Regression of Human Tumors Established in Nude Mice after Continuous Infusion of Thymidine

Shih-Shun Lee, Beppino C. Giovanella, John S. Stehlin, Jr., and Jan C. Brunn

ABSTRACT

A system for the continuous infusion of thymidine solutions in nude mice has been developed. High doses (0.5 to 1.0 ml/mouse/hr) of a 28.5-mg/ml thymidine solution (444 to 888 mg/kg/hr) can be administered continuously for 96 to 140 hr.

The preliminary results indicate that it is possible to induce total tumor regression of human heterotransplants established in nude mice of one human teratocarcinoma, five different human melanomas, and one human lung carcinoma and to inhibit to a large degree the growth of two human breast carcinomas by multiple (two to eight) cycles of infusion. The life span of the thymidine-treated animals has been significantly increased compared to that of control animals.

INTRODUCTION

dThd has demonstrated selective toxicity for cultured neoplastic cells (8) and inhibited the growth of human melanomas transplanted in nude mice (9). Here, we report that by continuous infusion of dThd it is possible to induce regression of human tumors already established in nude mice. Some of these results have been published previously in a short note (10).

MATERIALS AND METHODS

For this study, 9 human tumors growing in nude mice as heterotransplants were used. The 5 melanomas, CA 1, BE 1, FO 1, MI 1, and HO 1, have been described in a previous paper (6). The teratocarcinoma, PA 1, has also been described previously (7). The lung carcinoma, DO 1, is an oat cell carcinoma of a 48-year-old man. It is called LX 1 in the tumor panel used by Division of Cancer Treatment, National Cancer Institute, for experimental chemotherapy. The first breast carcinoma, CL 1, is an infiltrating duct cell carcinoma of a 28-year-old woman. It is called BX 1 in the same tumor panel. The second breast carcinoma, EL 1, is an infiltrating duct cell carcinoma, Grade III, which developed in a 48-year-old woman. PA 1, CA 1, BE 1, FO 1, and MI 1 were originally cell-cultured human cell lines derived from human tumors and carried in tissue culture. HO 1, DO 1, CL 1, and EL 1 originated from human tumor biopsies that were directly transplanted into nude mice and then serially passed from nude mouse to nude mouse. dThd was obtained from the Sigma Chemical Co., St. Louis, Mo., and from Division of Cancer Treatment, National Cancer Institute. It was dissolved in minimum essential medium (Eagle's; Grand Island Biological Co., Grand Island, N. Y.) at 28.5 mg/ml as stock solution for infusion.

Nude mice bred and maintained in pathogen-free conditions in our laboratory have been used throughout (5). In all experiments, 2 nude mice from the same litter, of the same sex, over 3 months old, and weighing 30 to 35 g were paired and inoculated with the tumor cells in the lower back. Tumor was introduced as a cell suspension or as a tumor mince (1 x 10⁷ cells or approximately 50 mg (wet weight) of tumor). When the tumor grew to a palpable size, a small Silastic medical grade tubing (Dow Corning Corp., Midland, Mich.) was inserted s.c. in the upper back of each mouse and connected to a 60-ml syringe containing the infusion solution. The movement of the mouse was restricted by tying the tail behind the steel feeding rack (Figs. 1 and 2). One of the nude mice was connected to a syringe containing the solvent fluid Eagle's minimum essential medium, the other was connected to a syringe containing dThd solution. Both syringes were then inserted into the same infusion pump (Harvard Apparatus Corp., Dover, Mass.). The output from both syringes was identical (0.5 to 1.0 ml/hr). The tumor volume was obtained by the product of the 3 major diameters.

RESULTS

The tumor volumes of dThd-infused and control mice are summarized in Table 1 for a period of up to 150 days after inoculation.

Untreated tumors, PA 1, always grew exponentially. Occasionally, the tumor ulcerated and shrunk to a certain extent, but it always regrew in a very short time. No spontaneous regressions have ever been observed. In 3 of the 5 dThd-infused animals, the tumors completely regressed. In the other 2, the tumor of one was reduced in size considerably during the treatment; however, the tumor was not completely eradicated and, after suspension of treatment, it regrew and killed the animal. The other animal also had its tumor considerably reduced in size; the tumor ulcerated, and the animal died of bleeding from the ulcerated area.

CA 1 tumors also grew exponentially, although at a slower pace than those of PA 1. However, unlike PA 1 tumors, CA 1 tumors very seldom if ever ulcerated. Again, no spontaneous regressions of these tumors have ever been noted. In the mice infused with dThd, the volume of the tumors continued to increase for a few days after treatment was started and then regressed completely. Occasionally, some of these tumors recurred locally after some time. One of the animals treated died, presumably because of toxic products produced by total destruction of a large tumor due to the dThd treatment.

BE 1 untreated tumors grew exponentially. The 4 animals carrying dThd-treated BE 1 tumors behaved as follows: 2 had tumors that regressed completely; one died, presumably due to dThd toxicity, and one failed to respond.
to absorption of toxic products immediately after total disappearance of a large tumor; and one was lost due to an accident.

FO 1 untreated tumors invariably killed the animals which carried them before Day 150. All 3 dThd-treated animals were alive with no visible tumor at the end of 150 days. Untreated animals carrying tumor HO1 died before Day 140, whereas the tumors in dThd-treated animals grew much more slowly. One of the 3 tumors regressed completely after 150 days. dThd treatment of animals carrying MI 1 tumors induced tumor regression in one-half of the animals and inhibited considerably tumor growth in the other half.

In animals carrying lung carcinoma (DO 1), the effect of dThd was also dramatic. The tumor regressed completely in 4 of 6 of the animals. In the remaining 2, the tumors were reduced to a volume less than 5% of that seen in the control animals.

Breast carcinoma CL 1, under dThd treatment, grew to about one-quarter of the volume of the control.

Breast carcinoma EL 1, under dThd treatment, grew to less than one-half of the volume of the control.

Charts 1, 2, and 3 and Figs. 3 to 9 depict examples of randomized pairs of animals carrying teratocarcinoma PA 1, melanoma CA 1, and lung carcinoma DO 1 that were untreated and treated with dThd.

Table 2 shows the survival of dThd-treated mice compared with the survival of the control mice. Although the data are not yet complete (because some of the animals are still alive), it is apparent that the treatment with dThd considerably lengthens the life of the treated animals compared with the untreated controls.

**DISCUSSION**

It has been known for a number of years that high concentrations of dThd applied to tissue-cultured cells produce a block (dThd block) of cell division (2, 16). Such a block is reversed by addition of deoxycytidine to the medium or by removal of dThd. After reversal of the dThd block, cell cultures divide synchronously at least once. It was soon realized that dThd block, if prolonged for more than the length of a cell cycle, produced a loss of viability of the treated cultures (12).

Interest in dThd as a pharmacological agent began with a series of studies which demonstrated that this compound has some activity against pernicious anemia (11). In 1967, Foley...
has been found that dThd increases the antitumor activity of 5-fluorouracil if administered immediately before this drug (14).

It is important to note that all of the experiments mentioned involved low doses of dThd (8 mg/sq m/day when given with methotrexate) or very short periods of administration (30 to 45 min when administered before 5-fluorouracil, and the half-life of dThd in the human blood under such conditions is 10 min.

It has been our continuing observation that in our system

and Lazarus (4) noted that cultured human leukemia lymphocytes were very sensitive to the toxic action of dThd in vitro. Their results were confirmed in vitro by others (13), in murine leukemic cells. No one, however, tried to establish whether dThd did have a selective toxicity in vitro for tumor cells but not (or less) for nonneoplastic cells.

In 1968, Apple and Greenberg (1) described growth inhibition by dThd of murine tumors. This interesting observation was not followed up. In the meantime, dThd entered anticancer chemotherapy but not as an antineoplastic agent per se. It had been observed by Tattersall et al. (15) that administration of dThd to tumor-carrying animals treated with methotrexate reduced the toxicity of this drug for the host without affecting its antineoplastic activity. These observations in an animal model prompted further clinical studies that fully confirmed the experimental results, at least for reduction of toxicity (3). Also, it

<table>
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<tr>
<th>Tumor</th>
<th>Pairs of animals</th>
<th>Av. survival (days)</th>
<th>Range (days)</th>
<th>Ratio (dThd/control)</th>
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<tbody>
<tr>
<td>PA 1</td>
<td>5</td>
<td>dThd 255</td>
<td>113–346</td>
<td>1.5</td>
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<tr>
<td></td>
<td></td>
<td>Control 173</td>
<td>92–214</td>
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</tr>
<tr>
<td>CA 1</td>
<td>4^a</td>
<td>dThd 301</td>
<td>170–392</td>
<td>1.8</td>
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<td>Control 168</td>
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</tr>
<tr>
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<td>2^a</td>
<td>dThd 323</td>
<td>256–397</td>
<td>2.1</td>
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<tr>
<td></td>
<td></td>
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<td>150–162</td>
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</tr>
<tr>
<td>FO 1</td>
<td>3</td>
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<td>264–320</td>
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<tr>
<td></td>
<td></td>
<td>Control 135</td>
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<tr>
<td>HO 1</td>
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<td>51–277</td>
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<td></td>
<td></td>
<td>Control 69</td>
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</table>

^a Exclude death due to total tumor destruction or accidentally killed.
^b At least one animal in dThd treated still alive.
such small doses or such short periods of treatment do not allow for any antitumor effect of dThd by itself.

This study clearly demonstrates that it is possible by continuous infusion of dThd to achieve total tumor regression of human heterotransplants in nude mice of one human teratocarcinoma, 5 different human melanomas, and one human lung carcinoma and to inhibit to a large degree the tumor growth of 2 human breast carcinomas.

Mice tolerate well repeated infusions of dThd. Of the 40 pairs of animals used in these studies, one pair had been infused 8 times, 4 pairs 6 times, 4 pairs 5 times, 4 pairs 4 times, 15 pairs 3 times, and 12 pairs 2 times. Occasionally, a mouse might show slight loss in body weight or a skin rash, or some diarrhea might develop after infusion had continued longer than 5 days. However, as soon as the dThd infusion was discontinued, the animal recovered in less than 5 days and could be safely infused again. Sometimes, when the treated tumor was very large, its total destruction by dThd treatment caused the sudden death of the host. Different tumors differ in their sensitivity to dThd. Of the 5 melanomas tested, FO 1 is the most sensitive to dThd treatment. With this tumor, 2 or 3 cycles of dThd infusion were sufficient to completely eradicate tumors that had an initial volume of up to 1.5 cu cm. With the other melanomas, more intensive treatment with dThd was required to achieve the same results.

We are unable to obtain complete eradication of the breast carcinomas CL 1 and EL 1, although tumor growth was inhibited considerably in the dThd-treated animals. We are now exploring the possibility of eradicating these tumors also, starting with small tumors and using a very intensive treatment schedule. In vivo results have confirmed our in vitro results, i.e., that the killing effect of dThd is dose and duration dependent. We observed in vitro that a majority of the cells of CA 1 and BE 1 did not detach under dThd treatment but kept enlarging without dividing for a period of more than a month. In the dThd-infused animals, the volume of the tumors obtained by injecting CA 1 and BE 1 cells also increases for a period of time before the tumor starts regressing and dying. Although the survival data are incomplete, it is already apparent that dThd-treated animals have their life prolonged by the treatment. These results are particularly apparent with very fast-growing tumors, such as DO 1 and FO 1. We must emphasize that to see any regression of established tumors one must continue infusion of dThd for several cycles. Approximately 50% of the animals in which total eradication of the tumor was obtained recidivate locally or at a distance after periods of time varying from 14 to 150 days. We are now planning to continue treatment of animals in which the tumor has been macroscopically totally eradicated for at least 2 cycles, to eliminate this long-term recurrence of the tumor.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the invaluable assistance of Randall Shepard and Betty Mendoza.

REFERENCES

Fig. 2. Detail of Fig. 1 showing how infusion catheter is inserted and kept in place.
Fig. 3. Control animal carrying teratocarcinoma PA 1 (Chart 1, arrowhead A).
Fig. 4. Treated animal carrying teratocarcinoma PA 1 (Chart 1, arrowhead B).
Fig. 5. Same control animal as in Fig. 3 at 160 days (Chart 1, arrowhead C).
Fig. 6. Same treated animal as in Fig. 4 at 160 days (Chart 1, arrowhead D).
Fig. 7. Control animal carrying melanoma CA 1 (Chart 2, arrowhead A).
Fig. 8. Treated animal carrying melanoma CA 1 (Chart 2, arrowhead B).
Fig. 9. Same treated animal as in Fig. 8 at 140 days (Chart 2, arrowhead C).
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