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Liver Lipids Profiles in Nude Mice Implanted Subcutaneously with Cells of Human Prostate Adenocarcinoma Grade IV

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Abstract

Liver lipid changes in male BALB-c nude mice due to subcutaneously implanted human prostate metastatic grade IV adenocarcinoma was studied. The prostate cancer cells were cultured in F12 plus 7.5% horse serum and 25% fetal calf serum medium. When they reached confluence, some of these cells were fixed with glutaraldehyde and thoroughly washed with buffer then $4 \times 10^7$ cells were implanted into four mice. Four more mice were implanted with $4 \times 10^6$ viable, unfixed cells. Four uninjected mice served as controls. All the mice were sacrificed 18 days later. The total liver lipids (TLL) from each liver were extracted with chloroform:methanol and dried in separate vials under a stream of gaseous nitrogen. While being dried, the TLL of each liver sample were weighed intermittently until their weight remained constant. The weight of TLL in each vial was then divided by the wet weight of each corresponding extracted liver. This was done to normalize the liver lipids in terms of one gram of liver because livers of different wet weights were extracted. The TLL per gram of wet liver were similar in the control and livers of mice injected with viable cancerous cells and decreased in the livers of mice injected with glutaraldehyde-fixed cells. When the total lipids were fractionated into polar and nonpolar lipids on silicic gels, entirely different changes were seen. The TLL of the mice injected with untreated viable cancer cells and those injected with glutaraldehyde-fixed cells were similar while the control liver lipids classes were significantly different. There was a decrease in the percentage of neutral lipids and an increase in the polar fractions in the two groups of mice injected with either cells. The growth of the viable injected prostate cells did not cause any unique lipid changes in these two lipid class profiles. However, the triglycerides and total cholesterol showed wide variations among the livers of mice injected with viable cancer cells. The ratio of the polar/neutral lipids did not differ between the mice injected with either cells. Thus, the present study does not support the accepted explanation that the rapidly growing cancer cells are mainly responsible for the lipid changes seen in animals implanted with other types of tumor cells.

Introduction

The idea that a growing tumor may influence metabolism in tissues remote from its site is a fascinating one since it implies the existence of a humoral agent that could possibly be intercepted to study and control tumor growth. Indeed, several investigators have obtained evidence that the turnover of liver lipids is profoundly changed in animals bearing a variety of tumors (Weber and Cantero, 1959; Stein et al., 1966; Stein et al., 1966; Wood, 1975; Ruggieri et al., 1976; Redgrave et al., 1984). These altered turnover rates are reflected in the composition of the liver lipids and may indeed reveal a major source of substrate and energy for tumor growth.

Stein and his co-workers (1965, 1966) considered that the adipose tissue may be the ultimate source of triglyceride fatty acids for this purpose, since it has been observed in many studies that this tissue is depleted during tumor growth (Wood et al., 1974). The consequent changes taking place in the liver may represent an intermediate transport mechanism by which the fatty acids are efficiently furnished to the neoplastic cells. These fatty acids could consist of the essential fatty acids necessary for membrane formation and cell growth as well as a general mixture of the usual 16-and 18-carbon acids for energy. Since fatty acids for these purposes may be derived from the diet, synthesized in other tissues, or released from stored triglycerides, it has not been easy to predict in many cases, or to interpret the changes in the fatty acids themselves, particularly since some of the enzymes involved in fatty acids alteration are missing or have escaped control in the tumor (Chiappe et al., 1974). However, the changes in the lipids, which must be synthesized in the tissues themselves by known pathways, may be very revealing.

Nakazawa and Mead (1976) measured the alterations in liver lipids in female BALB-c nude mice bearing transplanted cells from a human ovarian adenocarcinoma and reported significant changes in the phospholipid:triglyceride (PL:TL) ratio brought about by increases in the
phospholipid fraction and decreases in the triglyceride fraction. This system was proposed as a model for studying similar changes seen in the liver lipids of human patients (Nakazawa and Yamagata, 1971). However, in these earlier experiments, the degree of tumor metastatic grade, as well as the cell numbers implanted in the mice were not characterized and quantified. Such lack of precise number of the implanted cells as well as varying degree of grades of the tumor metastasis level might have been the cause for the disparities seen in different studies done using freshly obtained human tissues as the source of cells to implant into mice.

In order to obviate some of the problems and gain further information on the mechanisms producing liver changes, the present study was done using an established clonal cell line derived from a human metastatic prostate grade IV adenocarcinoma. This permitted a more quantitative injection and, in addition, treatment of some of the cells by glutaraldehyde.

Materials and Methods

Chloroform, methanol, petroleum ether, and pentane were reagent grade and were distilled before use. Ethyl ether was used directly from a freshly opened can of reagent grade ether. Other reagents were also reagent grade. Standards for gas-liquid chromatography were obtained from NU-Chek Prep, Elysian, Minnesota and Applied Science Laboratory (State College, Pennsylvania).

Treatment of Animals.—Male BALB-c nude mice 19 weeks old were divided into three groups and treated as follows. Four control mice (group 1) were not injected. In group 2 four experimental mice were injected subcutaneously on the right side with 0.2 ml (4 by 10⁶ cells) of cells from cell line PC₃, a human metastatic prostate grade IV carcinoma, cloned and cultured in F₁₂ plus 7.5% horse serum and 25% fetal calf serum. Four mice, making group 3, were injected with the same type of cells but which had been fixed for one hour with 2.5% glutaraldehyde in 0.1 M sodium cacodylate pH 7.4 buffer and rinsed 20 hr. in 0.1 M sodium phosphate buffer, pH 7.4. The injected and the control mice were kept for 18 days before sacrifice by cervical dislocation. The livers were removed and frozen at -80°C until extraction.

Extraction and Analysis of Lipids.—Lipids were extracted from liver tissue with chloroform:methanol (2:1) and, after washing, the solvent was removed in a stream of nitrogen at less than 40°C. The total lipids in each sample were separated into non-polar and polar fractions by elution from small silicic acid columns with chloroform and methanol, respectively.

Portions of neutral fractions were separated into components on thin-layer plates (silica gel GD. Merck, Darmstadt). The thin layer plates were developed using a mixture of solvents composed of ethyl ether (redistilled and collected between 60-70°C), petroleum ether, acetic acid (20:80:1), or chloroform. The developed plates were sprayed with cupric acetate-phosphoric acid reagent and charred in an oven at 140°C (Fewster et al., 1969). The relative proportions of the separated components were measured using a Kontes densitometer (K-495000 connected to K-495150 integrator), and quantified using comparison with standards spotted on the same plates.

Analysis of Component Fatty Acids.—Aliquots of lipid samples were converted into methyl esters by methanolysis, using 5% methanolic HCL. The methyl esters of the component fatty acids were separated and quantified on a Beckman gas chromatograph (6C-M) fitted with disc integrator and using a 6' x 3/8'' column of silicic 10C. The chromatograph was operated at 185°C. Areas under the peaks were read from the integrator.

Table 1. Distribution of lipids in livers of control and injected mice.

<table>
<thead>
<tr>
<th>Treatment of Mice</th>
<th>Group 1 Uninjected Controls</th>
<th>Group 2 Viable Growing Cells Injected</th>
<th>Group 3 Glutaraldehyde Fixed Cells Injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Liver Lipid</td>
<td>23.2±0.16</td>
<td>23.2±0.04</td>
<td>19.7±0.05</td>
</tr>
<tr>
<td>Percent of Polar Lipids</td>
<td>70±4.4</td>
<td>78±2.3*</td>
<td>77±2.5*</td>
</tr>
<tr>
<td>Percent of Neutral Lipids</td>
<td>32±2.1</td>
<td>21±2.1*</td>
<td>22±1</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>69±2.9</td>
<td>71±12.5*</td>
<td>65±4.4*</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>31±3.3</td>
<td>28±18.2*</td>
<td>35±4.1*</td>
</tr>
<tr>
<td>Ratio: Polar Lipid Neutral Lipid</td>
<td>2.2±0.2</td>
<td>3.6±0.09*</td>
<td>3.5±0.26*</td>
</tr>
</tbody>
</table>

All statistical comparisons were done using student's t test.

Values are mean ± S.D. of mg per g wet wt of livers.

Triglyceride expressed as percent of neutral lipids.

Number of mice in each group. The liver from each mouse was separately analyzed.

* Significantly different from controls at the 0.01 level.

Results

Distribution of Lipids.—The state of the injected cells did not change the total percentage of lipids profiles in the livers of mice (Table 1). However, there was a significantly higher proportion of polar lipid and a lower proportion of neutral lipid within the TLL in the mice injected with any of the cell preparations. This is reflected in a highly significant difference in the ratios of polar to neutral lipids between the controls and all treated groups.
Moreover, there were no significant differences in these values among the treated groups (Table 2).

Within the neutral lipid fractions, there was considerable variation in the proportions of triglycerides and total cholesterol as reflected in the standard deviations. This variability was also apparent in the distribution of free and esterified cholesterol. The meaning of these changes is not apparent. Among the fatty acids, there were no significant differences attributed to the treatment of the mice though the usual differences (Table 2) in neutral and polar lipids were seen.

### Table 2. Distribution of major fatty acids in polar and neutral lipids of control and injected mice (mean of relative percentages ± SD)

<table>
<thead>
<tr>
<th></th>
<th>16:0</th>
<th>18:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>20:4</th>
<th>22:6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Polar Lipids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1 (4)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninjected Control</td>
<td>22.8±4.6</td>
<td>1.03±0.7</td>
<td>18.6±1</td>
<td>11.6±0.95</td>
<td>20.6±5.01</td>
<td>17.2±0.24</td>
<td>4.6±0.91</td>
</tr>
<tr>
<td>Group 2 (4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viable Cells Injected</td>
<td>27.9±4.5</td>
<td>0.26±1.0</td>
<td>12.0±7.8</td>
<td>14.9±4.04</td>
<td>18.4±5.02</td>
<td>18.8±1.02</td>
<td>4.0±1.75</td>
</tr>
<tr>
<td>Group 3 (4)</td>
<td></td>
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</tr>
<tr>
<td>Glutaraldehyde Fixed Cells Injected</td>
<td>25.3±3.7</td>
<td>0.3±0.02</td>
<td>21.1±0.07</td>
<td>12.3±1.3</td>
<td>16.0±1.05</td>
<td>29.4±1.35</td>
<td>3.3±2.25</td>
</tr>
<tr>
<td><strong>B. Neutral Lipids</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Group 1 (4)</td>
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</tr>
<tr>
<td>Uninjected Control</td>
<td>30.9±2.08</td>
<td>2.02±1.25</td>
<td>3.08±0.43</td>
<td>39.4±4.57</td>
<td>16.8±2.13</td>
<td>1.2±0.78</td>
<td>0.0</td>
</tr>
<tr>
<td>Group 2 (4)</td>
<td></td>
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</tr>
<tr>
<td>Viable Growing Cell Injected</td>
<td>35.8±6.74</td>
<td>1.8±1.94</td>
<td>3.8±0.94</td>
<td>32.3±4.92</td>
<td>16.5±4.92</td>
<td>2.3±0.41</td>
<td>0.0</td>
</tr>
<tr>
<td>Group 3 (4)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Glutaraldehyde Fixed Cell Injected</td>
<td>32.1±1.85</td>
<td>1.5±1.66</td>
<td>2.65±0.35</td>
<td>33.6±1.35</td>
<td>22.6±4.85</td>
<td>1.5±0.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>

The number in parentheses is the number of mice receiving each treatment. Each liver was separately analyzed.

### Discussion

The results of these experiments appear to confirm those of Nakazawa and Mead (1976) since there is an increase in the ratio of polar lipid to neutral lipid in mice injected with the neoplastic cells. They do not however, confirm the very large difference between control and experimental values shown in the prior study. Explanations for these differences are not difficult to suggest. First, whole tissue from an ovarian adenocarcinoma was injected in the earlier study, while known numbers of cells from an established line derived from a metastatic prostate adenocarcinoma were used in the present study with male mice. Second, two months elapsed between injection and sacrifice in the prior experiment, thus allowing for considerable cell proliferation. Third, male animals were used in the present study while females were used in the previous one. In the present study, only two weeks elapsed between injection and sacrifice with the hope that changes occurring before profound generalized metabolic changes occurred could be seen. Moreover, since numbers of cells were not known in the previous work, an attempt was made in the present study to compensate for the proliferation of the viable injected cells by injecting larger numbers of the non-growing cells. Whether for this or other reasons, no significant differences in total liver lipids were seen between the livers of mice injected with proliferating cells and un.injected controls. However, there was a significant decrease of total liver lipids in the livers of mice injected with non-proliferating cells. But, when the total liver lipids from each group were fractionated into polar and non-polar components and each component expressed as a percent of the total liver lipids for each experimental group, there was no significant difference between the percentages in the two groups injected with cells (Table 1). The observed changes, therefore, cannot be a result of rapid growth of the injected neoplastic cells, but must depend on some other unknown cause. Such a cause might be attributed to the reactions of the injected cells with the host animal immune response. The reaction may be to increase the availability to the foreign cells of fatty acids needed for growth and energy demands. In the present case however, only in one of the groups were the foreign cells able to utilize the fatty acids. With the possible exception of 18:1 and 16:1 (Table 2) the lack of significant change in the fatty acids of neutral and polar lipids may indicate that there was no selective use of theses as opposed to utilization of the entire complement of the lipid involved.

Kampschmidt and Upchurch (1966) have called attention to the fact that the site of tumor transplant in an animal may lead to variation in the results obtained. We do not know whether this is also true as far as lipids are concerned. Total cholesterol in the control mice was constant, whereas in the mice bearing cells, there were some fluctuations as reflected in the large standard deviations (Table 2) with most variations in the mice injected with viable cells. Failure to find a substantial decrease of triglycerides in the lipids of mice injected with viable cells does not seem to support the notion frequently cited that the decrease is due to the presence of a tumor (Stein et al., 1965; Stein et al., 1966). These animals did not show any gross pattern of lipid loss. It was not possible to tell the experimental from the control mice in this respect.

### Literature Cited


