Oncogenic Studies on the Mongolian Gerbil

ALFRED H. HANDLER, SERGIO I. MAGALINI, AND DENISE PAV

Laboratories of Tumor Transplantation, Children's Cancer Research Foundation and Department of Pathology, Harvard Medical School at The Children's Hospital, Boston, Massachusetts

Summary

Possible use of the Mongolian gerbil as an experimental animal for oncologic studies was investigated. Several tumors were induced with X-irradiation and with 7,12-dimethylbenzanthracene. Homografts and perpetuation by serial transfer of these tumors were effected in this species. Human, mouse, and Syrian hamster tumors were successfully heterografted in gerbils conditioned with cortisone or X-ray. An induced lymphoma with conversion to leukemia was described and studied in detail. Preliminary studies suggested that this lymphoma may be used for assaying potential antitumor agents.

Hematologic values were determined and cytologic and histologic studies of bone marrow, spleen, and liver were made for comparison with those of the mouse and the hamster.

Introduction

The Mongolian gerbil, *Meriones unguiculatus*, a small rodent, ranging in size somewhere between the Syrian hamster and the mouse, is native to northeast China and eastern Mongolia. Since 1933, gerbils have been studied in several countries because of their susceptibility to many types of infection. Included in its broad spectrum of susceptibilities are brucellosis, salmonellosis, tuberculosis, leprosy, leptospirosis, rabies, ricetta diseases, and respiratory and neurotropic viruses (6-8, 10, 11, 13, 15).

Gerbils develop high blood serum cholesterol levels even on a diet containing a physiologic (4%) amount of fat (3, 4, 12). They also have a unique water metabolism in that they require almost no water and excrete only a few drops of highly concentrated urine per day (2). They are surprisingly tame, and seem to have more curiosity than fear. They make no attempt to escape, can be handled without the technicians being bitten or scratched, and they make such excellent pets that they are being used in research, we began an investigation in order to ascertain whether the gerbil might emulate the Syrian hamster and be a suitable animal for at least certain types of growth experimentation, especially in the field of transplantation (5).

As there were no spontaneous or transplantable gerbil tumors available, we induced tumors with a chemical carcinogen and with X-irradiation (9). Transplantation studies were conducted using these gerbil tumors to learn whether these animals would accept tumor homografts. These studies were extended to include heterografted human and animal tumors.

Since hematologic values and urine analyses of the normal gerbil were not available for base line comparisons such values were determined and compared with those of the mouse and hamster.

Materials and Methods

Young adult male and female gerbils weighing approximately 67–70 gm were fed *ad libitum* a diet of guinea pig pelleted food, sunflower seeds, and oat meal.

Tumors in gerbils were induced according to the following regimen: 7,12-dimethylbenzanthracene, 0.5% in mineral oil, was skin painted twice weekly for 7 weeks on the back and neck areas of 20 gerbils. Twenty additional gerbils were treated by 14 twice weekly injections in the right dorsal muscle with 0.25 ml of 0.5% 7,12-dimethylbenzanthracene. Groups of 20 gerbils received 1 exposure of total body irradiation in doses of either 300 r, 500 r, or 600 r.

Eight heterologous tumors established and routinely perpetuated by serial transfer were transplanted to gerbils. These included 2 human tumors grown in the Syrian hamster, embryoma of the kidney, Wilms' -N and myxofibrosarcoma, MFS-1; 2 hamster

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1 This research was supported in part by Grant CA 06156-04 from the National Cancer Institute, USPHS, Bethesda, Maryland, and the General Research Support of Grant FR 5451-04 (NIH) USPHS.

2 Present Address: Laboratories for Experimental Carcinogenesis, Department of Occupational Health, University of Pittsburgh Graduate School of Public Health, Pittsburgh, Pennsylvania.

3 Present Address: Research Department, St. Joseph's Hospital and Our Lady of Fatima Hospital, Providence, Rhode Island.

Received for publication August 25, 1965.


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A Comparison of Hematologic Values of the Gerbil with Those of the Mouse and the Hamster (Mean ± 1 S.D.)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Hemoglobin (gm %)</th>
<th>Hematocrit (%)</th>
<th>Platelet count/eu mm</th>
<th>WBC/eu mm</th>
<th>Neutrophils (%)</th>
<th>Eosinophils (%)</th>
<th>Basophils (%)</th>
<th>Lymphocytes (%)</th>
<th>Monocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gerbil</td>
<td>15.07 ± 0.87</td>
<td>37.0 ± 2.6</td>
<td>379,000 ± 45,814</td>
<td>9,260 ± 1,374</td>
<td>25.0 ± 6.9</td>
<td>0.1</td>
<td>0.4</td>
<td>73.0 ± 6.6</td>
<td>1.7 ± 1.3</td>
</tr>
<tr>
<td>Mouse</td>
<td>16.21 ± 7.46</td>
<td>43.1 ± 4.3</td>
<td>399,000 ± 92,100</td>
<td>10,820 ± 2,991</td>
<td>22.6 ± 5.8</td>
<td>0.3</td>
<td>0</td>
<td>76.1 ± 7.4</td>
<td>3.1 ± 1.5</td>
</tr>
<tr>
<td>Hamster</td>
<td>16.57 ± 2.44</td>
<td>45.2 ± 4.3</td>
<td>318,000 ± 106,300</td>
<td>4,930 ± 814</td>
<td>30.0 ± 5.9</td>
<td>1.1</td>
<td>0.2</td>
<td>68.3 ± 5.4</td>
<td>3.6 ± 2.7</td>
</tr>
</tbody>
</table>

URINALYSIS OF NORMAL GERBIL, HAMSTER, LAF-1 MOUSE

<table>
<thead>
<tr>
<th>Animal</th>
<th>Proteins</th>
<th>Glucose</th>
<th>Bilirubin</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gerbil</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hamster</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>±</td>
</tr>
<tr>
<td>LAF-1 Mouse</td>
<td>15 mg/100 ml</td>
<td>—</td>
<td>—</td>
<td>±</td>
</tr>
</tbody>
</table>

* +, positive, 30 mg/100 ml; ±, equivocal; —, negative.

Results

Hematologic Values

As shown in Table 1, hematologic values of the normal gerbil were determined and compared with those of the mouse and the hamster. Hematologic patterns in gerbils are generally similar to those of the mouse and the hamster, the only significant difference being that in the gerbil there is a lower hematocrit value and a slight increase in basophils. The total leukocyte count of the gerbil more closely resembles that of the mouse than that of the hamster. The bone marrow of the species is generally similar to the other species except that it contains a large number of basophils (0.7%).

As indicated in Table 2, normal values of urinary analyses of the gerbil were compared to those of hamsters and mice. Glucose and bilirubin were absent in all 3 species. Protein and ketone bodies were undetectable in gerbil urine while a small amount was observed in hamster and mouse urine.

Tumors Induced in Gerbils with Carcinogens

Seven of 60 gerbils treated with X-ray exhibited tumors in from 9 to 21 months following treatment. One was an ovarian carcinoma (500 r), 1 undifferentiated carcinoma (600 r), 3 malignant melanomas (600 r) 1 of which was metastatic, 1 metastasizing pleomorphic cell sarcoma of the lung (300 r), and 1 spindle cell sarcoma (600 r). Thus far, with the exception of 2 malignant melanomas 1 of which was lost due to death of the animal and the other which has not as yet grown in passage, all these tumors were readily transplantable.

Seventeen of 20 gerbils receiving carcinogen by skin painting exhibited papillomas in from 3 to 9 months following treatment. Two of these were well-differentiated squamous cell carcinomas and were transplanted to other gerbils.

Nine of 20 gerbils receiving carcinogen by i.m. injection have thus far exhibited tumors in from 4 to 9 months following treatment. All of these tumors are malignant and have been transplanted to other gerbils.

Included in the spectrum of induced tumors are: a well-differentiated squamous carcinoma, 6 anaplastic spindle cell sarcomas, and 2 malignant lymphomas.

GERBIL LYMPHOMA (A-1). A lymphoma (designated A-1) was observed in the liver and spleen of 1 of these 20 gerbils 135 days after termination of the injections of the carcinogen. The A-1 lymphoma was perpetuated by serial transfer with inoculations of 1:1 cell suspension in saline into the thigh musculature of gerbils using a 1-ml tuberculin syringe and a 20-gauge needle.

The A-1 lymphoma proved to be highly transplantable, becoming established in 100% of the animals inoculated. It showed marked invasion of the hematopoietic tissue with a lesser degree of invasion of other organs and tissues. The sequence of dissemination of transplanted lymphomatous cells was as follows: involvement of lymph nodes within 9 days, of liver and spleen within 15 days, and of bone marrow within 20 days. Approximately 30% of the animals showed neoplastic cells in peripheral blood approximately 1 month after the last injection of the carcinogen. Gerbils bearing transplants of this lymphoma died of dissemination of this disease in approximately 2 months.
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TABLE 3
HETEROLOGOUS TRANSPLANTATION OF HUMAN AND ANIMAL TUMORS IN GERBILS

<table>
<thead>
<tr>
<th>Tumors implanted*</th>
<th>No. of growths</th>
<th>No. of grafts in gerbil recipients</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Cortisone</td>
<td>X-ray</td>
</tr>
<tr>
<td>Human**</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Wilms, N, embryoma of kidney</td>
<td>0/6</td>
<td>1/6</td>
<td>4/6</td>
</tr>
<tr>
<td>MFS-1, myxofibrosarcoma</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Hamster</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAS-1, fibrosarcoma</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>AHT, lymphoma</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-241, pleomorphic cell sarcoma, C57BL/6 mouse</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>P-1534, leukemia DBA/2 mouse</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
</tbody>
</table>

* One-tenth ml of tumor suspension in saline was inoculated into the right leg muscle.
** Three to 5 mg cortisone acetate were administered s.c. twice weekly.
*** Three hundred r (150 r X 2) X-ray were administered to the total body.
**** Human tumors were transplanted from the Syrian hamster to the gerbil.

TABLE 4
SUMMARY OF CHEMOTHERAPY EXPERIMENTS WITH GERBIL LYMPHOMA (A-1)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dosage (mg/kg/day X 5)</th>
<th>Route</th>
<th>% Inhibition</th>
<th>Evaluation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Mercaptopurine</td>
<td>90, 40, 20, 10, 5</td>
<td>i.p.</td>
<td>16</td>
<td>-- (Toxic)</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>80, 40, 20, 10, 5</td>
<td>i.m.</td>
<td>84.8</td>
<td>++</td>
</tr>
<tr>
<td>D54 naponate</td>
<td>80, 40, 20, 10, 5</td>
<td>i.p.</td>
<td>82.06</td>
<td>++</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>150, 100, 50, 30, 15</td>
<td>i.p.</td>
<td>99.9</td>
<td>++</td>
</tr>
</tbody>
</table>

* Assays performed on 4 animals per dose level.
** Evaluation of tumor inhibition: ++, 75-100%; +, 51-75%; ±, 26-50%; –, 0-25%.

Heterologous tumor transplantation

Table 3 is a summary of the results of heterologous tumor implantation in gerbils. With the exception of P-1534 mouse leukemia, which failed to grow in all of the gerbils and AHT Syrian hamster lymphoma, which grew in only 1 gerbil, the other tumors appeared to grow quite well at least in the gerbils conditioned with X-ray plus cortisone. It was of interest to note that the human tumors which grew with regularity in conditioned Syrian hamsters grew in some of the gerbils and that T-241 pleomorphic cell sarcoma, which grows in several strains of mice appeared to grow as well in normal as in conditioned gerbils. Although a few of the transplants were observed earlier, the heterologous tumors which grew at all, grew progressively in gerbils for at least 3 weeks. Most of the animals were sacrificed at that time since this was but a preliminary investigation. However, in the case of MFS-1, the human myxofibrosarcoma, a large tumor was visible after 65 days. Tumors of each type which grew in gerbils were reinoculated from gerbil to animal species of origin. The human and hamster tumors grew in hamsters, and the mouse tumor grew in the mouse without apparent gross or microscopic alteration.

Chemotherapy

The possibility of utilizing the A-1 gerbil lymphoma for assaying potential anticancer compounds was investigated. In preliminary assays, gerbils with i.m. implants of A-1 lymphoma present for 28 days were treated, as shown in Table 4, with 4 compounds known to exhibit marked antitumor activity in other animal systems. The compounds were administered i.p., and in the case of 4,6-diamino-1-(3,4-dichlorophenol)-2,2-dimethyl-1,2-dihydropyrimidine, i.m., by means of 5 daily injections. The day after the last injection, the size of the implanted tumor was measured in the treated and control animals; 1 of each group was killed and postmortem studies were made. The other animals were permitted to remain alive for periodic observations. As indicated in the table, 6-mercaptopurine was completely without antitumor effect even though it was administered at toxic levels. The other compounds showed marked antitumor effects when given in nontoxic doses. In spite of the significant tumor regression observed during the use of these compounds, recurrence of a tumor occurred in all cases within 14 days after the termination of chemotherapy. In a single experiment, repeated administration of methotrexate following recurrences of tumor growth caused regression on 3 successive occasions; however, this was followed by the ultimate development of resistance of the tumor to this compound.

Discussion

The Mongolian gerbil has been presented as an animal which seems to be useful in cancer research. It has interesting anatomic and physiologic features which require investigation. It accepts homografted tumors in spite of the fact that no attempt has
been made at inbreeding the animal. Our preliminary experiments indicate that the gerbil accepts heterografted tumors, although not as well as the Syrian hamster. Heterografted tumors in gerbils retained species specificity on the basis of growth after reimplantation into the animal species of origin along with growth failure when implantations were performed in several other species. Gerbils require larger doses of cortisone than do hamsters to produce a leukocytopenia and especially a lymphocytopenia. It was obvious that 3–5 mg of cortisone acetate administered twice weekly, which effectively conditioned hamsters, has little effect on the gerbil. Additionally, it recently came to our attention that the 50% lethal dosage of X-ray in the gerbil is around 1300 r (13). Larger doses of X-ray may be necessary, therefore, in our attempts at conditioning the gerbil for transplantation of heterologous tumors.

Results of a preliminary chemotherapeutic assay on a transplantable lymphoma and the availability of several other (homologous and heterologous) transplantable tumors suggest that the gerbil may be a very useful animal for screening of potential antitumor agents.

Acknowledgments

The authors are indebted to Dr. Sidney Farber for his support and encouragement in the performance of this investigation and to Dr. G. P. Mascioli, Miss Deanne Rollins, and Mrs. Rhoda Pollack for technical assistance.

References

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*Cancer Res* 1966;26:844-847.

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