ABSTRACT

We have demonstrated that S-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721) given to mice prior to ionizing radiation inhibits development of radiation-induced sarcomas. The right hind legs of C3H/Kam mice were exposed to single doses of γ-rays ranging from 3400 to 5700 rads. Thirty min before irradiation, approximately one-half of the mice were given i.p. injections of WR-2721 (400 mg/kg). Mice were checked for development of radiation-induced tumors within the irradiated tissue of legs from 250 up to 786 days after irradiation. Tumors first appeared in both groups of mice at approximately 300 days after irradiation. Tumors in mice that received WR-2721 prior to irradiation first appeared at about 786 days. At that time, there were 40 mice that received WR-2721 and leg irradiation. Thereafter, the rate of tumor development was slower in mice that received WR-2721 and leg irradiation.

INTRODUCTION

Recently, there has been an increase in interest in research on radioprotective agents. The aim is to achieve preferential protection of normal tissues against injury inflicted by ionizing radiation or chemotherapy agents used to treat tumors. Among radioprotective agents, commonly the sulfhydryl-containing compounds, WR-2721 is currently the most widely used (8, 11, 17). WR-2721 is a potent protector of most normal tissues in rodents against both early and late radiation injuries (11, 17). In contrast, it is a poor protector of solid tumors (8, 9, 15). Because of its preferential radioprotection of normal tissues, WR-2721 has the potential to be effective in conjunction with tumor radiotherapy. A major mechanism underlying the radioprotective effect of WR-2721 is the scavenging of highly reactive free radicals induced by ionizing radiation (11, 17). Since damage inflicted by free radicals is a major event responsible not only for killing mammalian cells by radiation but also for malignant transformation of these cells (2, 3), it might be expected that WR-2721 would also protect against radiation carcinogenesis. We previously reported the effect of WR-2721 on tumor and normal tissue radioreponse (6, 9, 10). In this paper, we document protection by WR-2721 against the carcinogenic effects of ionizing radiation.

MATERIALS AND METHODS

Mice. Twelve-week-old C3H/Kam mice, bred and maintained in our own specific pathogen-free mouse colony, were used. The mice had transplants of a syngeneic methylcholanthrene-induced fibrosarcoma, designated FSA, growing in their right hind thighs, which were irradiated when 5 or 8 mm in diameter. Mice cured of their primary tumors were observed from 250 to 786 days after leg irradiation for development of radiation-induced tumors.

Leg irradiation. Tumor-bearing legs were locally irradiated with single doses of γ radiation ranging from 3400 to 5700 rads (individual radiation doses are listed in Table 1). Radiation was delivered with a small irradiator with 2 parallel-opposed 137Cs sources at a dose rate of 917 rads/min. During irradiation, the right hind thigh bearing the tumor was centered in the circular radiation field 3 cm in diameter (10).

Development of Radiation-induced Sarcomas. Mice that were cured of primary tumors by radiation were further observed for development of tumors in the irradiation field from 250 to 786 days after irradiation. Suit et al. (12) reported that tumors developing within the irradiated tissue later than 300 days after irradiation of primary tumors are radiation-induced and not late recurrences. Using the same strain of mice as we used, they observed that mice cured of mammary carcinoma, MCA-4, transplants in the leg developed new tumors in the irradiated field, and these were sarcomas. In addition, they found that irradiation of legs of normal mice also produced sarcomas in the irradiated field in the same time frame.

RESULTS

The incidence of induced tumors in the legs of mice that had been exposed to 3400- to 5700-rad single doses of γ radiation 500 and 786 days earlier are shown in Table 1. At 500 days after irradiation, 17 of 37 (46%) mice that received radiation only had tumors in the irradiated legs compared to a 5 of 46 (11%) tumor incidence in mice treated with both WR-2721 and radiation. There was a trend toward increased incidence of induced tumors with increase in radiation dose in mice exposed to irradiation only but not in those that received WR-2721 and irradiation.

Actuarial cumulative tumor incidence as a function of time is shown in Chart 1. Tumors started to appear at about 300 days after irradiation in both groups. At that time, there were 40 mice in the radiation-only group and 47 in the group that received WR-2721 and irradiation. The rate of tumor development was reduced in mice that received WR-2721 prior to irradiation of...
WR-2721 AND RADIATION CARCINOGENESIS

Table 1

<table>
<thead>
<tr>
<th>Irradiation</th>
<th>WR-2721 + Irradiation</th>
<th>WR-2721 + Irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiation dose (rads)</td>
<td>Irradiation only</td>
<td>Irradiation only</td>
</tr>
<tr>
<td>3400</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>3800</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>4000</td>
<td>1/4</td>
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<td>3/3</td>
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<td>4800</td>
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<tr>
<td>5200</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>5700</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>Total</td>
<td>17/37 (46%)</td>
<td>5/46 (11%)</td>
</tr>
</tbody>
</table>

a Number of mice with radiation-induced tumors/total number of mice surviving the period indicated.

Actuarial estimates (see Chart 1) indicate that 87% of mice developed tumors when exposed to irradiation, compared to only 26% when treated with both WR-2721 and irradiation.

Chart 1: Cumulative incidence of induced tumors as a function of time following irradiation (XRT) of the right hind legs of mice with or without pretreatment with WR-2721 (400 mg/kg i.p. 30 min before irradiation). The cumulative incidence was calculated using the Kaplan-Meier method and is significantly reduced in the WR-2721-treated group (p < 0.001).

legs. At 786 days after irradiation, nearly all surviving mice exposed to irradiation only had leg tumors (28 of 30; 93%), whereas only 11 of 37 (30%) of the surviving mice that received the drug and irradiation had tumors (Table 1). Corresponding actuarial estimates are 87 and 26%.

Fourteen of the 28 tumors that developed in irradiated mice only and 10 tumors that developed in mice treated with WR-2721 and radiation were examined histologically. In the former mice, 9 tumors were fibrosarcomas, 1 was an osteosarcoma, 1 was a liposarcoma, and 3 were unclassified sarcomas. Tumors of the latter group consisted of 8 fibrosarcomas and 2 unclassified sarcomas.

DISCUSSION

Radiation carcinogenesis is a complex multistep event to which a number of factors contribute. In its initial event, cellular DNA is injured either directly or by radiation-produced free radicals. However, many other factors, including cell proliferation, and nutritional, hormonal, and immune factors, will determine whether this induction process will proceed to frank cancer (1, 2, 5, 7). Interference with any of the events involved in radiation carcinogenesis can reduce or prevent development of tumors. It has been observed that in vitro radiation-induced carcinogenesis can be inhibited by protease inhibitors (1, 7), retinoids (1, 7), vitamin C (1, 7), or hypothyroid conditions (1).

Inhibition of the formation of free radicals or their action would be particularly effective at the initial phase of carcinogenesis. For example, superoxide dismutase is effective in reducing free radical formation and, through that mechanism, it inhibits in vitro radiation-induced transformation (4). Our present data show that the potent free radical scavenger WR-2721 can also greatly inhibit radiation carcinogenesis; it reduced a 93% rate of murine sarcoma to 30%. WR-2721 was effective against all radiation dose levels used in the study. An accurate protection factor value cannot be specified, since no radiation dose response relationship was apparent in either the radiation-only or the WR-2721-plus-radiation group. A dose dependence for radiation carcinogenesis was observed by Suit et al. (12), who reported that the incidence of murine sarcomas increased and their latency decreased as the dose of radiation increased. The range of doses used in their study was from 3000 to 7100 rads. Although the doses we used were in the same range, the number of mice at each radiation dose level was small, and this may explain the discrepancy between the 2 sets of data. Also, Suit et al. (12) and Urano et al. (13) reported a lower incidence of radiation-induced tumors than we reported here, although the length of observation period was similar in all 3 studies. In the final analysis of their data, these authors (12, 13) included all animals that succumbed from causes other than developing new tumors as if they survived the whole observation period and did not develop radiation-induced tumors. This underestimates the incidence of the latter tumors. In our analysis, we either excluded such animals (Table 1), which overestimates the incidence, or included them as censored data in our actuarial analysis (Chart 1).

Nonetheless, the results of this initial study on the anticarcinogenic effect of WR-2721 show that the compound can significantly reduce the development of radiation-induced tumors. Since WR-2721 has potential for increasing the therapeutic gain of radiotherapy because of its ability to protect normal tissues better than tumors, its anticarcinogenic activity could be an important additional benefit. Furthermore, since WR-2721 is a potent protector against tissue damage by alkylating agents (14, 16), it might also be useful in protection against chemical carcinogenesis.

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REFERENCES


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Inhibition of Radiation Carcinogenesis in Mice by S-2-(3-Aminopropylamino)-ethylphosphorothioic Acid

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