Ascorbic Acid Analog in Experimental Leukemia*

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Nichol and Welch (9, 10), Broquist et al. (1), Hill and Scott (6), and Welch et al. (16) found that ascorbic acid, a reducing agent, enhanced the formation of citrovorum factor (CF) from folie acid (PGA). Nichol and Welch (9, 10), indicated that glucoascorbate augments the enzymic conversion of PGA to CF by liver tissue in vitro, while Welch et al. (16), administering glucoascorbate parenterally to rats, disclosed that the analog is as active as ascorbic acid in this respect. Sokoloff et al. (14) found that a Sherman-LaMer diet, supplemented with 1 or 2 per cent of D-glucoascorbic acid, did not produce any apparent toxic effect on rats and mice but lowered the ascorbic acid concentrations of the blood plasma and certain tissues to nearly zero in 15-20 days and decreased the urinary excretion of the citrovorum factor in rats.

The present investigation concerns the effect of continuous administration of nontoxic doses, 0.75 and 1 per cent, of D-glucoascorbic acid on acute transplanted lymphocytic leukemia and spontaneous lymphocytic leukemia in mice.

MATERIALS AND METHODS

The Ak-lm strain of mice, with a high incidence of lymphocytic leukemia, and hybrid mice, Ak-Bc, were used for the experiments with D-glucoascorbic acid.

The mice were kept on a Sherman-LaMer scorbutogenic diet which was composed of: 18 gm. vitamin-free casein; 65 gm. corn starch; 5 gm. hydrogenated vegetable oil (Crisco); 2 gm. cod liver oil; 6 gm. dried brewers' yeast; and 5 gm. salt mixture #1. Control animals were kept on Ralston Purina Chow.

The technic of Farmer and Abt (2–4) for blood ascorbic acid determination was employed. Blood was mixed with potassium oxalate, centrifuged, deproteinized with fresh 5 per cent metaphosphoric acid solution, mixed, centrifuged again, and titrated with 2,6-dichlorophenolindophenol. For tissue ascorbic acid determination, the various fresh organs were weighed, extracted with cold 4 per cent trichloroacetic acid, ground in a Ten Broeck tissue grinder, diluted, centrifuged, and filtered. Ascorbic acid was determined by the method of Roe and Kuether (13) with dinitrophenylhydrazine and checked by the method of Ponting (12). The animals kept on D-glucoascorbic acid were fasted for 24–36 hours before ascorbic acid determination.

For the transplanted lymphocytic leukemia, the spleen of the leukemic donor, Ak-lm mouse, was minced in 2 cc. of isotonic saline solution, and the mince was filtered through three layers of cheesecloth. The resulting suspension of cells was counted and diluted in such a manner that 0.1 cc. of isotonic saline contained 1,000,000 cells. The injections were made intraperitoneally in the hybrid mice Ak-Bc of similar genetic constitution, age, and weight.

All dead animals were examined, and if the spleen was considerably enlarged and the size of lymph nodes increased they were considered leukemic.

D-Glucoascorbic acid was used in a powdered form added to the Sherman-LaMer diet.1

RESULTS

The ascorbic acid concentration of blood plasma.—Forty-five healthy mice Ak-lm, of an average age of 5 months, were divided into three groups. Group I received Purina chow; Group II, the Sherman-LaMer scorbutogenic diet to which 0.75 per cent of D-glucoascorbic acid was added; and Group III, the Sherman-LaMer diet with 1 per cent of D-glucoascorbic acid. Forty-five Ak-lm mice, averaging 8 months of age and showing first symptoms of lymphocytic leukemia, were divided into three

1 We wish to thank Dr. Phillip P. Gray and Dr. Harold E. Smith of the Wallerstein Company, New York, for generous gifts of D-glucoascorbic acid.

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groups which received the same diet as the healthy mice. The figures of Table 1 show that d-glucoascorbic acid added to the Sherman-LaMer diet brought the ascorbic acid concentration of blood plasma down from an average of 1.05 mg/100 cc to close to zero in 15 days. The figures for the normal values of ascorbic acid concentrations obtained by us for mouse were somewhat lower than the ones given by Leise et al. (7).

**TABLE 1**

**BLOOD PLASMA ASCORBIC ACID CONCENTRATION**

(Av. ascorbic acid concentration in mg/100 cc, 15 mice/group)

<table>
<thead>
<tr>
<th></th>
<th>Days</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purina chow</td>
<td>1.05±0.088</td>
<td>1.1±0.055</td>
<td></td>
</tr>
<tr>
<td>Sherman-LaMer+ 0.75 per cent glucoasc.</td>
<td>0.68±0.04</td>
<td>0.024±0.008</td>
<td></td>
</tr>
<tr>
<td>Sherman-LaMer+ 1.0 per cent glucoasc.</td>
<td>0.63±0.058</td>
<td>0.018±0.004</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leukemic mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purina chow</td>
<td>1.22±0.03</td>
<td>1.18±0.055</td>
<td></td>
</tr>
<tr>
<td>Sherman-LaMer+ 0.75 per cent glucoasc.</td>
<td>0.63±0.04</td>
<td>0.05±0.024</td>
<td></td>
</tr>
<tr>
<td>Sherman-LaMer+ 1.0 per cent glucoasc.</td>
<td>0.42±0.038</td>
<td>0.027±0.024</td>
<td></td>
</tr>
</tbody>
</table>

* Three mice died before the experiment was completed.
† Three mice died before the end of the experiment.
‡ Two mice died.

**TABLE 2**

**THE ASCORBIC ACID LEVELS OF SPLEEN AND ADRENAL**

(Ascorbic acid concentration expressed as mg/gm of dry tissue; 15 mice/group)

<table>
<thead>
<tr>
<th></th>
<th>Spleen</th>
<th>Adrenal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mice:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purina chow</td>
<td>3.93-0.55</td>
<td>19.45-1.42</td>
</tr>
<tr>
<td>Sherman-LaMer+ 1 per cent of glucoasc. after 15 days diet</td>
<td>0.51-0.09</td>
<td>5.19-0.87</td>
</tr>
<tr>
<td>Leukemic mice:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purina chow</td>
<td>4.18-0.65</td>
<td>22.53-1.78*</td>
</tr>
<tr>
<td>Sherman-LaMer+ 1 per cent glucoasc. after 15 days diet</td>
<td>0.38-0.21</td>
<td>7.35-0.82†</td>
</tr>
</tbody>
</table>

* Four mice died before termination of experiment.
† Five mice died before the end of experiment.

The ascorbic acid levels in spleen and adrenals. — Thirty normal Ak-1m mice, of an average age of 4 months, were divided into two groups. Group A was placed on Purina chow and Group B on the Sherman-LaMer diet supplemented with 1 per cent of d-glucocortic acid for 18 days. In another series of experiments, 30 Ak-1m mice of an average age of 8 months were divided into two groups placed on similar diets as the previous two groups. Table 2 summarizes the results. The ascorbic acid levels both in the spleen and adrenals of leukemic mice were higher than those of normal mice. D-Glucoascorbic acid added to the Sherman-LaMer diet in the amount of 1 per cent brought down the ascorbic levels in the spleen and adrenals both in normal and leukemic mice.

Acute lymphocytic leukemia. — Sixty hybrid mice, Ak-Bc, of an average age of 5 months, were divided into three groups. Group I remained on Purina Chow after having received intraperitoneally 0.1 cc. of spleen saline emulsion containing 1,000,000 lymphatic cells. Group II was placed on the Sherman-LaMer diet supplemented with 0.75 per cent of d-glucocortic acid a day prior to the injection. Group III was kept for 15 days prior to receiving the injection on the same diet as Group II. Table 3 gives the results of this trial. The results indicate that a diet containing 0.75 per cent d-glucocortic acid has no apparent effect on mouse acute lymphocytic leukemia.

Spontaneous lymphocytic leukemia. — Three groups, each of 40 Ak-1m mice, were used for this experimentation. Group I was kept on Purina Chow. Group II was placed at the age of 5 months on the Sherman-LaMer diet to which 0.75 per cent of d-glucocortic acid was added. Group III was placed on the same diet at the age of 2 months. Analyzing the figures of Table 4, one may conclude that d-glucocortic acid did not alter the incidence of lymphocytic leukemia in the strain Ak-1m mice but increased the average survival time by 26.8 per cent.

In another series of experiments, cortisone and d-glucocortic acid were administered simultaneously. In these experiments, we used 100 Ak-1m mice of 5 months of age, dividing them into three groups. Group I served as controls and remained on Purina chow. Group II, kept on Purina chow, was given 0.5 mg. daily of cortisone acetate, for 5 consecutive days, once every month. Group III was placed on the Sherman-LaMer diet supplemented with 0.75 gm. of d-glucocortic acid.

* One mouse did not "take," surviving 60 days after the inoculation.
† Started 1 day before inoculation.
‡ Started 15 days before inoculation.
§ Two mice survived over a 60-day period. No "take."

**TABLE 3**

**EFFECT OF D-GLUCOASCORBIC ACID ON ACUTE LYMPHOCYTIC LEUKEMIA IN MICE**

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. mice</th>
<th>Survival time av. (days)</th>
<th>Survival increase (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purina chow</td>
<td>20</td>
<td>10.3 (7-12)*</td>
<td>0.8</td>
</tr>
<tr>
<td>Sherman-LaMer diet+0.75 per cent glucoasc.</td>
<td>20</td>
<td>11.2 (9-15)</td>
<td>0.8</td>
</tr>
<tr>
<td>Sherman-LaMer diet+0.75 per cent glucoasc.</td>
<td>20</td>
<td>12.3 (9-15)§</td>
<td>19.4</td>
</tr>
</tbody>
</table>

* One mouse did not "take," surviving 60 days after the inoculation.
† Started 1 day before inoculation.
‡ Started 15 days before inoculation.
§ Two mice survived over a 60-day period. No "take."

We wish to thank Dr. Augustus Gibson of Merck & Co. for the generous supply of cortisone acetate.
Table 5 gives the summary of these trials. It appears that cortisone administered at a dose of 2.5 mg/month to 5-month-old mice reduced the incidence of leukemia from 75 to 50 per cent. When cortisone acetate was given in the same dose to the mice of the same age but kept on the Sherman-LaMer diet plus 0.75 per cent of D-glucoascorbic acid, the incidence of leukemia was reduced to 40 per cent, and the life span of leukemic mice was considerably prolonged.

In another series of experiments, the Ak-1m mice showing the first symptoms of leukemia were submitted to a combined treatment of cortisone and D-glucoascorbic acid. Cortisone acetate was administered in doses varying from 0.5 mg. to 4 mg/month. In these experiments which included 96 animals, the survival time was increased by 38 per cent, but all mice subsequently died from leukemia.

The recent work of Woolley and Peters (17) who, by administering cortisone to AKR mice beginning at the age of 1 month, 1 mg. for 8 consecutive days once every month, were able to reduce the incidence of leukemia to a considerable degree, points to the value of cortisone as a preventive measure against mouse spontaneous leukemia. The low ascorbic acid concentrations in blood plasma, induced by the analog, seemed to enhance this property of cortisone, as far as the Ak-1m strain was concerned.

**TABLE 4**

**EFFECT OF D-GLUCOASCORBIC ACID ON SPONTANEOUS LYMPHOCYTIC LEUKEMIA IN MICE**

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. mice</th>
<th>Survival time* (days)</th>
<th>No. leukemic mice†</th>
<th>Increase in survival time (per cent)</th>
<th>Per cent mice survived†</th>
<th>Dead from other causes (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purina chow</td>
<td>40</td>
<td>280 (255–310)</td>
<td>30</td>
<td>17.5</td>
<td>17.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Sherman-LaMer diet + 0.75 per cent glucoasc.§</td>
<td>40</td>
<td>282 (265–330)</td>
<td>28</td>
<td>18.4</td>
<td>15.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Sherman-LaMer diet + 0.75 per cent glucoasc.¶</td>
<td>40</td>
<td>555 (272–380)</td>
<td>26</td>
<td>26.8</td>
<td>17.5</td>
<td>17.5</td>
</tr>
</tbody>
</table>

* Nonleukemic mice are not included in these figures.
† Only the mice actually diagnosed as leukemic are included.
‡ Were alive 8 months after the termination of experiments and free of leukemia.
§ Started at age of 5 months.
¶ Started at age of 8 months.

**TABLE 5**

**EFFECT OF CORTISONE AND D-GLUCOASCORBIC ACID ON SPONTANEOUS LYMPHOCYTIC LEUKEMIA IN MICE**

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. mice</th>
<th>Survival time* (days)</th>
<th>No. leukemic mice†</th>
<th>Increase in survival time (per cent)</th>
<th>Per cent mice survived†</th>
<th>Dead from other causes (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purina chow</td>
<td>20</td>
<td>263 (224–305)</td>
<td>15</td>
<td>10.0</td>
<td>15.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Purina chow, 2.5 mg of cortisone/month</td>
<td>40</td>
<td>360 (286–350)</td>
<td>20</td>
<td>37.0</td>
<td>30.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Sherman-LaMer + 0.75 per cent glucoasc. and 2.5 mg of cortisone/month</td>
<td>40</td>
<td>420 (340–440)</td>
<td>16</td>
<td>67.5</td>
<td>40.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

* Nonleukemic mice are not included.
† Only the mice actually diagnosed as leukemic are included.
‡ Were alive 8 months after the termination of the experiments.

DISCUSSION

The work of Nichol and Welch and others showed that low ascorbic acid concentrations in blood plasma and tissue inhibit the conversion of PGA to CF, thus exerting certain antifolic properties. While in man, who does not synthesize ascorbic acid and depends entirely on dietary vitamin C, a clear-cut picture can be obtained, in mice and rats we are confronted with a much more complicated phenomenon. Although D-glucoascorbic acid, administered in nontoxic doses of 0.75, 1, or 2 per cent of ration brings the ascorbic acid concentrations in blood close to zero in 15–20 days, as was demonstrated by us, the synthesis of ascorbic acid is not suppressed completely.

Apparently, a sufficient supply of ascorbic acid is available in the mouse or rat for the conversion, partially reduced, of PGA to CF, in spite of the administration of D-glucoascorbic acid. This might explain the relatively slight effect of the analog on lymphocytic leukemia in mice.

Since D-glucoascorbic acid exerts no antagonistic activity to dietary vitamin C, it has no clinical application for abating the conversion of PGA to CF. On the other hand, a diet low in ascorbic acid accomplished this purpose satisfactorily. In a series of clinical trials on patients with chronic lymphocytic leukemia kept on a diet containing no more than 15 mg. of vitamin C per day, there was a drop in urinary excretion of citrovorum fac-
tor, according to Sokoloff et al. (15). Although the average CF content of normal urine is very small according to Broquist et al. (1) and Gabuzda et al. (5), the urine of leukemic patients kept on a vitamin C-free diet for a period of 1 month or more showed no trace of CF (8). These patients, nine altogether, observed for a period of about 2 years and manifesting no signs of scurvy syndrome, have been receiving 4 or 5 gm. of cortisone acetate each month, during the first 4 or 5 days of the month. Their condition remained more or less stationary (8). One may conclude that a diet low in ascorbic acid exerts certain, although mild, antifolic properties.

Ascorbic acid in the formation of the glycosidic bond between pentose/desoxypentose and the nucleic acids apparently plays a role in the formation of the DNA. Peterman and Schneider (11) and others reported that there was, in acute lymphocytic leukemia, an increase of about 50 per cent in the nuclear content of DNA in the spleen, as compared to normal mouse spleen. According to our investigation, the results of which will be reported in the near future, there is a decline in the nuclear content of DNA in the spleens of scorbutic guinea pigs. The DNA content was elevated by administration of ascorbic acid. This observation might be indicative of a certain involvement of ascorbic acid in the formation of the glycosidic bond between pentose/desoxypentose and the nucleic acids.

**SUMMARY**

D-Glucoascorbic acid added to a Sherman-LaMer scorbutogenic diet at a level of 0.75 or 1.0 per cent produced no apparent toxic effect on mice, but on this diet the ascorbic acid concentrations of the blood plasma, spleen, and adrenals were considerably lowered in 12–15 days.

Continuous feeding of a Sherman-LaMer ration containing 0.75 per cent of D-glucoascorbic acid for 2 weeks had no effect on the course of transplanted acute lymphocytic leukemia in the hybrid mice Ak-Bc.

Continuous feeding of a Sherman-LaMer ration containing 0.75 per cent of d-glucocascorbic acid to Ak-1m mice of 2 and 5 months of age did not affect the incidence of lymphocytic leukemia but prolonged the life span of leukemic mice by 26.8 and 18.4 per cent, respectively.

Administration of cortisone acetate, 0.5 mg. daily, for 5 consecutive days, once every month, to the 5-month-old Ak-1m mice kept on a Sherman-LaMer diet containing 0.75 per cent of D-glucocascorbic acid, decreased the incidence of leukemia from 75 to 50 per cent and prolonged the life span of mice by 67.5 per cent.

**REFERENCES**


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