Liv 02

DNA CYTOPHOTOMETRY OF HEPATOCYTES IN INDIVIDUAL ATPase-DEFICIENT PUTATIVE PRENEOPLASTIC FOCI IN RAT LIVER

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Single cell DNA cytophotometry of ATPase-deficient putative preneoplastic foci in rat liver was performed. These foci were induced by treatment of male Wistar AF/Han rats with a single dose of N-methyl-N-nitrosourea (25 mg/kg or 50 mg/kg) administered 25 hr after partial hepatectomy and 1 hr after hydroxyurea infusion from 14 to 24 hr after operation. By this procedure the carcinogen acts during the most sensitive period of the cell cycle i.e. early S-phase and induces altered hepatic foci at a high rate (Rabes et al., Cancer Res., in press). Preneoplastic foci were marked by overlaying ATPase-stained sections on 20 µm thick, methanol-fixed, unstained cryostat sections, which were subsequently Feulgen-stained. For hepatocytes in each enzyme altered focus, its surrounding hepatocytes or cells of normal control liver, the integrated light absorption and the surface area of 200 to 300 nuclei were measured as previously described (P. Dörmer, K. Pachmann, B. Lau, Histochem. 59:17-27, 1978). DNA measurements were performed separately for each focus in sequential sections and histograms were drawn. The size of the foci was determined by means of Videoplan planimetry (Kontron, Echting) in the sections which were stained for ATPase activity.

DNA histograms of ATPase-deficient foci revealed three different types: foci composed of an almost exclusively diploid cell population, others consisted of preferentially tetraploid cells. A few large foci contained a mixture of di-, tetra- and octaploid hepatocytes and some cells between these main ploidy stages. Ploidy distribution differed in small and large foci. Those ATPase-deficient foci which contained preferentially diploid or tetraploid hepatocytes, were significantly smaller than foci with three ploidy populations. The ploidy distribution of cells adjacent to ATPase-deficient foci was almost identical to the pattern in normal hepatocytes composed of the three well-known ploidy stages in di-, tetra- and octaploid cells.

The results of this selective measurement of individual preneoplastic foci indicate that not only diploid hepatocytes are a target for the transforming action of a carcinogen. Tetraploid cells, which are peculiar to the liver parenchyma, also give rise to altered hepatic foci. Both initiated cell types are capable of expanding as a homogeneous clone (Rabes et al., Cancer Res. 42: 3220-3227, 1982). However, clonal homogeneity appears to be lost when foci enlarge. Those preneoplastic foci which are heterogeneous with respect to DNA content indicate a more advanced state of development. These foci show an increased proliferative potential and may represent cell populations at high risk of further development into malignancy.

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Liv 03

HEPATOCELLULAR CARCINOMA: BIOCHEMICAL AND ANTIGENIC MARKERS OF THREE ESTABLISHED CELL LINES

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Hepatocellular carcinoma (hep.ca.) is one of the most frequent cancers. However the biology of these tumors has not been studied extensively. Recently we established the first carcinogen associated hep.ca. Mz-Hep-1 in tissue culture (Dippold et al. Hepatology in press). Our goal is now to define new markers for these tumors.

Three hepatoma cell lines Mz-Hep-1, SK-Hep-1, PLC/PRF/5 were studied. Biochemical markers were determined according to standard methods of the German Society of Clinical Chemistry or by commercially available tests. Cell surface antigens of cultured cells were tested by a rosette-formation Ig-mixed hemadsorption and an ELISA-test. New monoclonal antibodies (mAb) were generated against Mz-Hep-1 and SK-Hep-1-cells.

LDH was significantly elevated in the cell free culture supernatants and in the cell extract of all three hepatoma cells. In contrary SGPT and GLDH, were only detected in the cell extract of all three hepatomas: AP in the cell extracts of Mz-Hep-1 and SK-Hep-1 cells only. Mz-Hep-1 cells (1×10^6) accumulate 30 ng CEA in the supernatant during 4 days of culture. AFP and hepatitis B markers are only detectable in PLC/PRF/5 cells. Mz-Hep-1 and SK-Hep-1 hepatoma cells express HLA determinants and gp95, a glycoprotein molecule, which is related to transferrin. Only Mz-Hep-1 cells show a strong reaction with mAb to blood group B determinants and CEA. New mAbs to Mz-Hep-1 and SK-Hep-1 cells show a range of specificities in ongoing studies. With regard to liver tissue, one mAb reacts strongly with liver carcinomas, but weakly or not with normal liver tissue. Other mAbs recognize selectively different liver cell types.

Defining new markers for human hepatocellular carcinoma and normal liver cells will lead to a better understanding of the biology of these tumors.

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Liv 04

5-FU TISSUE PHARMACOKINETICS IN A RAT HEPATOMA MODEL AFTER INTRAARTERIAL HEPATIC CHEMOTHERAPY.

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The dismal prognosis and disappointing results of systemic chemotherapy in liver metastases have caused major interest in locoregional chemotherapy.

High tumor doses and at least partial sparing of systemic drug toxicity seem to be the main advantages of this modality. However, mode of administration (push injection versus infusion, use of microspheres) remains controversial. In this study, tissue pharmacokinetics of 5-FU were studied in Sprague-Dawley rats bearing Novikoff hepatoma implanted surgically under the liver capsule. 5-FU (30 mg/kg with 10 microCurie of ^{14}C -5-FU) was injected into the hepatic artery over an indwelling catheter. 5-FU tissue concentrations in the liver and the tumor nodules were measured at 15', 6 h, 12 h, 18 h after start to therapy using ^{14}C scintillation counting. Intraarterial push injection was compared to intraarterial push with microspheres (MAA) and to a 6 h continuous infusion. Additionally, the fraction of macromolecularly bound 5-FU was determined using TCA-precipitation. Both in intraarterial push injection and in continuous infusion, the concentration of 5-FU is about threefold higher in the normal liver than in the tumor. Continuous infusion leads to higher 5-FU tissue concentrations than push injection at 12 and 18 hours after therapy and to increased concentrations of macromolecularly bound 5-FU. Use of microspheres seems to allow for better drug exposure to the tumor with similar drug uptake in the hepatoma and normal liver. Total drug concentration and concentration-time product are close to those achieved by 6-hour-infusion of 5-FU without microspheres.

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Liv 05

COMPUTER TOMOGRAPHIC EXAMINATION OF HEPATIC METASTASIS WITH ARTERIAL INFUSION OF CONTRAST MEDIUM

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In 15 patients with hepatic metastases of gastrointestinal tumors, mostly colo-rectal carcinomas, an arterial catheter was implanted in the a. hepatica for intra-arterial chemotherapy. This catheter was also used for arterial infusion of contrast medium during computer tomography of the liver.

Method: A native whole liver ct-scan was carried out first. Then 50-70 ml contrast medium (Telebrix 300[®]) were infused through the implanted catheter with an electric infusion pump (Perfusor[®], Braun-Melsungen) with a constant flow rate of 600 ml/hour. 2-4 min after onset of arterial contrast infusion the ct-examination of the liver was repeated. This procedure was performed before the first course of i.a.-chemotherapy and repeated after each second or third course of therapy.

Results: In 13/15 patients the whole liver was perfused by the contrast medium. In one patient only the right lobe showed increased density, the left lobe was excluded from perfusion. In another patient the arterial catheter was dislocated and the contrast medium was found intra-peritoneal. In three patients there was contrasting of the whole liver but with non-uniform distribution, which was not clearly explained. In one patient a partial thrombosis of the a. hepatica was detected after the third course of therapy. The chemotherapy was stopped. In two patients primary hyperdense margins around the metastases disappeared after two courses of therapy. This could be an early sign of response.

Conclusions: 1. Computertomography of liver metastases with arterial contrasting is suitable for examination of exact arterial liver perfusion and provides greater security for correct i.a.-chemotherapy. 2. Disappearance of primary hyperdense margins of metastases could be an early sign of response.

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