Effect of Corticosteroids on Ribonuclease and Nucleic Acid Content in Lymphosarcoma P1798*

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SUMMARY

Administration of 9a-fluoroprednisolone to mice bearing the corticoid-sensitive strain of Lymphosarcoma P1798 resulted in a prompt regression of tumor size. This regression was accompanied by a selective decrease in tumor RNA. Tumor DNA concentration remained at control levels. These changes were preceded by an increased tumor ribonuclease activity in homogenates of tumor after the in vivo administration of corticosteroids. Regression of tumor size and activation of tumor ribonuclease were not observed in the resistant strain of tumor after steroid administration.

Lampkin-Hibbard has described two strains of Lymphosarcoma P1798 (7, 8). Strain I showed marked regression of tumor following corticoid treatment and was resistant to treatment with 5-fluorouracil (7). Strain II showed marked regression following treatment with 5-fluorouracil and was resistant to treatment with corticoids. This paper is concerned with a ribonuclease found in homogenates of P1798. It will be shown that the activity of this enzyme is increased by the administration of corticoid to mice bearing strain I. Similar treatment of mice bearing strain II failed to increase the activity of the enzyme. Selective loss of tumor RNA accompanies increased nuclease activity.

MATERIALS AND METHODS

BALB/c mice, obtained from Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and Battel Memorial Institute, Columbus, Ohio, were given inoculations of the appropriate strain of P1798 by the subcutaneous injection of a 30 per cent tumor mince into the inguinal region. After 2 weeks' implantation the tumors were approximately 2 cm. in diameter and were used for experimentation. Both strains of P1798 were obtained originally from Dr. J. M. Lampkin-Hibbard, University of Miami.

RNA, DNA, and protein analysis of tissues and homogenates were performed by the methods of Ceriotti (2), Seibert (16), and Lowry et al. (13), respectively. RNA-P32 was prepared from tumors of mice given injections 18 hours previously of 0.5 mc. P32 phosphate. The RNA was prepared by the method of Rosenbaum and Brown (14), and fraction B described therein was used for these experiments, since it contained the bulk of the precipitable RNA from this tumor. The aqueous solution contained 2–3 mg RNA/ml and was free of detectable DNA and protein. The specific activity was 1–3 x 10⁶ counts/min/mg RNA.

Ribonuclease activity was assayed by a modification of the method of Kalnitsky et al. (5). A 10 per cent tumor homogenate was prepared in 0.05 M acetate buffer, pH 5.7, containing 0.05 M EDTA by means of a conical glass homogenizer. The homogenate was centrifuged at 500 × g for 10 minutes and 0.1–0.2 ml. of the supernatant incubated with 0.1 ml. of the RNA-P32, 0.05 ml. of 0.05 M EDTA, and 0.45 ml. 0.05 M acetate buffer, pH 5.7 at 37° C. The reaction was stopped

Abbreviations used are: RNA = ribonucleic acid; DNA = deoxyribonucleic acid; 9a-FP = 9a-fluoroprednisolone; PCA = perchloric acid; 5-FU = 5-fluorouracil; and EDTA = ethylenediaminetetraacetate, tri-sodium salt.

Radioactive phosphate P32 was obtained from Oak Ridge National Laboratory, Oak Ridge, Tennessee, and was neutralized before use.
by the addition of 0.2 ml. of a 25 per cent per-
chloric acid solution containing 0.75 per cent
uranyl acetate and cooled in ice. The mixture was
centrifuged, and the radioactivity of a 0.10 ml.
 aliquot of supernatant was measured in a gas-flow
counter. The absorbancy of the supernatant was
measured at 260 m\textmu.

The ribonuclease assay was run at pH 5.7,
which is near the optimal pH of 5.9. The amount
of P\textsuperscript{32} rendered acid-soluble under the conditions
employed was proportional to the volume of the
500 \times g supernatant added to the incubation mix-
ture. RNA and protein were measured in the
500 \times g supernatant of tumor homogenates. Ribon-
uclease activity was expressed as the change in
absorbancy measured at 260 m\textmu/mg protein or
as:

\[
\frac{\text{counts/min P}^{32} \times (\text{mg. RNA substrate} + \text{mg. RNA in homogenate})}{\text{mg. P}^{32} \text{RNA} \times \text{mg. protein}}
\]

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. animals</th>
<th>Gm. tumor</th>
<th>Mg RNA/gm tumor</th>
<th>Mg DNA/gm tumor</th>
<th>RNA/DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain I:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>27</td>
<td>2.07 ± .22</td>
<td>7.39 ± .24</td>
<td>29.3 ± .8</td>
<td>.25</td>
</tr>
<tr>
<td>9a-FP</td>
<td>25</td>
<td>0.55* ± .09</td>
<td>5.05* ± 1.15</td>
<td>56.4 ± 1.6</td>
<td>.14</td>
</tr>
<tr>
<td>5-FU</td>
<td>8</td>
<td>1.95 ± .16</td>
<td>4.28* ± 1.0</td>
<td>22.1* ± 1.2</td>
<td>.19</td>
</tr>
<tr>
<td>Strain II:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>19</td>
<td>2.61 ± .27</td>
<td>5.89 ± .17</td>
<td>34.3 ± 1.3</td>
<td>.17</td>
</tr>
<tr>
<td>9a-FP</td>
<td>12</td>
<td>3.70* ± .54</td>
<td>5.59 ± .31</td>
<td>30.7 ± .7</td>
<td>.18</td>
</tr>
<tr>
<td>5-FU</td>
<td>6</td>
<td>0.66* ± .22</td>
<td>3.01* ± .05</td>
<td>24.4* ± 1.0</td>
<td>.18</td>
</tr>
</tbody>
</table>

* P < .01. Values are mean ± standard deviation.
† This apparent increase in tumor size after 9a-FP treatment is not real. External tumor measurements
revealed that only a normal amount of growth occurred during the experiment.

25 mg/kg body weight of 9a-FP or 5-FU was injected subcutaneously once daily for 2 days and sacrificed
18 hours after last injection.

This expression corrects for the decrease in radio-
activity released by the enzyme as a result of
dilution of the specific activity of the substrate by
added RNA in the enzyme under assay. When 15 \mu g.
crystalline pancreatic ribonuclease was
added to this assay system, the release of acid-
soluble radioactivity was proportional to the time
of incubation at 37\textdegree, and in 20 minutes 56 per
cent of the total radioactivity of the substrate was
converted to an acid-soluble form. Crystalline
deoxyribonuclease did not release any acid-soluble
radioactivity. A crude tumor ribonuclease prepa-
ration (0.4 mg.) released 75 per cent of the total
radioactivity into an acid-soluble form in 30
minutes. The release of radioactivity was linear
with time.

The differences in tumor size between control and
corticoid-treated groups were obtained at the start
of the experiment. Administration of 5-FU to
animals bearing strain I tumor resulted in no
change in tumor size and a fall in RNA and DNA.
The RNA/DNA ratio decreased to a lesser extent.
Similar treatment of mice bearing strain II tumor
resulted in striking tumor shrinkage and a fall in
both nucleic acids. The fall in the RNA/DNA
ratio reflects the greater fall in RNA.

**Effect of corticoid treatment on ribonuclease activ-
ity.**—Chart 1 shows the rise in ribonuclease specific
activity in the tumors 18 hours following a single
subcutaneous injection of 9a-FP, 25 mg/kg, to
mice bearing strain I tumors. Within 5 hours a
rise in enzyme activity was noted which became
maximal within 8 hours. Under these circumstances tumor regression is not evident before 24 hours. No such increase in enzyme activity was noted in strain II tumor (Chart 2). RNA-P₃₂ prepared from either strain I or II behaved in a similar fashion.

Table 2 shows that the administration of 5 mg. or more 9α-FP/kg body weight was followed 18 hours later by a significant increase in enzyme specific activity. The administration of 25 mg/kg body weight of progesterone and deoxycorticosterone also increased tumor ribonuclease activity;

**TABLE 2**

<table>
<thead>
<tr>
<th>Dose (mg/kg body weight)</th>
<th>Counts/min × total RNA mg P₃₂ RNA/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2820 ± 168*</td>
</tr>
<tr>
<td>0.1</td>
<td>2870 ± 60</td>
</tr>
<tr>
<td>1.0</td>
<td>2810 ± 181</td>
</tr>
<tr>
<td>5.0</td>
<td>3760 ± 128</td>
</tr>
<tr>
<td>10.0</td>
<td>3745 ± 289</td>
</tr>
<tr>
<td>25.0</td>
<td>4760 ± 292</td>
</tr>
</tbody>
</table>

* Mean ± S.E.M.

Six mice were studied for each dose level. Steroid was injected at 5 P.M., and the animals were sacrificed 18 hours later.

**TABLE 3**

<table>
<thead>
<tr>
<th>Steroid</th>
<th>No. mice</th>
<th>Mg steroid/ kg body wt.</th>
<th>O.D. 860 μg Mg protein/ min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>25</td>
<td>.195 ± .009</td>
</tr>
<tr>
<td>Progesterone</td>
<td>3</td>
<td>25</td>
<td>.370 ± .036</td>
</tr>
<tr>
<td>Progesterone</td>
<td>4</td>
<td>10</td>
<td>.319 ± .020</td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td>3</td>
<td>25</td>
<td>.380 ± .055</td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td>4</td>
<td>10</td>
<td>.200 ± .024</td>
</tr>
<tr>
<td>Testosterone</td>
<td>3</td>
<td>25</td>
<td>.194 ± .061</td>
</tr>
<tr>
<td>Estradiol-17β</td>
<td>3</td>
<td>25</td>
<td>.241 ± .052</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3</td>
<td>25</td>
<td>.179 ± .050</td>
</tr>
</tbody>
</table>

The indicated steroids were injected once daily for 2 days, and the mice were sacrificed 18 hours after the last injection, however, at a lower dose both of these steroids were inactive. These steroids did not produce any decrease in tumor size. Estradiol-17β, cholesterol, and testosterone were without effect on enzyme activity or tumor size (Table 3).

**Effect of 5-FU on tumor ribonuclease**—Charts 1 and 2 also contrast the effect of 5-FU with 9α-FP on tumor nuclease levels. These data represent typical ribonuclease assays 18 hours after the administration of 25 mg/kg of these agents to animals bearing strain I or II tumors, respectively. A small increase in strain I tumor nuclease activity following 5-FU compared with the greater response...
following corticoid administration is demonstrated in Chart 1. 5-FU administration to animals with strain II tumors did not influence nuclease activity.

The increase in nuclease following administration of 5-FU to mice with strain I tumor was not due to enhanced adrenal corticoid secretion caused by the chemotherapeutic agent. BALB/c mice were adrenalectomized and maintained on saline-sucrose solution. These mice were given inoculations of either strain I or II tumor and treated with 5-FU or 9a-FP as before. Strain II tumor showed considerable regression following 5-FU treatment, whereas strain I tumor was unaffected. No increase in ribonuclease activity was observed in strain II tumor, whereas strain I showed a slight increase in activity (Table 4). Corticoid treatment of such animals resulted in regression of strain I tumor only, and this strain showed a greater enhancement of tumor nuclease activity.

**Intracellular distribution of nuclease.**—The effect of corticoid treatment on the intracellular distribution of tumor ribonuclease was studied. Tumors were homogenized in 0.25 M sucrose by means of Potter-type glass homogenizers with a conical pestle. Aliquots were removed, frozen, and thawed in a dry ice-acetone mixture 3 times, then assayed for enzymatic activity. The major portion of the whole homogenate was centrifuged at 500 × g for 10 minutes, and the residue was discarded, since preliminary experiments revealed that this fraction had little or no activity. An aliquot was removed from the supernatant and assayed after the freeze-thaw technic described above. The remainder of the 500 × g supernatant was centrifuged at 10,000 × g for 10 minutes. The residue was resuspended in 0.25 M sucrose and recentlyrifuged, the supernatant being discarded. The residue was once again resuspended in the original volume of 0.25 M sucrose by means of a Teflon homogenizer and was assayed after freezing and thawing. The 10,000 × g supernatant was centrifuged at 100,000 × g for 60 minutes and the residue was resuspended in the original volume of 0.25 M sucrose and assayed without prior freezing and thawing. The 100,000 × g supernatant was also assayed without prior freezing and thawing.

The data presented in Table 5 indicate that the corticoid treatment of mice bearing Lymphosarcoma P1798 increased the total activity of tumor ribonuclease threefold in the 500 × g supernatant fraction. The total activities in the 10,000 × g residue, 100,000 × g residue, and the 100,000 × g supernatant were increased 4.2-, 3.3-, and 2.3-fold, respectively. In homogenates of control tumor, enzymatic activity was located principally in the 10,000 × g residue with lesser amounts in the

**Table 4**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Observations</th>
<th>Δ O.D. 860 μM/min mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>0.138 ± 0.016</td>
</tr>
<tr>
<td>5-FU</td>
<td>9</td>
<td>0.240 ± 0.011</td>
</tr>
</tbody>
</table>

25 mg 5-FU/kg body weight was administered daily for 2 days to mice that had been adrenalectomized 8 days earlier. Sacrificed 18 hours after last injection. Reaction mixture described in the text.

**Table 5**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Δ O.D. 860 μM/min mg tumor</th>
<th>Per cent of total activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 × g supernatant</td>
<td>4.03</td>
<td>52</td>
</tr>
<tr>
<td>10,000 × g residue</td>
<td>12.18</td>
<td>73</td>
</tr>
<tr>
<td>100,000 × g residue</td>
<td>2.11</td>
<td>8</td>
</tr>
<tr>
<td>100,000 × g supernatant</td>
<td>3.33</td>
<td>9</td>
</tr>
</tbody>
</table>

Tumor from each of four mice per group pooled. 25 mg/kg body weight of 9a-FP injected daily for 2 days, with mice sacrificed 18 hours after last injection. Tumors from each group were pooled and homogenized as described in text. The reaction mixture contained 0.1 ml. enzyme (0.085-0.08 mg protein), 1.65 ml. 0.05 M acetate buffer containing 0.435 mg. RNA, 0.05 ml. 0.01 M EDTA, and was incubated at 37° C. for 10 minutes in duplicate.
100,000 × g supernatant. The 100,000 × g residue had little activity. After corticoid treatment no significant change in the intracellular distribution of tumor ribonuclease activity was observed.

DISCUSSION

Tumor regression and enhanced nuclease activity occur in strain I and not strain II tumors following administration of corticoid to BALB/c hosts. The enzyme has been designated a ribonuclease, because it hydrolyzes purified RNA preparations which are devoid of measurable DNA and protein. Entirely similar results were obtained by measurement of the acid-soluble nucleotides produced by incubating yeast RNA with tissue extracts.

One simple interpretation of these data is that tumor regression is necessarily accompanied by increased ribonuclease activity; but this is not true, since strain II tumors show striking regression following 5-FU therapy and no increased ribonuclease activity. 5-FU therapy does not cause regression of strain I tumors, and the small increase in tumor ribonuclease under these circumstances is probably insufficient in amount to shrink the tumors. Out of a variety of steroids, only 9a-FP produced tumor shrinkage and an increase in ribonuclease activity. Progesterone and deoxycorticosterone, at a dose of 25 mg/kg body weight, stimulated ribonuclease activity without causing tumor shrinkage. 9a-FP, 10 mg/kg body weight, increased tumor ribonuclease activity and caused regression of tumor mass, whereas progesterone and deoxycorticosterone did not increase enzyme activity at this dose level. Testosterone, estradiol-17β, and cholesterol were not effective in stimulating increased ribonuclease activity. Increased tumor ribonuclease activity results from 9a-FP administration only in strain I tumor.

The RNA content of strain I tumors from corticoid-treated mice is selectively decreased with respect to DNA. No such effect is evident in strain II tumors. These data are consistent with the effect of increased ribonuclease activity as a result of corticoid action. Kass et al. (6) observed a selective fall of RNA with respect to DNA in rabbit lymph nodes following cortisone administration. Lowe and Williams (11, 12) noted a fall in RNA, DNA, and decreased polymerization of RNA in rat liver following cortisone administration. These results are consistent with a decreased RNA tissue content as a result of a corticoid-stimulated ribonuclease action. The results of Feigelson et al. (4) are at variance with those reported by Lowe and Williams. However, corticoid-stimulated ribonuclease action is not the only mechanism for selective decrease in tissue RNA. 5-FU produced this effect in strain II tumors without concomitant increase in ribonuclease activity.

Increased tumor ribonuclease activity following corticoid action could be due to enzyme induction or activation from an inactive state. This question remains unanswered. Administration of corticoid increases the ribonuclease activity found in the 10,000 × g residue and the 100,000 × g supernatant fraction of sucrose homogenates. This latter observation is suggestive of lysosomal release. However, after such treatment the total activity of tumor ribonuclease increases in a manner consistent with enzyme synthesis. The intracellular distribution of tumor ribonuclease is similar to that of a ribonuclease found in rat liver (3, 15). Cortisone administration, however, did not affect the activity of the liver enzyme. Studies are in progress to determine the identity of the enzymatic activity in tumor before and after treatment. It is tempting to speculate that a corticoid-enhanced ribonuclease activity is the cause for tumor regression. This concept is consistent with the precedence of enhanced enzymatic activity over tumor regression.

The administration of crystalline pancreatic ribonuclease to mice has resulted in retarded growth of some transplanted tumors (1, 9, 10). Increased ribonuclease activity in tumor might result in hydrolysis of messenger or soluble RNA with consequent reduction of protein synthesis. The purification, specificity, and effect on protein synthesis of the tumor ribonuclease are under active study.

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