Restriction of Tumor Growth in Mice by Sodium-deficient Diet

Burton P. Fine, Nicholas M. Ponzio, Thomas N. Denny, Elizabeth Maher, and Thomas R. Walters

ABSTRACT

Generalized malnutrition results in inhibition of tumorigenesis and tumor growth in experimental animal models. Neither the specific nutrient deficiency nor the mechanism has been definitely elucidated. We have shown previously that dietary sodium deprivation in rapidly growing rats retards protoplasmic growth. This effect was correlated to the extracellular fluid (ECF) volume expansion which is dependent on sodium accumulation. Since solid tumors are composed of a large quantity of ECF (which includes plasma volume) it was postulated that preventing the accumulation of new ECF by means of sodium restriction would influence tumor growth. The present study was designed to determine the effects of salt restriction on tumor growth and to relate these effects to ECF volume. Approximately $10^6$ viable B16 melanoma cells were injected into C57BL/6 × DBA/2 F1, and C57 mice. A salt restricted diet (sodium less than $3 \mu g/g$) was provided ad libitum. The drinking solution was distilled water for the experimental group and 0.45% saline solution for the controls. There was a significant decrease in tumor growth rates during ECF volume restriction. The total body ECF volume increased when dietary sodium was supplied but did not change during salt restriction. Therefore, the only source for the ECF in the tumor mass was from nontumorous tissue. We conclude that during dietary sodium restriction solid tumor growth is retarded and can proceed only to the extent that ECF is released from cachectic body tissues.

INTRODUCTION

Generalized malnutrition is a well recognized inhibitor of tumor growth in experimental animal models. Over 70 years ago Rous (1) demonstrated that a reduction of food intake inhibited the growth of both transplanted and spontaneous tumors in mice. This effect appears to be dependent mostly on caloric intake; supplementing the restricted diet with either starch or fat to augment the caloric intake restores the growth of the tumors to control values (2). Reduction of food intake also decreases carcinogenesis (tumorigenesis) of both spontaneous and experimentally induced tumors (3). Many nutritional factors, besides calories, can alter initiation or promotion of tumorigenesis, but only a few have been shown to retard growth of an established tumor. Low protein diets (4, 5), diets deficient in certain amino acids (6), and low zinc intake (7) have been associated with inhibition of tumor growth.

Sodium is an essential nutrient in the growing animal; a minimal amount is necessary for normal protoplasmic growth (8–10). Although a fraction of the sodium is utilized for bone mineralization and intracellular stores, most is located in the ECF. In fact, the quantity of sodium present in tissues determines the volume of the extracellular fluid. Because a large proportion of a solid tumor is composed of ECF, this study was designed to investigate tumor growth response to a diet deficient in sodium in which expansion of the ECF volume needed for new growth would be restricted.

MATERIALS AND METHODS

Study 1. Tumor Growth Rates. Thirty male 6-week-old, C57BL/6 × DBA/2 F1, (hereafter called B6D2F1) mice were randomly assigned to two groups and housed in polypropylene cages containing five mice from one group per cage. They were fed ad libitum a complete synthetic diet, American Institute of Nutrition (AIN-76) (11) without added sodium chloride; the measured sodium content was less than $3 \mu g/g$. One group received distilled water (experimental) and the other received 0.45% saline (75 meq Na+/liter) (controls) as their drinking solution. This concentration of saline is a palatable solution for mice which at their mean fluid intake (6.3 ml/mouse/day) supplied the dietary sodium required for growth (12).

A previous study showed growth retardation with the RCS syngeneic melanomas in SJL/J mice in response to sodium restricted diets (13). The present study sought to investigate the role of the ECF volume on tumor growth; therefore, we chose the more vascular tumor, melanoma B16. On the first day of the study $10^6$ viable B16 melanoma cells were injected into the s.c. flank tissue of all the mice. Viability was determined using trypan blue. Tumor size was measured with vernier callipers. The size was calculated as

$$\text{Tumor size (cm)}^2 = \frac{(\text{Length} \times \text{width})^2}{2}$$

The study was terminated on day 24 because of breakdown of the skin covering many of the tumors.

Study 2. Mortality Rates. Forty male 6-week-old B6D2F1 mice were assigned to two groups and housed and fed as in study 1. One group received drinking solution as distilled water and the other received 0.45% saline. On the first day $10^5$ viable B16 melanoma cells were injected into the peritoneum of each mouse. On the 34th day the study was terminated. The surviving mice were killed by exsanguination and the tumors were removed, weighed, and dried at 80°C until weight was stable (48 h).

Study 3. Alterations in Extracellular Fluid Volume. Since transplantation of virtually all B16 melanoma tumors is successful in syngeneic C57BL/6 mice, this strain was used to test the effects on food intake and changes in body composition due to both the tumor and sodium intake. Eighty male C57 mice, 6 weeks old, were randomly divided into 4 groups and housed and fed as in the previous studies. Two groups received distilled water and the other two received 0.45% saline as drinking solution. One million viable B16 melanoma cells were injected into the flank s.c. tissue of each mouse of one group receiving distilled water and one group receiving saline. The two factor study design was

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Dietary sodium</th>
<th>0</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

On the 20th day after transplantation bromide space was evaluated in surviving mice. A solution containing 0.5 μCi of $^{36}$Br was injected into the peritoneum and, after a 3-h equilibration, the mice were killed by exsanguination. The tumors were removed, weighed, counted for $^{36}$Br, and dried for 48 h at 80°C. The carcasses and pelts were dried, fat was removed with petroleum ether and homogenized, and a sample of each was counted for $^{36}$Br. Tissue ECF volume was estimated as

$$\text{Bromide space (ml/g) = } \frac{\text{Bromide (cpm)/g tissue}}{\text{Plasma } ^{36}\text{Br (cpm/ml)} \times \text{1.05/0.93}}$$
The 2 factors in the denominator account for the Gibbs-Donnan equilibrium and for plasma water, respectively.

Statistical Methods. Because of the threshold effect in detecting the tumor in study 1 there would be an artificial inflation of the standard error; therefore, the tumor size was evaluated by the nonparametric test (Wilcoxon two sample test). Frequencies were tested by χ² analysis. The main effects of dietary sodium and the tumor and their interactions were evaluated by the two factor analysis of variance. Computations were performed by the SAS statistical package for general linear models and for NPAR1WAY (14). The significance level used for treatment effect was 0.05.

RESULTS

Study 1. The incidence of measurable tumors in the control group was 12 of 15 (80%), whereas in the group on low sodium intake it was 7 of 14 (50%). The mean size of the palpable tumors at 24 days in the control group was 1.90 ± 0.62 cm³ and in the group on low sodium diet the size was 0.92 ± 0.26 (SE), P = 0.040 (Fig. 1). Two tumors from the low sodium and in the group on low sodium diet the size was 0.92 ± 0.26 24 days posttransplant.

The range was 0.48-8.53 g for the control and 0.04-2.96 for the experimental group. There was no difference in food intake between the groups.

Study 2. After i.p. injection of the tumor into B6D2F, mice the incidence of tumor "takes" was significantly less in the group on low sodium intake (control, 16 of 20; experimental, 10 of 20; P = 0.047). The mortality rate at 34 days posttransplant was 8 of 20 for the control group and 3 of 20 for the low sodium intake group (P = 0.077). In the survivors the wet tumor weight was 2.94 ± 0.49 g for the controls and 1.01 ± 0.35 g for the low sodium group (P = 0.002, Student's t test). The range was 0.48–8.53 g for the control and 0.04–2.96 for the experimental group. There was no difference in food intake between the groups.

Study 3. The tumor grew in all C57 mice given injections of B16 melanoma cells. The incidence of death at 20 days was the same in both groups (7 of 20). However, the tumor weight (wet and dry) was significantly less in the experimental group (Table 1, Lines 4 and 7). The difference could not be accounted for by the water content or the ECF volume, which were similar in both groups (Table 1, Lines 5 and 6). There was only minimal gross evidence of necrosis in the largest tumors.

The decreased food intake and the lower weights of the carcasses and pelts in the controls are shown to be due solely to the tumor and not to the sodium intake (Table 1, Lines 12 and 13). There were no interactions between the effects of the tumor and dietary sodium. The smaller tumor size in the salt deprived group was not caused by decreased food intake, which was similar in both sodium intake groups.

The whole body ECF volume was increased by dietary sodium and by the presence of the tumor (Table 1, Line 16). There was also an interaction between these two factors; i.e., the increase was greater than their sum. In the low sodium group with the tumor there was a decrease in the mean amount of ECF in the carcasses and pelts (0.70 ml) compared to the low sodium group without tumor (Table 1, Lines 14 and 15). This amount accounts for the mean ECF volume within the tumors (0.70 ml) (Table 1, Lines 4 and 6). As shown in Fig. 3, there was a significant correlation in the experimental group between the dry carcass plus pelt weights and the dry tumor weights (r = 0.83, P < 0.01). This relationship was not present in the control group.

Plasma sodium concentration was similar in all groups.

DISCUSSION

This study demonstrates decreased growth rates of transplanted melanoma tumors in mice during dietary sodium deprivation. That finding is consistent with the recognized necessity of dietary sodium for normal tissue growth in the young (8–10) and also for the replenishment of wasted protoplasm in adults (15). The mechanism for sodium deprivation induced growth failure is unknown, although it has been shown to be independent of food intake (10), serum sodium concentration (10), and potassium balance (9). The type of growth was not evaluated in this study, but we have previously reported a decrease in hyperplasia of RCS5 lymphoma during dietary sodium deprivation (13).

Decreased food intake retards tumor growth mostly by a substantial decrease in caloric intake. More than a 30% reduction in food intake causes only a slight inhibition of growth, whereas over 50% reduction is necessary to markedly reduce tumor growth rates (2). In our study the decreased food intake and lower carcass weights were caused by the tumor and not by sodium intake. Within the two tumor groups the intake of other
DIETARY SODIUM AND TUMOR GROWTH

Table 1 Tumor mass and fluid spaces in C57 mice 20 days after B16 melanoma transplantation

<table>
<thead>
<tr>
<th>Tumor dietary sodium</th>
<th>0</th>
<th>+</th>
<th>Main effect of</th>
<th>Interaction (tumor x sodium)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tumor (P)</td>
<td>Sodium (P)</td>
</tr>
<tr>
<td>1. No. of mice</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>2. Mortality</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3. Tumors in survivors</td>
<td>13</td>
<td>13</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>4. Wet wt (g)</td>
<td>3.6 ± 0.10 (20)</td>
<td>3.7 ± 0.10 (20)</td>
<td>2.6 ± 0.10 (13)</td>
<td>2.5 ± 0.20 (8)</td>
</tr>
<tr>
<td>5. ECF volume (ml)</td>
<td>2.5 ± 0.07 (17)</td>
<td>2.5 ± 0.14 (18)</td>
<td>2.5 ± 0.06 (12)</td>
<td>2.5 ± 0.20 (8)</td>
</tr>
<tr>
<td>6. Mean ± SEM</td>
<td>1.6 ± 0.07 (8)</td>
<td>1.9 ± 0.07 (9)</td>
<td>1.2 ± 0.07 (7)</td>
<td>1.1 ± 0.07 (4)</td>
</tr>
<tr>
<td>7. Whole body ECF vol-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-ume (ml)</td>
<td>5.1 ± 0.15 (9)</td>
<td>5.5 ± 0.45 (9)</td>
<td>5.4 ± 0.18 (8)</td>
<td>7.2 ± 0.08 (4)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of animals.

Fig. 3. Effect of dietary sodium on the relationship between the dry mass of carcass plus pelt and tumor, which in the group on low sodium intake reflects the redistribution of the extracellular fluid volume.

The mechanism whereby restricted sodium intake retards growth may be either direct (sodium concentration) or indirect (change in fluid spaces). In cultured cells there is a dose related response between extracellular sodium concentration and DNA synthesis (16, 17); this relationship is most pronounced below a sodium concentration of 100 mM. However, in our study serum sodium concentration was unchanged by diet, as has been shown previously in studies of retardation of normal growth by dietary sodium restriction (10). Therefore, altered sodium concentration, a reflection of water metabolism, is not the mechanism of the tumor growth inhibition.

Availability and retention of dietary sodium are necessary for expansion of ECF and plasma volumes during growth. The melanoma used in this study was composed of more than 50% ECF, regardless of sodium intake. Since in the sodium restricted mice there could be no production of new ECF, we postulated that the tumor could grow only to the extent that ECF freed from other tissue was available. The results of this study support this concept; almost all the ECF volume in the tumor of the sodium deprived mice could be accounted for from the decrease in volume from the cachexia of the carcass and pelt (the locations of the bulk of body ECF) (Table 1; Fig. 3). A similar inverse relationship was not observed during sodium feeding where tumor growth appears to be independent of carcass and pelt mass. An analogous situation has been shown previously in which about 50% of the carbon and nitrogen incorporated into tumor came from the host's muscle mass (18).

The mechanism by which restriction of ECF volume expansion by dietary sodium deprivation retards normal or neoplastic growth is unknown. The movement of sodium into cells via the Na+/H+ antiporter has been implicated as an early event in the proliferation of both normal and neoplastic cells (19, 20). The antiporter is activated by serum and circulating growth factors (21). There are various hormonal and nervous system alterations during sodium deprivations. Some of these factors have been shown to affect growth, but whether any are operative in altering tumor growth is not addressed by our study.

The postulate that growth of neoplastic tissue requires an increase in ECF volume, which in turn is dependent upon retention of dietary sodium, was investigated in this study. The findings support the tenet that dietary sodium restriction retards tumor growth and that the amount of growth appears to be dependent on the volume of ECF released from cachectic body tissues.

ACKNOWLEDGMENTS

The authors thank Marie Ellis for her secretarial skills and Diane L. Fine for her technical assistance.

REFERENCES


Downloaded from cancerres.aacrjournals.org on April 20, 2017. © 1988 American Association for Cancer Research.
DIETARY SODIUM AND TUMOR GROWTH

Restriction of Tumor Growth in Mice by Sodium-deficient Diet

Burton P. Fine, Nicholas M. Ponzo, Thomas N. Denny, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/12/3445

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.