Chemotherapy of a Human Malignant Melanoma Transplanted in the Nude Mouse

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SUMMARY

The effects of single agent therapy with 1-[(chloroethyl)-3-cyclohexyl-1-nitrosourea, 5-fluorouracil, and 5-(3,3 dimethyl-1-triazeno)-imidazole-4-carboxamide on a human malignant melanoma transplanted and passed serially in the thymusless nude mouse were studied.

Tumor response varied. A single dose of 1-[(chloroethyl)-3-cyclohexyl-1-nitrosourea induced initial tumor regression, but thereafter growth resumed at a rate similar to that in the untreated control animals. When 1-[(chloroethyl)-3-cyclohexyl-1-nitrosourea was given in divided dosage at an interval of 8 days, marked and persistent tumor regression was observed. 5-Fluorouracil had no effect. Treatment with 5-(3,3 dimethyl-1-triazeno)-imidazole-4-carboxamide was always reflected by almost total regression of tumors, an effect that was independent of dose within the range tested in this study.

The results resemble those reported from clinical practice in patients with disseminated malignant melanomas treated with the same agents.

This suggests that the pattern of drug susceptibility is preserved after transplantation of tumors in the nude mouse. The human tumor-nude mouse system is advocated as a new in vivo model for determination of individual tumor response to chemotherapeutic agents, and its potential as a model for the proving of new chemotherapeutic agents is suggested.

INTRODUCTION

A practical method for screening new chemotherapeutic agents and for determining individual tumor sensitivity before institution of treatment is of great potential value.

Recent work with a new in vivo model has given promising results (13, 16). The model is the nude mouse, a mutation that displays recessive thymic aplasia (5, 11). A variety of human tumors have been successfully transplanted and serially grown in the nude mouse (6, 7, 12, 14, 15, 19, 21), a phenomenon dependent on the defect in the animal's cell-mediated immune response.

It is a prerequisite that the original nature of the tumor be preserved after transplantation, so that the pattern of response to chemotherapeutic agents is preserved.

To study one aspect of this problem, 3 chemotherapeutic agents in frequent clinical use were tested on a human tumor transplanted in the nude mouse.

MATERIALS AND METHODS

Mice. Six- to 8-week-old nude mice of both sexes were bred at the Laboratory Animals Breeding and Research Centre, G1. Bomoholtgaard, 8680 Ry, Denmark. The animals were the outcome of 5th and 6th back-cross cycles in a gene transfer to BALB/c mice. Breeding and rearing are conducted at the Centre under specified pathogen-free conditions, but during the study period the animals were housed in individual cages under conventional conditions at the Pathological Anatomical Institute, Kommunehospitalet, Copenhagen, as previously described (18).

Tumor. The test tumor chosen was an amelanotic malignant melanoma serially transplanted in the nude mouse, originally deriving from a s.c. femoral metastasis. The patient was a 60-year-old woman who had undergone surgery 2 years previously for malignant melanoma of the leg. The patient never received chemotherapy.

Method of Inoculation and Measurement of Tumor Size. Solid tumor blocks (1 x 2 x 2 mm) were inoculated into the s.c. space on the flank of the mice. Tumor take was 90 to 100%, thereafter growth was rapid. Spontaneous regression was not observed. The tumors assumed spherical or ellipsoidal form, and displayed the same growth patterns irrespective of the sex of the recipient. Tumor growth was local; gross or histological metastases to lymph nodes or organs were never observed. Histological appearances of the serially transplanted tumor were identical to those of the original tumor, and chromosome analyses showed human karyotype (22, Series 12 and 13). At the time of the study, the tumor was between the 15th and 35th passage in nude mice. After inoculation, animals were observed daily, and the tumor was measured twice weekly in 2 dimensions (length and breadth) with slide calipers. Measurement of tumor area was preferred because the 3rd dimension could not be measured with accuracy. The animals were weighed at the time of measurement, and their general condition was evaluated.

Experimental Plan. The animals in each treatment group and the corresponding control animals were always inoculated with tumor tissue derived from 1 mouse donor. One control group served 1 to 3 treatment groups, as shown in...
Tumor-bearing nude mice were treated with CCNU, 5-FU, and DTIC in shown dosages. Survivors in treatment and control groups are listed. Percentage tumor reduction at the conclusion of experiments, 31 to 32 days after initiation of treatment, was calculated from the formula:

\[
100 \times \frac{\text{mean weight of treated tumors}}{\text{mean weight of control tumors}} - 100
\]

Tumor cellular changes are as described in the text.

<table>
<thead>
<tr>
<th>Cancer chemotherapeutic agent</th>
<th>Dose (mg/kg body wt)</th>
<th>Treatment schedule i.p.</th>
<th>Treatment groups (survivors/total)</th>
<th>Control groups (survivors/total)</th>
<th>% tumor reduction</th>
<th>Cellular changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCNU</td>
<td>10</td>
<td>Single dose</td>
<td>4/4</td>
<td>3/4</td>
<td>12.5</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Single dose</td>
<td>4/6</td>
<td>3/6</td>
<td>66.8</td>
<td>Slight</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Single dose</td>
<td>6/7</td>
<td>5/6</td>
<td>52.5</td>
<td>Slight</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>Single dose</td>
<td>7/7</td>
<td>6/7</td>
<td>86.6</td>
<td>Slight</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Single dose</td>
<td>6/7</td>
<td>6/7</td>
<td>85.4</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>15 x 2</td>
<td>8-day interval</td>
<td>5/6</td>
<td>4/6</td>
<td>86.4</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>25 x 2</td>
<td>8-day interval</td>
<td>4/6</td>
<td>5/6</td>
<td>96.5</td>
<td>Severe</td>
</tr>
<tr>
<td>5-FU</td>
<td>10</td>
<td>Daily for 10 days</td>
<td>5/7</td>
<td>5/5</td>
<td>-4.8*</td>
<td>Slight</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>Every 2nd day (5 doses)</td>
<td>6/8</td>
<td>6/8</td>
<td>99.3</td>
<td>Slight</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Every 2nd day (5 doses)</td>
<td>5/8</td>
<td>5/8</td>
<td>98.9</td>
<td>Slight</td>
</tr>
<tr>
<td>DTIC</td>
<td>25</td>
<td>Every 2nd day (5 doses)</td>
<td>6/8</td>
<td>5/8</td>
<td>99.4</td>
<td>Slight</td>
</tr>
</tbody>
</table>

* The minus sign indicates that tumor size increased.

One tumor regressed completely.

Table 1. Sex distribution in the treatment and control groups was similar. Chemotherapy was begun when tumor growth was certain, in general when tumor length and breadth were about 5 mm. The period between tumor inoculation and institution of chemotherapy varied from 14 to 20 days. Experiments were concluded 31 to 32 days after the day on which treatment was begun. The experimental period was determined by the short life-span of the nude mouse (4 to 6 months) when kept under conventional conditions (18).

Chemotherapeutic Agents. All agents were administered i.p. Dosages are summarized in Table 1. Doses were selected with reference to toxicity studies in which the LD₁₀ and LD₅₀ were determined for each agent.

CCNU. This preparation was provided by Lundbeck & Co., Copenhagen, Denmark. It was stored at -20°C. Immediately before use, it was brought into a suspension vehicle (benzyl alcohol, 9 mg; Tween 80, 4 mg; sodium carboxymethyl cellulose, 5 mg; sodium chloride, 9 mg; and sterile water up to 1 ml), and was administered in doses of 10, 20, 30, 40, and 50 mg/kg body weight as a single dose to the animals of the 5 respective treatment groups. Control animals were given 0.1 ml suspension vehicle alone. Previous toxicity studies gave LD₁₀ of 37 mg/kg and LD₅₀ of 62 mg/kg body weight (13).

5-FU. A commercially available preparation was used (Hoffmann-LaRoche & Co., Basel, Switzerland). Immediately before use the preparation was dissolved in 0.9% NaCl solution, and administered in a dose of 10 mg/kg body weight daily for 10 days. Control animals received 0.1 ml 0.9% NaCl solution alone.

DTIC. This preparation was provided by the National Cancer Institute, Bethesda, Md., and was stored at -20°C. Immediately before use, it was dissolved in sterile water and was administered in doses of 25, 50, and 75 mg/kg body weight every other day for 10 days to the animals of the 3 respective treatment groups. Control animals received similar injections but of 0.9% NaCl solution. The toxicity studies gave LD₁₀ of 11 mg/kg and LD₅₀ of 17 mg/kg body weight.

Autopsy and Histological Studies. Autopsies were performed in all animals. The tumors, or remnants thereof, were dissected free and weighed. Preparations were made from all tumors and from any organ that showed evidence of macroscopic change. The tissues were fixed in formalin and embedded in paraffin wax. Seven-μm sections were cut and stained with hematoxylin and eosin.

Statistical Methods. Results are presented either as analyses of tumor growth curves or as percentages of reduction in tumor weight at the conclusion of experiments.
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Only animals that survived the whole of the experimental period are considered.

**Tumor Growth Curves.** Measurements of tumors in the control groups indicated that tumor area (length x breadth) squared with time, thus showing a linear relationship when the square root of growth is plotted against time. Conventional linear regression analysis of tumor growth curves was made. This model was chosen because a model in which spread increased in proportion with time did not better describe the data. Each treatment group was compared with its corresponding control group.

For the treated animals, the hypothesis was proposed that the tumor growth curve could be described by an angled line comprising 2 or 3 segments. The location of the angles was determined graphically. Regression analysis of each line segment was made. To determine whether an angle was statistically apparent, the slopes of neighboring line segments were compared. If no angles were apparent, a joint regression line was calculated. Slope of the line segments thus derived was compared with the slope in the corresponding control group. The t test was applied to compare the slopes of 2 line segments (9).

**Percentage Tumor Reduction.** This was determined by the formula:

\[
100 - \frac{\text{Mean weight of treated tumors}}{\text{Mean weight of control tumors}} \times 100
\]

**RESULTS**

**Tumor Growth Curves.** For CCNU (10 mg/kg body weight) given as a single dose 14 days after tumor inoculation, the slope of the regression line was not significantly
different from that of the control group. Chart 1 shows the results for CCNU given in doses of 20, 30, and 40 mg/kg body weight. CCNU (50 mg/kg body weight) given as a single dose 14 days after tumor inoculation initially yielded a negative slope of the regression line. Thereafter, from Day 14, the slope became positive but significantly less than in the control group ($p < 0.0005$). The slope of the regression line for CCNU (15 mg/kg body weight) given twice with an 8-day interval was significantly less than that in the control group ($p < 0.0005$). CCNU (25 mg/kg body weight) given twice with an 8-day interval produced the results shown in Chart 2.

Chart 3 gives the results for 5-FU (10 mg/kg body weight) and Chart 4 shows results for DTIC in doses of 25, 50, and 75 mg/kg body weight.

**Percentage Tumor Reduction.** After single doses of CCNU, a dose-dependent tumor response was observed (Table 1; Chart 5). Response was more marked when the same total quantity of CCNU was given in divided doses at 8-day intervals, at least so far as it appears from comparative studies with total doses of 30 and 50 mg/kg body weight. 5-FU had no therapeutic effect, while DTIC had marked effect. Tumor reduction was emphatic, an effect that was independent of doses within the range tested in this study.

**Changes in Body Weight and Mortality.** Table 1 shows the number of survivors in treatment and control groups. CCNU dosage was tolerated with no weight loss, and there were only slight changes in the body weight of 5-FU-treated animals. All animals treated with DTIC lost weight, but this loss (mean value) never exceeded 10%.

In both treatment and control groups there were sporadic deaths of no specific pattern.

**Histological Study of Tumors.** All tumors of control animals showed histological appearance as in the original human tumor (Figs. 1 and 2).

In the experimental animals that displayed tumor regression, characteristic changes in tumor cells were observed. Changes classified as slight (Fig. 3) were swollen tumor cells with occasional vacuolization of the cytoplasm, swollen pale nuclei, and reduced number of mitotic figures (0 to 1/high-power field as compared with 4 to 5 in untreated control animals). Changes classified as severe were marked swelling of cells with frequent cytoplasmic vacuolization and grossly swollen nuclei that were much varied in form, pale, and with finely dealt chromatin. Such cells were separated by abundant extracellular substance. No mitotic figures were seen (Fig. 4).

These changes were seen in both CCNU- and DTIC-treated animals, but not in those treated with 5-FU.
animal treated with CCNU (40 mg/kg body weight), no tumor remnant could be identified but only macrophage aggregations. Results of the histological studies are given in Table 1. Histological findings and tumor growth curves were consistent in that most severe changes were observed in tumors that showed the most regression.

Histological Study of Organs. Metastases to lymph nodes or organs were not seen. The histological appearances of bone marrow in the treated animals were always normal with no signs of hypoplasia.

DISCUSSION

This study shows the different sensitivity of a transplanted human malignant melanoma to 3 chemotherapeutic agents.

Comparison of the percentage of tumor reduction with doses under the LD_{50} suggests that DTIC was most effective. Single doses of CCNU induced regression of tumors, which was more marked when the same quantity was given in divided doses at an 8-day interval. Tumors continued to grow in animals treated with 5-FU.

The pattern of response was similar to that seen in clinical studies of patients with disseminated malignant melanoma. Pugh et al. (17) found objective response in 12% of patients treated with CCNU. Carter and Friedman (4) report results of single agent therapy with DTIC and 5-FU. There was objective response in approximately 20% of patients treated with DTIC, but in only 2.5% of those treated with 5-FU. This suggests that the pattern of drug susceptibility after transplantation is preserved or at least not greatly impaired.

Previous studies of the effect of bleomycin on epidermoid carcinoma (13) and of cyclophosphamide on Burkitt’s lymphoma (16), both tumors transplanted in the nude mouse, also gave results consistent with clinical experience.

The same malignant melanoma that is the subject of this study was also studied earlier, in its 11th passage in nude mice (13). The response to CCNU was similar despite 5 further serial transplantsations of the tumor. The patient from whom the original tumor derives never received chemotherapy, so that a direct comparison of clinical and experimental results was not possible. No such comparisons have been made as yet, but they are clearly necessary before any evaluation can be made of the human tumor-nude mouse system as a screening test for selection of the most effective chemotherapeutic agent.

Other in vivo systems using heterotransplanted human tumors have been proved (13, 8, 10, 20).

The cheek pouch in unprepared or cortisone-treated hamsters has been the most used transplantation site, and similarity between clinical tumor response and transplant tumor response to various chemotherapeutic agents has
been observed. Direct comparisons have been infrequent, but Burt et al. (3) report consistent response of a human bladder carcinoma treated in situ and as a transplant in the cheek pouch of a cortisone-prepared hamster. In this hamster system, however, tumor growth is predictable only over a short period, and the cortisone preparation might not be without effect on the results.

Berenbaum et al. (2) studied the effects of various chemotherapeutic agents on a number of human tumors transplanted in thymectomized, irradiated, and antilymphocyte serum-treated mice. Chemotherapeutic effects were evaluated histologically. Measurement of tumors was not possible. Transplants were small, growth was slow, and transplant content of fibrous connective tissues varied extremely. These factors prohibited quantitative evaluation of tumor growth.

The human tumor-nude mouse system is rewarding to study and meets many of the requirements of a system for in vivo screening of the sensitivity of human tumors to chemotherapeutic agents. A variety of human tumors are accepted, and many can be serially transplanted. To date, no differences between tumors after many serial passages and the original donor tumors have been found. Specifically, histological appearances, chromosome patterns, isozymes, and antigens remain unchanged (6, 7, 12, 14, 15, 19, 21, 22). Tumor growth is localized and can be simply measured. The congenital immunological defect is sufficient. No further time-consuming, skilled, and costly preparation is required, and, of greater significance, interpretation of results is not complicated by the need to take preparation procedures into account.

Data are insufficient at present to allow evaluation of the model's utility in primary or secondary screening studies of chemotherapeutic agents.

The presented results suggest that the system is suitable for screening of individual human tumor sensitivity to chemotherapeutics and for detailed study of the mode of action of such agents. Such studies are in progress.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Section from human donor material. Note similarity to Fig. 2. H & E, × 350.
Fig. 2. Microscopic appearance of untreated human malignant melanoma after 17 passages in nude mice. H & E, × 350.
Fig. 3. Slight cellular changes in heterotransplanted, human malignant melanoma 32 days after treatment with CCNU (15 mg/kg body weight) given twice at an 8-day interval. For detailed description see text. H & E, × 350.
Fig. 4. Severe cellular changes in heterotransplanted, human malignant melanoma 32 days after a single dose of CCNU (50 mg/kg body weight). For detailed description see text. H & E, × 350.
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