Ultrastructural Changes of Mitochondria of the Neoplastic Cells following the Administration of Corticosteroids

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SUMMARY

The in vivo effect of corticosteroids on neoplasia was investigated morphometrically, with special reference to the ultrastructure of tumor mitochondria. The incidences of degenerative mitochondria with a moderate change (Grade 1) and with a severe change (Grade 2) in the cristae structure were found to increase with the progression of hormonal action in a cortisone-sensitive tumor, but not in an insensitive tumor: i.e., the incidences (%) of Grades 1 and 2 mitochondria 6 hr after the hormone administration divided by the corresponding values for the control (0 hr) were 38.5/1.4 and 15.2/1.9 in the ascitic Ehrlich tumor; 78.2/83.7 and 22.7/8.8 in the solid Ehrlich tumor; 0/1.2 and 0/2.9 in human leukemia Case 1; and 7.8/0 and 20.0/0 in human leukemia Case 2. The clinical course of these leukemias correlates well with the above parameter in regard to the responsiveness of malignant leukocytes to the corticosteroid therapy. The significance of mitochondria was discussed in relation to the action mechanism of cortisone on cancer.

INTRODUCTION

Differential responses to hydrocortisone between the liver parenchymal cells and the thymus lymphocytes were noted in the mitochondrial region, and they were related to the dual actions of hydrocortisone on the metabolism of these receptor organs (14). A question arises as to whether or not the lytic effect of hydrocortisone on mitochondria, as observed in the thymus lymphocyte, can be extended to a cortisone-sensitive neoplasm.

The present study was initiated to investigate the mitochondrial change of cortisone-sensitive and -insensitive tumor cells following hormone administration. A good correlation was established between the incidence of degenerative mitochondria and the hormone sensitivity of a neoplasm in the ascitic and solid Ehrlich tumor and in the 2 cases of human leukemia.

MATERIALS AND METHODS

The hypotetraploid Ehrlich tumor, supplied from the Second Department of Pathology, Nagoya University School of Medicine, was used for the preparation of ascitic as well as solid Ehrlich tumors. The tumor cells, $5 \times 10^6$/mouse, were inoculated i.p. or s.c. into SMA male mice. Ascitic tumor cells were collected on the 4th day after inoculation, and a solid tumor was excised on the 10th day after inoculation for electron microscopic study. Hydrocortisone acetate, Merck, Sharp and Dohme, West Point, Pa., was used for the animal experiment. The hormone, 1 mg/mouse, was injected i.m. at regular intervals prior to the collection of specimens. The tissues from 2 to 3 mice for each group were subjected to morphometric analysis. Samples of human blood cells were processed according to the method of Watanabe (23). In the clinical study, paramethasone and β-methasone (Syntex, S.A., Mexico City, Mexico) were given in doses of 90 and 45 mg/sq m body surface, respectively.

The details of the morphometric procedures for the electron micrographs are described in the preceding paper (14).

Results

Effect of Hydrocortisone Acetate on the Ultrastructure of Ehrlich Tumor Cells. On the basis of the findings with liver and thymus, emphasis was placed on the morphometric analysis of mitochondria in the tumor experiment. The time course of cortisone action was followed with the ascitic and solid Ehrlich tumor cells in SMA male mice. In the case of the ascitic tumor, the specimens were collected on the 4th day after inoculation, at intervals of 1 to 48 hr following the injection of 1 mg hydrocortisone acetate. Solid tumors were excised on the 10th day after inoculation, and the hormonal effect was investigated with the same time schedule that was used for the ascitic tumor. Tables 1 and 2 summarize the morphometric change of tumor mitochondria under the influence of the hormone. As in the thymus, the incidence of defective mitochondria increased with the lapse of time after injection in both the ascitic and solid tumors. The incidence of Grade 1 mitochondria of solid tumor cells was equally high in all groups, and the action of hydrocortisone was reflected only on the incidence of Grade 2 mitochondria. The mitochondrial size was not much affected by the hormone, and no giant mitochondria were observed within the tumor cell. A decrease in the matrix density was often associated with the defective cristae structure. Thus, the response of Ehrlich tumor cells may well be classified as the thymus type.

Effect of Paramethasone or β-Methasone on the Ultrastructure of Human Leukemia Cells. To answer the question of whether the cytolytic effect of
Table 1
Ultrastructural change of mitochondria of ascitic Ehrlich tumor cells following the injection of 1 mg hydrocortisone acetate

<table>
<thead>
<tr>
<th>Group</th>
<th>Geometric mean of mitochondrial sections (X 10^2 sq μm)</th>
<th>Incidence of defective mitochondria (%)</th>
<th>Grade 1</th>
<th>p^c</th>
<th>Grade 2</th>
<th>p^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.49 (208)§</td>
<td>1.4</td>
<td>n.s.</td>
<td>1.9</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>1 hr</td>
<td>3.12 (181)§</td>
<td>3.9</td>
<td>S</td>
<td>2.8</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>3 hr</td>
<td>1.80 (338)§</td>
<td>22.8</td>
<td>S</td>
<td>8.9</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>6 hr</td>
<td>2.25 (257)§</td>
<td>38.5</td>
<td>S</td>
<td>15.2</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>12 hr</td>
<td>1.99 (404)§</td>
<td>70.3</td>
<td>S</td>
<td>6.9</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>24 hr</td>
<td>2.08 (319)§</td>
<td>53.3</td>
<td>S</td>
<td>26.2</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>48 hr</td>
<td>3.14 (275)§</td>
<td>62.8</td>
<td>S</td>
<td>24.9</td>
<td>S</td>
<td></td>
</tr>
</tbody>
</table>

a No. in parentheses, mitochondrial particles counted.

b Grades 1 and 2 mitochondria, those that lack the crista structure in part and in toto, respectively.

c The statistical significance was calculated by χ^2 test. S, statistically significant (p < 0.05); n.s., not significant (0.05 < p).

Table 2
Ultrastructural change of mitochondria of solid Ehrlich tumor cells following the injection of 1 mg hydrocortisone acetate

<table>
<thead>
<tr>
<th>Group</th>
<th>Geometric mean of mitochondrial sections (X 10^3 sq μm)</th>
<th>Incidence of defective mitochondria (%)</th>
<th>Grade 1</th>
<th>p^c</th>
<th>Grade 2</th>
<th>p^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.73 (410)§</td>
<td>83.7</td>
<td>n.s.</td>
<td>8.8</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>1 hr</td>
<td>2.56 (305)§</td>
<td>87.2</td>
<td>n.s.</td>
<td>5.9</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>3 hr</td>
<td>2.55 (237)§</td>
<td>82.7</td>
<td>n.s.</td>
<td>15.2</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>6 hr</td>
<td>1.88 (348)§</td>
<td>78.2</td>
<td>n.s.</td>
<td>22.7</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>12 hr</td>
<td>2.85 (334)§</td>
<td>68.3</td>
<td>S</td>
<td>31.1</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>24 hr</td>
<td>2.63 (398)§</td>
<td>79.1</td>
<td>n.s.</td>
<td>15.3</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>48 hr</td>
<td>4.15 (344)§</td>
<td>66.6</td>
<td>S</td>
<td>31.7</td>
<td>S</td>
<td></td>
</tr>
</tbody>
</table>

a No. in parentheses, mitochondrial particles counted.

b Grades 1 and 2 mitochondria, those that lack the crista structure in part and in toto, respectively.

c The statistical significance was calculated by χ^2 test. S, statistically significant (p < 0.05); n.s., not significant (0.05 < p).

cortisone on the thymus lymphocytes or on the Ehrlich tumor cells is specifically related to the appearance of defective mitochondria, the ultrastructural changes of leukemia cells during the cortisone treatment were investigated with 2 patients. The clinical course of the leukemias is summarized in Charts 1 and 2. Case 1 was essentially nonresponsive to the steroid therapy, and promyelocytes predominated in the periphery as well as in the bone marrow. In spite of massive steroid therapy, the proportion of leukemia cells in the bone marrow and peripheral blood was not
Cortisone and Tumor Mitochondria

### Table 4

**Ultrastructural change of mitochondria of human leukemia cells in the course of the corticosteroid therapy, Case 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Geometric mean of mitochondrial sections ((\times 10^6 \text{ sq mm})^a)</th>
<th>Incidence of defective mitochondria (%)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Grade 1</td>
</tr>
<tr>
<td>Control</td>
<td>1.43 (227)</td>
<td>0</td>
</tr>
<tr>
<td>1 hr</td>
<td>1.09 (204)</td>
<td>1.5</td>
</tr>
<tr>
<td>3 hr</td>
<td>1.67 (240)</td>
<td>2.9</td>
</tr>
<tr>
<td>6 hr</td>
<td>1.12 (295)</td>
<td>7.8</td>
</tr>
<tr>
<td>24 hr</td>
<td>1.54 (273)</td>
<td>5.9</td>
</tr>
<tr>
<td>2 wk</td>
<td>1.10 (248)</td>
<td>4.8</td>
</tr>
</tbody>
</table>

\(^a\) No. in parentheses, mitochondrial particles counted.

\(^b\) The statistical significance was calculated by the Student’s t test for the geometric mean of mitochondrial sections and by \(\chi^2\) test for the incidence of defective mitochondria. S, statistically significant \((p < 0.05)\); n.s., not significant \((0.05 < p)\).

\(^c\) Grades 1 and 2 mitochondria, those that lack the crista structure in part and in toto, respectively.

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**Chart 1. Clinical course of human leukemia, Case 1.**

Patient T. H., female, 8 years old, with promyelocytic leukemia; has been treated with paramethasone alone for the 1st 20 days. Death on January 13, 1971, by intracranial bleeding. PLC, platelet count. Shaded portions in the histobar (myelogram) and in the line graph (peripheral blood), share of immature leukocytes.

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**Chart 2. Clinical course of human leukemia, Case 2.**

Patient S. H., male, 1 year, 4 months old, with acute, undifferentiated leukemia; has been treated with paramethasone and \(\beta\)-methasone for the 1st 4 weeks. Up to date (August 1, 1971), the patient is hematologically well controlled. PLC, platelet count. Shaded portions in the histobar (myelogram) and in the line graph (peripheral blood), share of immature leukocytes. For detailed sequence of the hormonal treatment, see text.

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affected throughout an observation period of 2 weeks. In contrast, paramethasone administration caused a complete remission in Case 2, as shown in Chart 2, which cytologically was classified as acute, undifferentiated leukemia. In Case 1, the 1st 24 mg paramethasone were administered p.o. immediately after the collection of the 0-hr specimen, and the 2nd 24 mg and 3rd 30 mg paramethasone were given between 6- and 24-hr exsanguinations. In case 2, the 1st 14 mg paramethasone were given p.o. and the 2nd and 3rd doses were given between 6- and 24-hr checkings. Because of the technical error, there was some loss in the course of p.o. administration, and the quantity of the 2nd and 3rd hormone doses was not estimated exactly. From the 4th administration on, paramethasone was replaced by \(\beta\)-methasone, and a total of 22 mg hormone (11 mg in 2 doses) were injected every day. The morphometric changes of mitochondria of leukemia cells are summarized in Tables 3 and 4. The mitochondria of the 1st case increased in size at 1 and 3 hr after hormone administration, but the incidence of degenerative mitochondria was very low throughout the experiment. In
contrast, the same parameter in the 2nd case apparently increased at 6 hr after the 1st administration of paramethasone. Very often, the membrane structure could hardly be resolved within mitochondria, as shown in Fig. 3b. The mitochondria of the cortisone-sensitive leukemia cells are larger in size, less compact in the matrix, and more irregular in shape, as compared with those of the cortisone-insensitive leukemia cells. The fact that the incidence of degenerative mitochondria declined at 24 hr might be related to the technical failure in the 2nd and 3rd p.o. administration of paramethasone. The incidence of Grade 2 mitochondria of the thymus lymphocyte in the 1 shot experiment reaches a peak at 6 hr and declines gradually from 12 through 48 hr. There was a fluctuation in mitochondrial size after hormone administration, but the change was bidirectional and less distinct in extent than that of liver mitochondria.

DISCUSSION

The clinical use of corticosteroids for the suppression of leukemia is an indirect outcome of earlier studies concerning the lympholytic action of ACTH and cortisone. Some palliative effects of cortisone could also be expected in the treatment of cancers of the breast and prostate. Biochemically, the catabolic action of cortisone on neoplasia was analyzed in terms of the incorporation of labeled precursors into the cellular nucleic acid and protein. The expression of the hormonal effect was somehow mediated by the presence of glucose (2, 19, 27). There were some discrepancies between the lymphocytolytic action in vivo and the metabolic activities in vitro of a given steroid for the respiration and for the process of nucleic acid-protein synthesis (2). Munck (17) showed, however, that the in vitro effect of a steroid at a physiological concentration was in good agreement with the in vivo effect for the same receptor tissue. The report of Schrek (20) on the viability test indicates that the cytolytic action in vitro of prednisolone might be correlated to the in vivo effect for the normal and malignant lymphocytes. The relation between cortisone sensitivity and cell structure is not clear as yet. The participation of membrane permeability is suspected in the volume change of an animal cell as a whole and of subcellular organelles under the influence of cortisone action in vivo (1, 15, 25) and in vitro (5). The responsiveness of the liver mitochondria to cortisone, as evidenced by electron microscopy, was also confirmed in the biochemical analysis of isolated liver mitochondria (3, 4, 6, 9, 24). The mitochondria of neoplastic cells are generally more fragile than those of the liver cells (8, 16, 18, 21). Graff and Bielka (7) stated that a tumor cell contained a relatively small number of mitochondria, which were scanty of crista structure and irregular in shape. The predominance of glycolysis over respiration in the energy metabolism of a tumor cell may have some relevance to the above findings.

Yamada et al. (26) mentioned the possible relationship between the antileukemic action of cortisone and morphological changes in the mitochondria of leukemia cells. So far, there is no systemic information available concerning the chronological and morphometric analysis of the leukemia cells under the influence of cortisone. The present study revealed a lytic action of cortisone on the mitochondria of a cortisone-sensitive tumor cell. The suspicion that the morphological change of mitochondrial membranes (Fig. 3b) might be due to an accidental artifact in the preparation of the electron micrographs seems improbable, because the specimens of the control and hormone-treated cells were prepared under similar conditions, and the comparison was made on the basis of statistical analysis. The effectiveness of hydrocortisone on Ehrlich tumor cells had already been confirmed in our previous studies (10—13). Degenerative changes in the mitochondria of Ehrlich tumor cells and cortisone-sensitive leukemia cells were tentatively related to the chemotherapeutic effect of cortisone. In light of the historical work by Warburg (22), the energy metabolism of a cancer cell is of interest, not only from the viewpoint of pure science but also from that of cancer chemotherapy. Further exploitation of the mitochondrial function may be of use in the elucidation of the action mechanism of cortisone on neoplasia.

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REFERENCES


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Fig. 1. Electron micrographs of ascitic Ehrlich tumor cells. a, portion of a nontreated tumor cell; b, portion of a cortisone-treated tumor cell, Cell 1, 1 hr after the hormone injection. The dislocation of crista structure and the decrease of matrix density are noted in the enlarged mitochondria; c, portion of a cortisone-treated tumor cell, Cell 2, 48 hr after the hormone injection. The crista structure is often missing. X 20,000.
Fig. 2. Electron micrograph of a nontreated human leukemia cell from Case 1. Compact mitochondria with fine cristae structure and dense matrix substance are intermingled with cytoplasmic granules.

Fig. 3. Electron micrographs of human leukemia cells from Case 2. a, portion of a nontreated leukemia cell; b, portion of a cortisone-treated leukemia cell; 6 hr after the p.o. administration of paramethasone. The mitochondrial membranes are disappearing with concomitant loss of matrix substance.
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