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Cancer Res 1970;30:309-311. Published online February 1, 1970.

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[CANCER RESEARCH 30, 309–311, February 1970]

The Biocleavage of Alkyl Glyceryl Ethers in Morris Hepatomas and Other Transplantable Neoplasms[1]

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SUMMARY

This study compares the ether-cleaving ability of normal rat liver homogenate with various transplantable tumor homogenates. The results show that the cleavage system is the most active in normal rat liver and Morris Hepatoma 7794A, much less active in Hepatoma 7777, and very low or absent in the other tumors tested. These data demonstrate that neoplasms that contain high levels of *O*-alkyl-diacylglycerols do not have an active alkyl ether cleavage system.

INTRODUCTION

In 1964, Tietz *et al.* (13) first described a cell-free system from rat liver for the cleavage of alkyl glyceryl ethers. Work in our laboratory has shown that in the rat the glyceryl ether cleavage system is located primarily in the liver (5). Glyceryl ether cleavage activity in rat intestines was approximately one-sixth of that in liver, whereas the activity in rat brain and perirenal fat was one-twentieth of that in liver. The level of activity in the mitochondrial supernatant of the whole slug, an organism rich in alkyl glyceryl ethers (12), was found to be similar to the level in the perirenal fat of the rat. Glyceryl ether cleavage activity was not detectable in the mitochondrial supernatant of Ehrlich ascites carcinoma cells (5).

The occurrence of ether-linked lipids in various tissues has been reviewed (6); neoplastic cells contain abnormally high concentrations of ethers in both neutral and phospholipid fractions (9, 10). The correlation of distribution patterns of glyceryl ethers and of the glyceryl ether cleavage system among tissues has led to the suggestion that the "highest activities of glyceryl ether-cleaving enzymes occur in cells that contain the lowest concentrations of ether-containing lipids" (5). The only apparent exception reported has been for the liver of *Squalus acanthias*, a tissue that has a high percentage of glyceryl ethers and one in which these lipids are oxidized and resynthesized rapidly (3). However, the study with *Squalus acanthias* was carried out *in vivo* and cannot be properly compared with the enzymatic biocleavage system studied *in vitro*.

Analysis of 2 Morris transplantable hepatomas and normal rat liver has revealed that the *O*-alkyl-diacylglycerol concentrations are significantly different in these tissues (7). The *O*-alkyl-diacylglycerol concentration was high in the 7777 hepatoma, but essentially none was found in the 7794A hepatoma and normal rat liver. However, *O*-alk-1-enyl-acylglycerylphosphorylethanolamine was found to be elevated in both hepatomas compared with normal liver. Although these tissues have a common origin, an alteration in metabolism resulting in a new lipid pattern has occurred.

This communication presents the results of a survey of the glyceryl ether-cleaving system in homogenates of normal rat liver, host rat liver, Morris hepatomas, and a series of other transplantable tumors that contain ether-linked lipids (9). Evidence was sought to substantiate or refute the suggestion that neoplasms that have the highest concentrations of ether-containing lipids have the lowest alkyl glyceryl ether-cleaving enzyme activities. The study was designed to determine whether the biocleavage enzyme for glyceryl ethers is absent from most tumors and whether this might explain the buildup of ether-linked lipids that occurs in neoplastic cells.

MATERIALS AND METHODS

The reagents used in this survey and their commercial sources were as follows. NAD^+, $NADP^+$, and glucose 6-phosphate dehydrogenase were obtained from P-L Biochemicals, Inc., Milwaukee, Wis.; glucose 6-phosphate was purchased from Calbiochem, Los Angeles, Calif.; 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine hydrochloride was purchased from Regis Chemical Company, Chicago, Ill.; sodium phosphate was obtained from Fisher Scientific Company, Pittsburgh, Pa. Oswald *et al.* (4) synthesized the chimyl-1-^{14}C alcohol.

The neoplastic tissues assayed for enzymic activity were obtained from transplantable tumors maintained in host rats and mice at this laboratory. The sources of the tumors have been listed previously (9). The hepatomas were received as a gift from Dr. H. P. Morris, Department of Biochemistry, College of Medicine, Howard University, Washington, D. C. 20001. The 7777 and 7794A hepatomas have been maintained through approximately 48 and 29 transplantations,

[1]This work has been supported in part by the American Cancer Society Grant P-470, and NIH Grant GM12562-06.

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Received April 24, 1969; accepted June 19, 1969.

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respectively. The specific tumors and their hosts are listed in Table 1. The normal livers were obtained from female Buffalo strain rats.

Homogenates of the tissues were prepared by grinding in 0.25 M sucrose with a Potter-Elvehjem homogenizer fitted with a Teflon pestle. Final dilution of homogenates was 15 ml/g original tissue, wet weight.

The standard incubation mixture at pH 7.4, which was used for evaluating the ether-cleaving enzyme system, contained, in a final 1.0-ml volume, 40 μmoles sodium phosphate, 0.60 μmole NAD^+, 0.38 μmole chimyl alcohol, 0.25 μmole 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine hydrochloride, 0.60 μmole $NADP^+$, 7.5 μmoles glucose 6-phosphate, 1 unit glucose 6-phosphate dehydrogenase, and tissue homogenate. These cofactor requirements were determined in earlier investigations (5, 13). The chimyl-1-^{14}C alcohol (specific activity, 0.14 μCi/μmole) was added in 10 μl propylene glycol. Incubations were carried out for 20 min in a thermostatically controlled water bath at 37° with shaking at a rate of 100 oscillations/min. Previous work has shown that the rate of the reaction is linear with time for at least 20 min in homogenates (13) or in subcellular fractions (5) of normal rat liver.

Lipids were extracted from the incubation mixture by the method of Bligh and Dyer (1). An aliquot of the lipid extract and standards were applied to various lanes on a 250-μ layer of Silica Gel G on a thin-layer chromatography plate; chromatographic development was carried out in a solvent system of hexane:diethyl ether:acetic acid (60:40:1, v/v). All samples were visualized with iodine vapor; and 4 areas of the thin-layer plate corresponding to glyceryl ethers, fatty alcohols, fatty acids, and fatty aldehydes were scraped directly into liquid scintillation vials. Radioassay of each vial was done in a Packard Tri-Carb liquid scintillation spectrometer. The percentage of ^{14}C in the area of the thin-layer plate corresponding to chimyl alcohol represented the percentage of chimyl alcohol that was not cleaved during the incubation. Protein concentrations in the tissue homogenates were estimated by a modification of the biuret method (2).

RESULTS AND DISCUSSION

The data presented in Table 1 show the ether cleavage activity of homogenates of the various neoplastic tissues in comparison with normal rat liver. The only tissues exhibiting appreciable glyceryl ether cleavage activity are normal rat liver and Hepatoma 7794A. The activity level of the 7777 hepatoma is only slightly higher than that of the other neoplastic tissues tested except that found in the 7794A hepatoma.

The suggestion that high levels of *O*-alkyl-diacylglycerols occur in neoplasms that have the lowest ether cleavage enzyme activity is confirmed in the series of neoplasms tested in these experiments. Rat livers and 7794A hepatomas are essentially free of *O*-alkyl-diacylglycerols, but the 7777 hepatoma has approximately 2% of its lipid as *O*-alkyl-diacylglycerols (7). The hepatomas resemble each other more than liver with respect to ether-linked phosphoglycerides, each with about 7% of the phosphatidylethanolamine as alk-1-enyl ether linkages, whereas normal liver from the Buffalo strain rats has approximately 3% alk-1-enyl ether-linked phospholipids (7). Similarly, the other tumors have larger quantities of ether-linked lipids than normal rat liver in both the neutral lipid and phospholipid fractions (9).

Because the 7777 hepatoma was originally derived from a tissue with the glyceryl ether cleavage system, the possibility exists that this tissue has lost the function of an essential component of the cleavage system through genetic aberra-

Table 1

Cleavage of chimyl alcohol by normal liver and neoplastic tissues

Tissues	Host animals	Cleavage enzyme activity[a]
Rat liver	Buffalo[b]	7.3
Host rat liver[c]	Buffalo[b]	5.8
Morris Hepatoma 7794A	Buffalo[b]	5.8
Morris Hepatoma 7777	Buffalo[b]	1.4
Preputial gland Tumor ESR-586	C57BL/6[d]	1.0
Sarcoma 180	HA/ICR[d]	0.42
Friend virus leukemia	DBA/2[d]	0.40
Melanoma B-16	C57BL/6[d]	0.31
Pituitary tumor	Wistar/Fu (females)[b]	0.30
Sarcoma T-241	C57BL/6[d]	0.30
Carcinoma E-0771	C57BL/6[d]	0.24
Fischer R-3259	Fischer 334[b]	0.19
Taper liver tumor	HA/ICR[d]	0.15
Ehrlich ascites carcinoma	HA/ICR[d]	0.14
KHZ mammary tumor	C3H[d]	0.11
Walker 256	Carworth Farms Nelson[b]	0.10

[a]Expressed as mμmoles chimyl alcohol cleaved/20 min/mg protein. Each value is the average of duplicate assays on a single sample.
[b]Rat strains.
[c]Host rat liver refers to pooled samples of livers of rats bearing 7794A and 7777 hepatomas.
[d]Mouse strains.

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tion. Whether this is due to the loss of a specific enzyme protein or some other factor remains to be investigated. The 7777 hepatoma is a unique neoplastic tissue in that it is similar to normal liver in morphology and function, but different in that it possesses significant levels of ether-linked lipids (7) and very low levels of alkyl ether cleavage activity.

Recent work in this laboratory (8, 11) has demonstrated that a number of the neoplasms included in this study contain a microsomal enzyme system that is capable of synthesizing alkyl glyceryl ether bonds. The presence of glyceryl ethers in tumors reflects their biosynthetic ability as well as their lack of degradative ability.

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