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## Abstract

Experimental hepatocellular carcinoma (HCC) in rats did not take up intravenously administered indocyanine green (ICG) and trypan blue, while surrounding tissue did. The lack of ICG uptake was also observed by peritoneoscopy in patients with HCC. The contrast between ICG-stained cirrhotic nodules and HCC tumors was intensified with infrared photography. Non-uptake of dyes by HCC cells may enable discrimination between tumors and normal cells.

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## LACK OF UPTAKE OF INDOCYANINE GREEN AND TRYPAN BLUE BY HEPATOCELLULAR CARCINOMA

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**Abstract.** Experimental hepatocellular carcinoma (HCC) in rats did not take up intravenously administered indocyanine green (ICG) and trypan blue, while surrounding tissue did. The lack of ICG uptake was also observed by peritoneoscopy in patients with HCC. The contrast between ICG-stained cirrhotic nodules and HCC tumors was intensified with infrared photography. Non-uptake of dyes by HCC cells may enable discrimination between tumors and normal cells.

**Key words :** indocyanine green, hepatocellular carcinoma, peritoneoscopy, trypan blue.

The plasma disappearance rate of indocyanine green (ICG) has been used clinically for the evaluation of liver function. The dye is taken up mostly by hepatocytes (1). In the course of studying lobular markings on the liver after administration of ICG and trypan blue (2), it was found that hepatocellular carcinoma (HCC) nodules do not take up the dyes.

### MATERIALS AND METHODS

**Tumor induction.** HCC was induced in male Fischer-344 rats weighing 150-160 g each (Charles River Japan Inc., Atsugi, Japan) by administering a basal diet (Oriental Yeast Co. Ltd., Tokyo) containing 0.02 % of 2-acetylaminofluorene (AAF) for 45 days. For the subsequent 6 months, the rats were given drinking water with 0.05 % phenobarbital *ad libitum* (3). The experiments were performed 10-11 months after the start of the AAF-administration.

**Staining.** Trypan blue (Merck, Darmstadt, Germany) was dissolved in a 5 % albumin solution (Plasmanate, Midori-Juji Co., Osaka), and 100 mg/kg body weight in 1 ml of solution was injected in the tail vein under ether anesthesia. ICG (Diagnogreen, Daiichi Pharm. Co., Tokyo), 25 mg/kg body weight, was injected in 1 ml of the albumin solution (2). Ten min after the stain was administered, the liver was observed with a laparoscope (CL-25, Shinko Optimal Co., Tokyo) under a second anesthesia. For perfusion-fixation, 1,000 IU heparin was injected into the heart to prevent blood coagulation, followed by infusion of 500 ml of Ringer solution into the thoracic aorta. The rats were perfusion-fixed with 1 % glutaraldehyde dissolved in 0.1 M phosphate buffer (pH 7.4). Before processing the liver for light microscopy, peritoneoscopy was performed. In some experiments, infrared photographs were taken (TV camera : C-158, Hamamatsu TV Co. Ltd. (4), film Kodak high speed infrared film 2481).

*Clinical observation.* Five patients were examined by peritoneoscopy. Computer assisted tomography, ultrasonography, hepatic arteriography, peritoneoscopy and elevation of alpha fetoprotein established the diagnosis of HCC and liver cirrhosis. Investigational methods included intravenous injection of ICG in Plasmanate solution (5 mg/kg body weight). The liver was examined for 60 min with a laparoscope (a-5213, Olympus Optical Co., Tokyo).

### RESULTS

All rats treated with AAF and phenobarbital developed HCC tumors and cysts. ICG injection resulted in green staining of the normal tissue surrounding the tumors, which were unstained and whitish brown (Figs. 1, 2). In the absence of ICG injection, the livers presented a brown surface. Small patches in the liver, shown to be hyperplastic nodules by light microscopy, did not take up ICG (Figs.

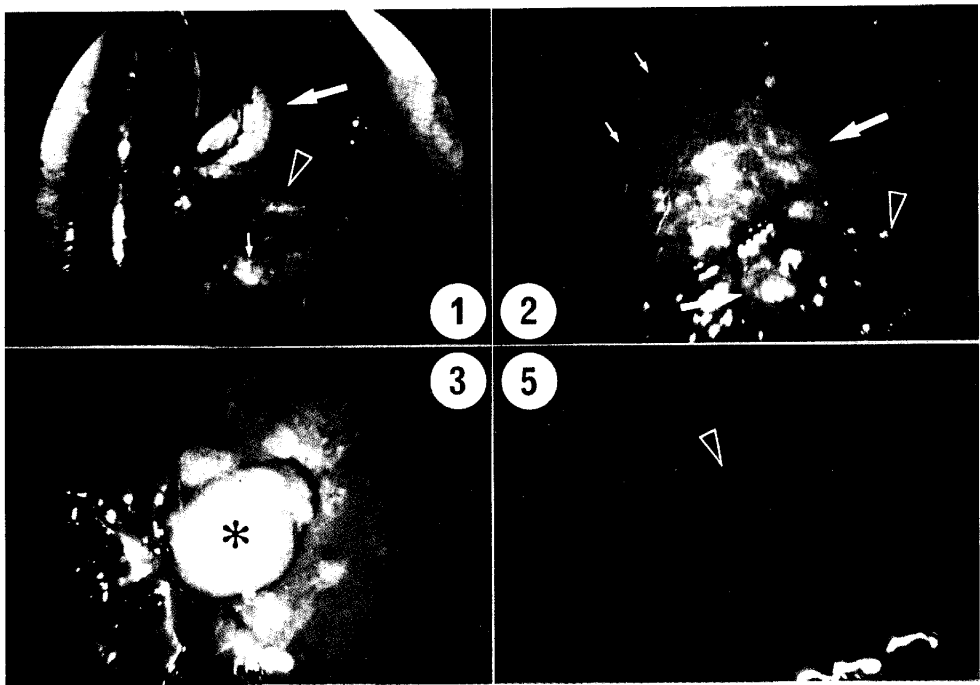


Fig. 1. HCC tumor-bearing rat liver. Normal tissue is stained greenish-brown from intravenous injection of ICG. The tumor (large arrow) and the hyperplastic nodules (small arrow) did not take up ICG. The arrowhead designates the hepatic vein.

Fig. 2. HCC tumor bearing rat liver. The tumor (large arrow) and the hyperplastic nodules (small arrow) are not stained after ICG administration. Arrowhead, cyst.

Fig. 3. Perfusion-fixed liver from the same rat in Fig 1. The white area is the HCC (asterisk), and surrounding normal tissue is stained green.

Fig. 5. Peritoneoscopic photograph of a liver of a patient with HCC and liver cirrhosis after ICG administration. HCC tumor (arrow) is not stained green in contrast with the surrounding cirrhotic tissue which stained green.

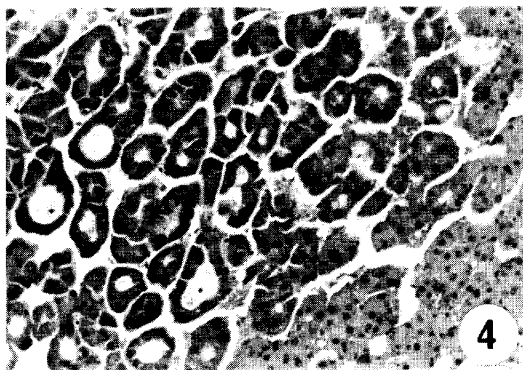


Fig. 4. Hepatocellular carcinoma in a rat. Hematoxylin and eosin. 100  $\times$ .

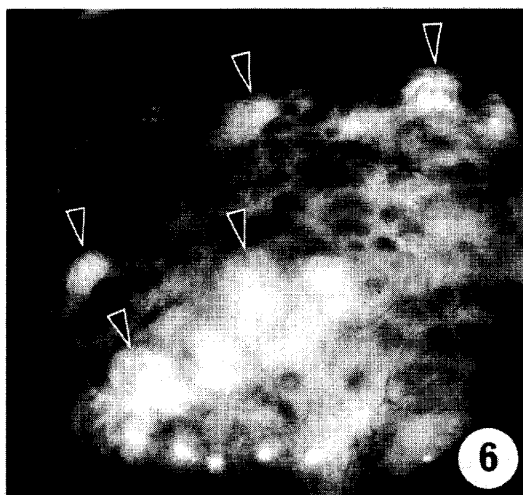


Fig. 6. Peritoneoscopic infrared photograph of a liver of a patient with HCC and liver cirrhosis after ICG staining. Arrows point to nonstaining tumors.

1, 2). Perfusion fixation, removing excess blood, presented well-demarcated unstained nodules (Fig. 3). The nodules were confirmed to be HCC by light microscopy (Fig. 4). The contrast between ICG-stained normal tissue and unstained HCC nodules was also demonstrated by infrared TV-photography. Trypan blue injection yielded identical results as with ICG.

ICG was injected into five patients suffering from HCC and liver cirrhosis. The liver, except the HCC tumor, stained green (Fig. 5). Color contrast between the tumor and cirrhotic tissue became clear in 30 min. The tumor surface presented well-developed vessels, a peritoneoscopic finding characteristic of HCC

tumors (5). The contrast between ICG-stained cirrhotic nodules and HCC tumors was intensified with infrared photography (Fig. 6).

#### DISCUSSION

HCC nodules in this study lacked the ability to accumulate ICG and trypan blue. The hepatocyte uptake-mechanism of organic anions is not well known (6). ICG is known to bind with ligandin, though that is not the major determinant of anion selectivity (7). The selectivity is thought to reside in the Z protein or in the cell membrane (7-9). The mutant Southdown sheep have impaired accumulation of BSP, bilirubin, Rose Bengal, and ICG (9). There is no difference in the amount of ligandin between normal and mutant sheep, therefore, the mutant defect is probably in the plasma membrane rather than in the cytoplasmic organic anion acceptor proteins (9). Constitutional ICG excretory defects with normal serum bilirubin and near normal BSP retention have been reported (10, 11). In the latter situation the basic defect resides in the uptake of ICG.

Most experimentally induced HCC tumors express markedly reduced amounts of ligandin compared to normal, regenerating and preneoplastic liver tissue (12-14). However, the present lack of uptake of ICG and trypan blue by HCC cells probably is caused by a defect in the plasma membrane. The HCC tumors did not show even low-level staining although AAF-induced HCC have decreased, but definite, ligandin levels, 0-35 % of normal (14).

We have not determined the stage of hepatocarcinogenesis at which hepatocytes lose the ability to take up organic anions. It is possible that hyperplastic cells also possess this defect, as small patchy areas were not stained by ICG (Figs. 1, 2). Reuber observed that all poorly differentiated HCC and 2/3 to 3/4 of well-differentiated HCC and hyperplastic nodules lost the ability to stain pink with Rose Bengal (15).

The lack of ICG uptake was also demonstrated in human HCC tumors. Most human HCC, except bile-secreting hepatomas, probably lose the ability to take up bilirubin and other dyes, because most HCC tumors are white and not stained by bilirubin, while HCC do not have functioning bile ducts (16). The lack of ICG uptake by HCC tumors may facilitate early peritoneoscopic diagnosis. The color difference against normal tissue simplifies enucleation of the tumor. The clinical cases did not always demonstrate sharp demarcation between HCC nodules and surrounding tissue, probably due to the small amount of ICG injected. The distinction was sharpened by infrared imaging (Fig. 6) (4).

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