

ARTICLE

Marker Enzymes of Rat Chemical Hepatocarcinogenesis in Human Liver Tumors

Ilona KOVALSZKY, Zsuzsa SCHAFF, Károly LAPIS, András JENEY

1st Institute of Pathology and Experimental Cancer Research, Semmelweis University of Medicine, Budapest; Hungary

Reduced glucose-6-phosphatase, increased GGT activity and reduction of cytochrome P-450 content are considered to be markers of chemical hepatocarcinogenesis in rats. The significance of these changes were studied in certain human liver lesions; adenoma, focal nodular hyperplasia and hepatocellular carcinoma all developed in non-cirrhotic livers. Enzymes showed normal values in 4 out of 5 adenomas, in 2/13 FNH and in 4/18 HCC samples. The decreased cP-450 content in HCC

Key words: liver tumor; GGT; G-6-Pase; cytochrome P-450

proved to be the most consistent alteration (12/18). Only 3 HCC samples possessed changes off all enzymes. These data suggest that at least those enzymes which are used as markers in rat chemical hepatocarcinogenesis have little or no biological significance in human liver tumors, primarily due to the intertumoral heterogeneity of enzyme activity. Such heterogeneity was observed in the peritumoral "normal" liver tissue, too. (Pathology Oncology Research Vol 2, No1-2, 56-58, 1996)

Introduction

Tumorigenesis is widely considered a multistep process.^{1,2} In several models of hepatocarcinogenesis, induced in rat liver by various chemicals, characteristic changes, including those in enzyme activity (cytochrom P-450, glucose-6-phosphatase, gamma glutamyl transferase)^{3,4,5} have been described as markers for the sequential events. It is a question to what extent such changes are relevant to human disorders, especially hepatocellular carcinoma (HCC) or conditions which can be putative forerunners of HCC (e.g. adenoma or focal nodular hyperplasia).

Materials and Methods

Samples

Five adenomas, 13 FNH, 18 HCC and 20 peritumoral tissues of human livers were studied. The average age of female patients, 4 with adenoma, 12 with FNH, and 11

with HCC, were 53, 32 and 38.5 years, respectively. The average age of 7 men with HCC was 55 years. These lesions developed in non-cirrhotic livers without history of hepatitis or jaundice. All samples were free from HBsAg. The majority of the female patients under 30 used contraceptive pills for various time periods.

Measurements

Surgically removed samples were kept on dry ice during transportation and kept at -70°C until use. Samples for histology and biochemical analysis were taken from the lesions and surrounding, seemingly non-tumorous, "normal" tissue.

Biochemical studies. For determining GGT activity, the tissues were homogenized and incubated in the presence of gamma-glutamyl-p-nitroanilide as substrate and glycylglycine as acceptor for 15 min at 37°C . The p-nitroaniline released was measured at 405 nm.⁶ G-6-Pase activity was measured in whole tissue homogenate in the presence of glucose-6-phosphate by determining the liberated inorganic phosphorus.⁷ The amount of cP-450 was studied on isolated microsomes. One gram of wet tissue was washed and homogenized in ice cold 50mM Tris-HCl, pH 7.4, containing 1.15% KCl. Nuclei and mitochondria were separated by 600 and 15000 g centrifugation, respectively. The microsomes were pelleted from the postmitochondrial

Received: Dec 20, 1995, accepted Feb 6, 1996

Correspondence: Ilona KOVALSZKY, MD, PhD; I. Institute of Pathology and Experimental Cancer Research, Semmelweis University of Medicine, Üllői út 26., H-1085 Budapest, Hungary; Tel/fax (36)(1)133-1142; e-mail: koval@korkl.sote.hu

Abbreviations: cP-450: cytochrome P-450, FNH: focal nodular hyperplasia, GGT: gamma glutamyl transpeptidase, G-6-Pase: glucose 6 phosphatase, HCC: hepatocellular carcinoma.

supernatant by 105 000 g for 90 min. Spectral measurement on the microsomes resuspended in 50 mM Tris-HCl, pH 7.7, containing 1 mM EDTA were performed using a dual beam spectrophotometer. The cP-450 concentrations were determined from the reduced carbonmonoxide difference spectra.⁸ All the enzymatic data were related to mg of proteins as determined by phenol reagent.⁹

Due to the lack of true normal liver samples, "normal" values were measured in the peritumoral (host) tissues. G-6-Pase and cP-450 values for host livers below average activity minus a standard deviation were considered to be low. The average values for these two enzymes were in good agreement with the published data for human livers.^{10,11,12} The activity of GGT is supposed to be low in normal liver.^{13,14} Therefore the mean of the 6 lowest enzyme activities was used as a limit. GGT was evaluated as elevated when at least a 100% increase above this limit value was found. Using these guidelines, G-6-Pase under 15 nmol Pi/min/mg protein, cP-450 under 0.2 nmol/mg microsomal protein and GGT above 50 mU/mg protein were considered abnormal.

Results and Discussion

The enzyme activities are shown in *Table 1*. (means) and 2. (individual values). Probably the most important finding was the heterogeneity of the values obtained for all enzymes. We thought, that measuring enzyme activities in the peritumoral (non-cirrhotic!) tissue could provide "normal" activity levels. However, the range of values questions the existence of "normality", instead suggests that the tumors influenced individually the peritumoral cells, mainly hepatocytes. Nevertheless, the means (*Table 1*) were very similar with one exception: the GGT activity increased in the peri-HCC tissue.

Regarding the lesions, average enzyme activity in adenoma samples showed the same pattern as the "normal". This was the case in one group of FNH samples (FNH II), while in the other (FNH I), enzyme activities

Table 1. Cytochrome P-450 content, glucose-6-phosphatase and gamma glutamyl transpeptidase activity in human liver

	cP-450 nM/mg microsomal- protein	G-6-Pase mM/Pi/min/ mg protein	GGT nM p-nitro ani- line/min/mg protein
liver#	0.55±0.3	34.43±21.1	36.00±14.1
adenoma	0.32±0.2*	24.54±22.0*	35.75±16.5*
liver#	0.51±0.3	32.92±17.0	46.45±29.3
FNH I	0.03±0.03	2.50±1.9 ^b	233.90±179.6 ^b
FNH II	0.55±0.2*	30.78±14.3	49.22±33.80
liver#	0.48±0.3	36.76±20.1	87.7±69.3
HCC	0.16±0.2 ^b	20.20±18.4 ^b	127.04±187.9*

peritumoral (host, "normal") liver; S - significant, p<0.005; * - non significant

were similar to those observed in rat chemical carcinogenesis. A significant decrease of cP-450 and G-6-Pase appeared in HCC, together with elevated GGT activity. As mentioned, increased GGT was also characteristic for the peritumorous tissue surrounding HCC. On the other hand significant average value variability of individual tumors was found (*Table 2*). Increased GGT and decreased G-6-Pase was measured only in a fraction of HCC samples (10/18 and 5/18 respectively). The most consistent alteration was the decrease of cP-450 (12/18).

Table 2. Marker enzyme alteration in individual human liver tumors

Marker alteration	Adenoma		FNH		HCC	
	Host	Lesion	Host	Lesion	Host	Lesion
Decreased G-6-Pase (<15nmol Pi/min/mg prot.)	0/3	1/5	1/4	7/13	1/13	5/18*
Decreased cP-450 (<0.2nmol/mg prot.)	0/3	1/5	1/4	5/13	2/13	12/18
Increased GGT (>50mU/mg prot.)	1/3	0/5	1/4	3/13	11/13	10/18

The great variance in the activity of marker enzymes raises serious problems with our attempt to find a connection between preneoplastic and neoplastic liver lesions. Apparently, enzymatic changes in the tumors could be induced by inadequate blood supply, drugs consumed prior to surgery and other factors not associated with the neoplastic proliferation or progression. The etiology of a liver tumor also may influence the marker enzyme changes. In fact, it was documented in our previous study that only very modest changes in marker enzymes developed in MC 29 virus induced hepatoma.¹⁵ Hepatomas induced with peroxisome proliferators do not contain elevated GGT activity either.¹⁶ The presence of cirrhosis is also capable of modifying enzyme activities.¹⁷ Although several factors may determine the behaviour of these "marker" enzymes, their occurrence is just an illustration of tumor heterogeneity. In other words, it supports the idea that different tumors are built up from cells with diverse phenotypes.

As a whole, our study failed to show characteristic changes in the activity of certain enzymes in human liver adenomas, FNHs and HCCs. Although these enzymes might be useful markers in rat liver carcinogenesis, their changes are rather model-specific, and show little or no significance in the biological behaviour of human liver tumors.

References

1. Farber E: The sequential analysis of liver cancer induction. *Biochim Biophys Acta* 605:149-166, 1980.
2. Pitot HC and Sirica AE: The stages of initiation and promotion in hepatocarcinogenesis. *Biochim Biophys Acta* 605:191-215, 1980.

3. Roomi MW, Ho RK, Sarma DSR and Farber E: A common biochemical pattern in preneoplastic hepatocyte nodules generated in four different models in the rat. *Cancer Res* 45:564-571, 1985.
4. Mayer D, Moore M and Bannasch P: Biochemical correlation of glycogen content and activity of some enzymes of carbohydrate metabolism in rat liver during early stages of carcinogenesis. *J Cancer Res Clin Oncol* 104:99-108, 1982.
5. Kalengavi MMR and Desmet VJ: Gamma glutamyl transferase as oncofetal marker of experimental hepatocarcinogenesis in the rat. *Scand J Immunol (Suppl)* 8:547-556, 1978.
6. Szasz G: A kinetic photometric method for serum γ -glutamyl transpeptidase. *Clin Chem* 15:124-136, 1969.
7. Harper AE: Glucose-6-phosphatase. In: Bergmeyer HU, ed *Methods of enzymatic analysis*. Academic Press, New York, pp 788-792.
8. Omura T and Sato R: The carbon monoxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J Biol Chem* 177:751-766, 1964.
9. Hartree EF: Determination of protein: modification of the Lowry method that gives linear photometric response. *Anal Biochem* 48:422-427, 1972.
10. Taketa K, Shimamura J, Ueda M, Shimada Y and Kosaka K: Profiles of carbohydrate-metabolizing enzymes in human hepatocellular carcinomas and preneoplastic livers. *Cancer Res* 48:467-474, 1988.
11. Nelson EB, Raj PP, Belli KJ and Masters BSS: Oxidative drug metabolism in human liver microsomes. *J Pharmac exp Ther* 178:580-588, 1971.
12. Pelkonen O, Kalliala EH, Larmi TKI and Karki NT: Cytochrome P-450 linked monooxygenase system and drug-induced spectral interaction in human liver microsomes. *Chem-biol Interact* 9:205-216, 1974.
13. Selvaraj P and Balasubramanian KA: Soluble forms of γ -glutamyltransferase in human adult liver, fetal liver, and primary hepatoma compared. *Clin Chem* 29:90-95, 1983.
14. Miyazaki M, Wahid S, Miyano K, Yabe T, Sato J: Expression of gamma-glutamyltranspeptidase in cultures of spontaneously and chemically transformed rat liver cells. *Int J Cancer* 32:373-377, 1983.
15. Jeney A: Trends in pathobiochemical studies of tumor development and progression. *Acta Morph Hung* 34:311-323, 1986.
16. Rao MS, Kokkinakis DM, Subbarao V and Reddy JK: Peroxisome proliferator-induced hepatocarcinogenesis: levels of activating and detoxifying anzymes in hepatocellular carcinomas induced by ciprofibrate. *Carcinogenesis* 8:19-23, 1987.
17. Kovalszky I, Szeberényi Sz, Zalatnai A, Vincze I, Lapis K and Jeney A: Modification of DENA-induced hepatocarcinogenesis by CCl₄ cirrhosis. Comparison of the marker enzyme patterns. *Carcinogenesis* 13:773-778, 1992.