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Significance of β-Glucuronidase and Glucose-6-Phosphatase Dehydrogenase in Liver Injury and its Protection in Rats

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The status of β-glucuronidase and glucose-6-phosphatase dehydrogenase in liver injury has been studied in carbon tetrachloride treated rats after protection with agents like metallic zinc, vitamin B_{12} and glutathione. Observations on β-glucuronidase has also been discussed with changes in serum bilirubin.

Key Words: **β-glucuronidase, Glucose-6-phosphatase dehydrogenase liver, CCl_4, Rat**

Introduction

β-glucuronidase (EC 3.2.1.31) has a particular function in the metabolism of drugs and toxic compounds (Fishman et al. 1967, Williams 1959). A high enzymatic activity has been correlated with growth and tissue proliferation (Fishman et al. 1969), whereas the administration of various hepatotoxins is known to induce the activity of glucose-6-phosphatase dehydrogenase (EC 1.1.1.49) (Watanbe et al. 1976). These reports suggest that they serve as useful markers of liver injury. Conversely, they form a suitable parameter for liver protecting agents. Several dietary factors are known to influence CCl_4 toxicity (McLean & McLean 1969, Marchand et al. 1970 and Seawright & McLean 1967). Necrotropic behaviour of zinc, vitamin B_{12} and glutathione against CCl_4 toxicity as studied earlier (1981a, b) also shows their suitability as liver protecting agents. To confirm further, their effects on biochemical lesions, present study was made in the liver of CCl_4 poisoned rats, following protection with zinc, vitamin B_{12} and glutathione.

Since bilirubin, is an esterglucuronide, relationship between β-glucuronidase and serum bilirubin has also been studied.

Materials and Methods

Fifty albino rats *(Rattus rattus* albino), 90 days old, weighing 150 ± 10 gms were randomly selected from the laboratory stock. They were divided into five groups, each containing ten rats. Each rat was housed separately in a suitable cage, fed on a pelletted diet (Hindustan Lever Ltd., Bombay), tap water *ad libitum* and maintained under standard laboratory conditions.

Rats of first three groups, i.e. A, B and C were injected intramuscularly with 0.5 ml of CCl_4 (2% in olive oil) on each alternate day for 15 days. After 15 days the rats of group 'A' were orally fed (by gavage) on zinc acetate (0.1 gm/150 gms of body weight). Rats of group B were injected with 0.5 ml of vitamin B_{12} (each ml contained cyanocobalamin anhydrous as cyanocobalamin IP (1000 mg) and phenol as preservative (0.5% w/v). The rats of group 'C' were intramuscularly injected 0.2 ml (1% w/v in distilled water) of glutathione. The rats of group A, B and C received these treatments on alternate days for last 15 days following the period of CCl_4 administration. The rats of group 'D' were injected with 0.5 ml of 2% CCl_4 in olive oil on each alternate day for the last fifteen days. Rats of group 'E' received 0.5 ml of the olive oil

alone in the same manner and served as controls. All rats were starved for 24 hours and then sacrificed by decapitation. A portion of the liver (0.5 g) was homogenized gently in all glass homogenizer in cold 0.25 M sucrose/0.01 M phosphate buffer (*p*H 7.4), 1:7 (w/v). The activity of β-glucuronidase between the fraction sedimenting with the 15,000 g pellet (bound enzyme) and fraction present in the supernatant (free enzyme) was used to mark lysosomal stability as suggested by Fishman et al. (1967). Phenolphthalein glucuronide (BDH, England) was used as a substrate. The reaction mixture consisted of 0.1 ml sample, 0.1 ml substrate solution (0.01 M; *p*H 5.0), 0.8 ml acetate buffer (0.1 M; *p*H 4.5), 2.5 ml of alkaline glycine solution, 1.0 ml trichloroacetic acid solution (5% w/v) and 6.0 ml of distilled water. 1% solution of phenolphthalein was used as standard. Optical densities were read at Systronyx Spectrophotometer at 540 nm and enzyme activity was calculated in Fishman Units.

G-6-PDH activity was measured spectrophotometrically at 340 nm in terms of the rate of NADPH formation at 37°C (Lohr & Waller 1961). The reaction mixture consisted of 0.9 ml of 0.25 M glycylglycine buffer (*p*H 7.6), 0.07 ml of 0.1 M $MgCl_2$, 0.01 ml of 0.011 nM $NADP^+$. The supernatant fraction from 10% liver homogenate in 0.25 M sucrose (*p*H 7.6) was prepared by centrifugation at 30000 g for 60 min at 4°C. As used here, one unit of enzyme activity is equivalent to 1.0 μ mol of substrate transformation per minute at 37°C. The molar extinction coefficient on NADPH was taken to be 6300 M^{-1} cm^{-1}.

Protein was determined according to Lowry et al. (1951) with crystalline bovine serum albumin. Total serum bilirubin was estimated applying the method of White et al. (1958). The student 't' test (Fisher 1950) was applied to calculate the statistical significance between control and experimental values.

Results and Discussion

Enhanced activities of β-glucuronidase and glucose-6-phosphatase dehydrogenase were recorded in the liver after CCl_4 treatment (tables 1 & 2). Both these enzymes are known to increase during various liver diseases as well as in experimentally

Table 1 *β-Glucuronidase in the liver of experimental rats*

Group	β-Glucuronidase			
	Bound (OD/hr/g)	Level of significance	Free (% Total)	Level of significance
Control	675 ± 76	–	28.00 ± 2.50	–
CCl_4	498 ± 22	P<0.05	44.30 ± 3.90	P<0.05
CCl_4 + Zinc	615 ± 105	N.S.	35.60 ± 2.80	N.S.
CCl_4 + Glutathione	688 ± 64	P<0.05	30.20 ± 2.50	P<0.05
CCl_4 + Vitamin B_{12}	601 ± 34	N.S.	32.10 ± 2.00	P<0.05

Table 2 *Glucose-6-phosphate dehydrogenase in the liver of experimental rats*

Treatment	Glucose-6-phosphate dehydrogenase (Bucher Units/gm of liver)		
	Unit	Level of significance	% Alteration
Control	42.00 ± 5.0	—	—
CCl_4	79.00 ± 8.0	P<0.01	88.09(+)
CCl_4 + Zinc	55.00 ± 7.0	P<0.05	30.37(−)
CCl_4 + Glutathione	52.00 ± 4.0	P<0.05	34.17 (−)
CCl_4 + Vitamin B_{12}	49.00 ± 6.0	P<0.05	37.97 (−)

Values are mean ± Standard error (3 observations). % Alteration and significance difference by (Fisher's Student 't' test) were determined for CCl_4 treated rats v/s controls and zinc glutathione and vitamin B_{12} protected rats v/s CCl_4 treated rats (−) indicate % inhibition and (+) indicate % stimulation. N.S., indicates not significant.

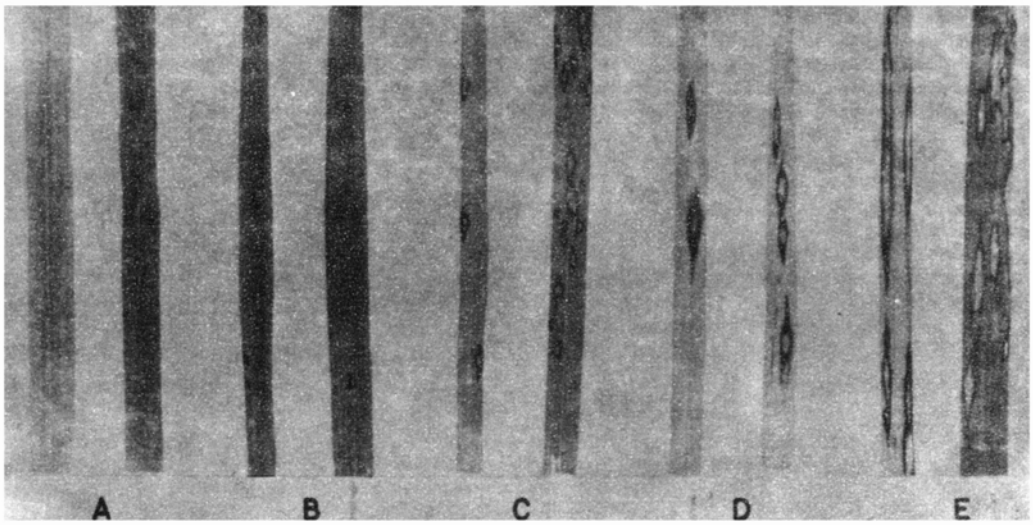

Figure 1 A–E. Bilirubin in the serum of control treated and protected rats. **A**, Controls; **B**, CCl_4 treated; **C**, Zinc protected; **D**, Plutothione protected; and **E**, Vitamin B_{12} protected rats.

induced injuries (Klassen & Plaa 1969, Kroes et al. 1975, Watanbe et al. 1976). The mechanisms involved have also been found different for different drugs/toxins (Mack et al. 1974). Treatment with vitamin B_{12} and glutathione significantly decreased β-glucuronidase level in the liver whereas non-significant effect of zinc was recorded. General mechanism of protection offered by these drugs against CCl_4 toxicity has been discussed at length by Tayal (1983). For β-glucuronidase, the explanation that seems to be valid is the stabilization of bio-membranes specially the lysosomes and endoplasmic reticulum, known to be destabilized by CCl_4 (Recknagel 1967, Dianzani 1978). For vitamin B_{12}, its glycogenetic activity seems to be the appropriate answer. Whereas glutathione participates in detoxication reactions by conjugation between nucleophilic thiol of glutathione and electrophilic site on another molecule.

The increase in G-6-PDH activity after CCl_4 administration can be regarded as a functional change attributed mainly to higher lipid synthesis which in turn is combined with higher rate of NADPH utilisation. A decline in its activity after protection by respective treatments indicates functional improvement in liver parenchyma.

Serum bilirubin in many experimental conditions and diseases reflects parenchymal injury. Therefore, it has been treated as a suitable marker of liver function. Present observations on bilirubin (figure 1) show different levels of protection offered by zinc, vitamin B_{12} and glutathione. Drugs can affect bilirubin metabolism at any stage from its production upto the transport in the blood through the liver, its conjugation and canalicular excretion (Sherlock 1971). CCl_4 aggravates serum bilirubin after affecting microsomal enzymes. Moreover, there is a close correlation between the extent of microsomal lipid peroxidation and extent of degradation of haem by cytochrome P-450 (Levin & Kuntzman 1969). Thus lipid peroxidation and bilirubinemia can be regarded as related phenomenae, however, any correlation between β-glucuronidase and bilirubin is not supported by present observations. A study on UDP-glucuronyl transferase that converts the unconjugated bilirubin into conjugated bilirubin is in progress. Nevertheless, observations on hepatic G-6-PDH are significant and suggest that it may be taken up as a marker of functional capacity of the liver parenchyma.

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