Quantitative Biochemical Differences between Tumor and Host as a Basis for Cancer Chemotherapy

IV. Niacin and 2-Ethylamino-1,3,4-thiadiazole*

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Neoplasms, in general, have considerably lower metabolite and enzyme concentrations than do most normal tissues (1, 5, 6, 8, 10, 12, 14, 15). Since competitive inhibition by an antimetabolite depends upon the concentration ratio of a metabolite and its antagonist, it is reasoned that an antagonist may interfere with some metabolic step to a greater degree in cancer tissue than in those normal tissues where higher concentrations of a sensitive metabolite or enzyme occur. In addition, it is our hypothesis that, by selectively increasing the number of antimetabolites in combination therapy, it may be possible to proceed stepwise from inhibition of tumor growth, to cessation of growth, to regression, and, finally, to complete eradication of a tumor, without serious host toxicity or prolonged therapy time.

Results of this chemotherapeutic approach, with coordinated biochemical and biological studies in a single tumor-host system, have been previously reported for pyridoxine (12) and riboflavin (10). This paper reports the results of similar studies on niacin metabolism with the use of the same tumor-host system.

Niacin metabolism was chosen as the next system in our series of studies because, like pyridoxine and riboflavin, it has been reported to be in lower concentration in the majority of tumor tissues than in normal tissues (8, 13). The niacin antagonist employed was 2-ethylamino-1,3,4-thiadiazole1 (7).

MATERIALS AND METHODS

General.—C57BL mice, 2–4 months old and weighing 18–25 gm., were housed in plastic cages in an air-conditioned, constant-temperature room (74° F.). All mice received, ad libitum, a diet of Rockland pellets and water. The neoplasm employed was Adenocarcinoma 755 (mammary), transplanted into the axillary region by the usual trocar technic.

Niacin analysis.—Young adult mice were killed 21–23 days after tumor implantation by decapitation, following light etherization, with the trachea clamped to prevent aspiration of blood. Tissues were removed, trimmed, blotted, and, in the case of stomach and intestine, cleaned and rinsed with isotonic saline. The tissues were homogenized in water, diluted, brought to l N with respect to H2SO4, and autoclaved for 80 minutes at 15-lb. pressure.

Biological.—Testosterone in sesame oil was injected intramuscularly, while deoxypyridoxine and 2-ethylamino-1,3,4-thiadiazole, dissolved in saline, were administered intraperitoneally. The consistent tumor inhibition obtained by the intraperitoneal injection of 8-azaguanine in weakly alkaline solution has been previously documented (4). The effects on the 755 tumor of deoxypyridoxine (11, 12) and testosterone, given alone and in combination with 8-azaguanine (9), have been described.

To objectively evaluate the presence or absence of antineoplastic effect, the tumors were removed, and wet weights were determined to the nearest milligram. Results were considered to have a probability of chance variation of 1 per cent or less when:

\[
\frac{m_1 - m_2}{\sqrt{(\sigma m_1)^2 + (\sigma m_2)^2}} = 2.5 \text{ or greater},
\]

where \(m_1\) and \(m_2\) are the average tumor weights of the two groups being compared.

RESULTS

Biological studies.—Representative experiments showing the carcinostatic effects of 2-ethylamino-


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1,3,4-thiadiazole, when administered alone at a level of 60 mg/kg twice daily at a 4-hour interval, are recorded in Table 1 (Groups 1 and 2). Continuation of this dose for a longer period of time, or utilization of higher doses, resulted in a marked increase of host toxicity with little or no increase of tumor inhibition.

In order to corroborate Oleson’s data\(^1\) that this compound might be acting as a niacin antagonist, attempts were made to counteract its carcinostatic effects by the concomitant administration of the vitamin in the form of nicotinamide. As noted in Table 1, it is apparent that nicotinamide counteracted the tumor inhibitory effects of 2-ethylamino-1,3,4-thiadiazole.

Multi-combination chemotherapy was explored by studying the effect of the addition of a noncarcinostatic dose of 2-ethylamino-1,3,4-thiadiazole tumor-bearing animals on the day therapy was begun. Under the experimental conditions employed, the addition of testosterone and deoxypyridoxine, or testosterone and 2-ethylamino-1,3,4-thiadiazole, to 8-azaguanine did not increase the carcinostatic effect beyond that produced by 8-azaguanine alone. However, the quadruple combination caused a significantly increased carcinostatic effect over that produced by 8-azaguanine alone in

### TABLE 1

<table>
<thead>
<tr>
<th>EXP. NO.</th>
<th>SEX</th>
<th>GROUP†</th>
<th>AV. TUMOR WT. (Mg.±S.E.)</th>
<th>NO. ANIMALS</th>
<th>PER CENT HOST WT. CHANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>♀</td>
<td>Control</td>
<td>553±77</td>
<td>0/19</td>
<td>+9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thdz.</td>
<td>173±26</td>
<td>0/20</td>
<td>+5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nic.</td>
<td>446±86</td>
<td>0/20</td>
<td>+5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nic.+Thdz.</td>
<td>437±64</td>
<td>0/20</td>
<td>+4</td>
</tr>
<tr>
<td>2</td>
<td>♂</td>
<td>Control</td>
<td>653±79</td>
<td>0/20</td>
<td>+5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thdz.</td>
<td>316±39</td>
<td>5/19</td>
<td>−7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nic.</td>
<td>587±57</td>
<td>0/20</td>
<td>+3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nic.+Thdz.</td>
<td>470±49</td>
<td>0/19</td>
<td>−4</td>
</tr>
</tbody>
</table>

* Injections were begun on a 7-day-old Adenocarcinoma 755 grown in C57BL mice. Animals received nine daily injections.

† Thdz. = 2-ethylamino-1,3,4-thiadiazole (60 mg/kg), administered I.P. twice daily at a 4-hr, interval. Nic. = nicotinamide (200 mg/kg), administered I.P. twice daily at a 4-hr, interval, 15 minutes prior to injection of 2-ethylamino-1,3,4-thiadiazole.

### TABLE 2

<table>
<thead>
<tr>
<th>EXP. NO.</th>
<th>SEX</th>
<th>GROUP*</th>
<th>No. INJECTIONS †</th>
<th>AV. TUMOR WT. (Mg.±S.E.)</th>
<th>NO. ANIMALS</th>
<th>PER CENT HOST WT. CHANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>♀</td>
<td>Sac. Cont.</td>
<td>292±49</td>
<td>0/20</td>
<td>+6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A+T</td>
<td>505±57</td>
<td>2/20</td>
<td>+8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A+T+D</td>
<td>355±43</td>
<td>0/20</td>
<td>+2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A+T+Thdz.</td>
<td>558±68</td>
<td>0/20</td>
<td>−1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A+T+D+Thdz.</td>
<td>244±42</td>
<td>2/20</td>
<td>−8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>♂</td>
<td>Sac. Cont.</td>
<td>237±36</td>
<td>0/20</td>
<td>+6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A+T</td>
<td>505±52</td>
<td>0/21</td>
<td>+5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A+T+D</td>
<td>405±56</td>
<td>1/21</td>
<td>+4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A+T+Thdz.</td>
<td>434±41</td>
<td>0/21</td>
<td>+3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A+T+D+Thdz.</td>
<td>276±27</td>
<td>0/21</td>
<td>−1</td>
<td></td>
</tr>
</tbody>
</table>

* Sac. Cont. = group sacrificed at onset of therapy to other groups; A = 8-azaguanine (50 mg/kg), injected I.P. once daily; T = testosterone (50 mg/kg), injected I.M. once daily; D = deoxypyridoxine (30 mg/kg) and Thdz. = 2-ethylamino-1,3,4-thiadiazole (90 mg/kg), injected I.P. twice daily at a 4-hr, interval.

† Injections were begun on a 14-day-old 755 mammary adenocarcinoma grown in C57BL mice.
eight out of ten experiments, and comparison of this group with the sacrificed control group reveals virtual stoppage of tumor growth.

Niacin analysis.—Data on total niacin concentration in the Adenocarcinoma 755 in relation to nine normal tissues of host male mice are summarized in Table 3. Testes had appreciably lower levels of niacin than did the tumor, which, in turn, had about the same amounts as did the intestine, lungs, stomach, and brain. Skeletal muscle, heart, liver, and kidney had significantly greater levels.

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**STOPPAGE OF TUMOR GROWTH BY A FOUR-COMPOND COMBINATION (4 Φ AND 6 Φ EXPS.)**

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The niacin content of the tumors in female mice (51.2 ± 0.27 μg/gm wet weight) were found to be very similar to those of the males.

**DISCUSSION**

The growth of the 755 tumor, when treated with 8-azaguanine, is consistently about one-fourth that of saline controls, and this inhibition is unaffected by the addition of testosterone (9). For any other chemical combination or addition to be considered to have chemotherapeutic value in our tests, it must inhibit the tumor to a significantly greater degree than did this double combination. Our studies have indicated that the three-compound combination of 8-azaguanine, deoxypyridoxine, and testosterone will exert a consistent inhibitory effect on the 755 tumor but only after ten or more daily injections (10, 12). This triple combination cannot attain the degree of inhibition, regardless of length of treatment, which can be obtained after only six or seven injections when the thiadiazole compound is added to it. The lack of significant carcinostasis in the groups receiving the triple combination of 8-azaguanine, testosterone, and deoxypyridoxine in this report is explained on the basis of a treatment period of less than ten daily injections. These data support the hypothesis that selectively increasing the number of antimetabolites used in multi-combination chemotherapy can concomitantly increase the degree of antineoplastic effect and lessen the therapeutic time period.

Although there is evidence of slight toxicity with this four-compound combination, particularly in male mice, it is not felt that the degree of weight loss recorded here is in any way responsible for the marked inhibitory effect on the tumor. Unpublished observations in this and another laboratory demonstrate that the growth of Adenocarcinoma 755 can be augmented to a point of pre-
enocarcinoma 755 is not significantly affected by host weight loss up to —10 per cent.

Several normal tissues contain niacin in the same low range as that found in tumor tissue (Table 1). This fact, together with the proved carcinostatic properties of a niacin antagonist (2-ethylamino-1,3,4-thiadiazole), may appear to contradict a cancer chemotherapeutic approach that is based on quantitative differences, particularly one placing emphasis on tumor vulnerability because of low metabolite concentrations.

There are several factors, however, that must be considered in this regard. From experience with total vitamin B₆ concentrations, vitamin B₆-requiring enzymes, and a vitamin B₆ antagonist, it is known that: (a) although several tissues may have the same total vitamin concentration, marked differences exist in the concentrations of specific enzymes requiring the vitamin for activity³ (8); and (b) an antagonist may not act as a general antagonist of all the biochemical functions of a vitamin (8). While total vitamin concentrations in tissues may afford some insight into quantitative host-tumor differences, a more detailed enzymatic examination along the lines initiated by the studies referred to (3) is more likely to lead to a better understanding of the presence and significance of quantitative differences and of the potential role and value of specific antagonists. Quantitative enzyme differences may exist with respect to certain niacin-dependent enzymes, despite the lack of a quantitative differential in the

TABLE 3
NIACIN LEVELS IN THE 755 MOUSE ADENOCARCINOMA AND IN NORMAL TISSUES OF MALE C57 MICE*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MEAN ± SEₐ (μg/gm)</th>
<th>MEAN ± SEₐ (μg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>53.3 ± 1.36</td>
<td>302 ± 7.73</td>
</tr>
<tr>
<td>Testes</td>
<td>54.7 ± 1.83</td>
<td>311 ± 10.7</td>
</tr>
<tr>
<td>Intestine</td>
<td>57.6 ± 2.52</td>
<td>300 ± 11.3</td>
</tr>
<tr>
<td>Lungs</td>
<td>60.5 ± 2.09</td>
<td>338 ± 12.9</td>
</tr>
<tr>
<td>Stomach</td>
<td>61.7 ± 2.10</td>
<td>339 ± 11.2</td>
</tr>
<tr>
<td>Brain</td>
<td>66.1 ± 1.89</td>
<td>315 ± 8.65</td>
</tr>
<tr>
<td>Muscle</td>
<td>75.3 ± 3.32</td>
<td>308 ± 16.7</td>
</tr>
<tr>
<td>Heart</td>
<td>158 ± 1.64</td>
<td>384 ± 7.11</td>
</tr>
<tr>
<td>Liver</td>
<td>159 ± 2.14</td>
<td>379 ± 7.70</td>
</tr>
<tr>
<td>Kidney</td>
<td>179 ± 1.55</td>
<td>739 ± 5.55</td>
</tr>
</tbody>
</table>

* Sacrificed 21–23 days after tumor transplantation. The averages reflect the values of at least ten animals except for heart, liver, and kidney, for which tissues of seven animals were analyzed.

† Standard error of the mean.

CHART 2.—Total concentrations of five vitamins in mammary adenocarcinoma 755 and nine normal tissues of the host C57BL mouse.

L. S. Dietrich and D. M. Shapiro, unpublished data.
total concentration and, thus, be responsible for the observed carcinostasis with 2-ethylamino-1,3,4-thiadiazole.

In these studies emphasis has been placed on combinations of antagonists to block several metabolic pathways simultaneously and not on the ability of one compound, alone, to cause serious damage to the tumor. With this in mind, it was considered of interest to compare normal and tumor tissue concentrations of niacin and four other vitamins. A study of Chart 2 will reveal that, while tumor tissue has a low concentration of every one of the five vitamins measured, this is not true for any other one tissue. Thus, no single normal tissue has the identical over-all complement of the low enzymatic levels found in the cancer cell. Therefore, it would seem reasonable to suppose that a battery of antagonists for all five vitamins would result in severe damage to the tumor and partial to no damage to the majority of normal tissues. Such multi-combination studies are in progress, and, since the above quadruple combination produces virtual stoppage of tumor growth, it is hoped that the addition of more antimetabolites will produce regression of the tumor.

SUMMARY

2-Ethylamino-1,3,4-thiadiazole, an analog of niacin, was observed to have a carcinostatic effect against the 755 tumor grown in either sex of the C57BL mouse.

The tumor-inhibitory effects of this compound were partially or completely prevented by the prior injection of nicotinamide. This supports other evidence that, at least in part, 2-ethylamino-1,3,4-thiadiazole acts as a niacin antagonist.

The addition of 2-ethylamino-1,3,4-thiadiazole to the triple combination of 8-azaguanine, deoxy-pyridoxine, and testosterone resulted in increased carcinostasis, and in a shorter time period, than can be obtained with the triple combination. Study of the data revealed that this four-compound combination stopped tumor growth during the therapeutic period.

Total niacin was measured in Adenocarcinoma 755 and in nine normal tissues of the host C57BL mouse. Differences in concentrations between tumor and a number of normal tissues were not marked. The pattern of this and four other vitamins in the tumor and host tissues has been discussed.

ACKNOWLEDGMENTS

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