Inconsistent Expression of Glycolipid Sulfotransferase Activity between Hepatoma and Serum

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KAMIO, K., JIN, T., GASA, S., OHHIRA, M., HONKE, K., KASAI, N. and MAKITA, A. *Inconsistent Expression of Glycolipid Sulfotransferase Activity between Hepatoma and Serum*. Tohoku J. Exp. Med., 1992, 168 (1), 29-35 — Glycolipid sulfotransferase activity in human and rat hepatocellular carcinoma tissues was studied, since an elevated level of the enzyme activity in serum had been demonstrated in cancer patients. The level of the enzyme activity in carcinoma tissues, however, could not be distinguished from that of normal controls. Similar observations were made for rat hepatoma. A higher level of enzyme activity was observed in the female than in the male. Inconsistent expression between hepatoma tissue and serum suggests that humoral factor(s) derived from hepatoma tissue stimulates production of the sulfotransferase, which is released into the blood-stream in the host. — glycolipid sulfotransferase; hepatocellular carcinoma; LEC rat

Glycolipid sulfotransferase (EC 2.8.2.11; abbreviated to GLST) catalyzes the formation of sulfated glycolipid (sulfatide) from a precursor glycolipid and 3'-phosphoadenosine 5'-phosphosulfate (PAPS). A high level of the enzyme exists in some tissues like brain and kidney where sulfatides are abundant. This GLST differs from other sulfotransferases such as arylsulfotransferases, steroid sulfotransferases (Jakoby et al. 1980) and glycosaminoglycan sulfotransferases (Inoue et al. 1986). As to intracellular distribution of GLST, it is known to be bound to Golgi membranes through examinations of rat brain (Benjamins et al. 1982) and kidney (Fleischer and Zambrano 1974).

Received January 13, 1992; revision accepted for publication September 3, 1992.

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Abbreviations: GLST, glycolipid sulfotransferase; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; GalCer, galactosylceramide.

In human cancer tissues, the elevation was observed in lung cancer adenocarcinoma (Gasa et al. 1979), gastric cancer (Mitsuyama et al. 1983) and renal cell carcinoma (Sakakibara et al. 1989) but not in Wilms' tumor (Sakakibara et al. 1991). The elevated level of this sulfotransferase is associated with increased contents of the sulfatides in the tumor tissues. Of these cancers, the serum level of the enzyme activity was significantly elevated in patients with renal cell carcinoma (Gasa et al. 1990). In an extended study of the GLST level in sera from patients with various types of cancer, we found that the serum level was significantly higher in hepatocellular carcinoma than in other cancers and non-cancer hepatic diseases examined (Gasa et al. 1991). Since liver is a tissue poor in sulfatide (Sweeley and Siddiqui 1977), it is presumed that hepatocarcinogenesis is associated with induction of GLST in the tissue followed by the appearance of GLST in serum. This led us to examine the GLST activity in hepatoma tissues of humans and rats.

MATERIALS AND METHODS

Materials

Unlabeled PAPS and [^{35}S] PAPS (25 Ci/mmol) were purchased from Sigma (St. Louis, MO, USA) and New England Nuclear (Boston, MA, USA), respectively. Galactosylceramide (GalCer) was purified from porcine brain and used as an acceptor substrate for GLST.

Human liver tissue

Hepatocellular carcinoma tissues were obtained at autopsy and separated into involved and uninvolved portions. Liver tissues of cirrhosis and chronic hepatitis patients and liver free from pathological changes were obtained at autopsy within 6h after death, characterized histopathologically and stored at -80°C until use.

Rat primary hepatoma tissues

3'-Methyl-4-dimethylaminoazobenzene (Tokyo Kasei, Tokyo) was fed to LEA rats for 20 weeks followed by feeding with normal food for 4 weeks. The liver tissue was removed and separated into cancerous and non-cancerous (uninvolved) parts. Normal liver and serum were obtained from untreated LEA rats (20–24 weeks after birth, raised in this school). LEC rats (approximately one year after birth) which generate hepatoma spontaneously were raised in this school (Yoshida et al. 1987).

Enzyme preparation

Approximately 1 g of the tissue was homogenized with a Polytron homogenizer in 3 volumes of 50 mM Tris-HCl buffer, pH 7.5, 0.25 M sucrose, 5 mM MgCl_2 and centrifuged at $600\times g$ for 10 min. The supernatant was employed for assay of the GLST. In all experiments, duplicate determinations were performed.

Enzyme assay

The GLST activity was assayed using GalCer, [^{35}S] PAPS, enzyme and cofactors as reported previously (Sakakibara et al. 1989). One unit of the enzyme was defined as one pmol of sulfate incorporated into GalCer per hour. Protein was determined with a BCA protein assay kit (Kanto Chemicals, Tokyo) using bovine serum albumin as the standard.

RESULTS

Although the GLST activity level in normal and diseased human liver tissues was considerably lower than that in human kidney tissues, the activities measured by the method adopted were significant. The enzymatic reaction depended on the amount of protein (0.05 to 1 mg) in enzyme preparations used (liver and hepatocellular carcinoma) and on the reaction time till 5 hr. The radiolabeled enzymatic product was confirmed by thin-layer chromatography on silica gel (data not shown).

The GLST activities of individual liver tissues from the control, hepatoma and other liver diseases are shown in Fig. 1, and the data are summarized in Table 1. The activity level in hepatoma was indistinguishable from the control and among respective disease groups. The enzyme activity in hepatoma tended to be slightly decreased compared with those in normals and other diseases, but the difference had no statistical significance. The activity in hepatoma did not correlate with the concurrence of cirrhosis, while the enzyme levels of involved and uninvolved tissues tended to be higher in females than in males.

As with human hepatoma, the sulfotransferase level was not increased in chemically induced primary hepatomas of rats (5 cases) compared to the uninvolved liver tissues except in one case (Fig. 2). The uninvolved liver tissues from the azo dye-treated rats appeared to have a lower level of sulfotransferase

Fig. 1. GLST activity of human liver tissues. "Normal" shows pathological change-free liver; HCC, hepatocellular carcinoma; U.I., uninvolved parts of the liver in hepatoma patients; LC, liver cirrhosis; CH, chronic hepatitis. Each bar indicates the mean value of the activity in respective groups.

TABLE 1. *Glycolipid sulfotransferase activities in human liver tissues*

Subjects	Activity ^a (units/mg protein)	No. of Cases
Normal	31.3±18.3	6
Uninvolved ^b	31.8±14.0	12
Hepatoma	25.5±13.4	15
Liver cirrhosis	31.1±13.8	8
Chronic hepatitis	30.0	2

^aMean±S.D.^buninvolved parts of the liver in hepatoma patients.

Fig. 2. GLST activity of rat liver tissues.

Upper panel, female; lower panel, male. □, mean value of normal rat livers; ■, hepatoma; ▨, uninvolved liver tissues in hepatoma rats; ND, not determined.

TABLE 2. *Glycolipid sulfotransferase activity of liver tissues and sera in LEC rats and chemically induced and uninduced LEA rats*

Rat	GLST activity ^a in			
	Liver tissue ^b		Serum ^c	
	Female	Male	Female	Male
Normal (uninduced) LEA	125.9±50.4(8) ^d	55.8±12.3(6)	83.5±65.2(6)	119.4±125.0(6)
Induced LEA				
Uninvolved part	42.2±20.4(6)	20.0±9.7(7)	N.D. ^e	N.D.
Hepatoma	3.9(1) ^f	14.6±16.3(5) ^f	N.D.	N.D.
LEC with hepatoma	30.6(1) ^f	16.1(1) ^f	5.9(1)	<0.1(1)

^aMean ± S.D.^bunits/mg protein^cunits/ml^dNumber of cases.^eNot determined.^fGLST activity in hepatoma.

than in age-matched, untreated rats. Interestingly, the activity level was higher in the female (normal, 125.9±50.4 units per mg protein [$n=8$]; uninvolved liver tissues, 42.2±20.4 units per mg protein [$n=6$]) compared to that in the male (normal, 55.8±12.3 units per mg protein [$n=6$]; uninvolved, 20.0±9.7 units per mg protein [$n=7$]), though the number of cases was insufficient for definitive estimation. The same tendency toward higher activity in females as in the uninvolved tissues was observed in normal livers (see below). With respect to the induction of hepatoma, lesser producibility of cancer was observed in the female rats after treatment with the azo dye.

In order to examine if rat GLST levels were inconsistent between hepatoma and serum, which was observed in hepatoma patients, the GLST activity was assayed in hepatoma tissue and the serum from LEC rats, which develop hepatoma spontaneously (Yoshida et al. 1987), together with those in normal LEA rats, from which the LEC is derived, as a control. As shown in Table 2, the enzyme activity of the hepatoma tissue in LEC rats was decreased irrespective of sex compared to that in LEA rat liver. The serum activity of the enzyme was at a low level in both LEA and LEC rats, and no elevation of the activity was observed in sera from the hepatoma-bearing rats, indicating a species-specific difference in respect to the expression of the sulfotransferase in hepatoma subjects. Of 6 samples of normal rat sera, the activities in 2 cases were hardly detectable, as in 1 case of male LEC serum, and those in the other 4 cases were widely distributed from 20 to 333 units/ml.

DISCUSSION

Tumor-associated substances often leak into the sera of patients from tumor

tissues, although the releasing mechanism is not necessarily understood in detail. This is also the case in such Golgi membrane-bound enzymes as β -galactosyl-transferase where the transmembrane domain is truncated (Podolsky et al. 1977; Ueshima et al. 1991), and N-acetyl-glucosaminylphosphotransferase (Ohhira et al. 1991; Uehara et al. 1991). Regarding GLST as well, there was an elevated serum enzyme level (Gasa et al. 1990) in patients with renal cell carcinoma where the enzyme activity was markedly elevated (Sakakibara et al. 1989). In human hepatocellular carcinoma, sera from many, but not all, patients had considerable elevation of the sulfotransferase level (Gasa et al. 1991). Nevertheless, the present study showed that the enzyme level in human hepatoma tissues was in the normal range. Rat hepatoma either of azo dye-induced or spontaneous generation had a low GLST level in the serum, being indistinguishable from normal controls.

The origin of serum GLST in hepatoma patients still remains to be determined. However, it is conceivable that human hepatoma produces a humoral factor which stimulates generation of GLST in some organs, concequently releasing it into the bloodstream. In fact, a steroid hormone, estrogen, was reported to stimulate the biosynthesis of sulfatide, the product of GLST, in human endometrium (Kubushiro et al. 1989). This observation could explain why a higher level of hepatic GLST was found in female rats than in male rats in the present study. Preliminary observation in our laboratory (Kobayashi, unpublished data) showed that hepatocellular growth factor enhanced GLST activity in kidney cells. Alternatively, higher expression of the activity in normal female rat liver may possibly be related to the lesser producibility of the hepatoma in the female rat. Since the levels of the activity in human hepatoma as well as in normal human liver were not distinguishable between female and male subjects, the discrepancies observed between humans and rats with hepatoma may be explained as different species specificities.

Acknowledgments

We are deeply indebted to Dr Taeko Miyagi, The research Institute for Tuberculosis and Cancer, Tohoku University for her suggestion on the preparation of chemically induced-rat hepatoma tissue, and Mr. Kim Barrymore for help in the preparation of the manuscript. This work was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture, Japan.

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