Effect of Dietary Stearic Acid On The Daily Growth Of Mouse Mammary Tumors

An Honors Thesis (HONRS 499)

by

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I. Introduction

Breast cancer is one of the most prevalent forms of cancer in women and is a great source of concern. Although huge amounts of research have been done about breast cancer, there is still no consensus on what causes it or how to effectively treat it. To date, the only way to reduce the risk of breast cancer is to alter the amount of dietary fat consumed. Studies on this are inconclusive, but it is generally thought that reducing the amount of certain fats will reduce the risk of breast cancer. The purpose of this study will be to study the effects of different dietary fats on daily tumor growth and time to tumor. It is presumed that diets that contain stearic acid, as their fatty acid, will have a slower daily growth and a longer time to tumor.
II. Review of Related Literature

In 1974 saturated fat, in terms of nutrient labeling, was defined as the sum of lauric, myristic, palmitic and stearic acids (16). This definition was adequate for that time because nutrient information was voluntary except for when a special claim was made. In 1990 the Nutrition Labeling and Education Act (NLEA) kept the same definition of a saturated fat but required all foods to display nutritional information (16). The 1974 definition of a saturated fat is not adequate for this day and age because not all saturated fats behave the same, and the nutritional information can be misleading. Stearic acid is one such fatty acid that does not behave in the same way as other fatty acids. When stearic acid is the major source of fatty acids in an animals diet, there is a decrease in tumor incidence and an increase in time to tumor as compared to animals whose fatty acids are obtained from other sources. Bennett showed that mice fed a high fat diet, in which stearic acid is the primary fatty acid, have a longer time to initial development of spontaneous mammary adenocarcinomas than do mice fed a low fat diet (1). Tinsley also showed that stearic acid was associated with decreased tumor incidence and increased time to tumor (16). Stearic acid is also unique from other saturated fatty acids in that it does not raise cholesterol
levels (9). In fact, Bonanome has shown that stearic acid reduces cholesterol levels by as much as 14 percent as compared to other saturated fats (2). This only happens when stearic is ingested alone and therein lies the problem. Most foods that contain stearic acid also contain the saturated fatty acids that are most people try to avoid. To combat the intake of saturated fats, people usually decrease their total intake of fats. This is not always a good thing. When a person reduces their fat intake greatly, they might be increasing their risk of other complications. In order to combat this, and breast cancer, effectively, a procedure must be devised where a person can increase their stearic acid intake without substantially increasing or decreasing their overall, non-stearic, saturated fat intake.
III. Materials and Methods

Strain A/St mice were maintained in our laboratory by brother sister inbreeding. The mice were kept in a controlled environment of 21-24 C and a 12 h light: 12 h dark cycle and fed a 4.5% fat stock diet of Purina Lab Chow (Ralston Purina, St. Louis, MO) and water ad libitum upon weaning. Originally, 10mg portions were cut from a 400 to 500mg perpetuated mouse tumor. These portions were then surgically implanted into the right number 4 mammary gland. The incision was then closed by two surgical steel staples and an ear tag was placed in their left ear. The mice were then sacrificed two weeks later, and the resulting tumors were removed and weighed. After the first attempt at this surgery, the procedure was altered slightly. Instead of implanting 10mg portions, a set of 50mg portions and a set of 20mg portions were implanted in the right number 4 mammary glands of mice. These mice were then sacrificed two weeks later, and the tumors were removed and weighed.

After receiving no clear data, a new approach was tried. The mice were randomly separated into four groups of five. These groups were then feed one of the following diets after they were injected with a tumor suspension: SF containing 15% safflower oil, SF-1 containing 1% safflower oil, PA containing or SA-1 containing 14% stearic acid and 1% safflower oil.
A mouse with a perpetuated tumor of about 500mg was sacrificed and the tumor was placed in a RPMI solution. The tumor was masserated until the solution was homogenous. The solution was then filtered through nylon mesh. This allows only single cells pass through. A 1:10 single cell suspension to trypan blue solution was made and counted on the hemocytometer. The resulting solution was diluted so that an injection of .2 ml of solution contained 600,000 single cancer cells. Twenty mice that were two months old were injected with .2 ml of the cell suspension. The mice were checked daily, and when the resulting tumor reached the size of approximately 1-2mm across, or about 150-200mg, they were removed and weighed.
IV. Results

The results from the original 10 mg implants showed that there was no direct link in the size of tumor implanted and resulting size of the final tumor. Final weights ranged from 140 mg to 780 mg. This was too broad of a range to draw any conclusive results.

The results from the altered procedure also showed that no link can be drawn from the tumor implant size to the final tumor size. The tumors removed from the two mice that had approximately 50 mg implants different by 1383 mg and, the tumors from the 20 mg implants were different by 2786 mg (Table 1). Both trials showed no consistency in the size of the tumor implant and the time amount of concurrent growth. An interesting phenomenon was observed in the two mice that received the 50 mg implants. When the tumors were removed, they had a hard capsule covering almost like that of a cyst. When the capsule was pierced and removed, there was a build up of fluid observed within the capsule. It was almost as though the mice's bodies had responded to the large 50 mg implants as a foreign object rather than a tumor.

After all the tumors were removed from the mice with the 600,000 cell injection, the mg of growth per day were calculated (Table 3). The averages of daily growth for each diet were
## Table 1: Weights of Tumors Two Weeks After Implants

<table>
<thead>
<tr>
<th>Implant weight (mg)</th>
<th>Age of Mouse (months)</th>
<th>Tumor Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>7</td>
<td>4795</td>
</tr>
<tr>
<td>53</td>
<td>7</td>
<td>4287</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>2009</td>
</tr>
<tr>
<td>47</td>
<td>6</td>
<td>2904</td>
</tr>
</tbody>
</table>
TABLE 2: WEIGHTS AND DAYS OF REMOVAL OF TUMOR INJECTIONS

<table>
<thead>
<tr>
<th>Days</th>
<th>SF</th>
<th>SF-1</th>
<th>SA-1</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>110mg</td>
<td></td>
<td>299mg</td>
<td>95mg</td>
</tr>
<tr>
<td>21</td>
<td>89mg</td>
<td>110mg</td>
<td>202mg</td>
<td>237mg</td>
</tr>
<tr>
<td>23</td>
<td>100mg</td>
<td>225mg</td>
<td></td>
<td>105mg 54mg</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>128mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
<td></td>
<td>136mg</td>
<td>147mg</td>
</tr>
<tr>
<td>29</td>
<td></td>
<td></td>
<td>137mg</td>
<td>194mg</td>
</tr>
<tr>
<td>30</td>
<td>161mg</td>
<td></td>
<td></td>
<td>184mg</td>
</tr>
</tbody>
</table>
then found (Table 3). The averages were very different from the expected. The SA-1, or stearic acid rich, diet was found to have the highest average daily growth. There was also a different result than was expected in the unsaturated fatty acid diets. The diet that held only the minimum daily requirements of fatty acids had a higher average growth rate than the high fat diet.
Table 3: TUMOR GROWTH PER DAY AND AVERAGES IN mg

<table>
<thead>
<tr>
<th>Diets</th>
<th>Tumor 1</th>
<th>Tumor 2</th>
<th>Tumor 3</th>
<th>Tumor 4</th>
<th>Tumor 5</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF</td>
<td>6.11</td>
<td>4.24</td>
<td>4.00</td>
<td>5.37</td>
<td></td>
<td>4.93</td>
</tr>
<tr>
<td>SF-1</td>
<td>5.24</td>
<td>9.78</td>
<td>5.12</td>
<td>4.72</td>
<td>6.69</td>
<td>6.31</td>
</tr>
<tr>
<td>SA-1</td>
<td>16.60</td>
<td>9.78</td>
<td>12.48</td>
<td>5.23</td>
<td>5.65</td>
<td>10.26</td>
</tr>
<tr>
<td>PA</td>
<td>5.28</td>
<td>11.29</td>
<td>7.17</td>
<td>2.35</td>
<td>6.13</td>
<td>6.44</td>
</tr>
</tbody>
</table>
V. Discussion

According to the existing literature, stearic acid causes the least detrimental effects of all saturated fatty acids. This is not what we found, and in fact, we found the opposite to be true in our experiment. If this type of experiment was to be repeated, there should be more than 5 mice in each of the groups, and the entire experiment should be repeated more than once so that consistency could be proven. It has been shown many times in results from different research labs that stearic acid has different effects than other saturated fatty acids. According to our results, stearic acid is no different from other fatty acids in its effects on tumor growth.

Although we were trying to find a relationship between different dietary fats and daily growth of tumors, no data was found that showed us that one type of fatty acid is better for a person over another. Our tumor implanting technique was not successful at producing reproducible results. This inconsistent data may be the result of many avenues of error. First of all, we found that the size of the implant is crucial to its growth. If too big a piece of a tumor is implanted, the body acts strangely to it. The body encases the tumor in a cyst-like capsule and this probably impedes the true growth of the tumor. Second, we didn't use enough test subjects to get a very accurate
reading. If more test subjects were used, there might have been consistent results for the tumor growth per day. Also if we had put tumor implanted mice on the different fat diets, this might have gave us better results than the stock diets. For future reference, large amounts of test subjects and tumor implants under 50 mg should be used for this type of experimentation. We did find one interesting thing about the whole procedure. The tumors in the older mice, ones that were four months old, seemed to grow faster than the tumors in the younger mice, ones of two months old. The final tumor size in each of the age groups were about the same no matter what size of tumor was implanted. This could be an area that would be a source of good information.

Even after changing our procedure, we still came out the experiment without any data that specifically showed that one fatty acid is better than any other. The average tumor growth per day was far from what we expected. If the results had gone as expected, the PA diet would have yielded the highest average growth per day. As it turned out, PA yielded the second highest growth per day behind the SA-1 diet which should have yielded the lowest growth per day out of all of the diets. Not only were the saturated fats not as expected, but the results of the unsaturated diets was not as expected either. The high percentage saturated fat diet resulted in a lower daily growth than the diet that contained only the minimum daily requirements of fat. This data may not give consistent results because the a small group of each diet was used. This leaves a lot of room
for deviation from the normal.
VII. References


