol/g of tissue in the normal liver and 12.3 μ mol/g in the cirrhotic liver.

To determine the contents of desmosine and isodesmosine, the livers were homogenized in 0.15 M-NaCl. The insoluble part was suspended in hot 0.1 M-NaOH, kept in a boiling-water bath for 15 min and centrifuged at 20000 g for 30 min. The heating with 0.1 M-NaOH was repeated three times (Lansing *et al.*, 1952). After washing with distilled water, the residue was dried at 90°C, weighed and hydrolysed in 6 M-HCl for 72 h at 105°C.

Samples corresponding to 4 mg of the insoluble residue were applied on the columns (62 cm $\times$ 0.8 cm and 6 cm $\times$ 0.7 cm) of Ostion LG AN ion-exchange resin (Chemapol, Prague, Czechoslovakia) in a type 881 amino acid analyser (Mikrotechna, Prague, Czechoslovakia). The elution was performed with citrate buffers of pH 3.25, 4.25 and 5.28, consecutively. The columns were maintained at 53°C.

Elastase activity was determined with 3-carboxypropionyl-L-alanyl-L-alanyl-L-alanine *p*-nitroanilide as described by Kasafírek *et al.* (1976) and Bartík & Kasafírek (1980). The livers were homogenized in

0.05 M-Tris/HCl buffer, pH 8.0, and centrifuged at 10000g. The release of *p*-nitroaniline into solution caused by the activity in the supernatant was measured at 405 nm.

A portion of the liver was homogenized in 5 mM-sodium phosphate buffer, pH 7.4, and centrifuged at 10000g before the determination of elastase activity with Congo Red–elastin suspension. The assay was performed as described by Shotton (1970). The release of Congo Red into the incubation medium was measured at 595 nm.

Results and discussion

Table 1 shows the contents of desmosine and isodesmosine in normal and cirrhotic livers. The individual experiments included six to nine rats. The amount of elastin cross-links in the cirrhotic liver was severalfold higher than in the normal liver in each of the experiments. The increase was more than 4-fold on average when the results were expressed per g wet wt. of tissue. Since water content in the cirrhotic liver (73%) was only slightly higher than in the normal liver (69%), it did not influence the results significantly.

As desmosine contents are constant within species, they can be used to quantify mature insoluble elastin. Moreover, elastin is the only known protein to contain this cross-linking amino acid, with the exception of egg-shell membrane (Starcher, 1982). We can therefore suppose that the elastic fibres found previously by morphological methods in human cirrhotic liver (Liban & Ungar, 1959; Shikata & Sakai, 1974; Thung & Gerber, 1982) as well as in the liver of experimental animals (Kanta & Bartoš, 1978; Popper & Lieber, 1980) contain elastin.

Increased synthesis as well as decreased breakdown can cause elastin accumulation in the liver. Table 2 shows elastase activity in the liver as determined with two independent methods. Elastase activity in the cirrhotic liver was 17% lower than in the normal liver when 3-carboxypropionyl-L-alanyl-L-alanyl-L-alanine *p*-nitroanilide was used as substrate. No change was seen when Congo Red–elastin was used. Elastase activity thus seems to change very little in cirrhosis; increased formation of insoluble elastin is probably responsible for its accumulation in liver tissue. Neither of the two methods used in the present study is absolutely specific for elastase (Shotton, 1970; Stone *et al.*, 1982). It is also well-known that the properties of elastases in various cells and tissues differ (Ashe & Zimmerman, 1977; Banda & Werb, 1981). The activity and the properties of elastase, in respect of different cell types present in the cirrhotic liver, require further study.

Table 1. *Desmosine and isodesmosine contents in the liver*

The contents of desmosine and isodesmosine were determined in three independent experiments. The numbers of rats whose livers were pooled in the individual experiments are given in parentheses. The results of individual determinations are given together with means $\pm$ S.E.M. Asterisks indicate statistically significant results ($P < 0.05$).

Expt. no.	Desmosine (mmol/g of liver)	Isodesmosine (mmol/g of liver)
Control 1	0.35	0.26 (7)
2	1.33	1.39 (9)
3	0.53	0.44 (9)
Mean $\pm$ S.E.M.	0.74 $\pm$ 0.30	0.70 $\pm$ 0.35
Cirrhosis 1	1.94	2.12 (6)
2	5.58	4.82 (7)
3	3.13	3.41 (7)
Mean $\pm$ S.E.M.	3.55 $\pm$ 1.07*	3.45 $\pm$ 0.78*

Table 2. *Elastase activity in the liver as determined with A synthetic substrate and with Congo Red–elastin*

Means $\pm$ S.E.M. are given. Numbers of rats are shown in parentheses. Asterisk indicates a statistically significant result ($P < 0.05$).

	3-Carboxypropionyl-L-alanyl-L-alanyl-L-alanine <i>p</i> -nitroanilide (nkat/g of liver)	Congo Red–elastin (μ mol of Congo Red released/g of liver per g of substrate)
Control	996 $\pm$ 54 (15)	224 $\pm$ 29 (7)
Cirrhosis	831 $\pm$ 45 (13)*	224 $\pm$ 27 (6)

The presence of elastic fibres in hepatic fibrosis, particularly in cirrhosis, was repeatedly reported in the past. Although some of the publications have been almost forgotten, it is possible that elastin is a critical component in hepatic fibrosis (Popper & Piez, 1977). The changes in rat liver produced by carbon tetrachloride treatment may be a suitable model to study the role of elastin in liver cirrhosis.

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