Characteristics of the distribution of lectin receptors in intrahepatic cholangiocellular carcinoma

SHIMIN ZHANG, MENGCHAO WU, HAN CHEN, XIUZHONG ZHANG, WENMING CONG and HONGKUI SHO

Institute of Hepatobiliary Surgery, Changhai Hospital, 174 Changhai Road, Shanghai, People's Republic of China

Received 19 October 1988 and in revised form 17 January 1989

Summary
The receptors of peanut agglutinin (PNA), Dolichos biflorus agglutinin (DBA) and Ulex europaeus agglutinin I (UEA-I) were localized in intrahepatic cholangiocellular carcinoma, hepatocellular carcinoma, intrahepatic bile ducts and normal, cirrhotic and pericarcinomatous liver using the avidin-biotin-peroxidase complex method. It was found that epithelial cells of normal bile ducts had many UEA-I receptors, fewer DBA receptors and no PNA receptors. The positive rates of PNA, UEA-I and DBA receptors in 18 cases of intrahepatic cholangiocellular carcinoma were 88.9%, 61.1% and 33.3% respectively, which were significantly higher than those in hepatocellular carcinoma (16.0%, 4.0% and 4.0% respectively). Hepatocytes in normal, cirrhotic and pericarcinomatous liver had no receptors for these three lectins. It is suggested that lectin receptor distribution in intrahepatic cholangiocellular carcinoma is obviously different from that in normal bile duct cells and in hepatocellular carcinoma, and might be used as an auxiliary index in its clinical diagnosis.

Introduction
Liver cancers are common human malignant tumours. With the discovery of α-fetoprotein (AFP) as a tumour marker and the continuous improvement of measuring it, the diagnosis of AFP-positive hepatocellular carcinoma, which is the most common of liver cancers, has become relatively easy. The investigation of the AFP variants, abnormal prothrombin, α-L-fucosidase and γ-glutamyltranspeptidase (γ-GT) (Liebman et al., 1984; Dicioccio et al., 1985; Buamah et al., 1987) have led to an increase in the rate of diagnosis of hepatocellular carcinoma with a lower level of AFP or without AFP in the serum. However, the diagnosis of intrahepatic cholangiocellular carcinoma, another of the commoner liver cancers, is difficult because of the lack of a specific tumour marker.

Lectins are a group of proteins or glycoproteins isolated from plants, micro-organisms or animals. They can bind specifically to glycosyl residues on glycoconjugates. This binding has the specificity related to both monosaccharide and anomeric carbon on sugars, and is effected by the spatial conformation of glycoconjugates (Lis & Sharon, 1986; Damjanov, 1987). Therefore lectins can distinguish slight differences in saccharide structures of glycoconjugates. In this study, peanut agglutinin (PNA), Ulex europaeus agglutinin I (UEA-I) and Dolichos biflorus agglutinin (DBA) were used as probes. The lectin receptors in intrahepatic cholangiocellular carcinoma were detected histologically by the avidin–biotin–peroxidase complex (ABC) method. Normal intrahepatic bile ducts, hepatocellular carcinoma, and normal, cirrhotic and pericarcinomatous liver were also included. Changes in the glycoconjugate composition of bile duct cells after neoplastic transformation, and its clinical implication in the diagnosis of intrahepatic cholangiocellular carcinoma, were explored.

Materials and methods
Reagents
Biotinylated lectins (biotinyl-PNA, -UEA-I and -DBA) and an ABC kit were purchased from Vector Laboratories, USA; 3,3'-diaminobenzidine tetrahydrochloride (DAB) was from Fluka AG, Switzerland; N-acetylgalactosamine (GalNAc) was from J. T. Baker Chemical Co., USA; fucose (Fuc) was from Serva Fine Biochemica, West Germany. Galactose (Gal) was a product of Shanghai Second Reagents Factory, China.

Tissue samples and preparation of paraffin sections
Eighteen cases of intrahepatic cholangiocellular carcinoma (male, 10 cases; female, eight cases; with an average age of 45.3 years) were included in this study; among them, seven cases were well differentiated, six cases were moderately differentiated and five cases were poorly differentiated. Also 25 cases of hepatocellular carcinoma, five cases of liver...
cirrhosis and five cases of normal liver were studied with respect to their lectin receptors. These samples were obtained by surgical biopsies or resections in our institute, except for the normal liver tissues which were from normal subjects who had died in accidents. All tissues were fixed in formalin, dehydrated with graded ethanol and embedded in paraffin in the routine fashion to become paraffin blocks. Thirty serial sections, each 4-6 µm thick, were made from each paraffin tissue block.

Histological localization of lectin receptors
Using the ABC method of Hsu et al. (1981) with a slight modification, the procedure was as follows. (1) The sections were deparaffinized with xylene, and rehydrated with graded ethanol; (2) immersion of the sections in 0.3% H₂O₂ in methanol for 30 min to remove any endogenous peroxidase activity; (3) immersion in 3% normal sheep serum in TBS (0.85% NaCl, 0.05 M Tris/HCl buffer, pH 7.5) for 30 min to prevent non-specific absorption of proteins during labelling; (4) rinse three times with TBS, each for 3-5 min; (5) incubation with biotinyl-lectin (10 µg ml⁻¹) for 30 min at 37°C, followed by a TBS rinse as above; (6) incubation with ABC reagent (1:100 dilution) for 1 h at 37°C, and a TBS rinse as above; (7) coloration with 0.03% H₂O₂/0.75 mg ml⁻¹ DAB in TBS for 5 min at room temperature; (8) after being rinsed for 1 min with tap water, the sections were counterstained with Haematoxylin, dehydrated with graded ethanol, cleared with xylene and mounted with neutral balsam.

Control experiments
(1) Negative control experiment: biotinyl-lectin and ABC reagent were respectively replaced by TBS on two separate tissue sections.

(2) Non-specific staining: the treatment with 0.3% H₂O₂ in methanol and 3% normal sheep serum was respectively omitted on two separate tissue sections.

(3) Specific inhibition test: biotinyl-UEA-I + Fuc; biotinyl-UEA-I + Gal; and biotinyl-UEA-I + GalNAc. The solid hapten sugars were dissolved in biotinyl-lectin solutions and fully mixed. After standing for 20 min at room temperature, the mixtures were ready to be used.

(4) H&E staining: one or two sections of each sample were stained by H&E in order to make a pathological diagnosis.

Assessment of the labelling results of lectin receptors
After excluding non-specific staining, a section with brown staining was called positive, otherwise it was negative (−). When the average rate of brown-stained cells was less than 5%, the section was designated as ±; 5-30%, as +; 30-70% as ++; and more than 70%, as +++.

Results
The distribution of lectin receptors in epithelial cells of normal intrahepatic bile ducts
Study of the distribution of lectin receptors in epithelial cells of bile ducts in the normal, cirrhotic and pericarcinomatous liver tissues investigated here revealed that bile duct cells had no PNA receptor, and DAB receptors were present in a small amount only in multiplied bile duct cells and on endomembranes of a few bile ducts, while a relatively large amount of UEA-I receptor existed in epithelial cells of both normal and multiplied bile ducts. Most of these were distributed in cytoplasm adjacent to the lumen of the bile ducts, and on their membranes (Fig. 1). However, the amount and distribution characteristics of lectin receptors in the bile duct cells varied to some extent with the size of the bile ducts.

The distribution of lectin receptors in intrahepatic cholangiocellular carcinoma
Positive receptors for each of three lectins in this carcinoma were different. They are shown in Tables 1 and 2. The positive rate of PNA receptors was the highest; out of 18 cases, 16 were positive. UEA-I receptors were present in 11 cases. The positive rate was the lowest for DBA receptors; only six cases had this receptor. The labelling result for the three lectins in one specimen was not always the same. There were five cases that were all positive when they were labelled with the lectins. Five cases contained two types of lectin receptors, and eight cases had one type of lectin receptor. All cases had at least one receptor for these three lectins (Table 1).

Similarly, the amount and distribution characteristics of one type of lectin receptor varied in different specimens. The staining rate of cancer cells varied from 0 to 100%. In the well differentiated carcinoma, the lectin receptors were mainly distributed on the membranes of the acinar lumen and in the cytoplasm adjacent to the lumen (Fig. 2). Inclusions of many acini were also stained by this procedure. The cancer cells of the poorly differentiated carcinoma appeared to be evenly stained in their overall cytoplasm (Fig. 3) or locally stained (Fig. 4). Plasma membranes of some cancer cells were stained very obviously in some cases (Fig. 5).

In addition, there were also differences in the distribution of lectin receptors between the groups of cancer cells in the same specimen. Some cancer cells had many lectin receptors, while some had none (Fig. 6). The amount and distribution characteristics of lectin receptors were not absolutely related to the differentiation grade or size of tumour, nor to the age or sex of the patients.

The distribution of lectin receptors in hepatocellular carcinoma and other liver tissues
The hepatocytes in normal, cirrhotic and pericarcinomatous liver lacked PNA, UEA-I and DBA receptors. The endothelial cells of blood vessels had many UEA-I receptors. Of 25 cases of hepatocellular carcinoma, PNA receptors were present in four cases, UEA-I in...