Effect of Carbon Tetrachloride Poisoning on the Plasma Levels of Aspartate-Aminotransferase Isoenzymes in the Rat

A comparative study of aspartate-aminotransferase (AAT) isoenzymes serum levels during liver diseases might provide information on the severity of the underlying cellular injury[1-3]. However, the studies carried out to investigate the behaviour of the serum AAT isoenzymes levels, using acute carbon tetrachloride poisoning as experimental model, have given contradictory results.

In fact, while all the investigators have observed high increase in serum cytoplasmic AAT (AAT I) levels, as far as mitochondrial AAT (AAT II) is concerned, some authors[4] found high increases of its activity in the serum, others little[5] increase, and some[6] no increase at all. Because of these varied results, the behaviour of serum AAT isoenzymes in rats, after acute experimental poisoning with different doses of CCl_4 has been studied. AAT isoenzymes have already been characterized by several methods, such as electrophoresis, enzyme kinetics, chromatography, gel filtration and immunochemistry[1-21]. In the present study, column anion exchanger chromatography was used as characterization technique.

Materials and methods. In all experiments, male Wistar rats, weighing around 250 g, and fed with standard laboratory diet, were used. Without anaesthesia, the rats (6 animals per group and per dose) were treated by gastric intubation with 0.1 and 0.02 ml of carbon tetrachloride per 100 g of body weight, and then sacrificed at 3, 6, 12, 24, 36, 48 and 96 h after the treatment. Chemically pure CCl_4 was diluted with mineral oil. The rats were fasting for 12 h before being sacrificed by rapid exsanguination. Blood was collected in heparinized containers, then centrifuged, and the plasma was employed for enzyme assays.

AAT activity was determined according with Boyde[20]. AAT isoenzymes have been characterized by column anion exchanger chromatography. Columns had 7.5 cm length and 0.9 cm diameter. Absorbent: DEAE-Sephadex A 50 medium. After 6 h dialysis against 0.008 M Na phosphate buffer, pH 7.2, 1 ml of plasma is applied to the column. After the plasma has soaked into the column, elution is started with 10 ml 0.008 M Na phosphate buffer, pH 7.2, and then continued with 10 ml 0.2 M Na phosphate buffer pH 7.2 + 0.2 M NaCl. AAT II is eluted with the first fraction, while AAT I is contained in the second one.

After chromatography, the recovery of AAT was always over 90%. This method is very simple, quick, and well reproducible. A total of 95 animals were studied, including a group of 17 rats that received only mineral oil.

Results and discussion. Results are summarized in Tables I and II. AAT II contributes 21.6% to total

Table I. Activity of AAT isoenzymes in the plasma of rats treated with CCl_4 (0.02 ml/100 g body weight)

Hours after poisoning	0	3	6	12	24	36	48	96
AAT I								
Mean ± S.E.	58 ± 10	70.6 ± 11.1	–	74 ± 8	213 ± 35	213 ± 29	805 ± 121	32.8 ± 2.5
P	–	$P > 0.05$	–	$P > 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P > 0.05$
AAT II								
Mean ± S.E.	16.4 ± 4	24 ± 3.7	–	27 ± 4	30 ± 6	131 ± 18	37 ± 17	17.5 ± 5.3
P	–	$P > 0.05$	–	$P > 0.05$	$P > 0.05$	$P < 0.001$	$P < 0.025$	$P > 0.05$

The data are expressed as mU/ml of plasma

Table II. Activity of AAT isoenzymes in the plasma of rats treated with CCl_4 (0.1 ml/100 g body weight)

Hours after poisoning	0	3	6	12	24	36	48	96
AAT I								
Mean ± S.E.	58 ± 10	88 ± 16	258 ± 43	366 ± 48	6338 ± 658	952 ± 445	1221 ± 203	61.15 ± 7.2
P	–	$P < 0.1$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.02$	$P < 0.001$	$P > 0.05$
AAT II								
Mean ± S.E.	16 ± 4	35 ± 6.7	34 ± 7.4	61 ± 10	949 ± 80	319 ± 111	32 ± 4	22 ± 0.7
P	–	$P < 0.05$	$P < 0.05$	$P < 0.025$	$P < 0.01$	$P < 0.005$	$P < 0.025$	$P > 0.05$

The data are expressed as mU/ml of plasma.

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plasma activity in normal rats, according with FLEISHER and WAKIM[5]. Both the isoenzymes were remarkably increased in plasma, after poisoning with the 2 doses of CCl_4. Increases were directly related to the administered dose of CCl_4, according to the results given by ZIMMERMANN[22].

As far as AAT I is concerned, our data are in agreement with those of the other authors. An increase of plasma levels of AAT II has been also reported by FLEISHER and WAKIM[5], and by GABRIELI and ORFANOS[4], but not by HIRAYAMA et al.[6]. It is possible that negative findings reported by HIRAYAMA et al.[6] depend on a loss of AAT II activity, which is known to be more labile than AAT I[23]. This is supported also by the very low recovery (6–20%) of AAT activity that the authors obtained after the elution. The maximal activity peak of both the isoenzymes appeared in plasma at 24 h after poisoning with 0.1 ml of CCl_4, while with 0.02 ml the highest increase was observed 36 h (AAT II) and 48 h (AAT I), respectively, after the administration of the drug. This suggests that the administration of a high dose of CCl_4 causes a more severe and earlier injury than the administration of a lower dose.

On the other hand, we were able to find a relationship between isoenzyme behaviour and histological changes. These data agree with those of CORNISH and BLOCK[24], but are in contrast with those of DINMAN et al.[25] and ZIMMERMANN et al.[22], who found, respectively in rabbits and in rats, that the dose of CCl_4 has no influence on the time elapsing before the appearance of the peak serum levels of AAT, and of the maximal histological alteration in the liver. These different results may depend on the different animals employed by these authors, and, when rats were used, on the different mode of drug administration[26].

Riassunto. Mediante frazionamento cromatografico su colonna di resina a scambio anionico, abbiamo potuto documentare nel siero di ratti intossicati con dosi diverse di CCl_4, incrementi assai significativi sia della componente mitocondriale che citoplasmatica della aspartato-aminotransferasi.

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An Inheritable Abnormal β-Chain in Rabbit Haemoglobin

By routine use of carboxymethylcellulose column for the separation of α- and β-chain of rabbit haemoglobin[1], we have found an abnormal globin composition in the haemoglobin of one rabbit. The additional globin peak was eluted after the normal β-chain. The abnormal globin is inheritable: the same pattern has been observed in one animal out of a kindred of 5 of the 'mutant' rabbit. Data are presented which indicate that our findings can be accounted for by a mutation affecting the β-chain.

Materials and methods. Rabbit erythrocytes were collected and haemolyzed as previously reported for human red blood cells[2]. The concentration of the haemoglobin cyano derivative (CN Met Hb), used[3] for all the chromatographic and electrophoretic separations, was determined at 541 nm according to SCHNEK and SCHROEDER[4].

Horizontal starch gel electrophoresis of the various haemoglobins was carried out at 4 °C in Na borate-Na bicarbonate buffer pH 9.3 in *Tris*-EDTA-borate buffer pH 8.6, pH 8.3[5] and pH 7.3 and in *Tris*-succinate buffer pH 5.4. Starch gels were stained using benzidine or Amino Black. Electrophoresis of haemoglobin was also performed on cellulose acetate at 18 °C using the same buffers. The strips were stained with Ponceau Red. Chromatography of cyanomethaemoglobin on Amberlite IRC-50 was accomplished according to ALLEN et al.[3].

Free globin chains were prepared by acid-acetone precipitation[6] and α- and β-chains were separated according to DINTZIS[1]. Electrophoretic separation of Hb subunits was carried out on starch gel in 6 *M* urea and 0.05 *M* mercaptoethanol in *Tris*-EDTA-borate buffer pH 7.3[7] and *Tris*-K phosphate buffer pH 6.1.

Results. A typical chromatographic separation on CM cellulose column of the α- and β-globins, prepared from 'normal' rabbit haemoglobin, is reported in Figure 1a. Only 2 peaks can be detected.

The chromatographic pattern of globin from the 'mutant' rabbit haemoglobin reveals on the contrary the presence of 3 peaks well separated one from another (Figure 1b). The first peak has a migration corresponding to that of the α-chain, and the second peak to that of the normal β-chain. In fact, if purified β-chain uniformly labelled with L-Valine C^{14} (U) (New England Nuclear Corp., 0.05 mc/0.0293 mg) is added to the 'mutant' globin before chromatography, the peak of radioactivity is coincident with the second peak (Figure 2). The sum of the β- and the additional chain, as mg of protein (determined by the Folin technique), is equal to the α-chain.

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