Alpha-1-antitrypsin deficiency and hepatocellular carcinoma

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SUMMARY Forty-two cases of hepatocellular carcinoma (HCC) were examined for the presence of the inclusions of alpha-1-antitrypsin (AAT), which indicate a carrier state for the Pi Z gene. These were found in the non-neoplastic liver tissue of two cases of HCC and in one of the 98 control livers, a difference that is not statistically significant.

Typical globules of AAT deficiency were not found in HCC cells. One-quarter of HCCs, however, contained cells which showed diffuse cytoplasmic staining for AAT, a pattern seen also in the non-neoplastic livers.

It has been established that alpha-1-antitrypsin (AAT) deficiency due to the homozygous Pi Z state may give rise to cirrhosis and hepatic fibrosis, although the mechanism whereby this occurs is obscure. Also there are numerous reports of hepatocellular carcinoma (HCC) developing in such cases (Ganrot *et al.*, 1967; Berg and Eriksson, 1972; Eriksson and Hägerstrand, 1974).

The position of the heterozygous Pi Z state as a precursor of cirrhosis and HCC is, however, still undecided. A phenotyping study (Morin *et al.*, 1975) and an investigation of AAT serum levels with selective phenotyping (Fisher *et al.*, 1976) have shown no excess of Z heterozygotes in cirrhosis as compared with controls; but immunocytochemical and histological studies, using intracytoplasmic liver AAT globules as markers, have suggested that there is an association with cirrhosis (Eriksson *et al.*, 1975; Blenkinsopp and Haffenden, 1977). Similar immunocytochemical studies have also indicated that there is an increased incidence of the Z allele in cases of hepatocellular carcinoma (Blenkinsopp and Haffenden, 1977; Palmer and Wolfe, 1976), but phenotyping studies have shown only a slight increase not reaching statistical significance (Peters, 1976) or no increase (Theodoropoulos *et al.*, 1976). It has also been suggested that HCC may produce AAT globular inclusions (Palmer and Wolfe, 1976). In order to clarify the relationship between HCC and AAT status an immunocytochemical survey of necropsy cases of HCC was carried out.

Material

Paraffin-embedded liver blocks from 42 cases of primary liver cell carcinoma from the necropsy files of Manchester Royal Infirmary during the years 1959 to 1977 were used. The number of blocks per case varied from 1 to 10, the average being 3.7. Thirty-four cases were cirrhotic, 29 micronodular, and 7 macronodular; 2 had hepatic fibrosis; 3 had haemochromatosis; 36 were male and 6 female. The tissue was fixed in either formol sublimate or 4% formol saline. A control series of 98 sequential postmortem livers was studied similarly.

For immunocytochemistry, rabbit anti-AAT (R-AAT) (Dakopatts and Behringwerke), swine anti-rabbit IgG (SWAR), and peroxidase-rabbit-anti-peroxidase complexes (PAP) (Dakopatts) were used.

Methods

Sections were stained with haematoxylin and eosin and PAS with and without prior diastase digestion. AAT was localised by the Sternberger (1970) immunoperoxidase technique. Normal swine serum was used to saturate non-specific protein binding sites, and the sections were treated sequentially with R-AAT (45 min, 1/120 dilution), SWAR (15 min, 1/20 dilution), and PAP (20 min, 1/60 dilution). Peroxidase was stained with 3,3' diaminobenzidine tetrahydrochloride by the method of Graham and Karnovsky (1966).

Control sections included the use of R-AAT absorbed with purified AAT. Other control sections

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were replacement of R-AAT by normal rabbit serum and replacement of SWAR by normal swine serum.

Results

In two cases the non-neoplastic liver contained typical AAT globules which were located around portal zones and stained most strongly at the globule periphery by the immunoperoxidase technique. Absorption controls were unstained. In neither case was there evidence of AAT in the HCC. Both patients were alcoholics. Histologically one was cirrhotic; the other showed fibrosis and fatty change without true cirrhosis. One of the 98 control livers contained AAT globules. The difference between the HCC and control series was not statistically significant ($P = 0.22$). The frequency of antitrypsin globules in controls does not differ significantly from the expected frequency of 3.2% (Cook, 1974; Geddes *et al.*, 1977).

In three cases in the non-neoplastic liver and in one HCC, PAS-positive, diastase-resistant globules resembling those produced in AAT deficiency were present in hepatocytes at the edges of zones of centrilobular congestion and necrosis. They were

multiple in each cell which contained them and they were absent from the periportal zones. On immunocytochemical staining most were negative but a few were weakly stained. The cytoplasm surrounding the globule was often stained in a finely granular pattern. These atypical globules were considered not to indicate a Z gene. Phenotyping was not possible on any of these cases.

In the non-neoplastic liver sections, AAT was present in the cytoplasm of variable numbers of liver cells in nine cases with HCC and in 28 controls. The pattern of staining was diffuse and very finely granular throughout the whole cytoplasm (Fig. 1). The strength of the positive staining varied from cell to cell and from case to case. Eleven HCCs showed this staining pattern, usually in a focal distribution and confined to well-differentiated areas only (Fig. 2).

This staining pattern was reproducible and absorption controls were unstained.

Discussion

It is now considered that intrahepatic AAT globules are indicative of alpha-1-antitrypsin deficiency resulting from the presence of the Pi Z allele (Sharp,

Fig. 1 AAT in normal hepatocytes from a liver containing a hepatocellular carcinoma elsewhere. Individual liver cells show diffuse cytoplasmic staining (dark), varying in intensity from cell to cell, while other hepatocytes are totally negative. (Immunoperoxidase-DAB-haematoxylin $\times 635$).

Fig. 2 AAT in well-differentiated hepatocellular carcinoma cells. Individual cells show diffuse cytoplasmic staining of variable intensity similar to that in Fig. 1, while adjacent cells are unstained. (Immunoperoxidase-DAB-haematoxylin $\times 635$).

1971; Gordon *et al.*, 1972). There are rare exceptions in that globules may be present in other uncommon variant phenotypes (Lieberman *et al.*, 1976) but these are also associated with deficient AAT levels.

The present morphological study indicates that in the UK there is no connection between heterozygous Pi Z AAT deficiency and HCC. This finding is in contrast to other histological studies in the UK and USA (Palmer and Wolfe, 1976; Blenkinsopp and Haffenden, 1977) but confirms the work of Theodoropoulos *et al.* (1976) and of Peters (1976).

In this study two types of staining pattern occurred in hepatic cells with the immunoperoxidase method of AAT detection, but only the globular staining with peripheral accentuation should be regarded as indicating AAT deficiency states. The atypical PAS-positive globules may be giant lysosomes (Kerr, 1969). Their location at the junctions of viable and necrotic zones is consistent with autophagic vacuole formation in damaged cells. These atypical globules are difficult to distinguish from the true AAT globules on cursory examination. It is possible that the claimed increased incidence of AAT in cirrhosis and HCC in previous studies may be due to these atypical bodies.

A possible explanation for the diffuse AAT stain-

ing, which was present in both neoplastic and cirrhotic hepatocytes, is that it could represent selective uptake by individual cells of AAT from the blood stream, but as the staining pattern is similar to that of albumin (Feldmann and Maurice, 1977) it is more probable that it represents normal synthesis of AAT. If this is so, it is of interest that it is also synthesised by malignant liver cells. It is unlikely that the presence of this AAT staining pattern would be of any diagnostic value in HCC as the pattern is too focal and inconsistent.

A possible explanation for the presence of this staining pattern in only a proportion of normal livers is that AAT is an acute phase reactant and the rate of synthesis will consequently vary from person to person at any time. The quantity within hepatocytes at any time in the normal person probably reflects the rate of synthesis and would reach stainable levels in cells with higher synthetic rates. If this is so, post-mortem staining patterns should increase in parallel with postmortem serum levels.

This diffuse AAT staining is identical with the finely granular pattern described by Palmer *et al.* (1977) in oral contraceptive-associated hepatic tumours. However, Palmer *et al.* did not find this pattern in non-neoplastic liver adjacent to the

tumours; this may reflect synthesis of adequate quantities of AAT by the tumours with suppression of synthesis in the normal liver.

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