Tumoricidal Effects of Sodium Hexachloroiridate on an Ascitic Tumor in Mice

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ABSTRACT

Sodium hexachloroiridate injected 1 day after i.p. injection of 10^6 mouse ovarian tumor cells prevents the appearance of ascitic tumors in mice. Mice given injections of tumor cells all die at 20 to 30 days after tumor injection. Mice treated with sodium hexachloroiridate after tumor injection either have significant prolongation of life span without ascites or show no evidence of cancer even up to 7 months after tumor injection.

INTRODUCTION

In studies on the effects of SHI on tissue culture cells, it was found that this compound could arrest cell division in the G1 stage of the cell cycle (5). Cultures stopped dividing, and very large giant cells were produced. Because of the growth-inhibiting properties of SHI, investigations were conducted on the effects of SHI on a mouse tumor in vivo. The MOT is easily passaged by i.p. injection and results in a large quantity of ascitic fluid (4). This tumor arose spontaneously as an embryonal cell carcinoma in a female C3H mouse and has been maintained by serial i.p. transplantation in female C3HeB/FeJ mice. There is a regular relationship between the size of the tumor cell inoculum and the median time to death (1-4). SHI was found to have significant tumoricidal effects on this tumor in vivo.

MATERIALS AND METHODS

MOT cells (1) were passaged in female BALB/c mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.). After harvesting, the cells were centrifuged, washed once, and resuspended in PBS. MOT cells, 10^6, in 1 ml of PBS, were then injected into the peritoneal cavity of 6- to 7-week-old female mice weighing 18 to 22 g. In some cases, cells were pretreated with varying concentrations of SHI (NaIrCl_6·6H_2O, M, 558.99) (Alfa Inorganics, Beverly, Mass.). One day after injection of cells, mice were given i.p. injections of either 1 ml of 0.025 M SHI in 1 ml of Dulbecco's serum-free medium (Grand Island Biological Co., Grand Island, N. Y.) or 1 ml of medium without SHI as controls. In other experiments, SHI was added to the drinking water of the mice rather than injected. Mice were also treated with i.p. injections of SHP, cisplatin, SHR, SHO, or SHPD 1 day after injection of MOT cells.

Four mice were treated with i.p. injections of 1 ml of 0.05 M SHI in PBS. Sixteen hr later, whole organs and portions of kidney, liver, brain, bone, lung, muscle, and heart were excised from these animals as well as from 2 untreated animals. These tissues were rinsed 3 times in PBS and dried at 90°C for 72 hr. The tissues were weighed and then boiled separately in concentrated nitric acid until all of the tissues were dissolved. The samples were then analyzed for iridium content by inductively coupled argon plasma emission spectrometry (Jarrell-Ash, Waltham, Mass.).

RESULTS

SHI Toxicity. Four groups of mice (each group consisting of 5 mice) were treated with varying doses of SHI to determine the toxicity of a single i.p. injection of this compound. The mice were given i.p. injections of 1 ml of either 0.01, 0.025, 0.05, 0.075, or 0.1 M SHI. This corresponds to doses of between 0.25 and 2.5 g/kg. All of the mice treated with 0.05 M concentrations or greater of SHI were dead within 24 hr. Mice treated with lower doses survived for periods of up to 7 months after injection and appeared healthy. There was no significant weight loss in these animals in comparison with control animals as a result of SHI treatment. Both SHI and untreated animals weighed between 20 and 24 g at 25 days after SHI inoculation. A group of 5 mice were fed for 2 weeks with water containing 1.2 x 10^{-3} M SHI. The concentration of SHI was then raised to 4 x 10^{-3} M for 2 additional weeks and finally to 0.01 M for 2 weeks. These concentrations of SHI had no noticeable effect on the animals. The final p.o. dose represented a daily intake for each mouse equal to 12 times the amount which would kill the animal when injected i.p. in a single dose. This indicates that the p.o. route of administration is significantly less toxic than is the i.p. route.

In Vitro SHI. MOT cells, 10^6, in serum-free medium, were incubated for 2 hr at 37°C with either 10^{-2} M SHI or 3 x 10^{-4} M SHI. One ml of cells was then injected i.p. into 5 mice; 5 control mice were given injections of cells not preincubated with SHI. Although all of the control mice were dead within 25 days, all of the mice given injections of the cells treated with SHI have survived for up to 7 months and appear healthy.

In Vivo SHI. Twelve experimental mice and 8 control mice were each given i.p. injections of 10^6 MOT cells in 1 ml of PBS. One day later, the 12 experimental mice were each given an injection of 1 ml of 0.025 M SHI in PBS. All of the control mice were dead with a large amount of ascitic fluid 24 to 31 days after injection. Two of the experimental animals died 33 and 56 days after MOT injection without evidence of tumor or ascitic fluid. Three animals died 48, 54, and 59 days after injection with large solid flank tumors but without visible ascites. The remaining 7 animals are alive, healthy, and without visible tumor 7 months after MOT injection. These results have been repeated with an additional total of 19 mice given injections in separate experiments. Mice treated with 1 ml of 0.025 M SHI 1 day after i.p. injection of MOT cells uniformly do not develop ascites and have
in all cases demonstrated a longer life span than untreated animals. Histological sections of major organs of cured animals done at 3 months after tumor inoculation showed no evidence of tumor and were unremarkable when compared to normal mice. Previous studies (2) of median and absolute survival of animals given injections of increasing numbers of tumor cells have shown that the injection of more than $10^6$ MOT cells i.p. will kill 100% of the animals. Amounts of $10^5$ and $10^6$ cells will kill more than 90% of animals given injections. Even injection of only 10 tumor cells results in greater than 40% mortality of animals. Median survival is also correlated with the number of cells injected. Our results with $10^6$ injected cells clearly indicate, therefore, significant tumor cell killing with SHI.

Four animals were given injections of MOT cells and 0.01 M SHI added to their drinking water for 1 day prior to injection and for the succeeding 12 days. Three of the mice died 27 days after tumor injection, and 1 mouse died 31 days after tumor injection. There was no significant difference between the life span of the control animals and that of these animals treated with SHI p.o.

**In Vivo Platinum Compounds.** Considerable previous work (6) has been done in evaluating the tumoricidal effects of another heavy metal agent, cisplatin, which contains platinum. To evaluate whether SHI may be exerting its effects on MOT cells in a manner similar to that of cisplatin, animals were given injections of SHP or cisplatin 1 day after MOT injection.

To first determine the toxicity of SHP, 7 groups of animals with 5 animals in each group were given i.p. injections of $2.5 \times 10^{-2}$, $5 \times 10^{-3}$, $2 \times 10^{-4}$, $10^{-4}$, or $5 \times 10^{-5}$ M SHP. Mice treated with doses of $10^{-3}$ M SHP or greater died within 24 hr of injection. One of the mice given an injection of $2 \times 10^{-4}$ M SHP died within 6 hr of injection, while the other mice appeared healthy for 25 days after injection. All of the mice treated with $10^{-4}$ or $5 \times 10^{-5}$ M SHP were alive and well 2 weeks after injection.

Five mice were therefore given i.p. injections of 1 ml of $10^6$ MOT cells and 1 day later were given injections of 1 ml of $10^{-4}$ M SHP. Five control animals died 25 to 30 days after injection of MOT cells. The SHP-treated animals died 24 to 31 days after SHP injection. There was thus no demonstrable tumoricidal effect of SHP at doses which were not toxic to the animals.

Ten mice were therefore given injections of $10^6$ MOT cells. One day later, 0.3 mg of cisplatin [cis-dichlorodiaminineplatinum (II)] in 0.3 ml of an aqueous solution containing mannitol (20 mg/ml) and NaCl (9 mg/ml) were injected into 5 of these mice. The mice which were not treated with cisplatin died between 23 and 25 days after tumor injection. The cisplatin-treated animals died between 31 and 56 days after tumor injection with an average life span of 43 days after injection of MOT cells. Cisplatin therefore had a significant tumoricidal effect on MOT cells in vivo.

Because of the marked tumoricidal effect of SHI on MOT cells in mice, we investigated the effects of other related heavy metal compounds: SHPD, SHO, and SHR. Results with SHP are presented above. Injection i.p. of 1 ml of SHR (0.025 m or greater) was found to be lethal to the mice within 24 hr of injection. Mice were therefore given injections of SHR at 0.01 and 0.005 m. No significant life span prolongation was found with animals given injections of these levels of SHR after MOT cell treatment.

Animals given injections of 1 ml of SHO or SHPD at concentrations of 0.1 m or greater died within 24 hr. Mice given injections of $10^6$ MOT cells followed 24 hr later by treatment i.p. with 1 ml of 0.025 or 0.05 m SHO or SHPD did not live significantly longer than did control animals not treated with SHO or SHPD.

The toxic effects of SHI appear within 24 hr of injection. Heavy metal poisoning is usually caused principally by renal effects. However, renal damage would not be expected to cause death within 24 hr of administration. Cardiac or nerve damage may have played a role in the rapid toxicity that was observed. To assess the extent of tissue localization of the administered SHI, animals were treated i.p. with toxic doses of 1 ml of a 0.05 m solution of SHI, and organs of the dead animals were assessed 16 hr later for iridium content. Table 1 lists the content of iridium for the tissue examined. The highest iridium concentration was found in the kidney. However, all of the organs examined showed significant iridium content, including the heart and brain.

### DISCUSSION

Our results show a significant tumoricidal effect of SHI on MOT cells. These experiments with SHI have been repeated several times in the same format, and the results are consistent. In experiments with MOT-injected animals treated with SHI, animals lived much longer than did controls and failed to develop ascitic tumors. However, occasional animals did develop solid flank or abdominal tumors at the site of MOT cell injection and eventually died because of the large ulcerating tumor mass. These animals were cured of their ascitic tumor. However, cells introduced into the flank during i.p. injection presumably gave rise to the solid tumors observed. This suggests that SHI injected i.p. did not result in a SHI blood level high enough to affect the solid tumors. In preliminary experiments, tumoricidal effects were observed even when injection of SHI was delayed up to 5 days after tumor inoculation. Further experiments are planned to assess the effects of delay in treatment with SHI for longer periods after tumor inoculation.

In the comparative experiments of the effects of the other heavy metal agents other than SHI, we first have presented toxicity data to arrive at concentrations of the agents which were just below those required to produce death. These doses were then used to treat animals given injections of MOT cells. It is possible that slightly higher doses than those used for treatment might have produced an improvement in the tumoricidal effect of the agent and yet still not cause death of the animals. However, the therapeutic ratio of such agents would still have been much smaller than that of SHI.

Although iridium is similar to platinum in its chemical properties, our results documenting different responses of MOT cells to SHI and platinum compounds suggest that SHI may possibly be
acting by mechanisms different than those which are responsible for the antitumor properties of cisplatin. With regard to MOT cells in mice, our data suggest that SHI has a far greater tumoricidal effect than does cisplatinum, while SHP had no detectable tumoricidal effects. None of the animals treated with these platinum-containing agents survived longer than 60 days, and all eventually developed ascites.

The tumoricidal effects of SHI on a mouse tumor suggest that SHI may possibly have similar effects on human tumors. Further animal experimentation is planned with a view toward ultimately investigating human tumors. Additional experiments will be necessary to better define the therapeutic ratio, to investigate the effects of SHI on other mouse tumors, to optimize the dose rate schedule, and to investigate the effects of SHI on DNA and its constituent mononucleotides.

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