The Evaluation of Thermal Cisplatin Sensitization in Normal and XP Human Cells Using Mild Hyperthermia at 40 and 41°C

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Abstract. The effect of protracted mild hyperthermia treatment at 40 and 41°C given, concurrently with cisplatin, was evaluated in human normal AG1522 and human mutant XPA cells. While mild hyperthermia itself for up to 6 hours showed little to no toxic effects, it did result in significant sensitization of response to cisplatin treatment. Sensitization for the normal and mutant cell line was comparable, indicating that nucleotide excision repair (NER) probably does not have a role in this process. For the 41°C heating, thermotolerance developed and heating times greater than 4 hours resulted in protective effects from cisplatin cytotoxicity. This was not observed for heating at 40°C for up to 6 hours.

Cisplatin is a chemotherapy agent that is widely used in the treatment of cancer and is also used in combination with other treatment modalities (1-10). A number of studies have shown that the response of cells to cisplatin can be influenced by their ability to repair cisplatin damage, and repair systems such as nucleotide excision repair (NER) and homologous recombination repair have been demonstrated to influence cisplatin sensitivity (11-16). Thus, the modulation of cellular repair pathways might influence the response to cisplatin.

Many studies have shown that hyperthermia can inhibit cellular recovery and DNA repair after irradiation (17, 18). It was also shown that mild protracted hyperthermia was more effective in sensitizing cells to protracted radiation treatment due to a greater effect on cell damage accumulation and inhibition of its repair (19).

Hyperthermia has already been shown to be a good sensitizer for cisplatin treatment (20-26). We also showed that hyperthermia combined with cisplatin and radiation resulted in enhanced inhibition of sublethal damage repair (27). These findings have led us to evaluate the effect of mild hyperthermia on concurrent low dose cisplatin treatment to determine if this would be a more effective treatment due to the inhibition of damage repair by hyperthermia. In addition, we used two human cell lines, one with no known repair deficiencies and the other deficient in NER (28), to determine if the thermal sensitization by concurrent protracted treatments would be influenced by a deficiency in NER.

Materials and Methods

The cells used in these experiments were normal human fibroblasts, AG1522, and human xeroderma pigmentosa cells, XPA, deficient in NER. The AG1522 cells have been used in our laboratory for many years and the XPA cells were kindly donated by Dr. B. Mackay. All cells used for the experiments were less than passage 15 in order to avoid deterioration due to senescence. The cells were grown in a 1:1 mixture of DMEM and F-12 medium, supplemented with 7.5% foetal bovine serum, 7.5% newborn calf serum, 0.1 mM MEM non-essential amino acids, 10 mM sodium bicarbonate and 20 mM HEPES, and incubated at 37°C in a humidified atmosphere of 2% CO₂ and 98% air. The experiments were done for cells grown into the plateau phase. For the plateau phase, cells were fed with fresh medium two days before the experiment; the plating efficiencies ranged from 15-20% and 10-15% for the AG1522 and the XPA cell lines, respectively.

Cells were grown to the plateau phase and treated in this state in order to avoid complex cell cycle redistributions during protracted treatments. In addition, the confluent cell density is more representative of tissue cell cycle redistributions during protracted treatments. The cells used in these experiments were normal human fibroblasts, AG1522, and human xeroderma pigmentosa cells, XPA, deficient in NER. The AG1522 cells have been used in our laboratory for many years and the XPA cells were kindly donated by Dr. B. Mackay. All cells used for the experiments were less than passage 15 in order to avoid deterioration due to senescence. The cells were grown in a 1:1 mixture of DMEM and F-12 medium, supplemented with 7.5% foetal bovine serum, 7.5% newborn calf serum, 0.1 mM MEM non-essential amino acids, 10 mM sodium bicarbonate and 20 mM HEPES, and incubated at 37°C in a humidified atmosphere of 2% CO₂ and 98% air. The experiments were done for cells grown into the plateau phase. For the plateau phase, cells were fed with fresh medium two days before the experiment; the plating efficiencies ranged from 15-20% and 10-15% for the AG1522 and the XPA cell lines, respectively.

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covering the cells. At the end of the exposure period, the medium containing cisplatin was aspirated, the cells were rinsed twice with isotonic citrate saline and new medium was added. Hyperthermia treatments were performed by sealing T-25 flasks with parafilm and immersing them in a circulating water bath with the temperature controlled to ±0.05°C. The flasks were placed in a 37°C water bath for 5 minutes at the end of the hyperthermia treatment period. For concurrent treatments, cisplatin was added 10 minutes before hyperthermia was started and removed 10 minutes after the completion of hyperthermia. Cell survival after treatment was determined by the colony-forming assay. Briefly, the cells were rinsed with isotonic citrate saline, trypsinized (0.2% w/v trypsin in citrate saline for 5 minutes at 37°C), counted using an electronic cell counter, and plated into 60-mm dishes containing fresh medium. Colonies larger than 50 cells on day 16 were scored as survivors. Three replicate dishes were plated for each point. The plotted points represent the mean of 3 replicate experiments. Error bars are standard error of the mean.

Results

The response of human normal and XPA fibroblasts to cisplatin is shown in Figure 1. The data show that, for a 1-hour treatment, the XPA cells deficient in NER are more sensitive than the normal cells and confirm earlier results (12-14).

Figure 2 shows the response of the two human cell lines to hyperthermia alone (40°C), cisplatin alone and to cisplatin given concurrently with mild hyperthermia. The treatment at 40°C alone did not have any effect on either cell line when treated for up to 8 hours. For cisplatin alone we tried to match the toxic effect and treated AG1522 cells with 1.0 µg/ml and XPA cells with 0.5 µg/ml. The figure shows comparable results, with XPA showing slightly greater cell killing. For the combination treatment, the results for both cell lines show substantial thermal sensitization to cisplatin with 40°C hyperthermia. The degree of thermal sensitization is shown in Table I and indicates that, at the 10 and 5% survival levels, the sensitization was about the same.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Survival level</th>
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<tbody>
<tr>
<td></td>
<td>10%</td>
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<tr>
<td>AG1522 40°C</td>
<td>1.44</td>
</tr>
<tr>
<td>AG1522 41°C</td>
<td>1.80</td>
</tr>
<tr>
<td>XPA 40°C</td>
<td>1.81</td>
</tr>
<tr>
<td>XPA 41°C</td>
<td>2.12</td>
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1. TER. Thermal enhancement ratio was calculated by dividing the incubation time for cisplatin alone by the incubation time for the combined treatment. Note values were interpolated from the fitted curves.
2. The error on the TER values is ±10%.
for both cell lines. The slightly higher thermal cisplatin sensitization (TER) for the XPA cells may be due to the fact that the cisplatin treatment was more toxic in these cells compared to the AG1522 cells.

Hyperthermia at 41°C was also tested and Figure 3 shows that there was very little cell killing by hyperthermia alone. The exposure to cisplatin alone for AG1522 (1.0 μg/ml) and XPA (0.5 μg/ml) cells again showed that the survival results are comparable for both cell lines. For concurrent treatment with hyperthermia and cisplatin, both cell lines showed an initial thermal sensitization at 2 hours, but at longer treatment times sensitization decreased. For the longest treatment time of 6 hours, the combination treatment was less effective than the treatment with cisplatin alone. The results for thermal sensitization at the 10 and 5% survival levels are shown in Table I.

In order to determine whether the results in Figure 3 for the combination treatment were due to thermodurability, the cells were first exposed to hyperthermia at 41°C for up to 6 hours and then given a 1-hour treatment of cisplatin at 2 and 1 μg/ml for the AG1522 and XPA cells, respectively. The data in Figure 4 show initial sensitization at 2 hours, which then levelled off to very little additional sensitization. The plateauing effect occurred at 2 hours for XPA and at 4 hours for AG1522. These times agree with the development of the plateaus observed in Figure 3.

**Discussion**

Our data confirm that NER deficiency, such as found in XPA, results in greater sensitivity to cisplatin (12-14) and, in order to match toxicity, the cisplatin concentration for the XPA cell treatment (0.5 μg/ml) was half of that used for the normal human fibroblast line AG1522 (1.0 μg/ml). When TER at 40°C was compared under these conditions the results were similar for both cell lines, indicating that the mutation XPA did not affect TER. Thus, if thermal sensitization acts through DNA repair inhibition (17, 18), it appears that the NER pathway may not play an important role in this process.

Our results with protracted concurrent mild hyperthermia show thermal enhancement ratios that indicate significant TER. These results agree with the sensitization observed for acute treatments at higher temperatures (20-26, 31) and show the potential of mild hyperthermia as a sensitizer. It is also of interest to note that earlier studies by Mitchel et al. (32) showed that thermal radiosensitization occurred in both normal and XP cells, also indicating that the NER pathway may not play a role in thermal sensitization.

Also noteworthy was that for the 41°C treatment, the combination treatment curves reached a plateau at 2-hour and 4-hour treatment times for the XPA and AG1522 cells, respectively. In fact, the curves crossed over the results for
treatment with cisplatin alone, indicating a sub additive effect. Further studies shown in Figure 4 indicate that this effect is due to the development of thermotolerance. Earlier studies by others (33, 34) have in fact shown that the development of thermotolerance after acute hyperthermia treatments at 42°C can reduce or provide protection for cisplatin sensitization, especially at the longer heating times. However, one study showed no effect at higher heating temperatures of 45°C (35), indicating that there may well be a temperature dependence. The protection of thermal drug sensitization by the development of thermal tolerance has been reviewed for a wide range of drugs and potential mechanisms have been discussed (36). Our data confirm the thermotolerance effect in human cells and indicate that it can occur for protracted mild hyperthermia at 41°C, while for the heating times used at 40°C it was not evident. The data also show that this effect occurred in both AG1522 and XPA cells to the same degree, thus ruling out any influence of NER.

In summary, our data show that mild hyperthermia has good potential for cisplatin sensitization and that this effect is not affected by deficiencies in the NER pathway. Our data indicate that, for protracted treatments, the protection of thermal drug sensitization by the development of thermal tolerance has been reviewed for a wide range of drugs and potential mechanisms have been discussed (36). Our data confirm the thermotolerance effect in human cells and indicate that it can occur for protracted mild hyperthermia at 41°C, while for the heating times used at 40°C it was not evident. The data also show that this effect occurred in both AG1522 and XPA cells to the same degree, thus ruling out any influence of NER.

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