In Vivo Potentiation of Radiation Response by Topotecan in Human Rhabdomyosarcoma Xenografted into Nude Mice

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ABSTRACT

The lack of new highly efficacious drugs for cancer treatment promotes the search for innovative therapeutic modalities. The authors reported the results leading to the definition of parameters needed to demonstrate a possible radiopotentiation by topotecan (TPT) on two representative human rhabdomyosarcomas (RMSs) xenografted into nude mice. Experimental studies of radiopotentiation with different doses of topotecan showed that concomitant association of topotecan and RT for 5 consecutive days provided a synergistic therapeutic effect. Response rates were statistically higher with the radiochemotherapeutic combination (P < 0.001). Efficacy enhancement factors of this combination compared with the sum of the antitumoral activity of these treatments separately administered were 1.54 and 1.60, respectively, on both rhabdomyosarcomas. Moreover, the efficiency of the combination of radiotherapy at the dose of 20 Gy with topotecan (12.5 mg/kg) was not statistically different from that of radiotherapy at the dose of 40 Gy. According to microscopy results, the analyses performed at different periods after topotecan treatment alone, radiotherapy alone, and their combination seemed to show that tumoral repopulation by malignant cells is as fast as the dose of radiotherapy and/or topotecan is low. Furthermore, lesions observed with the dose of 40 Gy were similar to those obtained with the association of topotecan at the dose of 12.5 mg/kg and radiotherapy at the dose of 20 Gy. In conclusion, all clinical and pathological results are consistent with a radiopotentiation effect of topotecan on the two xenografted human rhabdomyosarcomas and are currently leading to the design of clinical studies.

INTRODUCTION

Topoisomerase I nuclear enzyme relaxes supercoiled DNA and appears to be important for semiconservative replication of double helical DNA, transcription, and chromosomal decondensation (1). Topoisomerase I is the target of topoisomerase I inhibitors, represented by camptothecin and its analogues. These agents stabilize the cleavable complex resulting from the enzyme and DNA. The existence of a synergistic cell-killing effect between ionizing radiation and camptothecin derivatives has been demonstrated in vivo on different cell lines (2, 3). TPT2 [SKF 104864A, NSC 609699; (S)-9-dimethylaminomethyl-10-hydroxy-camptothecin hydrochloride], a camptothecin analogue, demonstrated in vitro radiopotentiation on Chinese hamster ovary, P388 murine leukemia cultured cells (4), human carcinoma cells (5), human squamous carcinoma of the head and neck (6), melanoma (7), non-small cell lung carcinoma (8), and malignant gliomas (8, 9). In vivo potentiation of response has been shown in murine fibrosarcoma and MCA-4 mammary carcinoma (3, 6, 10). To our knowledge, radiopotentiation by TPT has not been demonstrated in vivo in human cancers yet. The diagnosis of RMSs, which are the most frequent soft tissue sarcomas in children and young adults, has increased over the last two decades, and both chemotherapy and RT are required in most cases.

It has been shown, both on in vitro and in vivo preclinical models and in Phase II studies in children, that TPT is efficient on RMSs (11, 12). The present study was designed to evaluate the interaction of TPT with ionizing radiation in vivo in two RMSs, one from a child and one from an adult, xenografted into nude mice, in a once-daily schedule of fractionation over 5 days.

MATERIALS AND METHODS

Animals. Athymic NCr/Sed nude (nu/nu) mice, 7–8 weeks of age, were obtained from Iffa Credo (Lyon, France). Over the experimental period, the mice used in this study were maintained in microisolators, housed one mouse to a cage, fed with nonirradiated laboratory pellets, and nonfiltered water ad libitum. Experiments were carried out under the conditions established by the European Community (Directive No. 86/609/CEE). No whole-body irradiation was used to immunosuppress the nude mice further.

Tumor. The first RMS (RMS1) was derived from a previously untreated stage III RMS of a 16-year-old girl. The second RMS (RMS2) was derived from a previously untreated tumor located around the right knee in a 82-year-old woman. The pathological and immunohistochemical characteristics were unchanged with the successive passages.

The abbreviations used are: TPT, topotecan; RMS, rhabdomyosarcoma; IDU, iododeoxyuridine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GST, glutathione S-transferase; RT, radiation therapy (radiotherapy); TGD, tumor growth delay.

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2 The abbreviations used are: TPT, topotecan; RMS, rhabdomyosarcoma; IDU, iododeoxyuridine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GST, glutathione S-transferase; RT, radiation therapy (radiotherapy); TGD, tumor growth delay.
These tumors were directly transplanted into nude mice and maintained in vivo by sequential passages in nonirradiated nude mice. For the experiments, the solid transplanted tumors, from the 17th generation for RMS1 and the 9th generation for RMS2, were excised, cleaned from necrotic tissue, cut into small chunks, and s.c. transplanted into each experimental mouse. Tumor growth was determined two to three times per week by measuring three perpendicular diameters with a caliper. Tumor volume \( V \) was calculated as 
\[
V = (\text{length} \times \text{width} \times \text{depth})/2
\]

Pathology, Immunohistochemistry, and Labeling

Index: Pathological and Biological Studies. These studies were designed to assess the relationship between tumor volume evolution and pathological modifications. Microbiopises were performed at different periods after each treatment in comparison with controls. For us to study cell kinetics, mice received, 24 h prior to biopsy, an i.p. injection of IDU at the dose of 0.013 mg/g. Fragments were fixed in 10% formol, embedded in paraffin, cut into 5-mm sections, and stained with H&E for pathological assessment. Immunohistochemistry studies were performed using antibodies specific to vimentin, actin, desmin, and anti-IDU antibody using peroxidase-antiperoxidase technique to identify nuclei labeled with IDU. The IDU labeling index was calculated in each fragment as the percentage of cells labeled with IDU, excluding vascular components and hematogenous cells, in three to six microscopic fields (<400; approximately 1000–3000 cells) in viable tissue within 200 mm of the surface in areas where labeled cells were distributed evenly.

RNA Isolation and Reverse Transcript-PCR Analysis. Expression of genes associated with drug resistance were determined by reverse transcription-PCR. Isolation of RNA was performed using TRIZol (Life Technologies, Inc.). cDNA synthesis was performed with 1 \( \mu \)g of total RNA in a reaction volume of 20 \( \mu \)l containing 100 ng of random primers, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl\(_2\), 0.5 mM deoxy nucleotide triphosphate, 10 mM DTT, and 200 units of SuperScript II reverse transcriptase and incubated for 10 min at room temperature, 50 min at 42°C, followed by 15 min at 70°C. RNase H (2.5 units) was added into each sample, then incubated for 20 min at 37°C and then stored at -20°C. PCR reactions were performed with 1 \( \mu \)l of the cDNA reaction mixture in a volume of 20 \( \mu \)l containing 16 mM (NH\(_4\))\(_2\)SO\(_4\), 67 mM Tris-HCl (pH 8.8), 0.01% Tween 20, 2 mM MgCl\(_2\), 0.2 mM deoxynucleotide triphosphate, 5 \( \mu \)M of each 5'- and 3'- primers, and finally 0.5 unit of Taq polymerase. Quantification was performed by UV transillumination using a gel Doc 1000 system (Bio-Rad, Ivry-sur-Seine, France). For each cDNA sample, a relative expression ratio was calculated as fluorescence intensity of the target gene band/fluorescence of the \( \beta \)2-microglobulin or GAPDH band.

Semiquantitative PCR Analysis. p53 primers correspond to sequences published by Aguilar-Santelises et al. (13). GST-p primers were synthesized as follows: 3'-CCC TTT ATC TGG TGC CAC AT-5' and 5'-CTG TTT CCC GTT GCC ATT GAT-3'. \( \beta \)2-Microglobulin primers correspond to sequences published by Gussow et al. (14). GST-\( \pi \) primers were adapted from Bröckmüller et al. (15), as already described by Dubessy et al. (16), and mdr1 primers from Noonan et al. (17). mdr primers were adapted from Bordow et al. (18). Semiquantitative PCR conditions were optimized using \( \beta \)2-microglobulin or GAPDH as reference genes. p53, GST-\( \pi \), and GST-\( \mu \) were coamplified with \( \beta \)2-microglobulin (14), whereas mdr1 and mrp were co-amplified with GAPDH (18). Analyses were performed on untreated xenografts (control) and on irradiated xenografts at the doses of 20 or 40 Gy.

**TPT.** TPT was a gift from Dr. M. E. Boutin-Tranchant (SmithKline Beecham, France). It was made fresh for each experiment by dissolving the compound in sterile water. TPT was injected i.p., once daily over 5 days.

**RT.** Two doses, 20 and 40 Gy, were administered. An 85-kV orthovoltage apparatus was used for the irradiation with a 1.25 aluminum filter and a collimator of 1.5 cm in diameter, in oxic conditions. The dose rate was 985 cGy/min. The dose was calculated on the skin (100%).

The animals were anesthetized with ketamine administered by i.p. injection at a dose of 0.05 mg/g body weight. The RT was administered once daily over 5 days. During radiation treatments, the mice were immobilized with tape, on a plastic plate.

**Assays of TPT Tolerance.** The maximum tolerated dose was determined from experiments using four different doses of topotecan: 10, 12.5, 15, and 20 mg/kg administered on 5 consecutive days. Weight loss and mortality were assessed each day for a period of 3 weeks.

**Determination of Parameters for the Demonstration of a Potential Synergistic Effect between TPT and RT.** We conducted a study on 63 tumors to determine the dose of TPT and RT required to manage to demonstrate a potential synergistic effect between topotecan and RT. When tumors reached a volume of 200 ± 100 mm\(^3\), the animals were randomly assigned into six groups: control; topotecan 10 mg/kg; topotecan 12.5 mg/kg; irradiation at the dose of 20 Gy; irradiation at the dose of 40 Gy; and irradiation at the dose of 60 Gy. TPT was administered 0.5 h after irradiation.

**Assays of Antitumor Activity of TPT Alone, Irradiation Alone, and Combination of Both Treatments.** The parameters of complete (no palpable tumor) and partial (decrease in tumor volume >50%, over two successive measurements) response rates, and TGD (defined as the difference in days between the time for the tumor of treated and control animals to reach four times the volume recorded at the time of original treatment) were used to assess the influence of TPT, RT, and the combination of TPT and RT.

The effect of the combined treatment was compared with the amount of the effect of the two treatments given separately to assess a potential synergistic antitumor activity. Statistical analyses were performed using the \( \chi^2 \) test and a nonparametric Wilcoxon test with a significance limit set at 0.05. The radiosensitization ratio was calculated as: TGD (RT + TPT)/TGD (RT) + TGD (TPT).

**RESULTS**

**Determination of Parameters for the Demonstration of a Potential Synergistic Effect between TPT and RT**

**Determination of the Maximum Tolerated Dose.** Forty-two nude mice were used, divided into five groups: control; TPT 10 mg/kg; TPT 12.5 mg/kg; TPT 15 mg/kg; and TPT 20 mg/kg. The details of weight loss, time for recovering of initial weight,
and mortality according to treatment are presented in Table 1. None of the three autopsies ascertained the cause of death.

Efficacy: RMS1. The efficiency of TPT alone and RT alone was tested on 68 tumors. Nude mice were randomly assigned to six therapeutic groups: control; RT 20 Gy; RT 40 Gy; RT 60 Gy; TPT 10 mg/kg; and TPT 12.5 mg/kg. The results of TPT or RT efficacy according to the dose are presented in Table 2.

According to those results, the dose of 12.5 mg/kg for TPT, which seemed to have a moderate efficacy, was selected to be combined with 20 Gy RT to demonstrate a potential radiopotentiation effect of TPT.

Determination of Radiopotentiation

The results obtained with TPT alone, RT alone (20 and 40 Gy), and combination of TPT (12.5 mg/kg) and RT (20 Gy) are reported in Table 3. The theoretical response rate corresponding to the cumulative effect of TPT and RT alone (20 Gy) was 20%, whereas it was 67% for the observed response rate corresponding to the combination of TPT and RT. This difference was statistically significant ($P < 0.0001$). The theoretical TGD corresponding to the cumulative effect of TPT and RT alone (20 Gy) was 34 ± 10 days, and the observed effect was 50 ± 9 days.

No statistically significant difference was observed between the efficacy of RT at the dose of 20 Gy combined with TPT and RT at the dose of 40 Gy (Fig. 1). The combination of TPT and RT gave a supraadditive effect with a radiopotentiation ratio of 1.54.

RMS2. The efficacy of TPT alone and RT alone was tested on 64 tumors. Nude mice were randomly assigned to seven therapeutic groups: control; RT 20 Gy; RT 40 Gy; RT 60 Gy; TPT 7.5 mg/kg; TPT 10 mg/kg; and TPT 15 mg/kg. The results of TPT and RT efficacy according to the dose are presented in Table 4.

According to these results, the dose of 3 mg/kg for TPT was selected to be combined with 10 Gy RT to demonstrate a potential radiopotentiation of topotecan.

The theoretical response rate corresponding to the cumulative effect of TPT and RT alone (20 Gy) was 0%. The observed response rate corresponding to the combination of TPT and RT was 40% (Table 5). This difference was statistically significant ($P < 0.05$).

The theoretical TGD corresponding to the cumulative effect of TPT and RT alone was 27.1 ± 2 days. The observed TGD was 47.3 ± 2.5 days. The radiopotentiation ratio was 1.7.

Table 1 Determination of the maximum tolerated dose

<table>
<thead>
<tr>
<th>TPT (mg/kg)</th>
<th>No. of mice</th>
<th>Weight loss (mean % ± SE)</th>
<th>Weight recovery</th>
<th>TGD (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>7</td>
<td>0 ± 2</td>
<td>10 ± 2</td>
<td>0</td>
</tr>
<tr>
<td>12.5</td>
<td>7</td>
<td>18 ± 3</td>
<td>14 ± 2</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>25 ± 7</td>
<td>18 ± 2</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>26 ± 9</td>
<td>17 ± 3</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>0</td>
</tr>
</tbody>
</table>

In a second experiment, we used the same doses as for RMS1, on 68 tumors, to compare the efficacy of equivalent treatment on two different tumors from the same histotype. The theoretical response rate corresponding to the cumulative effect of TPT and RT alone was statistically different (12 of 25 versus 8 of 8; $P < 0.05$; $x^2$ test) from the observed response rate with the combination of TPT and RT. The theoretical TGD corresponding to the cumulative effect of TPT and RT alone was 73 ± 4.4 days. The observed effect was >75 days but could not be calculated because six of six mice died in complete response because of extra-experimental reasons. Thus, the radiopotentiation ratio could not be evaluated. The mean tumor volume according to time and treatment is given in Fig. 3.

Pathology

RMS1. Tumors were studied 32, 56, and 68 days after the end of different treatments, corresponding to approximately the time of tumor re-evolution, at doses of 20, 40, and 60 Gy, respectively. Four groups of three tumors were analyzed: control; RT 20 Gy; RT 40 Gy; and RT 60 Gy. Briefly, on post-treatment day 32, most of the round cells from the control group were small, with necrosis areas; few intermediate cells and no large cells were observed. The small and intermediate cells were desmin, vimentin, and actin positive; large cells were vimentin positive, with many of vimentin-type intermediate filaments, using electron microscopy. These cells correspond to RMS components as demonstrated by using in situ hybridization with ALU probes, which are specific for human DNA.

The number of small cells decreased proportionally to the dose of RT, progressively replaced by intermediate and large cells. The IDU labeling index was about 30–35% in small cells and about 2–3% in large cells.

On day 56, small cells were seen to replace intermediate and large cells as rapidly as the dose of RT was low. Large cell DNA synthesis was less pronounced. Necrotic areas occurred, just as in untreated tumors. On day 68, tumors irradiated at the dose of 20 and 40 Gy had the same pathological aspect as untreated tumors, whereas tumors irradiated at the dose of 60 Gy included a great majority of small tumoral cells with a high labeling index. On day 58, tumors treated with the combination of TPT and RT at the dose of 20 Gy had the same pathological and histological characteristics of tumors irradiated at the same period, at the dose of 60 Gy.

Table 2 Response rates, response durations, and TGD of RMS1 with different doses of RT and TPT alone

<table>
<thead>
<tr>
<th>TGD (days)</th>
<th>n tumors</th>
<th>CR$^a$</th>
<th>CR duration</th>
<th>PR</th>
<th>PR duration</th>
<th>TGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 Gy</td>
<td>16</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>5 ± 2 d</td>
<td>42 ± 6</td>
</tr>
<tr>
<td>40 Gy</td>
<td>16</td>
<td>1</td>
<td>8</td>
<td>8</td>
<td>27 ± 14 d</td>
<td>52 ± 7</td>
</tr>
<tr>
<td>60 Gy</td>
<td>16</td>
<td>4</td>
<td>8</td>
<td>5</td>
<td>31 ± 8 d</td>
<td>57 ± 7</td>
</tr>
<tr>
<td>TPT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12.5 mg/kg</td>
<td>15</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>12 ± 4 d</td>
<td>5 ± 5</td>
</tr>
</tbody>
</table>

CR, complete response; PR, partial response.
The cells were more differentiated than in RMS1. They were desmin, actin, and vimentin positive. The effects of RT alone (60 Gy) were assessed 6 days after the end of the treatment. We observed a great reduction in small cell numbers (5%), replaced by large (10%) and intermediate (75%) cells. The IDU labeling index was 3%. There was no necrosis. The effects of TPT alone were assessed for the doses of 7.5, 10, and 15 mg/kg, 6 and 28 days after the end of treatment. On day 6, intermediate cells composed the majority of cells (75%) at the dose of 10 and 7.5 mg/kg. Large cells were predominant at the dose of 15 mg/kg; then came intermediate cells. IDU labeling index was about 20–25%. On day 28, 90% of the cells were small cells at the dose of 7.5 mg/kg, whereas they were less frequently observed at the doses of 10 and 15 mg/kg.

The effects of the combination of TPT (3 mg/kg) and RT

### Table 3 Response rates, duration of responses, and absolute TGD of RMS1

<table>
<thead>
<tr>
<th></th>
<th>No. of tumors</th>
<th>CR</th>
<th>CR durations</th>
<th>PR</th>
<th>PR durations</th>
<th>CR + PR</th>
<th>TGD (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPT</td>
<td>15</td>
<td>0</td>
<td>—</td>
<td>1</td>
<td>12 d</td>
<td>7</td>
<td>5 ± 7</td>
</tr>
<tr>
<td>RT 20 Gy</td>
<td>15</td>
<td>0</td>
<td>—</td>
<td>5</td>
<td>5 ± 2 d</td>
<td>33</td>
<td>29 ± 8</td>
</tr>
<tr>
<td>RT 40 Gy</td>
<td>15</td>
<td>2</td>
<td>17 ± 3 d</td>
<td>12</td>
<td>24 ± 4 d</td>
<td>93</td>
<td>62 ± 9</td>
</tr>
<tr>
<td>(TPT) + (RT 20 Gy)</td>
<td>30</td>
<td>0</td>
<td>—</td>
<td>6/30</td>
<td>20</td>
<td>20</td>
<td>34 ± 10</td>
</tr>
<tr>
<td>Theoretical effect</td>
<td>15</td>
<td>2</td>
<td>54 ± 20 d</td>
<td>8</td>
<td>16 ± 3 d</td>
<td>67</td>
<td>50 ± 9</td>
</tr>
<tr>
<td>Observed effect</td>
<td>15</td>
<td>2</td>
<td>—</td>
<td>15</td>
<td>15 ± 3 d</td>
<td>7</td>
<td>5 ± 7</td>
</tr>
</tbody>
</table>

CR, complete response; PR, partial response.

### Table 4 Response rates, response durations, and TGD of RMS2 with different doses of RT and TPT alone

<table>
<thead>
<tr>
<th></th>
<th>n tumors</th>
<th>CR</th>
<th>CR duration</th>
<th>PR</th>
<th>PR duration</th>
<th>CR + PR</th>
<th>TGD (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT 20 Gy</td>
<td>10</td>
<td>3</td>
<td>15 ± 3 days</td>
<td>5</td>
<td>22 ± 3 days</td>
<td>80</td>
<td>48</td>
</tr>
<tr>
<td>RT 40 Gy</td>
<td>10</td>
<td>4</td>
<td>28 ± 9 days</td>
<td>6</td>
<td>24 ± 1 days</td>
<td>100</td>
<td>81</td>
</tr>
<tr>
<td>RT 60 Gy</td>
<td>12</td>
<td>12</td>
<td>&gt;80 days</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>&gt;101</td>
</tr>
<tr>
<td>TPT 7.5 mg/kg</td>
<td>12</td>
<td>1</td>
<td>44 days</td>
<td>2</td>
<td>6 ± 2 days</td>
<td>25</td>
<td>19</td>
</tr>
<tr>
<td>TPT 10 mg/kg</td>
<td>12</td>
<td>0</td>
<td>—</td>
<td>5</td>
<td>22 ± 11 days</td>
<td>42</td>
<td>25</td>
</tr>
<tr>
<td>TPT 15 mg/kg</td>
<td>8</td>
<td>0</td>
<td>—</td>
<td>1</td>
<td>11 days</td>
<td>12.5</td>
<td>30</td>
</tr>
</tbody>
</table>

a CR, complete response; PR, partial response.

### RMS2. The cells were more differentiated than in RMS1. They were desmin, actin, and vimentin positive. The effects of RT alone (60 Gy) were assessed 6 days after the end of the treatment. We observed a great reduction in small cell numbers (5%), replaced by large (10%) and intermediate (75%) cells. The IDU labeling index was ~3%. There was no necrosis. The effects of TPT alone were assessed for the doses of 7.5, 10, and 15 mg/kg, 6 and 28 days after the end of treatment. On day 6, intermediate cells composed the majority of cells (75%) at the dose of 10 and 7.5 mg/kg. Large cells were predominant at the dose of 15 mg/kg; then came intermediate cells. IDU labeling index was about 20–25%. On day 28, 90% of the cells were small cells at the dose of 7.5 mg/kg, whereas they were less frequently observed at the doses of 10 and 15 mg/kg.

The effects of the combination of TPT (3 mg/kg) and RT
(10 Gy) were assessed on posttreatment day 36. For tumors treated with TPT, 40% of the cells were large, 35% were medium, and 15% small. The IDU labeling index was ~40%. At the tumor periphery, there were small cells with an IDU labeling index of ~60%. The majority of cells corresponding to irradiated tumor were small, whereas 20–30% of the remainders were large. The IDU labeling index was ~50% in small cells and ~30% in large cells. In tumors that received both treatments, 15% of cells were large, and 40% were intermediate. There was an important loss of differentiation (actin and desmin negative). Pathological alterations were similar to those observed with TPT at the dose of 15 mg/kg and RT at the dose of 60 Gy. Thus, early after RT and/or TPT treatment, small cells were progressively disappearing and being replaced by intermediate and large cells, fibromyoblasts, and hyalinosis. After a period of time as short as the doses of RT and TPT were low, repopulation by small cells occurred, while intermediate and large cells and stroma disappeared.

The Mdr1 gene was not significantly expressed in RMS1 tumor tissue; its expression level was not modified after irradiation. Similar findings were observed for RMS2 tumor (Table 6).

DISCUSSION

The therapeutic effect of RT has been demonstrated in childhood tumors and is used in most cases. However, it often leads to the impairment of irradiated tissue growth. This clinical situation led us to investigate radiopotentiative chemoradiotherapy associations that could allow us to keep the effect of RT while decreasing the radiation dose and therefore RT-related side effects.

TPT, a camptothecin analogue, is a topoisomerase I inhibitor the efficacy of which has been demonstrated in adulthood cancers (19). In childhood cancers, high response rates have also been reported in metastatic neuroblastomas (39%) and RMSs (45%; Refs. 11 and 20).

In vitro studies reported radiopotentiation by topoisomerase I inhibitors (4–9). In murine tumor cells, radiopotentiation ratios ranging from 1.4 to 2 were achieved with both camptothecin and TPT (4). In human tumor cells, such as non-small cell lung carci-

\[
\begin{array}{|c|c|c|c|c|c|}
\hline
\text{No. of tumors} & \text{CR}\text{a} & \text{RP} & \text{PR durations} & \text{PR + CR \%} & \text{TGD} \\
\hline
\text{TPT 3 mg/kg} & 10 & 0 & 0 & 0 & 8 \pm 1 \\
\text{RT 10 Gy} & 10 & 0 & 0 & 0 & 18 \pm 1.8 \\
\text{(TPT 3 mg/kg) + (RT 20 Gy)} & 20 & 0 & 0 & 0 & 27 \pm 2 \\
\text{Expected effect} & 10 & 0 & 4 & 19 \pm 4 \text{ days} & 40 & 47 \pm 2.5 \\
\text{Observed effect} & 10 & 0 & 4 & 19 \pm 4 \text{ days} & 40 & 47 \pm 2.5 \\
\hline
\end{array}
\]

CR, complete response; PR, partial response.

Fig. 2 Effects of radiation and/or TPT on the tumor growth delay of a human RMS (RMS2) xenografted into nude mice. TPT (3 mg/kg) and RT (10 Gy) were administered separately over 5 consecutive days. They were also administered in combination, concomitantly over 5 consecutive days. Each group consisted of a minimum of 10 tumors. Data represent the mean tumor volume; bars, SE.
noma, glioblastoma, and melanoma, radiopotentiation was also reported, with radiopotentiation ratios from 1.2 to 3.2. (2, 7–9). As opposed to in vitro studies, radiopotentiation is difficult to assess in vivo. To our knowledge, synergistic interaction between TPT and RT was only reported in murine tumors (3, 6). In the present study, radiopotentiation was investigated in comparing: (a) the theoretical additive effect of TPT and RT (calculated from data achieved with each treatment given separately); and (b) the observed effects of the combined treatment, to a double dose of RT alone. In addition, the histological consequences of separate and combined treatment were compared.

The results presented in this report first confirmed the potency of TPT in RMS. When used experimentally at the same schedule as most frequently used in clinical practice, TPT alone at the maximum tolerated dose yielded high TGD (29 days) and response rate (42%). However, within the same histotype, high variations were observed, either in TGD (5 and 29 days, respectively, for RMS1 and RMS2) or in the response rate (7 and 42%). This intertumor variability was consistent with the results reported previously in vivo on RMS xenografts and on patients (19). It is worth noticing that the systemic exposures at the 12.5 mg/kg level in mice are unlikely to be achieved in patients.

High radiosensitivity was observed in both RMS. However, RMS1 was found to be less radiosensitive than RMS2 with respect to TGD and response rates at 20 Gy of 38 days and 31% and 48 days and 80%. This difference led us to select different doses of TPT and RT (12.5 mg/kg and 20 Gy for RMS1 and 3 mg/kg and 10 Gy for RMS2). These differences in chemo- and

Table 6  Expression levels of p53 and MDRa MRP, GST μ and GST π genes for RMS1 and RMS2, before (control) and after treatment (TPT or radiotherapy)

<table>
<thead>
<tr>
<th></th>
<th>RMS 1</th>
<th></th>
<th>RMS 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDR (SE)</td>
<td>MRP (SE)</td>
<td>p53 (SE)</td>
</tr>
<tr>
<td>Control</td>
<td>0.03 (0.01)</td>
<td>5.79 (1.0)</td>
<td>0.77 (0.09)</td>
</tr>
<tr>
<td>TPT</td>
<td>0.36 (0.04)</td>
<td>4.30 (0.37)</td>
<td>1.55 (0.50)</td>
</tr>
<tr>
<td>TPT</td>
<td>0.23 (0.02)</td>
<td>4.76 (0.65)</td>
<td>0.84 (0.40)</td>
</tr>
<tr>
<td>RT 20 Gy</td>
<td>0.26 (0.01)</td>
<td>3.74 (0.19)</td>
<td>3.13 (0.76)</td>
</tr>
<tr>
<td>RT 40 Gy</td>
<td>0.03 (0.00)</td>
<td>4.81 (0.50)</td>
<td>0.77 (0.11)</td>
</tr>
<tr>
<td>Control</td>
<td>0.03 (0.00)</td>
<td>3.36 (0.06)</td>
<td>1.07 (0.88)</td>
</tr>
<tr>
<td>TPT</td>
<td>0.03 (0.00)</td>
<td>4.71 (0.52)</td>
<td>0.84 (0.40)</td>
</tr>
<tr>
<td>RT 20 Gy</td>
<td>0.16 (0.01)</td>
<td>4.65 (1.7)</td>
<td>3.36 (0.36)</td>
</tr>
<tr>
<td>RT 40 Gy</td>
<td>0.13 (0.01)</td>
<td>3.72 (0.69)</td>
<td>1.95 (0.79)</td>
</tr>
<tr>
<td>RMS 2</td>
<td>0.14 (0.01)</td>
<td>3.95 (0.27)</td>
<td>1.95 (0.79)</td>
</tr>
<tr>
<td>Control</td>
<td>0.13 (0.01)</td>
<td>4.71 (0.46)</td>
<td>0.93 (0.31)</td>
</tr>
<tr>
<td>TPT</td>
<td>0.08 (0.01)</td>
<td>4.11 (0.39)</td>
<td>0.53 (0.09)</td>
</tr>
<tr>
<td>RT 20 Gy</td>
<td>0.08 (0.00)</td>
<td>4.22 (0.61)</td>
<td>1.04 (0.72)</td>
</tr>
<tr>
<td>RT 40 Gy</td>
<td>0.04 (0.01)</td>
<td>2.14 (0.61)</td>
<td>0.53 (0.09)</td>
</tr>
</tbody>
</table>

a MDR, multidrug resistance; MRP, MDR-associated protein.
radiosensitivity could not be explained by any difference in the expression of resistance genes, as already observed in head and neck carcinoma cells (16).

The present study reports the radiopotentiation achieved with TPT in two human RMS models. Macroscopic examination showed a significant enhancement of the response rate and TGD both for RMS1 (53 versus 20% and 50 versus 34 days) and RMS2 (40 versus 0% and 47 versus 27 days), with radiopotentiation ratios of 1.5 and 1.6, respectively. Moreover, the TGD achieved in combining TPT (12.5 mg/kg) and RT (20 Gy) was not found to be statistically different from the TGD achieved using 40 Gy RT alone, thus suggesting that the addition of TPT enabled radiation dose reduction by 50%

Interestingly, these results were confirmed by the histological data showing that the lesions obtained after 40 Gy irradiation are similar to those observed with combined therapy using TPT at 12.5 mg/kg and 20 Gy. Additionally, at different times after separate or combined treatment administered at different doses, the repopulation by malignant cells appeared as rapid as the radiation and/or TPT dose is low.

Mechanistically, the molecular pathways involved in the radiopotentiation by topoisomerase I inhibitors remain unclear. Thus far, inhibition of postirradiation repair and cell cycle redistribution have been proposed as the mechanisms for the induction of radiosensitization (2, 4). However, at this time, there is no evidence that topoisomerase I could be involved in DNA repair. Recently, Chen et al. (1) characterized a mechanism of radiosensitization by camptothecin derivatives in cultured mammalian cells, implying the involvement of DNA topoisomerase I in mediating such an effect. They also demonstrated that camptothecin derivatives radiosensitized cells in a highly schedule-dependent way, because the phenomenon was observed when drug treatment was used concurrently or immediately prior to radiation but not after radiation, even within 5 min after completing ionizing radiation. Kim et al. (10) showed that best results were obtained when topotecan was administered 2 and 4 h before RT, whereas no potentiation was observed when TPT was administered 2 h after RT. In this study, the tumor (murine fibrosarcoma) was much less chemosensitive than in our case because the dose of 20 mg/kg, administered over a 24-h period, was inefficient. In the present study, the scheduling was not investigated. Nevertheless, enhancement of radiation response was observed in both RMSs with TPT being injected, for technical reasons, 30–45 min after RT. Moreover, we showed in two other different studies that the schedule for TPT administration (ranging from 4 h before to 2 h after RT) had no influence on the radiopotentiation effect (21, 22).

In conclusion, this study demonstrates that TPT significantly potentiates the radiation response of two human RMS tumor models xenografted into nude mice, assessed using clinical and histological parameters. These results are very encouraging for designing clinical trials and should be extended to other histotypes.

REFERENCES


In Vivo Potentiation of Radiation Response by Topotecan in Human Rhabdomyosarcoma Xenografted into Nude Mice

P. Chastagner, J. L. Merlin, C. Marchal, et al.


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