Addition of 2-Nitroimidazole Radiosensitizers to cis-Diamminedichloroplatinum(II) with Radiation and with or without Hyperthermia in the Murine FSA1IC Fibrosarcoma

Terence S. Herman, Beverly A. Teicher, Sylvia A. Holden, M. Raphael Pfeffer, and Steven M. Jones

ABSTRACT

We have examined the ability of misonidazole (MISO) or etanidazole (ETA) to improve the antitumor efficacy of cisplatin (CDDP), hyperthermia, and radiation in the FSA1IC murine fibrosarcoma. A growth delay of about 25 days was produced with CDDP (5 mg/kg) and hyperthermia (43°C, 30 min) prior to radiation (3 Gy daily for 5 days) on day 1. The addition of MISO (1 g/kg) on day 1 resulted in a tumor growth delay of about 28 days. The addition of ETA at 0.5 g/kg or 1 g/kg resulted in tumor growth delays of about 33 and 43 days, respectively. Tumor cell survival assay showed that MISO was additive with CDDP either at 37°C or with hyperthermia (43°C, 30 min). In contrast, ETA at both 0.5 g/kg and 1 g/kg was dose modifying over the CDDP dosage range at 37°C or 43°C. Analysis of tumor cell killing in Hoechst 33342 selected bright (presumably oxic) and dim (presumably hypoxic) tumor cell subpopulations demonstrated that the addition of MISO to the CDDP trimodality regimen increased killing in the dim cell subpopulation, while the addition of ETA increased tumor cell killing in both subpopulations, although the greater effect was in the dim cell subpopulation. These results indicate that ETA may add to the efficacy of the CDDP trimodality in the clinic and may be of value as a chemosensitizer with CDDP.

INTRODUCTION

The use of radiosensitizing drugs (1), chemotherapeutic agents (2), and hyperthermia (3) in conjunction with radiation therapy has shown promise in the clinic. The rationale for the use of radiation, hyperthermia, and a chemotherapeutic agent in combination for the treatment of local disease is 2-fold. First, some chemotherapeutic agents (4–6) and hyperthermia (7, 8) can effectively attack radioresistant subpopulations of the tumor such as hypoxic cells at low pH. Second, some chemotherapeutic drugs interact strongly with both radiation and hyperthermia (9).

We are currently testing CDDP, hyperthermia, and radiation in patients with superficial tumors which can be heated adequately with an external hyperthermia applicator (9–11). CDDP is a particularly promising agent for use in this trimodality approach because it is known to interact positively with hyperthermia both in vitro (12–14) and in vivo (15–17), and the normal tissue effects of this combination have been found to be acceptable (18, 19). In addition, CDDP is also known to interact positively with radiation both in vitro (20, 21) and in vivo (22–24) and is thought to sensitize normal tissue less than tumor tissue to the effects of radiation (24). In proceeding with further development of the trimodality approach, the addition of misonidazole or etanidazole to the current regimen would be reasonable especially due to the hypoxic cell cytotoxic selectivity of these agents.

In this study, we have examined the addition of misonidazole or etanidazole to CDDP, with hyperthermia and radiation, by tumor growth delay and tumor excision assay using the FSA1IC fibrosarcoma murine tumor system. In addition, we have used the Hoechst 33342 dye diffusion method to define the effect each of these treatment combinations has on the survival of putative normally oxygenated and hypoxic FSA1IC tumor cells from tumors treated in vivo.

MATERIALS AND METHODS

Drugs. cis-Diamminedichloroplatinum(II) was a gift from Drs. Donald Picker and Michael Abrams, Johnson Matthey, Inc. (West Chester, PA). Misonidazole and etanidazole (SR-2508) were obtained as gifts from the Drug Synthesis and Chemistry Branch of the Developmental Therapeutics Program of the National Cancer Institute, Bethesda, MD. Tumor. The FSA1IC fibrosarcoma (25) adapted for growth in culture (FSAlIC) (26) was carried in male C3H/He mice (The Jackson Laboratory, Bar Harbor, ME). For the experiments, 2 x 10^6 tumor cells prepared from a brei of several stock tumors were implanted i.m. into the legs of male C3H/He mice 8–10 weeks of age.

Tumor Growth Delay Experiments. When the tumors were approximately 100 mm^3 in volume (about 1 week after tumor cell implantation), treatment was initiated. In those groups receiving drugs, CDDP (5 mg/kg), MISO (1 g/kg), and/or ETA (0.5 or 1 g/kg) in 0.9% phosphate-buffered saline (0.2 ml) were injected i.p. as single doses alone or in combination on the first day of treatment. In those groups receiving hyperthermia, heat was delivered as a single dose on day 1 of the treatment locally to the tumor-bearing limb for 30 min by immersion in a specially designed Plexiglas water bath at 44°C which allowed the centers of tumors to reach 43 ± 0.2°C as measured by a digital readout thermometer (Sensortech Inc., Clifton, NJ) placed into the center of the tumor in selected control animals as previously described (26). In those groups receiving radiation, X-rays were delivered locally to the tumor-bearing limb at a dose of 3 Gy daily for 5 days. In all cases the sequence and timing of the treatments were drugs administered i.p., followed within 2 min by immersion of the limb in the hyperthermia bath for 30 min, and then radiation delivery at ambient temperature within 5 min. No anesthetic was used. The progress of each tumor was measured three times/week until it reached a volume of 500 mm^3. Tumor growth delay was calculated as the days taken by each individual tumor to reach 500 mm^3 compared to the untreated controls. Each treatment group had 7 animals and the experiment was repeated 3 times. Days of tumor growth delay are the means ± SE for the treatment group compared to the control.

Tumor Excision Assay. When the tumors were approximately 100 mm^3 in volume (about 1 week after tumor cell implantation), various doses of CDDP (0, 5, 10, or 20 mg/kg), MISO (1 g/kg), and/or ETA (0.5 or 1 g/kg) were injected i.p. into the animals as single agents or combinations alone or immediately followed by hyperthermia (43°C, 30 min), as described above, to the tumor-bearing limb. Mice were sacrificed and soaked in 95% ethanol 24 h after treatment to allow for full expression of drug cytotoxicity and repair of potentially lethal damage. The tumors were excised under sterile conditions in a laminar flow hood and minced to a fine brei with 2 scalpels. Four tumors were pooled to make each treatment group.

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2 To whom requests for reprints should be addressed, at Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115.

3 The abbreviations used are: CDDP, cis-diamminedichloroplatinum(II); MISO, misonidazole; ETA, etanidazole (SR2508); αMEM, α-minimal essential medium; FBS, fetal bovine serum; TGD, tumor growth delay; BT cells, bright cell subpopulation; D cells, dim cell subpopulation.
Approximately 500 mg of tumor brei were used to make each single-cell suspension. All reagents were sterilized with 0.22-μm Millipore filters and were added aseptically to the tumor cells. Each sample was washed in 20 ml of αMEM, after which the liquid was gently decanted and discarded. The samples were resuspended in 450 units/ml collagenase (Sigma, St. Louis, MO) and 0.1 mg/ml DNase (Sigma) and incubated for 10 min at 37°C in a shaking water bath. The samples were centrifuged at 200 x g, and the supernatant was discarded. The samples were resuspended as described above and incubated for another 15 min at 37°C. One ml of 1 mg/ml DNase was added, and incubation was continued for 5 min at 37°C. The samples were then filtered through 2 layers of sterile gauze. The samples were washed twice, then resuspended in αMEM supplemented with 10% FBS. These single-cell suspensions were counted and plated at 3 different cell concentrations in duplicate for the colony-forming assay. No significant difference was observed in total cell yield from the pooled tumors in any treatment group. One week later the plates were stained with crystal violet, and colonies of >50 cells were counted manually. The results are expressed as the surviving fractions ± SE of cells from treated groups compared to untreated controls (27).

Tumor Subpopulation Studies: Tumor Growth and Hoechst 33342 Labeling. FSaIC fibrosarcoma tumors were grown as described above. Animals received the various CDDP, MISO/CDDP, ETA/CDDP, hyperthermia, and radiation treatments as described for the tumor excision experiments. Hoechst 33342 (2 mg/kg, Aldrich Chemical Co., Milwaukee, WI) dissolved in sterile 0.9% phosphate-buffered saline was administered by injection into the tail vein (0.25 ml) of tumor-bearing mice at 24 h posttreatment. Tumor cell suspensions were prepared by excising the tumor 20 min after the i.v. administration of the dye (28–30). The single-cell suspensions of the tumor cells were prepared as described for the tumor excision assay. To remove contaminating erythrocytes, 0.17 M NH4Cl was added to the tumor cell pellets for 3 min at room temperature just after filtering through gauze. The cells were then washed once with αMEM supplemented with 10% FBS, filtered through a syringe fitted with a 40-μm nylon mesh filter to remove cell clumps, and then counted. After centrifugation at 200 x g, cells were resuspended at a concentration of 2 x 10^6 cells/ml in αMEM supplemented with 10% FBS to be sorted (31–33).

Flow Cytometry and Sorting. Cells from tumors were analyzed and sorted using the Coulter Epics V instrument. Hoechst 33342 intensity was measured using excitation at 350–360 nm (40 mW power) and emission monitored with a 449 ± 10-nm band pass filter. The fluorescence intensity of Hoechst 33342-stained cells was divided by the peripheral light scatter signal from the 488-nm laser beam (using a 488 ± 10-nm band pass filter) for each cell to obtain an estimate of cellular “concentration” of Hoechst 33342. The fluorescence distributions were generally divided into 10 fractions (sort windows) based on the Hoechst 33342 intensity or concentration, with each fraction containing 10% of the population. Two sort fractions of cells were collected, one which contained the 10% brightest cells and one which contained the 20% dimmest cells. The fluorescence distribution of the cells sorted was not significantly different at 24 h following any of the treatments administered as compared with cells from untreated control tumors. The cells were then washed once with αMEM containing 10% FBS, counted, and plated at different cell concentrations in αMEM containing 10% FBS for colony formation. After 1 week colonies were stained with crystal violet, and colonies of ≥50 cells were counted manually. The plating efficiencies for the unsorted, 10% brightest, and 20% dimmest populations were 15.7 ± 2.5%, 10.3 ± 1.5%, and 7.2 ± 1.5%, respectively. Results are expressed as the surviving fractions ± SE of the treated bright and dim fractions compared to the bright and dim untreated controls, respectively.

Data Analysis. Data on the delay of tumor growth were analyzed with a Basic program for an Apple microcomputer. The program derives the best fit curve for each set of data, then calculates the median, mean, and SE for individual tumor volumes, and the day on which each tumor reached 500 mm^3. Statistical comparisons were carried out with the Dunn multiple comparisons test after a very significant effect was found by analysis of variance (27).

Quantitative analysis of dose-response curves for cell survival was performed using the log-probit iterative least squares method. This approach is a statistically rigorous and objective means for determining the slope of the dose-response curve. Correlation coefficients for the linear log-probit line and χ² analysis for goodness of fit were also calculated. The dose-modifying factors were calculated as the ratio of the slopes of the survival curves obtained with CDDP alone or with hyperthermia (minus the heat alone point) and those treatments plus ETA (0.5 g/kg or 1 g/kg).

Because the tumor subpopulation studies were carried out only at single doses of each treatment, additivity could not be rigorously determined. The product of the surviving fractions was used as an indicator of additivity.

RESULTS

For the combination treatments, a dose of 5 mg/kg CDDP, which produced about 4.4 days of TGD was used (Table 1). Hyperthermia alone (43°C, 30 min), MISO (1 g/kg), and ETA (0.5 or 1 g/kg) produced minimal TGDs. At treatment at 43°C was immediately preceded by CDDP administration, a TGD of about 6 days resulted. The addition of hyperthermia to treatment with MISO (1 g/kg) or ETA (1 g/kg) resulted in about 3.3 and 4.4 days of TGD, respectively. The combination of CDDP (5 mg/kg)/MISO (1 g/kg) produced a TGD which was a 1.5-fold increase over CDDP alone. On the other hand, ETA/CDDP (5 mg/kg) produced TGDs at the lower (0.5 g/kg) and higher (1 g/kg) doses of ETA which were 2.1- and 2.7-fold increases over CDDP alone, respectively. When treatment with CDDP/MISO (1 g/kg) was followed by hyperthermia (43°C, 30 min), the TGD was about a 1.2-fold increase over the effect of CDDP/hyperthermia. The addition of hyperthermia immediately following administration of CDDP/ETA (0.5 g/kg) resulted in about 16.1 days of TGD, while CDDP/ETA (1 g/kg) with hyperthermia resulted in about 20.9 days of TGD. These two tumor growth delays represent about a 2.7- and about a 3.5-fold increase over the effect of CDDP/hyperthermia, respectively.

Preceding the radiation fraction on day 1 of the treatment regimen with hyperthermia (43°C, 30 min) or CDDP (5 mg/kg) increased the TGD to about 8.4 and 11.7 days, respectively. MISO (1 g/kg) or ETA (1 g/kg) administered prior to radiation delivery on day 1 increased TGD to about 7.4 days and 8.1 days, respectively. MISO (1 g/kg) on day 1 with CDDP/X-rays resulted in a 1.4-fold increase in TGD over CDDP/X-rays. ETA (1 g/kg) in combination with CDDP/X-rays resulted in a TGD of about 22.1 days which was a 1.9-fold increase over CDDP/X-rays.

The trimodality therapy was administered in the sequence which was found to be optimal for CDDP, hyperthermia, and radiation (21). When radiation was given after CDDP/heat on the first day of treatment, the resulting TGD was about 25 days. When hyperthermia was preceded by CDDP plus MISO (1 g/kg) on the first day of treatment, the TGD was about 28.6 days. ETA (0.5 g/kg) and CDDP/hyperthermia on the first day of treatment resulted in about 33 days of TGD. Increasing the dose of ETA to 1 g/kg with the complete combination treatment produced about 43.1 days of TGD. Therefore, ETA was more effective in combination with CDDP in the trimodality therapy regimen than was MISO.

FSaIC tumor cell killing by CDDP increases linearly with increasing dose of the drug as measured by in vitro tumor cell survival following treatment of the tumor in vivo (Fig. 1). Hyperthermia (43°C, 30 min) killed only about 20% of the tumor cell population. However, treatment with CDDP fol-
Table 1 Growth delay of the FSal1C fibrosarcoma produced by combinations of CDDP, heat, and X-rays

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Tumor growth delay, (days)*</th>
<th>No addition</th>
<th>MISO (1 g/kg)</th>
<th>ETA (0.5 g/kg)</th>
<th>ETA (1 g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>43°C, 30 min®</td>
<td>1.4 ± 0.7</td>
<td>6.4 ± 1.2</td>
<td>9.3 ± 1.8</td>
<td>11.9 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>CDDP (5 mg/kg)</td>
<td>4.4 ± 0.9</td>
<td>6.4 ± 1.2</td>
<td>9.3 ± 1.8</td>
<td>11.9 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>CDDP → Heat</td>
<td>5.9 ± 1.1</td>
<td>6.9 ± 1.4</td>
<td>16.1 ± 1.9d</td>
<td>20.9 ± 2.3d</td>
<td></td>
</tr>
<tr>
<td>X-rays (5 × 3 Gy)</td>
<td>6.3 ± 1.5</td>
<td>6.3 ± 1.5</td>
<td>6.3 ± 1.5</td>
<td>6.3 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Heat → X-rays</td>
<td>8.4 ± 2.2</td>
<td>16.0 ± 1.7d</td>
<td>21.7 ± 2.3d</td>
<td>22.1 ± 2.5d</td>
<td></td>
</tr>
<tr>
<td>CDDP → X-rays</td>
<td>11.7 ± 1.8</td>
<td>33.0 ± 3.4</td>
<td>43.1 ± 5.2d</td>
<td>43.1 ± 5.2d</td>
<td></td>
</tr>
<tr>
<td>CDDP → heat → X-rays</td>
<td>25.2 ± 2.8</td>
<td>28.6 ± 4.3</td>
<td>33.0 ± 3.4</td>
<td>43.1 ± 5.2d</td>
<td></td>
</tr>
</tbody>
</table>

* Growth delay is the difference in the number of days for treated tumors to reach 500 mm³ compared to untreated control tumors. Data are presented as the means of 14 animals ± SE.

® Heat was delivered as a single dose on day 1 of the treatment locally to the tumor-bearing limb by immersion in a water bath at 44°C which allowed the tumors to reach 43°C.

† CDDP was injected in normal saline as a single dose i.p. on day 1 of the treatment.

¶ Significant differences from CDDP → heat or CDDP → x-rays or CDDP → heat → x-rays (P < 0.001).

* X-rays were delivered locally to the tumor-bearing limb at a dose of 3 Gy daily for 5 days. No anesthetic was used.

lowed by hyperthermia (43°C, 30 min) led to markedly increased tumor cell killing. The addition of MISO (1 g/kg) to treatment with CDDP at 37° or 43°C (30 min) increased tumor cell killing only slightly.

Dose modification was evident with ETA in combination with CDDP at both doses of ETA (Fig. 2). The dose-modifying factor obtained with 0.5 g/kg of ETA was about 2.1, while that obtained with 1 g/kg of ETA was about 1.6. At the lowest dose of CDDP (5 mg/kg), there was about a 2.4-fold increase in tumor cell killing with 0.5 g/kg of ETA and about a 4.3-fold increase in tumor cell killing with 1 g/kg of ETA. A similar pattern was obtained when ETA was added to treatment with CDDP/hyperthermia. The dose-modifying factor produced by 0.5 g/kg ETA was about 1.8 and that produced by 1 g/kg was about 1.3.

To determine the effectiveness of these various treatments on environmentally determined tumor subpopulations, tumor-bearing animals were treated as described above, and then single-cell suspensions of the tumors were sorted into Hoechst 33342 defined subpopulations (Fig. 3). The 10% brightest (BT) cells represent a population near the tumor vasculature (enriched euoxic) and the 20% dimmest (D) represent a cellular population distal from the tumor vasculature (enriched hypoxic) (31-33). Hyperthermia (43°C, 30 min) was more cytotoxic to the D cells than to the BT cells. A single dose of radiation (10 Gy) was about 2-fold more toxic to the BT cells than to the D cells and CDDP (10 mg/kg) was also about 2-fold more toxic to the BT cells than to the D cells. Hyperthermia immediately after CDDP resulted in about a 37-fold increase in the killing of the BT cells and about a 25-fold increase in the killing of the D cells, so that the relative sparing of the D cells increased to about 2.8-fold. In the BT cells the combination CDDP/radiation was essentially additive; however, in the D cells the tumor cell killing by CDDP/radiation was slightly greater than would be predicted by the product of the surviving fractions. The
trimodality combination therapy of CDDP/hyperthermia/ radiation produced about 4 logs of cell killing in the BT cells and about 3.5 logs of cell killing in the D cells so that about a 5-fold sparing of the dim cells (hypoxic cell subpopulation) was evident.

The addition of MISO (1 g/kg) to CDDP (10 mg/kg) did not significantly increase the cell killing in either subpopulation over that produced by CDDP alone (Fig. 4). The addition of MISO to CDDP/hyperthermia, however, resulted in about a 1.8-fold increase in the killing of BT cells and about a 2.1-fold increase in the killing of D cells. The combination MISO/CDDP with radiation was 1.8-fold more effective in the BT cells and about 1.5-fold more effective in the D cells than CDDP/radiation. In the trimodality regimen the addition of MISO resulted in no change in the killing of the BT cells, however, there was about a 1.9-fold increase in the killing of the D cells. Therefore, when the trimodality therapy included MISO/CDDP the sparing of the D cells was reduced to about 2.3-fold versus about 5-fold without MISO.

ETA (0.5 g/kg) did not significantly alter the killing of the BT cells produced by CDDP (10 mg/kg) but did increase the killing of the D cells by about 1.8-fold (Fig. 5). ETA (0.5 g/kg) increased the BT cell killing by CDDP/hyperthermia by about 2.3-fold and increased the killing of the D cells by about 3.3-fold. The combination of ETA (0.5 g/kg) with CDDP/radiation was about 2.6-fold more cytotoxic than CDDP/radiation in the BT cells but only about 1.8-fold more effective in killing the D cells. As with MISO, ETA (0.5 g/kg) did not change the killing of the BT cells produced by CDDP/hyperthermia/radiation; however, ETA (0.5 g/kg)/CDDP/hyperthermia/radiation was about 2.7-fold more toxic to the D cells than was CDDP/ hyperthermia/radiation. Therefore, with the addition of ETA (0.5 g/kg) to the trimodality regimen the sparing of dim cells was reduced to about 1.5-fold from about 5-fold.

Increasing the ETA dose to 1 g/kg resulted in marked increases in tumor cell killing in both tumor subpopulations (Fig. 6). The addition of ETA (1 g/kg) to CDDP (10 mg/kg) increased the killing of the BT cells by about 5.5-fold and the killing of the D cells by about 9.6-fold resulting in essentially equal cell killing in both tumor subpopulations at 37°C. ETA (1 g/kg)/CDDP/hyperthermia was about 9.2-fold more toxic to the BT cells and about 12.6-fold more toxic to the D cells than was CDDP/hyperthermia. Treatment with ETA (1 g/kg)/CDDP resulted in about a 1.5-fold sparing of the D cells. ETA (1 g/kg) in combination with CDDP/radiation produced about a 5.2-fold greater increase in the killing of the BT cells than of the D cells. Overall, although there was increased cytotoxicity in both tumor subpopulations there was about a 5.8-fold sparing of the D cells with the combination of ETA (1 g/kg)/CDDP/hyperthermia/radiation. The trimodality regimen of ETA (1 g/kg)/CDDP/hyperthermia/radiation reduced the sparing of the D cells to about 1.9-fold from about 5-fold and produced a significant increase in tumor cell killing in both D and BT cells.

In order to discern whether the dose modification observed on CDDP cytotoxicity with ETA in the whole tumor cell survival studies (Fig. 2) was due to a larger effect in either the BT or D cells, the effect of increasing the dose of CDDP to 20 mg/kg was examined with ETA (0.5 g/kg) (Fig. 7). CDDP (20 mg/kg) was about 2-fold more toxic to both tumor cell subpopulations than was CDDP (10 mg/kg). When ETA (0.5 g/kg) was added to CDDP (20 mg/kg), there was about a 10.6-fold increase in the killing of the BT cells and about a 5.6-fold
RADIOSENSITIZERS WITH CDDP/HYPERThERMIA/X-RAYS

Fig. 5. Survival of subpopulations based on Hoechst 33342 fluorescence intensity of F8AIC cells from F8AIC tumors treated with a single dose of hyperthermia (43°C, 30 min), radiation (10 Gy), CDDP (10 mg/kg) and ETA (0.5 g/kg), or combination treatments. Points, means of three independent determinations ± SE (bars).

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increase in the killing of the D cells. Hyperthermia with CDDP (20 mg/kg) killed 4 times as many BT cells and 4 times as many D cells as did CDDP (10 mg/kg)/hyperthermia. The addition of ETA (0.5 g/kg) to CDDP (20 mg/kg)/hyperthermia increased the killing of the BT cells about 9.1-fold and the killing of the D cells about 5.2-fold. The combination CDDP (20 mg/kg)/radiation produced about 2-fold greater killing of both tumor subpopulations than would be expected by the product of the surviving fractions. The relative sparing of the D cells by ETA (0.5 g/kg)/CDDP (20 mg/kg)/radiation was about 3-fold. Overall, the trimodality regimen including ETA (0.5 g/kg)/CDDP (20 mg/kg)/hyperthermia/radiation resulted in about a 5-fold sparing of the dim (hypoxic) tumor subpopulation (31-33). MISO was first described as a chemosensitizer in 1980 by Rose et al. (35) and Clement et al. (36). In both of these studies as well as in other early reports (37,38), although substantial enhancement of the antitumor actions of melphalan, cyclophosphamide, and nitro-}


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DISCUSSION

Improvements in the therapy of solid tumors require both increased total tumor cell killing and increased killing in viable, therapeutically resistant tumor cell subpopulations. Using the diffusion of Hoechst 33342 and fluorescence-activated cell sorting to separate F8AIC tumor subpopulations near to and distal from tumor vasculature, we found that the trimodality treatment combination of CDDP/hyperthermia/radiation resulted in about a 5-fold sparing of the dim (hypoxic) tumor subpopulation (31-33). The 2-nitroimidazole radiosensitizers MISO and ETA seemed suitable additions to the current trimodality regimen because these agents are more cytotoxic to hypoxic cells and interact positively with radiation (34). MISO was first described as a chemosensitizer in 1980 by Rose et al. (35) and Clement et al. (36). In both of these studies as well as in other early reports (37,38), although substantial enhancement of the antitumor actions of melphalan, cyclophosphamide, and nitro-}

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in the chronically hypoxic cells of the tumor. In contrast, however, in the subpopulation studies with CDDP (10 mg/kg) plus heat and radiation, ETA (1 g/kg) clearly resulted in more killing of the D cells. Surprisingly, the relatively modest increases in tumor cell killing evident in both BT and D cells resulting from the addition of ETA (1 g/kg) translated into a large increase in TGD (about 43 days for the ETA/CDDP trimodality versus about 25 days for the CDDP trimodality).

We are nearing completion of a phase I-II trial with CDDP, hyperthermia, and radiation in patients with superficial malignancies (20). The addition of ETA to this multimodality protocol is our intended next step based on the very positive results reported here.

REFERENCES


22. Stephens, T. C., Adams, K., Peacock, J. H., and Steel, G. G. Temporal administration and other treatments until the Hoechst 33342 is injected into the tumor-bearing animals, we chose 24 h posttreatment as a uniform point of comparison at which to examine the effects of these treatments in the tumor subpopulations (31-33). The trimodality treatment regimen including CDDP (10 mg/kg)/hyperthermia/radiation produced about 4 logs of killing in the BT (oxygenated) cells of the FSallC tumor. Adding MISO (1 g/kg) or ETA (0.5 g/kg) to the trimodality regimen resulted in no change in the killing of this tumor subpopulation, and ETA (1 g/kg) had only a small positive effect increasing tumor cell killing about 1.6-fold. In the D (hypoxic) cell subpopulation, however, there was a small but progressive increase in tumor cell killing with the addition of 2-nitroimidazoles. MISO (1 g/kg) increased D cell killing by about 1.9-fold, ETA (0.5 g/kg) increased D cell killing by about 2.7-fold, and ETA (1 g/kg) increased D cell killing by about 4.3-fold. Therefore, the effect of the 2-nitroimidazoles in conjunction with the other components of the trimodality therapy seems to be primarily in the hypoxic subpopulation of the tumor.

Increasing the dose of CDDP from 10 to 20 mg/kg resulted in a 2-fold increase in the killing of both BT and D cells in the presence and absence of hyperthermia. Addition of ETA (0.5 g/kg) to CDDP (20 mg/kg) produced a greater increase in the killing of the BT (oxygenated) cell subpopulation, both in the presence and absence of hyperthermia, than of the D cell subpopulation indicating that the dose modification observed in the whole tumor cell survival study may have resulted to a greater extent from an interaction of ETA and CDDP in the oxygenated (or intermittently hypoxic) cells of the tumor than


Addition of 2-Nitroimidazole Radiosensitizers to cis-Diamminedichloroplatinum(II) with Radiation and with or without Hyperthermia in the Murine FSaIIC Fibrosarcoma

Terence S. Herman, Beverly A. Teicher, Sylvia A. Holden, et al.


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