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Distinctive Biochemical Pattern Associated with Resistance of Hepatocytes in Hepatocyte Nodules during Liver Carcinogenesis

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Hepatocyte ("hyperplastic") nodules induced in the liver by initiation with diethylnitrosamine and selected by dietary 2-acetylaminofluorene plus partial hepatectomy ("resistant hepatocyte model") have a special pattern of biochemical behavior and metabolic activity different than that seen acutely with many xenobiotics including many promoting agents and carcinogens. The nodule cells show a very low uptake of 2-acetylaminofluorene, relative to surrounding and normal liver, low levels of activity in the cytochromes P-450 and aryl hydrocarbon hydroxylase, high levels of activity in γ-glutamyltransferase, microsomal epoxide hydrolase, soluble glutathione-S-transferase and soluble UDP-glucuronyltransferase (UDP-GT_1) and elevated levels of glutathione. This metabolic pattern appears to maximize the resistance of the nodules to xenobiotics generally, such as 2-acetylaminofluorene, and thereby may account for the resistant behavior of nodule hepatocytes to the inhibition of cell proliferation and the cytotoxicity by 2-acetylaminofluorene and other carcinogens. The possible importance of this seemingly new metabolic program in carcinogenesis is discussed briefly.

Introduction

It is becoming increasingly attractive to consider that at least two patterns of development of liver cancer probably exist in rats with several chemical carcinogens. These may be designated (a) the chronic enzyme induction model, and (b) the resistant hepatocyte model.

The chronic enzyme induction model utilizes a brief exposure to one of several carcinogens to induce initiation, followed by a relatively long (2 to 7 months or longer) exposure to one of several different promoters (*1-3*). The latter are by themselves either noncarcinogenic or at most weakly carcinogenic. So far, all the promoters such as phenobarbital (PB) (*1, 4-6*), polychlorinated biphenyls (PCBs) (*7-9*), DDT (*10*), butylated hydroxytoluene (BGT) (*11* and others (*3*) have been shown to be very effective enzyme inducers in the liver. This is associated often with a considerable enlargement of the whole liver, due mainly to hypertrophy but also to some hyperplasia (*3*).

The resistant hepatocyte model utilizes a brief exposure to an initiating dose of one of many different carcinogens to induce resistant liver cells (*12, 13*) followed by the selection of resistant hepatocytes to form focal proliferations variously designated as hyperplastic nodules, neoplastic nodules, adenomas or simply hepatocyte nodules. The selection or "clonal expansion" is accomplished by a short exposure to another carcinogen, such as 2-acetylaminofluorene (2-AAF) (*14-16*) and others (*15, 16*) coupled with a stimulus for liver proliferation, such that the resistant cells scattered apparently randomly throughout the liver undergo 10 or more cycles of cell proliferation while the surrounding cells are largely inhibited by the carcinogen. This brief exposure (1 to 2 weeks) to the second carcinogen is sufficient not only to induce nodule formation but also to set in motion a sequence of unknown changes in a small subset of nodules ("persistent nodules") that leads to hepatocellular carcinoma in 70 to 90% of rats by 8 to 10 months (*17*). A direct linear sequence between resistant hepatocytes, nod-

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ules and metastasizing cancer has been established, although there is yet no way to predict which particular expanded clone (nodule) will evolve into cancer (*18*). Two particularly valuable properties of this model are the relatively high degree of synchrony of the resistant cells during the genesis of nodules and the predictability in the biological findings and behavior as a function of time during the first 8 to 10 weeks.

It is noteworthy that there is an apparent antagonism between the two models. Several of the agents that are effective promoters when used after initiation are also effective inhibitors of liver cancer development when used with carcinogens. Thus, they can be either promoters or anticarcinogens, depending upon the conditions of their use. The mechanisms of these dual, seemingly contradictory, effects have yet to be clarified.

The present study was designed to obtain more insight into the biochemical basis for the resistance in the new population of hepatocytes induced by diethylnitrosamine (DENA) or 2-AAF and selected by dietary 2-AAF plus partial hepatectomy (PH). Since resistant hepatocytes generated during initiation cannot as yet be harvested until they are stimulated to proliferate, the entire emphasis on resistance has focused on nodules. The special and perhaps unique biochemical or metabolic pattern associated with this resistant hepatocyte population is the subject of this communication.

Experimental

Male Fischer 344 or Wistar rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) initially weighing 150 to 170 g were used. The rats were maintained on a high protein (24%) basal diet (Bio-Serv. Inc., Frenchtown, NJ, and Dyets Inc., Bethlehem, PA) and water *ad libitum* with a 12-hr light and dark daily cycle and they were acclimated to their environment for at least 1 week before the start of the experiment.

The experimental regimen using DENA was that used in previous studies (*19*). Briefly, the rats were given DENA (Eastman Kodak Co., Rochester, NY) 200 mg/kg IP dissolved in 0.9% NaCl solution. After a 2-week recovery period, they were placed on the basal diet containing 2-AAF for 1 week, then subjected to a standard PH and continued for an additional week on the 2-AAF-contained diet. The rats were returned to the basal diet or a stock rat laboratory chow until the termination of the experiment. The regimen in the Wistar rats used intermittent periods of feeding diets containing 2-AAF and basal diet without 2-AAF (*20*).

At various time periods up to 31 weeks, animals were anesthetized with ether and the livers rapidly removed and chilled. Individual or pooled nodules were dissected free of the surrounding liver and used for glutathione or glutathione-S-transferase assays. The surrounding liver and normal controls on the same diets without the carcinogens were used.

Results and Discussion

As summarized in Figure 1, hepatocyte nodules show a metabolic pattern quite different than that seen in normal or surrounding liver or in livers following exposure to many different inducers including carcinogens (*3, 21-23*). Virtually all xenobiotics lead to either no change or most often increases in the activities of one or more of the microsomal cytochromes P-450, epoxide hydrolase and the various "drug-metabolizing" enzymes. In addition, one often sees elevations in glutathione concentrations and in activities of one or more glutathione-S-transferases and UDP-glucuronyltransferase, especially I.

In contrast, hyperplastic nodules induced by one of several different carcinogens have been found to have low levels of cytochromes P-450 and of several microsomal enzyme activities such as aminopyrine *N*-demethylase and aryl hydrocarbon hydroxylase (*20, 24-28*). Hyperplastic nodules showed a greater difference from control liver with a respect to *N*-hydroxylation and ring-3-hydroxylation of 2-AAF than other hydroxylations, indicating a nonuniform decrease in the various cytochromes P-450 in the nodules (*28, 29*). Also, diminished significantly are the levels of interaction of DMN and 2-AAF with proteins, RNA and DNA (*30*).

Coupled with the decreases in cytochromes P-450 and in activities of some drug-metabolizing microsomal enzymes are large elevations in selective microsomal enzymes as epoxide hydrolase (EH) (*23, 31*) and in cytosolic glutathione (*32*), γ-glutamyltransferase (γ-GT) (*33*), some glutathione-S-transferases (*34, 35*) and UDP-glucuronyltransferase I (*34, 36, 37*).

So far, no similar pattern of enzyme alteration has been found in induced livers (Fig. 1). Induced liver shows either increases or no effect of these several activities or liver components including microsomal monooxygenases.

γ-GT is high in fetal liver (*33*), but EH is not in neonatal rat liver (*38*). Fetal liver is low in cytochromes P-450. Also, the pattern of induction of microsomal 2-AAF-metabolizing enzymes with PB in fetal liver resembles that seen in nodules (*28*). However, glutathione is reported to be low in fetal liver (*39*) as is ligandin (*40, 41*), a major form of glutathione-S-transferase.

CONDITION	UPTAKE BY CELLS 2-AAF	MIXED FUNCTION OXYGENASES	DNA ADDUCTS — 2-AAF DMN	EPOXIDE HYDROLASE	GLUTATHIONE	GLUTATHIONE-S-TRANSFERASES (CDNB)	γ-GLUTAMYL TRANSFERASE	UDP-GLUCURONIDE TRANSFERASE
PHENOBARBITAL		↑		↑	↑	↑	⇡	↑
OTHER INDUCERS		↑		↑	↑	↑		↑
HEPATOCYTE NODULES	⬇	⬇	⬇	⬆	⬆	⬆	⬆	⬆

FIGURE 1. Summary of comparison of metabolic patterns in normal liver induced by phenobarbital or many other xenobiotics and in hyperplastic nodules induced by one of several different chemical carcinogens. The pattern in liver nodules would appear to be related to the acquisition of a new biochemical program associated with resistance to some inhibitory effects of carcinogens.

The pattern of properties of the nodules to date appears to be appropriate for a new population that is resistant to the inhibitory effects of at least some xenobiotics. The low uptake of at least one hydrophobic compound, 2-AAF, the low levels of activation, the high levels of EH and the elevated levels of glutathione, γ-GT, glutathione-S-transferase and UDP-glucuronyltransferase all favor minimal uptake, minimal metabolism and maximal conjugation of 2-AAF and probably other xenobiotics. This contrasts sharply with the patterns seen in fetal or neonatal liver or after enzyme induction where increased or equal metabolism is coupled with increasing conjugation.

The hypothesis of resistance based on these biochemical patterns is now worthy of more vigorous tests. Consistent with this formulation is the observation that regardless of the way in which the nodules are induced, the patterns are remarkably similar. This includes use of 2-AAF alone, DENA plus selection with 2-AAF plus PH or CCl_4, 3′-methyl-4-dimethylaminoazobenzene, prolonged ethionine feeding or initiation with DENA followed by promotion with with choline-methionine-deficient diet for 44 weeks (Ho and Roomi, unpublished results). The majority of studies were done many weeks or months after termination of exposure to any xenobiotic. Thus, it appears that the biochemical patterns seen in nodules to date are "permanently" imprinted and not due to any transitory induction.

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