Effect of 5-Fluorouracil on Early Teratomas in Mice

JOHN T. ALDRICH AND LEROY C. STEVENS

The Jackson Laboratory, Bar Harbor, Maine 04609

SUMMARY

Testicular teratomas were experimentally induced in Strain 129/Sv mice by grafting genital ridges from 12-day fetuses into the testes of adults. In approximately 80% of the grafts, teratomatous foci were observed histologically 6 days after grafting. They are discrete masses of cells within the seminiferous tubules and are similar in several environmental and genetic factors (10). In the induced tumor system the teratocarcinogenetic process is initiated within 24 hr. The tumors grew and were composed predominantly of neural tissue. This provided a well-defined oncogenic system in which to study the carcinostatic effects of compounds such as 5-fluorouracil (FU).

Host mice received a single injection of FU at 50 mg/kg on one of several days beginning with the day prior to grafting and ending with the 11th day following grafting. The development of teratocarcinomatous foci was markedly inhibited in grafts in mice treated on Days 1 through 6. Those in hosts treated on Days 7 through 11 had an increasing incidence of tumors approaching that of the controls (78%). FU at 25 mg/kg also prevented the growth of tumors when injected into host mice on Day 0 or Day 1.

INTRODUCTION

Testicular teratomas may be experimentally induced in Strain 129 mice by grafting genital ridges of fetuses after 12 days of gestation into the testes of adults (8). Approximately 80% of the grafts develop tumors that are morphologically and developmentally identical to the spontaneously occurring testicular teratomas found in approximately 2% of inbred Strain 129/Sv male mice (8). These spontaneous tumors develop congenitally, and there is considerable evidence that they are derived from primordial germ cells (9). The initiation and development of teratomas have also been shown to be under the influence of several environmental and genetic factors (10). In the induced tumor system the teratocarcinogenetic process is initiated within 24 hr following grafting. Twelve-day genital ridges of Strain 129 mice are susceptible to experimental teratocarcinogenesis, but 13-day genital ridges are resistant (8). The tumors may be observed histologically 6 days after grafting. They are discrete masses of cells within the seminiferous tubules and are similar in appearance to spontaneous tumors in 15- to 17-day fetuses (Fig. 1). At 2 weeks the tumors have ruptured the tubules and are similar to spontaneous tumors in 2- to 5-day-old mice (Fig. 2). These tumors contain ectodermal and endodermal epithelium and immature neuroepithelium in addition to undifferentiated embryonal cells (Figs. 5, 6). At 3 weeks the induced tumors contain a wide variety of tissues, but neural tissue predominates.

This tumor system is unique in that the carcinogenetic process takes place at a precisely known time, i.e., within 24 hr following grafting. No exogenous carcinogenic agent is required to induce these tumors, and the increase in tumor frequency over the spontaneous rate may reflect an intensification of the factors responsible for the development of the spontaneous tumors. This system offers advantages over other tumors used in chemotherapy studies, since drugs may be administered at precisely known stages of tumor development.

5-Fluorouracil (FU) is an anticancer agent that functions as an antimetabolite for uracil in nucleic acid biosynthesis (1). The aim of this investigation was to study the effect of FU on the development of early teratomas.

MATERIALS AND METHODS

The mice used in this study were Strain 129/Sv-Str1, which was derived from Strain 129/Sv by introducing the genes C, P, and Str1, a spontaneous recurrence of Str1 found in strain C3H/Hu by K. P. Hummel. The incidence of spontaneous teratomas in these mice is about 10% in males of second or later litters.

Genital ridges were removed from 12-day fetuses and grafted into the testes of adults. The testes were exposed through a median ventral incision, and the genital ridges were implanted with a micropipet with rubber tubing and mouthpiece. Most grafts were removed after 2 weeks, fixed in Vandegrift's solution, and sectioned serially at 7 μ and stained with hematoxylin and eosin. Some grafts were allowed to grow for 3 and 4 weeks before being removed. About one-half of the grafts developed into ovaries and were not included in the results.

Of the pair of genital ridges removed from each fetus, one was grafted into a host in the experimental group and the other into a host in the control group. Most mice in the treated group received 1 injection of FU at a dose of 50 mg/kg i.p. on one of several days starting with the day prior to the grafting procedure and ending with the 11th day following the grafting procedure. A few mice received 2 injections of FU at 50 mg/kg, and another small group received single injections of the drug at a dose of 25 mg/kg. FU was dissolved in distilled water, and control mice received injections of distilled water at the same time as the corresponding treated group. In order to determine the correct dosage of FU, each host mouse was weighed prior to injection. Mice were weighed again before they were killed.

RESULTS

Intratesticular grafts of 311 male genital ridges were examined. The incidence of teratomas in the treated and the corresponding
TABLE 1
Effect of 5-Flourouracil (FU) on the Incidence of Teratomas

<table>
<thead>
<tr>
<th>Day of i.p. injection</th>
<th>FU</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of grafts</td>
<td>FU</td>
</tr>
<tr>
<td>Mice receiving FU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(50 mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 and 2</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>2 and 3</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>–1</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>0</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Mice receiving FU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(25 mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>161</td>
<td>Total</td>
</tr>
</tbody>
</table>

control groups is presented in Table 1. The overall incidence of teratomas among the controls was 78%, which is similar to previous observations (8). A high incidence (11/19) of teratomas was observed in mice treated with FU on the day prior to receiving a graft (Day minus 1), and a low incidence (4/19) was observed in the group treated on the same day as grafting (Day 0). In groups treated from Day 1 through Day 6, the development of teratomas was almost completely inhibited, while groups treated on Days 7 through 11 showed an increasing incidence of teratomas approaching that of the control groups. No tumors were observed in the groups receiving 2 injections of FU at 50 mg/kg or in groups receiving 1 injection at 25 mg/kg.

Testes derived from grafts were histologically alike in both control and treated groups, and no difference in size was apparent. The size of tumors found in experimental groups treated on Days 7 through 11 was compared with the size of corresponding controls by counting the number of sections containing tumor in each graft. No difference was observed. Host mice treated with FU showed no adverse effects that could be detected by loss of weight, diarrhea, or fall in hematocrit.

DISCUSSION

This experimental situation exposes two kinds of rapidly proliferating tissues to the potentially inhibitory effects of FU. One is the normal tissue of the genital ridge, and the other is the teratomatous tissue within the graft. The first was not affected by the drug, whereas the second was markedly inhibited if treated during early stages of development.

This difference in response to the drug might be explained by one or both of the following mechanisms: (a) the number of cells composing an early teratomatous focus is so small that each cell might be expected to be affected by the drug; and (b) the drug may be selectively absorbed by the tumor cells.

Pierce et al. (5), Kleinsmith and Pierce (3), and Stevens (7) have shown that teratomas arise from single pluripotent cells, probably primordial germ cells. The teratocarcinogenetic process is initiated in germ cells of testes developing from intratesticular grafts of 12-day genital ridges within 24 hr after grafting. Teratomatous foci are histologically identifiable within control grafts at 6 days after grafting. At this time foci composed of approximately 5 cells may be seen (Fig. 3). Foci observed on Day 7 are much larger, containing 10-30 cells (Fig. 4). Until 6 days the tumors are too small to be positively identified. Between Days 6 and 7 the tumor cells begin to proliferate actively, resulting in a small cluster of undifferentiated embryonal-type cells. The 1st 6 days of tumor development correspond to the period during which FU is highly effective in preventing the appearance of teratomatous foci. The small number of tumor cells during this period might make it likely that each cell might be affected by the drug. As the number of tumor cells rapidly increases after Day 6, more and more cells might escape the effects of the drug, and an increasing incidence of tumors is noted in succeeding days.

It is also possible that the tumor cells are selectively absorbing the drug. Rutman et al. (6) showed that exogenous uracil is utilized for nucleic acid formation during the process of hepatic carcinogenesis by 2-acetylaminofluorene in the rat, whereas no such utilization occurs in normal rats. Work by Heidelberger et al. (2) also supports the claim that tumors may utilize uracil to a greater extent than most other tissues.
The high incidence of tumors observed in grafts made to mice which had been treated the day prior to grafting presumably reflects the fact that FU is rapidly absorbed and excreted (4), leaving only low levels of the drug at the time of grafting.

The high incidence of tumors in grafts treated on the day of grafting may be due to the fact that levels of the drug are sufficiently reduced by the time cells undergo the teratocarcinogenic alteration so that such cells occasionally escape the effects of the drug.

The induction of testicular teratomas by this method may become a useful assay system for carcinostatic agents. For example, Storer (personal communication) found that X-irradiation markedly reduces the oncogenic potential of fetal genital ridges grafted into adult testes of Strain 129 mice. Experimentally induced testicular teratomas develop in a predictable manner, making it possible to correlate drug effects and histologic appearance of the tumors at various stages of development.

ACKNOWLEDGMENTS

The 5-fluorouracil was generously supplied by Dr. W. E. Scott of the Research Division of Hoffman-La Roche Inc., Nutley, New Jersey.

REFERENCES


Fig. 1. Spontaneous testicular teratoma in the testis of a 16-day fetus. Note that the central tubule is enlarged by the presence of a group of undifferentiated cells. These cells will give rise to embryonal germ layers and to the many kinds of immature and adult tissues characteristic of teratomas.

Fig. 2. Spontaneous teratoma in the testis of a 4-day-old Strain 129 mouse. Note an endodermal vesicle in the upper part of the tumor and two ectodermal vesicles in the lower. The tumor is surrounded by normal seminiferous tubules.

Fig. 3. Experimentally induced teratoma (arrow) in a tubule of a testis derived from a 12-day genital ridge grafted to an adult testis 6 days previously. A portion of a seminiferous tubule of the host is at the upper left. This tumor arose in a control animal and is composed of about 5 cells.

Fig. 4. Experimentally induced teratoma (arrow) in a tubule of a testis derived from a 12-day genital ridge grafted to an adult testis 7 days previously. This focus was in a control mouse and contains about 25 cells. 5-Fluorouracil prevents the growth of tumors of this stage.

Fig. 5. Experimentally induced teratoma 14 days after grafting a 12-day genital ridge. This has several foci and is typical of control testes.

Fig. 6. Experimentally induced teratoma 12 days after grafting a genital ridge showing small vesicles of ectoderm (Ec) and endoderm (En). 5-Fluorouracil does not prevent the growth of tumors of this stage.
Effect of 5-Fluorouracil on Early Teratomas in Mice

John T. Aldrich and Leroy C. Stevens


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/27/5_Part_1/945

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.