Interaction with Hyperthermia of Tetrachloroplatinum(II)(Nile Blue)$_2$ and Tetrachloroplatinum(II)(Neutral Red)$_2$ in EMT6 Murine Cells and the Murine FSAIIC Fibrosarcoma

Terence S. Herman, Beverly A. Teicher, M. Raphael Pfeffer, Vrinda S. Khandekar, and Timothy T. Korbut


ABSTRACT

Complexes of the tetrachloroplatinum(II) dianion with positively charged nuclear dyes were prepared in an effort to produce agents which gain ready access into the nucleus and become very cytotoxic at clinically relevant hyperthermia temperatures. Pt(Nile blue)$_2$ and Pt(neutral red)$_2$ are complexes of tetrachloroplatinum(II) with two closely related $\mu$-quinonediimine dyes. Pt(Nile blue)$_2$ and Pt(neutral red)$_2$ were slightly cytotoxic towards exponentially growing hypoxic EMT6 cells in vitro at pH 7.40 and 37°C. At pH 7.40 and 42°C exposure to $\mu$-quinonediimine dyes either 95% air/5% CO$_2$ or 95% N$_2$/5% CO$_2$ for 4 h at 37°C increased the actual pH to 6.45 as measured by a bioprobe combination pH electrode (Diamond General, Inc., Ann Arbor, MI). Average readings of 2.6 ± 1.4 mm of Hg were observed.

INTRODUCTION

An ideal chemotherapeutic agent for use in combination with local hyperthermia and radiation therapy would be systemically nontoxic at normal temperature but would become highly cytotoxic in the tumor volume at hyperthermic temperatures (1). Furthermore, the best drugs for such clinical protocols would interact positively with the cytotoxicities of radiation and hyperthermia across the different environmentally determined regions present within tumors. Our laboratory has been studying complexes of the platinum tetrachlorodiaminodichloroplatinum(II), cis-diamminedichloroplatinum(II), and PtCl$_2$(neutral red)$_2$, two of these new platinum complexes. The cytotoxicities of these drugs were tested at normal and clinically achievable hyperthermic temperatures and at normal and acidic pH, under normally oxygenated and hypoxic conditions. In addition, both drugs were studied in vivo in the FSAIIC fibrosarcoma with and without hyperthermia using tumor growth delay and tumor cell survival as end points.

MATERIALS AND METHODS

Drugs. Nile blue A and neutral red were purchased from Aldrich Chemical Co. (Milwaukee, WI). Potassium tetrachloroplatinate was a gift from Drs. Donald H. Picker and Michael J. Abrams, Johnson & Johnson, Inc. (West Chester, PA). The platinum complexes of tetrachloroplatinate and the dyes were prepared in our laboratory by reaction of potassium tetrachloroplatinate with a slight molar excess of the dyes in water at room temperature. The precipitated complexes were washed with ice-cold water, methanol, and diethyl ether, then used for experiments (2, 12). Elemental analyses of the complexes for carbon, hydrogen, nitrogen, chlorine, and platinum was performed by Galbraith Laboratories, Inc. (Knoxville, TN) and found to be in agreement with calculated values within 0.5%.

Cell Culture. EMT6 mouse mammary tumor cells have been widely used for the study of hypoxia (13-15) and heat effects in vitro (16). The EMT6 tumor cell line was originally developed by Rockwell et al. (17). Cultures were maintained in exponential growth in Waymouth’s medium (I.S.I. Corp., Chicago, IL), supplemented with 15% newborn calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) (Grand Island Biological Co., Grand Island, NY). The doubling time of these cultures, growing at 37°C in a 5% CO$_2$/95% air atmosphere, was 16–19 h. In vitro plating efficiencies of control cultures were 65–80%.

Heat Treatments. Exponentially growing cells were exposed to a temperature of 37, 42, or 43°C for 1 h. Heating was accomplished in a Plexiglas water tank with a continuous inflow and outflow system controlled by a water temperature controller (Braun Thermomix 1460; Braun Instruments) (18). Cells underwent heating in sealed plastic flasks (Falcon Plastics) containing 5 ml of complete medium. Water temperature could be maintained at ±0.10°C (SD).

Production of Hypoxia. To produce hypoxia, the plastic flasks, containing exponentially growing monolayers in complete medium plus serum, were fitted with sterile rubber septums and exposed to a continuously flowing 95% N$_2$/5% CO$_2$ humidified atmosphere for 4 h at 37°C as previously reported (19, 20). Parallel flasks were maintained in 95% air/5% CO$_2$. At the end of 4 h, the drug or vehicle was added to the flasks by injection through the rubber septum without disturbing the hypoxia. The partial pressure of oxygen was measured in sample flasks at various points in these experiments with a Diamond 760 polarographic O$_2$ electrode (Diamond General, Inc., Ann Arbor, MI). Average readings of 2.6 ± 1.4 mm of Hg were observed.

pH Alterations. The pH of the medium was adjusted with a sodium bicarbonate (NaHCO$_3$)/5% CO$_2$ buffer system (21). For medium with serum, the lowest pH that could be achieved with 5% CO$_2$ without NaHCO$_3$ was 6.43 ± 0.01. The reduced solubility of CO$_2$ at 40–45°C increased the actual pH to 6.45 as measured by a bioprobe combination pH electrode (Orion Research, Cambridge, MA). For altered pH experiments, the original bicarbonate buffer medium (pH 7.40) was replaced with media without NaHCO$_3$, and flasks were purged with either 95% air/5% CO$_2$ 4 h before heating for normally oxygenated conditions or gassed with 95% N$_2$/5% CO$_2$ for 4 h at 37°C for hypoxic conditions.
experiments as stated above. At various points in the experimental procedures, the pHs of sample flasks were measured as above and were found not to vary by more than 0.08 unit.

Drug Treatments. Exponentially growing cells were exposed to varying concentrations of Pt(Nile blue)₂ or Pt(neutral red)₂ for 1 h at 37, 42, or 43°C. Drugs were prepared in sterile PBS immediately before use and were added to the flasks in a small volume (50–100 µl). Addition of the drug solution did not significantly alter the pH of the culture.

Cell Viability Measurements. Cell viability was measured by the ability of single cells to form colonies in vitro, as described previously (19, 20). Each experiment was repeated 3–5 times, and each data point per experiment represents the results of 3 different dilutions of cells plated in triplicate.

Platinum Diaminations. Solutions of CDDP, K₂PtCl₆, Pt(Nile blue)₂, and Pt(neutral red)₂ were prepared in media without serum. The final concentration of all four solutions was confirmed by flameless atomic absorption spectrophotometry (4). EMT6 cell growth was monitored with a 1:1 0.25% trypsin:EDTA solution and centrifuged at 500 x g for 4 min. The cell pellets were resuspended in media containing 15% newborn calf serum at a concentration of approximately 4 x 10⁶ cells/ml for 1 h. Tubes containing the drugs in media were preheated in water baths at 37 and 42°C. The cells were added to the drug-containing media at each temperature and were incubated for 1 h at a concentration of 1.15 x 10⁶ cells/ml of 25 µM drug solution. After incubation, the cells were placed on ice and washed 4 times with 0.9% PBS to remove extracellular drug. The final washings were determined by atomic absorption spectrophotometry to have below detectable levels of platinum. The final cell pellet was sonicated, and the mass of intracellular platinum was determined by atomic absorption spectrophotometry. The limit of detection of this method is 0.3 ng of platinum and the standard curve is linear between 0.3 and 9 ng of platinum. Standards were run with each experiment.

Flameless Atomic Absorption Spectrophotometry Procedure (5). Platinum from a 15-µl sample injection volume was atomized from the walls of electrolytically coated graphite tubes. A Perkin Elmer Model 2380 atomic absorption spectrophotometer was used in conjunction with a Perkin-Elmer Model 400 graphite furnace to measure the absolute mass of platinum in the cell samples (4, 22). Each measurement was made in triplicate in three independent experiments.

Tumor. The FSaII fibrosarcoma (23) adapted for growth in culture (FSaIIIC) (24) was carried in male C3H/He mice (The Jackson Laboratory, Bar Harbor, ME). For the experiments, 2 x 10⁶ tumoral cell suspensions were prepared from a brei of several stock tumors implanted i.m. into the legs of male C3H/He mice 8 to 10 weeks old.

When the tumors were approximately 100 mm³ in volume, treatment was initiated. In those groups receiving the drug, CDDP (5 mg/kg), Pt(Nile blue)₂ (100 mg/kg), or Pt(neutral red)₂ (100 mg/kg) in 0.9% PBS (0.2 ml) was injected as a single dose i.p. In those groups receiving hyperthermia, heat was delivered as a single dose locally to the tumor-bearing limb 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 read-out thermistor (Sensortech, Inc., Clifton, NJ) placed into the center of the tumor in selected control animals as previously described (25). No anesthetic was used. Hyperthermia was delivered immediately following i.p. injections. The progress of each tumor was measured 3 times weekly until it reached a volume of 500 mm³. Tumor growth delay was calculated as the additional days taken by each individual tumor to reach 500 mm³ beyond that required for the untreated controls. Each treatment group had 7 animals, and the experiment was repeated twice. Days of tumor growth delay are the mean ± SE for the treatment group compared to the control.

Tumor Excision Assay. When the tumors were approximately 100 mm³ in volume (about 1 week after tumor cell implantation), the animals were given injections i.p. of various doses of either Pt(Nile blue), or Pt(neutral red)₂ (100, 250, or 500 mg/kg) alone or immediately followed by hyperthermia (43°C, 30 min), as described above, to the tumor-bearing limb. Twenty-four h after treatment, which allowed for full expression of drug cytotoxicity and repair of potentially lethal damage, mice were sacrificed and immersed in 95% ethanol. The tumors were excised under sterile conditions and single cell suspensions were prepared as described previously (25). The untreated tumor cell suspensions had a plating efficiency of 10–16%. The results are expressed as the surviving fraction ± SE of cells from treated groups compared to untreated controls (25).

RESULTS

The general structures of Pt(Nile blue)₂ and Pt(neutral red)₂ are shown in Fig. 1. Both the oxazin dye, Nile blue, and the azin dye, neutral red, bear diffuse positive charges and have found use in the past as nuclear stains (13). Reaction of 2 mol of the dyes with 1 mol of potassium tetrachloroplatinate results in formation of a tight ion pair complex which precipitates from a concentrated aqueous solution, so that the platinum complexes have the formulae (PtCl₄)(Nile blue)₂ and (PtCl₄)(neutral red)₂ (2, 12), and like Pt(Rh-123)₂ (2–5, 12), the complexes formed are neutral.

It has been shown that the cytotoxicity of platinum complexes such as CDDP is markedly affected by pH, oxygenation, and temperature (26). At normal pH (pH 7.40) and 37°C, Pt(Nile blue)₂ was only moderately cytotoxic (Fig. 2). Increasing the temperature during drug exposure to 42°C produced a small increase in the killing of normally oxygenated cells of about 1.5-fold with 500 µM Pt(Nile blue)₂. The effect of the increased
temperature on the kill of hypoxic EMT6 cells, however, was greater, reaching 5- to 7-fold at a 500 μM concentration of the drug. At 43°C and pH 7.40, approximately 4 logs of normally oxygenated EMT6 cells were killed by exposure to 250 μM Pt(Neutral red)2 for 1 h. Although there was also increased killing of hypoxic EMT6 cells under these treatment conditions, the effect was not as great as was seen for the normally oxygenated cells.

A very different pattern of cytotoxicity was seen when the extracellular pH was lowered to pH 6.45 (Fig. 2). At pH 6.45 and 37°C, exposure of EMT6 cells to 500 μM Pt(Nile blue)2 for 1 h resulted in the killing of only approximately 50% of the normally oxygenated cells and of approximately 40% of the hypoxic cells. Increasing the temperature during drug exposure to 42°C, however, produced a large increase in the kill of hypoxic EMT6 cells and only a moderate increase in the kill of normally oxygenated EMT6 cells. As was seen at pH 7.40 when the temperature during drug exposure was increased to 43°C, there was a marked increase in the kill of normally oxygenated EMT6 cells and a smaller increase in the kill of hypoxic EMT6 cells. At pH 6.45 and 43°C, exposure to 500 μM Pt(Nile blue)2 for 1 h resulted in the kill of nearly 5 logs of normally oxygenated EMT6 cells, an increase of approximately 4 logs compared with the cell kill observed with the same concentration of drug at 42°C.

Pt(Neutral red)2 was more cytotoxic than Pt(Nile blue)2 at 37°C and normal pH (pH 7.40) (Fig. 3). Exposure of normally oxygenated EMT6 cells to 250 μM Pt(Neutral red)2 for 1 h at normal pH and 37°C produced nearly 3 logs of cell kill. Hypoxic EMT6 cells were much less sensitive to Pt(Neutral red)2 under these environmental conditions. As was observed for Pt(Nile blue)2, increasing the temperature to 42°C during drug exposure produced only a small increase in the kill of normally oxygenated EMT6 cells and had a greater effect on the kill of hypoxic EMT6 cells. At 42°C and pH 7.40, exposure to 250 μM Pt(Neutral red)2 for 1 h killed approximately 3 logs of normally oxygenated EMT6 cells. Exposure to the same drug concentration under the same conditions resulted in the killing of 1.5 logs of hypoxic EMT6 cells. Increasing the temperature during drug exposure to 43°C at pH 7.40 resulted in greater than 5 logs of kill of normally oxygenated EMT6 cells exposed to 250 μM Pt(Neutral red)2 for 1 h, as opposed to 3.5 logs of hypoxic cells.

Adjusting the extracellular pH to pH 6.45 during exposure of normally oxygenated EMT6 cells to Pt(Neutral red)2 at 37°C did not significantly alter the level of cytotoxicity observed when normally oxygenated EMT6 cells were exposed to 250 μM Pt(Neutral red)2 for 1 h from the 3 logs of cell kill seen with the same treatment at pH 7.40 (Fig. 3). Increasing the temperature during drug exposure to 42°C at pH 6.45 resulted in an increase of about 8-fold in the kill of normally oxygenated EMT6 cells exposed to 100 μM Pt(Neutral red)2 compared to that seen at 37°C and pH 6.45. The increase in the kill of hypoxic EMT6 cells observed at elevated temperature (42°C) at pH 6.45 was similar to that seen in the normally oxygenated cells. Exposure of hypoxic EMT6 cells to 250 μM Pt(Neutral red)2 for 1 h at 42°C and pH 6.45 produced 2.5 logs of cell kill, a 1 log increase over the cell kill seen by the same treatment at 37°C. Although further increasing the temperature during drug exposure to 43°C resulted in no significant change in the cell kill seen in normally oxygenated EMT6 cells, there was an additional significant increase in the cytotoxicity of Pt(Neutral red)2 in hypoxic EMT6 cells exposed to the drug at 43°C and pH 6.45 compared to that seen at 42°C and pH 6.45. Whereas exposure to 50 μM Pt(Neutral red)2, for 1 h at pH 6.45 and 42°C killed approximately 1.5 logs of hypoxic EMT6 cells, at 43°C this same drug treatment killed 3.5 logs of hypoxic EMT6 cells.

The levels of intracellular platinum measured following exposure of normally oxygenated EMT6 cells to 25 μM CDDP, K2PtCl4, Pt(Nile blue)2, or Pt(Neutral red)2 for 1 h at pH 7.40 or pH 6.45 and at 37 or 42°C are presented in Table 1. Approximately 1 ng of platinum was found in 1 x 10⁶ EMT6 cells after exposure to 25 μM CDDP under each of the conditions tested. There was no significant difference in the amount of platinum present in EMT6 cells whether the pH during drug exposure was 7.40 or 6.45. There was a trend toward a slightly higher level of platinum content in the EMT6 cells after exposure of the cells to CDDP at 42°C, but this effect was not statistically significant. As has been reported previously (4, 11), platinum in the form of K2PtCl4 enters cells poorly. Under the conditions tested, approximately one-fifth as much platinum entered the EMT6 cells after exposure to 25 μM K2PtCl4 as compared to CDDP. Again, there was no statistically significant difference in the amount of platinum found in the EMT6 cells at either acidic or normal pH at either 37 or 42°C. At pH 7.40 and 37°C, there was approximately 200-fold more platinum found in EMT6 cells after exposure to 25 μM Pt(Nile blue)2 or Pt(Neutral red)2 for 1 h than found in the same cells exposed to Pt(Neutral red)2 at 37°C and normal pH (pH 7.40) (Fig. 3).

Table 1 Intracellular levels of platinum after exposure to various platinum complexes at 37 or 42°C for 1 h

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH 7.40</th>
<th>pH 6.45</th>
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<tbody>
<tr>
<td>CDDP</td>
<td>0.95 ± 0.13</td>
<td>1.17 ± 0.21</td>
</tr>
<tr>
<td>K2PtCl4</td>
<td>0.18 ± 0.06</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td>Pt(Nile blue)2</td>
<td>229 ± 28</td>
<td>597 ± 108</td>
</tr>
<tr>
<td>Pt(Neutral red)2</td>
<td>221 ± 42</td>
<td>441 ± 60</td>
</tr>
</tbody>
</table>

Pt(Neutral red)2 Concentration, μM

Fig. 3. Survival curve of exponentially growing normally oxygenated (●) and hypoxic (○) EMT6 cells exposed to the concentrations of Pt(Neutral red)2 indicated at 37, 42, or 43°C at pH 7.40 and pH 6.45. The survival value plotted on the y-axis represents heat alone killing at the conditions indicated. Points, means of 3 independent determinations; bars, SEM.
25 μM CDDP under the same conditions. At pH 6.45 the amount of platinum found in EMT6 cells exposed to 25 μM Pt(Nile blue)2 or Pt(neutral red)2 at 37°C was slightly greater than that seen at 37°C and pH 7.40. As opposed to CDDP and K2PtCl4, the amount of platinum in EMT6 cells exposed to 25 μM Pt(Nile blue)2 or Pt(neutral red)2 at either pH 7.40 or pH 6.45 was significantly increased when the cells were exposed to the drugs at 42°C as compared to 37°C. The amount of platinum in cells exposed to 25 μM Pt(Nile blue)2 increased approximately 2.5-fold when the drug exposure was carried out at 42°C as compared to 37°C. Similarly, the amount of platinum in cells exposed to 25 μM Pt(neutral red)2 increased approximately 1.8-fold when the cells were exposed to the drug at 42°C as compared to 37°C.

The efficacy of Pt(Nile blue)2 and Pt(neutral red)2 as antitumor agents alone and in combination with local hyperthermia was tested in the FSAIIC fibrosarcoma by assessment of TGD (Table 2) and tumor cell survival (Figs. 4 and 5). TGD was measured after a single dose of drug alone or immediately followed by hyperthermia (43°C, 30 min) administered locally to the tumor-bearing limb (Table 2). The hyperthermia produced 1.4 days of TGD. CDDP (5 mg/kg) produced a TGD of 4.4 days, and with hyperthermia this increased about 1.3-fold to 5.9 days. The dose of 100 mg/kg was chosen for Pt(Nile blue)2 and Pt(neutral red)2 because this dose of each agent produced a TGD similar to that of CDDP (5 mg/kg) alone. Pt(Nile blue)2 (100 mg/kg) produced a TGD of 4.6 days which was increased approximately 2.4-fold with the addition of hyperthermia to 10.9 days (7). Pt(neutral red)2 (100 mg/kg) was also an effective antitumor agent in the FSAIIC fibrosarcoma producing a TGD of 3.8 days. Treatment with Pt(neutral red)2 in combination with hyperthermia resulted in a TGD of 8.0 days which was a 2.1-fold increase over the TGD seen in the absence of hyperthermia. Pt(Nile blue)2, over the dosage range from 100 to 500 mg/kg killed increasing numbers of FSAIIC tumor cells in a log-linear manner (Fig. 4). With hyperthermia (43°C, 30 min) alone, the surviving fraction of FSAIIC tumor cells was 0.75. The combination of Pt(Nile blue)2 and hyperthermia produced a tumor cell-killing curve with a steeper slope than with Pt(Nile blue)2 alone, indicating dose modification of the drug by hyperthermia. Similarly, Pt(neutral red)2, over the dosage range from 100 to 500 mg/kg killed increasing numbers of FSAIIC tumor cells in a log-linear manner (Fig. 5). Hyperthermia also produced a dose-modifying effect on the toxicity of Pt(neutral red)2 toward FSAIIC tumor cells.

### Table 2 Growth delay of FSAIIC fibrosarcoma produced by combinations of CDDP, Pt(Nile blue)2, or Pt(neutral red)2, with hyperthermia

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Tumor growth delay (days)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>43°C, 30 min</td>
<td>1.4 ± 0.7</td>
</tr>
<tr>
<td>CDDP (5 mg/kg)</td>
<td>4.4 ± 0.9</td>
</tr>
<tr>
<td>CDDP → heat</td>
<td>5.9 ± 1.1</td>
</tr>
<tr>
<td>Pt(Nile blue)2 (100 mg/kg)</td>
<td>4.6 ± 0.7</td>
</tr>
<tr>
<td>Pt(Nile blue)2 → heat</td>
<td>10.9 ± 1.2</td>
</tr>
<tr>
<td>Pt(neutral red)2 (100 mg/kg)</td>
<td>3.8 ± 0.6</td>
</tr>
<tr>
<td>Pt(neutral red)2 → heat</td>
<td>8.0 ± 1.0</td>
</tr>
</tbody>
</table>

* Tumor growth delay is the difference in the number of days for the treated tumors to reach 500 mm3 compared to untreated control tumors. The data presented are the means of 14 animals ± SE.

DISCUSSION

Clinically, the addition of local hyperthermia to radiation therapy has been shown to substantially improve tumor control (1, 27-31). Treatment with hyperthermia and radiation therapy is not always successful, however, especially when tumors are large, radiation tolerance is limited, or achievable tumor temperatures are low (28). After a review of the pertinent preclinical and clinical literature, we concluded that CDDP was probably the best drug currently available to use weekly with local hyperthermia and daily fractionated radiation therapy (1). CDDP has been shown to become more cytotoxic to acidic cells at elevated temperatures (26, 32, 33). Since hypoxic and acidic conditions would be expected to occur in the poorly vascularized portions of tumors (33-35), we have previously examined the effect of these culture conditions on hyperthermic sensitization to CDDP in cells (32). For CDDP, marked hyperthermic sensitization was evident in normally oxygenated cells, but hypoxic cells showed essentially no sensitization to the cytotoxicity of CDDP at elevated temperature at normal pH. Low pH further increased the cytotoxicity of CDDP toward normally
oxygenated and hypoxic cells, restoring nearly the full sensitizing effect of hyperthermia on CDDP in the hypoxic cells.

We are searching for drugs which are relatively nontoxic at normal temperature but are capable of causing important degrees of increased cytotoxicity in conjunction with hyperthermia across the various environmental conditions present in solid tumors. Pt(Nile blue), at normal pH showed marked enhancement of cytotoxicity only at 43°C in normally oxygenated EMT6 cells but only moderate enhancement of cytotoxicity toward hypoxic EMT6 cells even at 43°C. At acidic pH (pH 6.45), however, marked enhancement in the cytotoxicity of Pt(Nile blue)2 toward hypoxic EMT6 cells occurred at 42°C, while marked sensitization of the normally oxygenated cells required 43°C as at normal pH. The reversal in cytotoxic selectivity of Pt(Nile blue)2 based on oxygenation status of the cells at pH 6.45 and 45°C indicates that these environmental factors are highly interactive and requires that the experiment be done under each set of conditions. Pt(Nile blue)2 would be expected to be highly effective in combination with hyperthermia if tumor temperatures of 43°C could be achieved. Pt(Nile blue)2 is also a highly effective radiosensitizing agent at a concentration of 100 μM in hypoxic EMT6 cells (7) and, therefore, should be an excellent candidate for combined modality treatment programs using both hyperthermia and radiation therapy.

The cytotoxicity of Pt(neutral red), in normally oxygenated EMT6 cells was only moderately enhanced even at 43°C and normal pH, but the killing of hypoxic EMT6 cells was markedly enhanced at 43°C but not at 42°C. Under acidic environmental conditions (pH 6.45), Pt(neutral red)2 cytotoxicity was further enhanced at 42°C and to a more marked extent at 43°C in both normally oxygenated and hypoxic EMT6 cells. Overall, therefore, Pt(neutral red)2 would also require a tumor temperature of 43°C to be a highly effective treatment in combination with hyperthermia. Pt(neutral red)2 is a moderately effective radiosensitizing agent of hypoxic EMT6 cells at a concentration of 100 μM Pt(neutral red)2 (7) and, therefore, would probably be a somewhat less favorable candidate for a combined hyperthermia and radiation treatment program than Pt(Nile blue)2.

Platinum levels achieved in cells by several platinum-containing agents have been measured at both normal temperature (37°C) and at 42°C. We have previously found that the amount of platinum which enters cells exposed to CDDP, K2PtCl4, Pt(Rh-123)2 (4), and Pt(fast black) (11) is not changed at elevated temperature (42°C). The amount of platinum found in cells exposed to 25 μM Pt(Nile blue)2 and Pt(neutral red)2 for 1 h, however, was approximately doubled at both normal and acidic pH. As opposed to several other platinum complexes examined, therefore, the increased cytotoxicity of Pt(Nile blue)2 and Pt(neutral red)2 produced by elevated temperature may be due, at least partially, to an increased level of drug in the cells. Other effects of hyperthermia on these drugs may be increased reactivity of these platinum complexes with DNA (4, 5, 7, 36) or a heat-induced inhibition of DNA repair (37).

Both Pt(Nile blue)2 and Pt(neutral red)2 at a dose of 100 mg/kg were well tolerated and produced approximately 4 days of tumor growth delay in the FSA1IC fibrosarcoma. The addition of hyperthermia to the drug treatment (43°C, 30 min) resulted in a slightly greater increase in the tumor growth delay with Pt(Nile blue)2 than with Pt(neutral red)2. This finding is in agreement with the results obtained in cell culture which indicated that there is an overall greater enhancing effect of hyperthermia on the cytotoxicity of Pt(Nile blue)2 than on the cytotoxicity of Pt(neutral red)2. In vivo, hyperthermia appears to have a dose-modifying effect on both Pt(Nile blue)2 and Pt(neutral red)2, since there is an increasing differential in tumor cell kill seen with increasing dose of drug. This is similar to the pattern of enhanced tumor cell kill observed with hyperthermia and Pt(fast black) (11) or hyperthermia and 1,3-bis(2-chloroethyl)-1-nitrosourea (38) in this tumor and different than the pattern of enhanced tumor cell kill seen with CDDP under the same conditions (25). In conclusion, Pt(Nile blue)2 and Pt(neutral red)2 are new platinum complexes which were designed based on the rationale that positively charged dyes which show some selectivity for staining nucleic acid when complexed with the tetrachloroplatinum diion would form a neutral complex capable of localizing greater amounts of platinum in the vicinity of the DNA (7). Pt(Nile blue)2 and Pt(neutral red)2 clearly carry greater amounts of platinum into cells than does CDDP. These new complexes are less cytotoxic than CDDP at normal temperatures but can be highly activated at clinically relevant elevated temperatures and, therefore, are potentially interesting agents for use on combined modality treatment regimens with hyperthermia with or without radiation therapy.

REFERENCES


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