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THE RELATIONSHIP BETWEEN PLASMA ASPARTATE AMINOTRANSFERASE ACTIVITY AND TISSUE DAMAGE IN ANIMALS

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SUMMARY

In order to establish the relationship between plasma aspartate aminotransferase (AST) activity and liver damage, the AST activity was determined in plasma from rats treated with carbon tetrachloride (CCl_4). The results were compared with AST activity in plasma from normal cattle and cattle with Lumpy Skin Disease (LSD) and East Coast Fever (ECF). In the rats a marked increase in plasma AST activity was well correlated with acute liver damage. The increase in AST activity in plasma from cattle with LSD and ECF implied leakage of the enzyme from damaged tissues other than the liver. It was concluded that measurement of plasma AST activity may serve as a test to evaluate the health status of animals in general.

INTRODUCTION

The health status of animals is greatly affected by pathological conditions which cause damage to the liver and other organs. Damage of tissues causes significant release of tissue enzymes into plasma because the ratio between tissue enzymes and plasma enzymes is very high (Whitby *et al.* 1975). It seems evident that the degree of increase of enzymes in plasma will be proportional to the degree of tissue damage.

Commonly used enzymes in evaluation of liver damage include aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltranspeptidase (GGT), sorbitol dehydrogenase (SDH) and glutamate dehydrogenase (GDH) (Stogdale 1981).

In order to establish the relationship between AST activity and liver damage, an experiment with rats treated with carbon tetrachloride (CCl_4) was carried out. The liver damage was indicated by changes in plasma bilirubin concentration, total lipid per g liver and histopathologic examination. The rat experiment was followed by preliminary comparison between AST values in healthy and sick cattle.

MATERIALS AND METHODS

Animals and treatment

Twelve rats raised under laboratory conditions were given a single dose of 1.5 ml CCl_4. The carbon tetrachloride was dissolved in maize oil to give a total volume of 5 ml and was administered orally by stomach catheter. A control group of other twelve rats raised under the same conditions was given an equal volume of maize oils.

Administration of CCl_4 to conscious rats

Oral administration of CCl_4 and maize oil was performed on conscious rats by placing a hand made oval-shaped piece of wood between the jaws just behind the front teeth. This prevented the rats from biting the catheter which was introduced into the stomach through a small hole in the center of the wood piece. Details of this technique are described by Waynforth, (1980).

Table 1: Criteria for grading hepatic damage.

Grade of damage	Histological features
0	Normal liver.
1	More or less normal liver architecture
2	Minor disorganization of hepatic cords around central veins
3	Most degenerated hepatocytes are replaced by granulation tissue.
4	Necrotic areas marked by intensely basophilic proliferating epithelial cells.
5	Severe centrilobular necrosis.

Sampling

Blood samples from two treated rats and from two non-treated ones were collected by heart puncture into heparinized tubes on days 2, 3, 5, 7, 9 and 13 after treatment according to the technique described by Waynforth (1980). Plasma was removed by centrifugation at 3000 rotation per minute for 20 minutes shortly after bleeding and frozen at -20°C until analysed. After collection of blood each rat was decapitated and two separate sections of the liver were taken. One of the sections was used for histopathologic examination and the other for determination of total lipid per g liver. The liver section for histopathology was fixed in 10 percent neutral - buffered formalin while that for determination of total lipid was frozen at - 20°C until analysed.

Sampling from healthy and sick cattle

Whole blood was sampled at the University farm, Morogoro, Tanzania from ten clinically healthy cattle and from two groups of three cattle with clinical cases of Lumpy Skin Disease (LSD) and East Coast Fever (ECF) respectively. Plasma samples were removed and preserved as described above.

Analytical methods

Plasma AST activity was determined by the kinetic method with commercially available reagent mixtures prepared by Merck according to the Scandinavian Committee on Enzymes (1974). Bilirubin concentration in plasma and total lipid per g liver were determined using the Merck photometric method and according to the method described by Folch *et al.* (1957) respectively.

Liver sections for histopathologic examination were embedded in paraffin, sectioned at about 6µm and stained with haematoxylin and eosin. The stained sections were systematically scanned in a microscope using a high dry objective. Each successive field was individually assessed for the severity of parenchymal damage and allotted a score between 0 and 5 using a predetermined scale of severity (Table 1).

RESULTS

Table 2 shows that AST activity correlated strongly with degree of liver damage. The activity was very high 2 days after treatment when there was also high bilirubin concentration and pronounced lipid infiltration of the liver. The AST values remained elevated after bilirubin values and lipid concentration in the liver had returned to normal. Table 3 shows that plasma from cattle with LSD and ECF had higher AST activity than plasma from healthy cattle.

Table 2: Mean plasma AST activity, bilirubin concentration, total lipid per g liver and degree of liver damage in CCl_4 treated and non-treated rats.

	Average values of determined parameters in treated and non-treated rats			
Days after treatment	AST (IU/1)	Bilirubin (mg/100 ml)	Lipid (mg/g liver)	Degree of Liver damage
2	3833 (60)	10.7 (3.0)	114.7 (45.4)	5 (0)
3	384 (58)	5.6 (2.6)	71.0 (48.9)	4 (0)
5	96 (67)	3.5 (2.5)	60.0 (64.5)	3 (0)
7	87 (62)	3.7 (3.1)	54.7 (43.7)	2 (0)
9	72 (67)	3.5 (3.4)	53.8 (50.3)	1 (0)
13	70 (61)	3.6 (3.9)	41.7 (45.2)	1 (0)

Values in Parenthesis are for non-treated rats.

Table 3: AST activity in the plasma of healthy and sick cattle

Plasma number	Healthy cattle	Cattle with Lumpy Skin Disease	Cattle with East Coast Fever
1	55	110	115
2	68	158	92
3	73	216	64
4	46		
5	71		
6	57		
7	49		
8	77		
9	55		
10	56		

Mean value of AST in health cattle = 61

DISCUSSION

The model experiment with rats treated with CCl_4 revealed that liver damage can be identified by changes in the concentrations of AST and bilirubin in plasma and total lipid in the liver. The magnitude of the change in AST activity corresponding to severe liver damage was more pronounced than the magnitude of the changes in bilirubin and lipid concentrations. This finding points to the high sensitivity of intracellular enzymes in identifying tissue damage.

The type of tissue damage which coincided with the peak of AST was severe centrilobular necrosis. This is quite in agreement with long established results on the pathogenesis of the liver lesion due to the administration of CCl_4 (Andrews and Maegraith, 1948; Boyd 1962). The damage observed lead to abnormal release of AST from the dying hepatocytes. The rather short time interval of 2–3 days after treatment during when the peak of AST was observed indicates that the enzyme is released during the active necrotic process. Kutas and Karsai (1961) have also observed AST values to be within the normal range during the recovery or chronic phase of CCl_4 – induced liver damage.

Incrimination of the increase of AST on the liver damage in the rat experiment has been based on the selective toxicity of CCl_4 on the liver (Castro and Gomez, 1972). However, this does not mean that AST is liver-specific. The enzyme is known to be present in the cytoplasm and mitochondria of many tissues in the body in all mammalian species (Stogdale 1981). The findings of increased AST activity in plasma from cattle with LSD and ECF indicate leakage of the enzyme from a number of tissues affected by these diseases. In LSD cutaneous nodules which become necrotic in the course of the disease, fatty liver degeneration, vasculitis and focal infarcts in the muscles (Ristic and McIntyre 1981; Jubb *et al.* 1985) are possible lesions from which AST leaked into blood. In ECF on the other hand, destruction of lymphoid cells and haemorrhagic lesions in many internal organs such as the spleen, the heart and the kidney (Robertson 1976) are likely to be the source of AST.

The strong relationship between AST activity and tissue damage in the rat experiment indicates that AST is a good indicator of tissue damage. Its use as one of the general tests could be of value in evaluating health condition and productional capacity in cattle. This might prove useful in areas with endemic occurrence of parasitic infections such as fascioliasis known to be associated with increases in AST activity and lowered performance (Maselle 1988).

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