Inhibition of Growth of Morris Hepatomas 7777 and 7800 by Corn Oil

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Abstract

Intraperitoneal injection of trace amounts of corn oil prior to and following the injection of 40–50 mg of tissue from hepatoma 7777 or 7800 into the thigh of adult male Buffalo rats resulted in a marked decrease in the growth rate of both tumors. Exhaustive extraction of the corn oil with water indicated that the active component was not water soluble. Similar injections of safflower oil or isotonic saline had no effect on tumor growth rate. Analysis of the tissue phospholipid fatty acids revealed that the injected corn oil caused no change in the esterified fatty acids in this lipid fraction.

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Introduction

The role of phospholipids as structural components of cell membranes is accepted. Recognition of their involvement in the function of membrane bound enzymes [1–10], in cell fusion [11–13], and the regulation of RNA synthesis is more recent [14, 15]. While these studies implicate lipids in a number of important physiological functions of the cell, only a few in-vitro attempts have been made to utilize individual lipids to control growth [16–20].

The results reported in this study differ from those noted above in that here we describe the effects of the in-vivo administration of trace amounts of corn oil on the growth of Morris hepatoma 7777 and 7800. The effects achieved with corn oil are contrasted to those observed following injection of safflower oil or isotonic saline.

Material and Methods

Male rats of the Buffalo strain (Simonsen Laboratories, Gilroy, California) were used for this study and for stock transplants of Morris hepatomas 7777 and 7800. The biology and growth properties of these hepatomas have been described [13]. Hepatoma 7777 between 100–105

generations and hepatoma 7800 between 67–68 generations were used. Throughout this study the animals received Purina rat chow and water ad libitum.

“Mazola” brand corn oil and “Saff.o.life” brand safflower oil were administered as described in table I and II. Tumors were transplanted by injecting 40–50 mg of tissue into each thigh muscle with a 14 gauge needle fitted with a plunger. Animals were killed by cervical dislocation and the livers and hepatomas quantitatively removed. The hepatoma was freed from its capsule, weighed and frozen prior to lipid extraction. Lipids were extracted from the tissue and phospholipids isolated [21]. Methyl esters were prepared from the phospholipid fraction by transmethylation [22] and qualitatively analyzed [23] on a Becker model 417 gas chromatograph.

Results

The effects of intraperitoneal injections of trace amounts of isotonic saline, safflower oil and corn oil on the growth rate of hepatoma 7777 is presented in table I. The three treatments had little effect on body or liver weight. However, treatment with corn oil resulted in a marked decrease in tumor weight as compared to the other two treatment regimes. Application of Scheffe’s test indicated that a significant difference did not exist between the mean tumor weight of the saline or safflower oil treated animals as compared to the controls, but that the tumor weight of the corn oil treated animals was significantly different from the saline, safflower oil or control groups at a $p < 0.01$ [14].

The effect of a longer corn oil pretreatment interval on tumor growth is described in table II. Again a significant difference was not noted between the average tumor weights of animals treated with safflower oil and the untreated controls, but the tumor weights of animals treated with corn oil was significantly

Table I. Effect of corn oil and safflower oil on the growth of hepatoma 7777. Experimental animals received daily intraperitoneal injections of 0.6 ml isotonic saline, safflower oil or corn oil. The control animals received no treatment. The treatment was initiated two days prior to tumor transplant and continued for a total of 14 days. Tissues were removed 21 days after transplantation.

Treatment	Number of animals	Weight, g of body	liver	hepatoma
None	6	273 ± 71	10.1 ± 2.1	13.5 ± 1.4
Saline	4	304 ± 27	10.5 ± 0.7	13.9 ± 1.8
Safflower oil	4	291 ± 24	10.8 ± 0.5	12.9 ± 1.7
Corn oil	12	271 ± 55	10.6 ± 2.5	7.4 ± 1.8

1 Values are the means ± SD. The hepatoma weight is the combined weight of the hepatomas from each thigh.

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Table II. Effect of pretreatment with corn oil on the growth of hepatoma 7777 and 7800.

Hepatoma 7777: Animals received daily intraperitoneal injections of 0.2 ml/100 g body weight corn oil or safflower oil for seven days prior to tumor transplant. Daily injections were continued for an additional seven days after transplant. Control animals received no treatment. Tissues were removed seven days after transplantation. Hepatoma 7800: Animals received daily injections of corn and safflower oil as above. The tumor was transplanted seven days after the

initiation of treatment. Daily injections were continued for an additional 14 days after transplant. Tissues were removed 14 days after transplantation.

nitrogen. The phases were separated by centrifugation and the water wasted. The washed n-heptane solution was dried over Na₂SO₄ and filtered; the solvent was removed under vacuum at 30 °C. In two separate experiments similar to those in table I it was observed that the washed corn oil had the same inhibitory effect on tumor growth as the untreated oil. Also, since safflower oil did not inhibit tumor growth it is unlikely that an oxidation product of a polyunsaturated fatty acid is involved as this oil contains a greater proportion of polyunsaturated fatty acids than corn oil [24].

Treatment

Weight, g'

Number

hepatoma

body

liver

of

animals

9.8 ± 1.8 10.7 ± 0.7 10.6 ± 1.8

6.2 ± 2.8 7.1 ± 2.8 3.6 ± 0.9

273: 315: 284:

50 12

:57

Hepatoma 7777none 6

safflower oil 4 corn oil 12

327 ± 4 292 ± 16

12.6 ± 0.5 5.9 ± 1.7 11.3 ± 1.0 2.4 ± 0.7

Hepatoma 7800safflower oil 3corn oil 4

1 Values are expressed in the same way as in table I.

different from the other two groups at a $p < 0.01$. Similar results were observed in animals inoculated with the slower growing tumor 7800. In this instance a statistical analysis was not carried out as the number of animals in the corn oil treated group was small.

Treatment with corn oil did not change the fatty acid composition of the phospholipids in either the host liver or hepatoma (table III). The esterified fatty acids of the phospholipids in the hepatoma varied considerably from those of the host liver; the monoenoic species were increased in the hepatoma while stearic and the polyunsaturated moieties were decreased. The possibility that the corn oil might contain oxidation products of polyunsaturated fatty acids was investigated since in a previous study it was reported that exposure of linolenic acid to oxygen and ultra violet light produced an oxidation product that was denser than water and insoluble in petroleum ether. This product reportedly inhibited the growth of Ehrlich ascites tumor cells in mice [17]. To evaluate the presence of similar compounds here, corn oil was dissolved in a five fold excess of n-heptane and the solution washed five times with an equal volume of distilled water previously equilibrated with

Discussion

From these results it is apparent that daily injections of corn oil initiated prior to tumor inoculation result in a marked decrease in tumor growth rate. In two experiments not reported

here corn oil treatment initiated on the day of or five days after transplant had little or no effect on tumor growth.

The injection of safflower oil, an oil containing a greater proportion of polyunsaturated fatty acids than corn oil, did not inhibit tumor growth indicates that oxidation products of polyunsaturated fatty acids are not involved in the response to corn oil. The opposite effects observed with corn oil and safflower oil also indicate that the calories obtained from the injected oil did not alter the caloric intake of the experimental animals such that the uptake of other nutrients essential for optimal tumor growth was restricted.

That the trauma of interperitoneal injection did not lead to the in-vivo release of a substance capable of inhibiting hepatoma growth is indicated by the contrasting results observed between corn oil and safflower oil or isotonic saline. While the animals in each treatment group were not weight matched, the body weights overlapped indicating that body size and age were not variables that affected the inhibition of tumor growth. That the response to corn oil was similar in animals inoculated with either hepatoma 7777 or 7800 indicates that the effect was fundamental to the tumor and not related to the tumor's growth rate.

The distinct difference in the fatty acid composition of the phospholipid from host liver and hepatoma (table III) is similar to previous results [25–27]. The fatty acid composition of the phospholipids from the host liver and tumor did not change as a result of the corn oil supplement. These data,

Table III. Phospholipid fatty acids of host liver and hepatoma from control and corn oil treated rats. Treated animals received daily intraperitoneal injections of 0.6 ml corn oil, control animals 0.6 ml isotonic saline for a total of 21 days. The values presented in the table are the average percent weight, the values in brackets are the observed range. Fatty acids are designated carbon number: number of double bonds.

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along with effects observed with safflower oil, indicate that a change in the fatty acid composition of cellular phospholipids was not a factor contributing to the inhibition of tumor growth. The molecular events accounting for the growth inhibition are obscure, but it is obvious that intraperitoneal injection of corn oil induces a marked decrease in the growth rates of hepatomas 7777 and 7800.

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