Cure of Fisher Rats Bearing Radioresistant F98 Glioma Treated with cis-Platinum and Irradiated with Monochromatic Synchrotron X-Rays

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Abstract

High-grade gliomas are usually of poor prognosis, and conventional radiotherapy, even combined with chemotherapy, still fails to improve the survival of patients. Here, we propose an innovative therapeutic approach combining synchrotron radiation with cis-diamminedichloroplatinum (II) (CDDP). As suggested previously, monochromatic synchrotron irradiation of CDDP at 78.8 keV, just above the 78.4 keV platinum absorption K-edge, leads to an enhanced photoelectric effect and an increased local toxicity. To select a particular radiation energy that could provide supra-additive effect, we used pulsed-field gel electrophoresis to assess yields of DNA double-strand breaks induced in rat F98 glioma cells after CDDP treatment combined with synchrotron X-rays. Thereafter, intracerebral CDDP injection combined with synchrotron X-rays was applied to Fisher rats bearing F98 glioma. CDDP concentrations were mapped by synchrotron X-ray microfluorescence. An extra number of more slowly repaired double strand breaks were observed when irradiating CDDP-treated F98 cells at 78.8 keV. In vivo treatments were then performed with different radiation doses and CDDP concentrations. All cell inoculations in rat brain resulted in tumor development, and tumor presence was controlled by computed tomography. Among all of the conditions tested, the combination of 3 μg of CDDP with 15 Gy resulted in the largest median survival time (206 days). After 1 year, about 34% of treated rats were still alive. This preclinical finding, validated by molecular analysis, represents the most protracted survival reported with this radioresistant glioma model and demonstrates the interest in powerful monochromatic X-ray sources as new tools for cancer treatments.

Introduction

The overall incidence rate of gliomas is about 5/100,000, and despite significant advances in cancer therapy, treatment of high-grade gliomas is still palliative. To date, the median survival for patients with glioblastoma is less than 1 year, and virtually no patients with glioblastoma multiforme survive 5 years after treatment (1, 2). Although radiotherapy significantly increases survival of patients, gliomas are some of the most radioresistant human tumors. Furthermore, adjuvant chemotherapy generally fails to improve patient outcome, because of inadequate drug delivery and/or concentration inside the tumor (1, 2). Hence, considerable efforts were provided to optimize chemo-radiotherapy treatments against brain tumors by increasing both chemotherapeutic drug concentration and radiation dose inside the tumor, while preserving healthy tissues (1).

Among recent therapeutic techniques, boron neutron capture therapy consists of accumulating a sufficient level of 10B in tumors before irradiating them with neutrons to increase local radiation absorption (3). Another promising technique, computed tomography, uses a modified X-ray scanner to irradiate brain tumors loaded with iodinated contrast agent. This technique was shown to result in a supra-additive response, perhaps because of an increased photoelectric effect (5–8). A monochromatic radiation beam would potentially enhance this physical effect. However, conventional X-ray medical irradiators are not manufactured with sufficient fluence to reach this requirement (9).

Synchrotrons might overcome this problem: their high fluence (105 times higher than conventional X-ray sources) makes possible a sufficient residual monochromatic beam tuned over tens of keV to allow medical imaging and radiotherapy applications (10). Over the last 5 years, several therapeutic techniques involving synchrotron radiation have been developed (10). For example, microbeam radiation therapy, based on multiple parallel collimated synchrotron radiation beams, results in several hundreds of Gy delivered to the tumor bed and has been successfully used on rat brain tumors (11, 12). Recently, our group developed a variant computed tomography therapy technique involving synchrotron radiation at the European Synchrotron Radiation Facility (ESRF) (13). Like microbeam radiation therapy, this technique also consists of delivering a radiation dose excess by targeting vascularization, but not necessarily the DNA of tumor cells.

Theoretically, the irradiation of high-Z elements at their K-edge absorption energy leads to emission of Auger electrons and photoelectrons, releasing a large amount of energy at their immediate vicinity. This phenomenon is called photoactivation (14–22). Hence, to irreversibly damage the DNA of tumor cells, an ideal anticancer treatment would consist of a chemo-radiotherapy combining monochromatic synchrotron radiation with DNA-binding photoactivatable high-Z compounds (19–22). Among the drugs of interest, molecules containing platinum atoms (Z = 78), such as cis-diamminedichloroplatinum (II) (CDDP), are used extensively (23, 24). CDDP molecules react with nucleophilic sites into DNA by forming DNA adducts (25). A number of in vivo studies involving platinum compounds and medical X-rays have already been performed, on either mice or rats bearing various tumors. However, these experiments demonstrate a small increase in life span (26–32).

By developing the synchrotron photoactivation of cis-platinum, we have recently proposed a molecular model for describing mechanisms implicated in the combination of CDDP treatment and irradiation with soft X-rays (33). This treatment was shown to inhibit the major DNA double-strand break (DSB) repair pathway and to induce an extra number of more slowly repaired DSBs, suggesting that CDDP treat-
ment combined with synchrotron X-rays above the K-edge of platinum produces supra-additive molecular effects (33). However, these data were obtained from experiments on human cells, and our model has not yet been verified in rodents. Hence, in this study, synchrotron CDDP photoactivation was investigated in vitro and in vivo on the radiosensitive and weakly immunogenic rat F98 glioma (34). Our findings show that the combination of CDDP treatment with synchrotron irradiation at 78.0 and 78.8 keV (i.e., just below and just above the platinum K-edge) leads to both an excess of unrepaird DNA damage in treated cells and the most protracted survival obtained with this brain tumor model.

Materials and Methods

Cell Culture. The F98 glioma cell line used in this study was originally established by Drs. A. Koestner and W. Wechsler (Ohio State University; Ref. 34). Cells were routinely cultured as monolayers with DMEM (Gibco-Invitrogen-France, Cergy-Pontoise, France) supplemented with 10% FCS, penicillin, and streptomycin for both in vitro and in vivo experiments. All of the experiments were performed at the ESRF.

CDDP Treatment and Irradiation of Cells. CDDP (Cysplatin; Rhône-Poulenc, Rorer, Montrouge, France) was kindly provided by Michallon Hospital (Grenoble, France). Cells were treated for 6 h with 30 μM CDDP in DMEM supplemented with 10% serum. They were irradiated in suspension in rotating plastic tubes. The beam size (0.85-mm high) and its homogeneous part (8-cm wide) allowed two tubes to be irradiated simultaneously by vertical scanning. To avoid any artifacts due to DNA repair during irradiation, cells were irradiated at 4°C by using a cooling system based on liquid nitrogen vapor diffusion in a polystyrene box containing the rotating tubes. Temperature was monitored by a thermocouple throughout the irradiation. A cylindrical ion chamber coupled with a UNIDOS electrometer and a high-purity germanium detector (Eurisys Mesure, Lingolsheim, France) were used for radiation dose calibration. The dose rate delivered by synchrotron radiation is directly proportional to the storage ring current and was set at 0.1 Gy/scan at the center of the tube. The beam energy was precisely tuned, either 400 eV above (78.8 keV) or below (78.0 keV) the K-edge absorption of platinum (78.4 keV), with an energy bandwidth of 80 eV (35). Other series of experiments have been performed at 30, 40 and 85 keV. A monochromator based on a pair of dispersive bent Laue Si (1.1) crystals mounted in fixed-exit geometry was used for all these settings (36).

DSB Assays. DSB induction and repair assays have been described previously in detail (37). Briefly, cells were irradiated at 4°C and incubated at 37°C at the indicated repair times, when required. Agarose plugs containing 2 × 10⁶ cells/ml were prepared and incubated at 50°C for 3 h in lysis solution containing t-laurilsarcosine and protease K (Sigma-Aldrich, St. Louis, MO). Migration of DNA fragments was performed by using pulsed-field gel electrophoresis (CHEF DRII; Bio-Rad, Hercules, CA) with a 4-day migration program discriminating the megabase-sized fragments (37). Under these particular conditions, only fragments of <15 Mb are able to migrate out of the well. To avoid any artifacts, we did not use radioactivity to label DNA. DSB data were therefore expressed as the fraction of DNA fragments migrating out of the well (FDM) after quantifying the light that each migration lane emitted in the ethidium bromide-stained gel by using a densitometer. Chromosomal S. pombe DNA size standards (Bio-Rad) were used in each experiment to evaluate DNA fragment size all along the migration lane.

Animals and Tumor Cell Inoculation. Male 7–8-week-old Fisher 344 rats (Charles River Laboratories France, L’Asbreles, France) were used in all experiments. Their weights ranged between 220 and 240 g. All of the operative procedures and animal care were in conformity with the Guidelines of the French Government (Decree 87-848 of October 19, 1987, License 7593 and A38071). Each rat was anesthetized by isoflurane inhalation followed by i.p. infusion of chloral hydrate 4% (0.1 ml/100 g body weight). This procedure maintains animals under anesthesia for about 2 h.

The tumor inoculation technique itself has been described elsewhere (38). Briefly, each rat was positioned on a stereotaxic frame. An incision was made in the skin 3.5 mm to the right of the bregma. A 0.5-mm-diameter hole for implanting tumor cells was drilled through the skull without breaking the dura. One thousand F98 cells diluted in 5 μl of DMEM were implanted stereotaxically at a depth of 6 mm in the cerebral parenchyma. The hole was sealed with bone wax, and the scalp was sutured thereafter. Tumors were allowed to grow for 13 days after implantation. Under our conditions, this procedure resulted in 100% tumor uptake and a median survival time (MeST) of 26 days for the sham untreated controls.

CDDP Injection in Rat Brain. CDDP inoculation was performed on day 13 after tumor implantation. As described above, the rats were anesthetized and set in a stereotaxic frame. The drug was intracerebrally injected at the tumor site. The rats received 3 or 5 μg of CDDP in 5 μl of NaCl isotonic solution. The untreated and irradiated controls were injected with isotonic solution only.

Platinum Content Mapping. The platinum contents were evaluated by sacrificing some rats 24 h after CDDP inoculation and others 14 days after tumor implantation. The synchrotron induced X-ray fluorescence (SR-XRF) microprobe at ID22 ESRF beamline allows the assessment of elemental concentrations (with Z > 13) in biological samples. SR-XRF set-up was used for intracellular analysis as described elsewhere (39, 40). Briefly, a Si (111) monochromator and a Kirkpatrick-Baez X-ray focusing system were used to generate a 14-keV monochromatic X-ray beam. This incident radiation energy allows the Kα fluorescence excitation of elements ranging between Al (Z = 13) and Br (Z = 35) and the Lα fluorescence excitation of Pt (Z = 78). Unstained sections of the entire rat brain (15-μm thick) were deposited on 4-μm thin film target (Ultratrace; Spex Certiprep, Metuchen, NJ). This type of backing optimized for SR-XRF analysis is free of contaminants. A spot size of 3 × 10-μm² (vertical × horizontal) was used. The sample was rastered through the focused beam with a 4-s dwell time, and the entire X-ray fluorescence spectrum was recorded for each map pixel using an energy-dispersive Si(Li) detector (150 eV energy resolution at 5.9 keV). A noncontiguous pixel step-size (50 × 100 μm²) was deliberately chosen because of the large scanned area required (7.6 × 6.4 mm²). This was the best compromise between spatial resolution, measurement stability, and reasonable mapping time (10–12 h). Consequently, some information was lost, which slightly degrades the resolu-
tion of the SR-XRF elemental images. The AXIL (analysis of X-ray spectra by iterative least squares) code was used for spectral evaluation to correct the background fluorescence contribution and the overlaps in the fluorescence peaks of neighboring elements (39). Elemental concentrations (μg cm⁻²) were then calculated from areas under peak from thin-film standards [SRM 1832 and 1833; National Institute of Standards and Technology (39, 40)].

Irradiation of Rats. Irradiation was performed stereotactically with monochromatic X-rays from the ESRF synchrotron source. The radiation beam energy was tuned at either 78.8 or 78.0 keV. As described elsewhere, rats were anesthetized at day 14 after implantation, set in a stereotactic frame, and irradiated while rotating. The rotation axis was positioned at the injection point (Fig. 1A; Ref. 15). The radiation beam was fixed at values of 10-mm wide and 0.85-mm high, centered on the injection point. The total scanned height was 12.75 mm, corresponding to fifteen 0.85-mm joined slices. The dose rate was 0.3 Gy s⁻¹ at the skin entrance. Each tumor was assumed to be surrounded by 1.5 cm of equivalent tissue. As an example, the attenuation coefficient (μg μm⁻¹) at 78.8 keV is 0.1818 cm⁻¹, corresponding to a 24% dose reduction (ICRU4). Hence, the device rotation speed was adjusted to deliver 15 Gy to the tumor, corresponding to 19.7 Gy at the skin entrance.

Postirradiation Imaging. After each irradiation, an image was taken with Iomeron (Bracco, Milan, Italy), an iodinated contrast agent, to verify whether rats bore tumors. It is noteworthy that imaging was performed after radiotherapy to avoid any potential synergy between contrast agent and radiation. Two ml of 350 mg ml⁻¹ Iomeron solution were injected into the tail vein. Images were taken at 35 keV to get maximal contrast. The radiation dose delivered during imaging is 0.2 Gy (about 0.1 Gy in the tumor). Fig. 1 shows a representative example of tumor imaging on a rat surviving 1 year after treatment.

Posttreatment Observations and Histological Studies. Feeding ability, external appearance, locomotion, and weights of rats were routinely controlled. Rats exhibiting unusual weakness, bleeding eyes, tremors, and loss of weight were euthanized by i.p. Doletal injection (150 mg kg⁻¹; Vetoquinol, Lure, France). The surviving rats were euthanized 1 year after treatment. The brain of each treated rat was removed and frozen in N-methylbutane. Tissues were then stored at −80°C. Ten-μm brain slices were prepared and stained with hematoxylin and erythrosin.

Statistical Methods. Each group of rats consisted of 7–18 animals bearing tumors. Survival times were determined from the day of tumor implantation to the day of death. For the euthanized rats, 1 day was added to the life span. All of the animals that died immediately after intracerebral drug injection because of either anesthesia or injection itself were excluded from the statistical study. The mean survival time and MeST were calculated for each group, and Kaplan-Meier survival data were plotted against times after tumor inoculation. The groups were compared using the log-rank test. The percentage increase in life span (% ILS) relative to MeST was defined by the following equation:

\[ \% \text{ILS} = 100 \left( \frac{\text{MeST}_t - \text{MeST}_u}{\text{MeST}_u} \right) \]

where \( t \) and \( u \) represent treated and untreated animals, respectively. The same formula can be applied to mean survival time to calculate percentage increase in life span relative to mean survival time.

Results

DNA DSB Induction and Repair. DSB induction was examined in rat F98 glioma cells treated with CDDP and irradiated at 30, 40, 78.0, 78.8, and 85 keV. Treatment of F98 cells with CDDP alone did not elicit any detectable DSBs (data not shown). Conversely, synchrotron radiation alone lead to a FDM of about 10%, whatever the radiation energies tested (Fig. 2A). When combined with 30 μM CDDP, synchrotron radiation resulted in a larger FDM than seen with the two above-mentioned treatments alone. CDDP-treated cells irradiated at 30, 40, and 85 keV showed two and three times lower FDM than at 78.0 and 78.8 keV, respectively, suggesting that DSB induction in CDDP treated cells is strongly enhanced when approaching the platinum K-edge (Fig. 2A). DSB repair rate was examined thereafter; when CDDP treatment was applied, DSBs induced at 78.8 keV were

![Image](image_url)
found to be 1.3, 1.5, and 2 times more slowly repaired than those induced at 78.0, 85, and 30 or 40 keV, respectively (Fig. 2). Even if differences in yields of induced and repaired DSB at 78.0 and 78.8 keV are smaller than those obtained previously in SQ20B cells, our findings are consistent with an excess of severe DSBs at 78.8 keV (33). Photoelectric effect and potential subsequent Auger cascades, more probable at this energy, should supposedly increase energy deposition at close vicinity of CDDP molecules. Consequently, this photoactivation phenomenon should consist of the production of numerous short DNA fragments close to DNA-bound CDDP molecules. To verify this assumption, the 10^6 fragments of DNA from 10^6 cells were analyzed; an excess of FDM was observed in CDDP-treated cells. Consequently, this finding suggests that to irradiate CDDP-treated cells at an energy above the platinum K-edge results in a supra-additive molecular effect. The biological relevance of these molecular findings was then examined in rats bearing brain tumors.

Preparation of Preclinical Trials and Platinum Content in Treated Tumors. From preliminary data with rats bearing brain tumors, the efficiency of the anticancer treatment appeared to be strongly influenced by the CDDP microdistribution within the tumor. Hence, by using SR-XRF, platinum content was assessed after CDDP injection in rat brain. To our knowledge, this sensitive technique has never been applied to brain histological samples at this scale. Thirteen days after brain tumor inoculation, rats received intracerebral injection with 3 μg of CDDP, and brain sections were prepared the next day. Fig. 3 shows the potassium, zinc, iron, and platinum distributions in the whole treated rat brain hemisphere. Potassium and zinc, which were more highly concentrated in the cortex part, reflected the structure of the brain tissue (Fig. 3, A and B). The needle route was revealed by iron contained in the large amount of red blood cells due to the injection wound (Fig. 3C). Platinum mapping showed that CDDP molecules were highly concentrated at the end of the needle pathway (average, 0.18 μg cm^{-2}; some hot spots at 0.62 μg cm^{-2}; Fig. 3D). An average platinum concentration of 0.08 μg cm^{-2} was assessed around the high concentration zone (50–100 times higher than that after i.p. injections), and 0.03 μg cm^{-2} was found in the edges of the inoculated hemisphere (Refs. 41 and 42; Fig. 3D). No traces of platinum were detected in the contralateral hemisphere. It is noteworthy that, by using argon-coupled plasma mass spectrometry, a constant level of platinum remains in the inoculated hemisphere 48 h after injection (data not shown). Taken together, these findings show

Table 1 Survival data from rats bearing F98 glioma and treated with the indicated protocols

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Median</th>
<th>Mean</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls</td>
<td>12</td>
<td>24–37</td>
<td>28 (5)</td>
<td>26</td>
<td>39%</td>
<td>42%</td>
</tr>
<tr>
<td>3 μg of CDDP a</td>
<td>10</td>
<td>28–60</td>
<td>39 (9)</td>
<td>37</td>
<td>39%</td>
<td>42%</td>
</tr>
<tr>
<td>Irradiated controls</td>
<td>10</td>
<td>33–70</td>
<td>48 (11)</td>
<td>48</td>
<td>71%</td>
<td>85%</td>
</tr>
<tr>
<td>3 μg of CDDP + 15 Gy (exp 1)</td>
<td>18</td>
<td>42–365 b</td>
<td>213 (128 b)</td>
<td>206.5</td>
<td>661%</td>
<td>694%</td>
</tr>
<tr>
<td>3 μg of CDDP + 15 Gy (exp 2)</td>
<td>11</td>
<td>49–365 b</td>
<td>184 (145 b)</td>
<td>110</td>
<td>557%</td>
<td>323%</td>
</tr>
</tbody>
</table>

a CDDP, cis-diammine dichloroplatinum (II); exp, experiment.
b Six and four rats still alive after 1 year for experiment 1 and 2, respectively.

Fig. 5. Kaplan-Meier survival curves of rats bearing F98 glioma and subjected to the indicated treatments. The survivals are plotted against time (days) after tumor inoculation. A: untreated controls; ▲, 3 μg of cis-diamminedichloroplatinum (II) (CDDP) alone; ○, 15 Gy alone; ▼, 3 μg of CDDP combined with irradiation at 78.0 keV; ●, 3 μg of CDDP combined with irradiation at 78.8 keV. B: intercomparisons between survival fractions obtained from two series of independent experiments with 3 μg of CDDP treatment combined with irradiation (15 Gy) at 78.0 keV or 78.8 keV. Data from experiment 1 and 2 are represented by solid and dotted lines, respectively. The gray confidence zone under the curve combines the survival fraction of untreated controls, rats treated with 3 μg of CDDP and rats treated with 15 Gy.
that CDDP molecules are very abundant in the region of interest and diffuse very slowly after injection.

**Survival of Rats Bearing F98 Glioma.** CDDP treatment combined with synchrotron irradiation at energies surrounding the platinum K-edge was applied to Fisher rats bearing F98 glioma. All of the F98 cell inoculations ($10^3$ cells) resulted in tumor development, clearly distinguishable by computed tomography on day 14 (Fig. 1). At this time, the average tumor volume was estimated to be $20 \pm 10$ mm$^3$, corresponding to about 15% of an average rat brain volume (1500 mm$^3$). To optimize rat survival, several CDDP and radiation doses (3--5 μg and 5--15 Gy, respectively) were tested. Fig. 4 shows MeSTs obtained from different combined treatments such as 5 μg of CDDP + 5 Gy, 5 μg of CDDP + 10 Gy, and 3 μg of CDDP + 15 Gy. The MeST of sham untreated rats was found to be 26 days (Table 1). Compared with controls, CDDP intracerebral injections of 3 and 5 μg significantly improved survival, with a MeST of 37 and 39.5 days, respectively ($P < 0.0018$; Fig. 4). When synchrotron radiation alone was applied (5, 10, and 15 Gy), the MeST was found to be 31, 36, and 48 days, respectively. When combined with CDDP treatment, a dose of 5 and 10 Gy provided a MeST of about 53 days. Increasing the dose up to 15 Gy at 78.0 and 78.8 keV drastically enhanced the MeST, which reached 214 and 194 days, respectively (Fig. 4). CDDP treatment (3 μg) combined with 15 Gy of synchrotron irradiation at 78.0 and 78.8 keV appeared to be the most efficient treatment tested and was thereafter systematically applied to rats bearing F98 gliomas. Fig. 5A shows the in vivo survival curves from the first series of experiments; an obvious gain of survival was obtained with the combined treatment by comparison with the 15-Gy irradiation alone ($P < 0.0001$). One year after the 3 μg of CDDP + 15 Gy treatment, 33% (6 of 18) of treated rats were still alive. The same experiment was performed again 4 months later. Although a higher early death rate was observed in the second experiment, survivals were not significantly different from those of the first experiment, whatever the radiation energies tested (78.0 and 78.8 keV; $P > 0.6$; Fig. 5B; Table 1). Similar to the first series, 36% (4 of 11) of treated rats were still alive 1 year after treatment.

**Histology.** Among the surviving rats, we investigated the histopathological changes in the brains of treated rats. To this end, the brains of some rats subjected to the 3 μg of CDDP + 15 Gy treatment were stained with hematoxylin and erythrosin. The brain from a rat that died at day 177 showed evidence of tumor growth with necrotic areas (Fig. 6). A similar result was thereafter systematically applied to rats bearing F98 gliomas. Fig. 6 shows absence of residual tumor with a cellular pseudo cyst cavity surrounded by a fibrosis margin but no trace of residual tumor (Fig. 6, C and D), suggesting its irreversible regression.

**Discussion**

In this study, we attempted to optimize a chemo-radiotherapy protocol against aggressive glioma consisting of CDDP treatment combined with monochromatic synchrotron radiation. Our in vitro findings suggest that CDDP treatment combined with irradiation just above the platinum K-edge results in a supra-additive molecular effect. In vivo experiments show that the combination of 3 μg of CDDP and 15 Gy monochromatic radiation resulted in the most protracted survival reported with the radioresistant F98 glioma.

**The Choice of the High-Z Photocifiable Compound.** Previous evidence has already suggested that chemo-radiotherapy treatment is

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**Table 2. Review of in vivo studies dealing with rats bearing brain tumors**

<table>
<thead>
<tr>
<th>Tumor model</th>
<th>Animals</th>
<th>Drug treatment</th>
<th>Radiation treatment</th>
<th>Radiation type</th>
<th>No. of inoculated tumor cells</th>
<th>MeST* of sham untreated controls</th>
<th>MeST of treated rats</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTB rat brain tumor</td>
<td>CD-Fisher rats</td>
<td>Single ip injection of 4 mg/kg CDDP</td>
<td>15 Gy 24 h after CDDP injection</td>
<td>140 kVp X-rays</td>
<td>$10^3$</td>
<td>14</td>
<td>30</td>
<td>44</td>
</tr>
<tr>
<td>9L glioma</td>
<td>Fisher 344 rats</td>
<td>3 μg intracranial injection CDDP</td>
<td>16 Gy 2 h after CDDP injection</td>
<td>Cs$^{137}$ γ</td>
<td>$10^4$</td>
<td>10</td>
<td>23</td>
<td>40</td>
</tr>
<tr>
<td>9L glioma</td>
<td>Fisher 344 rats</td>
<td>BNCT, boronophenilalanine + mannitol ip</td>
<td>MRT 250 Gy at the skin entrance</td>
<td>Neutrons</td>
<td>$10^4$</td>
<td>15</td>
<td>19</td>
<td>171</td>
</tr>
<tr>
<td>9L glioma</td>
<td>Fisher 344 rats</td>
<td>BNCT, boronophenilalanine + sodium borocaptate + mannitol ic</td>
<td>17 Gy (equivalent dose)</td>
<td>Neutrons</td>
<td>$10^4$</td>
<td>14</td>
<td>22</td>
<td>250*</td>
</tr>
<tr>
<td>F98 glioma</td>
<td>CD-Fisher rats</td>
<td>Single ip injection of 5 mg/kg F98-1</td>
<td>3 fractions of 7.5 Gy</td>
<td>300 kVp X-rays</td>
<td>$10^5$</td>
<td>10, 13, 15</td>
<td>31</td>
<td>55</td>
</tr>
<tr>
<td>F98 glioma</td>
<td>Fisher 344 rats</td>
<td>Iodinated contrast agent</td>
<td>10 Gy</td>
<td>Neutrons</td>
<td>$10^5$</td>
<td>8</td>
<td>12.5</td>
<td>18</td>
</tr>
<tr>
<td>F98 glioma</td>
<td>Fisher 344 rats</td>
<td>BNCT, boronophenilalanine + sodium borocaptate + mannitol ic</td>
<td>53.52 Gy (equivalent dose)</td>
<td>Neutrons</td>
<td>$10^3$</td>
<td>14</td>
<td>25</td>
<td>72</td>
</tr>
<tr>
<td>F98 glioma</td>
<td>Fisher 344 rats</td>
<td>3 μg intracranial injection CDDP</td>
<td>15 Gy 24 h after CDDP injection</td>
<td>Neutrons</td>
<td>$10^3$</td>
<td>14</td>
<td>26</td>
<td>206.5</td>
</tr>
</tbody>
</table>

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* MeST, median survival time; ip, i.p. injection; CDDP, cis-diaminedichloroplatinum (II); MRT, microbeam radiation therapy; BNCT, boron neutron capture therapy; iv, i.v. injection; ic, intracarotid injection.
- 100% still alive after 250 days.
more efficient than radiotherapy or chemotherapy alone. CDDP was deliberately chosen as a chemotherapeutic agent because of its DNA binding properties, the high-Z number of platinum, and its frequent use against ovarian, testicular, bladder, and squamous cell carcinomas and small cell lung carcinomas, especially in combination with conventional radiotherapy (43). However, the efficiency of CDDP treatment remains to be confirmed against primary and metastatic brain tumors (44–47).

Molecular mechanisms at the origin of the supra-additive effect observed with the combined treatment with CDDP and X-rays are not yet fully understood. CDDP induces DNA adducts, which are generally repaired by a recombination-like pathway. In parallel, ionizing radiation produces DSBs, which are more likely recognized by the Ku protein and rejoined by nonhomologous end joining, suggesting that CDDP treatment combined with radiation leads to the interplay of two major DNA repair pathways (25, 33, 48, 49). Moreover, concentrated CDDP treatment was shown to prevent the translocation of Ku all along the DNA, affecting the nonhomologous end joining pathway and contributing to cell lethality (50). Hence, particular efforts were provided to optimize the CDDP injection protocol resulting in the highest platinum concentrations in rat brains. These requirements were reached by intracerebral CDDP injection. The assessment of platinum concentrations with the SR-XRF technique under the conditions of in vivo treatment confirms that the highest CDDP levels are reached around the injection point and are clearly higher than those seen when CDDP is injected i.p. (Fig. 3).

**Molecular Findings.** In presence of CDDP, yields of radiation-induced DSBs were found to be 2–3 times larger at 78.0 and 78.8 keV than at any other energy tested (30, 40, and 85 keV). Our DSb data are therefore consistent with an excess of the delivered dose in the immediate vicinity of the CDDP molecules bound to DNA, the production of smaller DNA fragments at 78.0 and 78.8 keV than at the other energies, the generation of more severe damage with more DSBs surrounding DNA adducts, and the difficulty for tumor cells to repair these DSBs in conditions of CDDP photoactivation. Hence, it is shown here that the biological efficiency of synchrotron photoactivation of platinum is strongly dependent on monochromatic energy of radiation treatment.

When compared with the other energies tested, the efficiency of the combined treatment is maximal at 78.0 and 78.8 keV in CDDP-treated cells. However, significant differences also exist between these two energies: induced DSB yields were found to be higher at 78.8 keV than at 78.0 keV. Such an effect was reported previously (33), but with a 1:3 ratio, in human SQ20B carcinoma cells subjected to the same treatment. Unrepaired DSB yields were found to be 1.2 and 2 times higher at 78.8 keV than at 78.0 keV in F98 and SQ20B cells, respectively (33). These differences between SQ20B and F98 cells do not affect our general conclusion and may be attributed to species-dependent features (more condensed chromat and faster DSB repair rate in rodents than in humans; Ref. 51). The differences observed in vitro between 78.0 and 78.8 keV are not detectable in survival curves. The magnitude of the extra number of DNA damage between 78.0 and 78.8 keV are not detectable in survival curves. The magnitude of the extra number of DNA damage between 78.0 and 78.8 keV are not detectable in survival curves. The magnitude of the extra number of DNA damage between 78.0 and 78.8 keV are not detectable in survival curves. The magnitude of the extra number of DNA damage between 78.0 and 78.8 keV are not detectable in survival curves. The magnitude of the extra number of DNA damage between 78.0 and 78.8 keV are not detectable in survival curves. The magnitude of the extra number of DNA damage between 78.0 and 78.8 keV are not detectable in survival curves. The magnitude of the extra number of DNA damage between 78.0 and 78.8 keV are not detectable in survival curves. The magnitude of the extra number of DNA damage between 78.0 and 78.8 keV are not detectable in survival curves. The magnitude of the extra number of DNA damage between 78.0 and 78.8 keV are not detectable in survival curves. The magnitude of the extra number of DNA damage between 78.0 and 78.8 keV are not detectable in survival curves. The magnitude of the extra number of DNA damage between 78.0 and 78.8 keV are not detectable in survival curves. The magnitude of the extra number of DNA damage between 78.0 and 78.8 keV are not detectable in survival curves. The magnitude of the extra number of DNA damage between 78.0 and 78.8 keV are not detectable in survival curves.

The **Choice of Animal Tumor Model and Rat Survival Intercomparisons.** The F98 tumor model, which is known to be refractory to a number of therapeutic modalities, has been deliberately chosen because of its extreme radioresistance (surviving fraction at 2 Gy of about 75%) and its proliferation capacities (34). Furthermore, F98 glioma is a very aggressive model, perhaps due to its weak immunogenicity. Note that some unexpected immune responses have been reported with 9L gliosarcoma, one of the most widely used rat brain tumor models (34).

An impressive MeST (206 days) was found in rats treated with 3 μg of CDDP and irradiated at 78.0 and 78.8 keV (15 Gy). This result represents the most protracted survival reported with the radioresistant F98 glioma (Table 2). In the two series of experiments, about 34% of rats survived 1 year after treatment and appeared healthy. By using the same F98 tumor model, Kaneko et al. (31) reported a MeST of 31 days for the untreated controls (26 days here) and 55 days for rats treated with CDDP and irradiated with X-rays (206 days here). CDDP was injected i.p., which may lead to limited platinum levels inside tumors (31). Up to now, boron neutron capture therapy was considered the most efficient therapy when using the F98 model: with the same conditions (number of inoculated cells, day of irradiation) and very similar MeST of untreated controls, Barth et al. (53) reported a MeST of 72 days for the treated rats. Interestingly, boron neutron capture therapy applied to another model, the 9L gliosarcoma, provided an impressive 100% rat survival at 250 days after treatment (4). With a protocol involving both intracerebral injection of 3 μg of CDDP and 16 Gy of ionizing radiation, Kimler et al. (32) reported a MeST of 40 days for 9L-bearing rats. Note that in all of the above studies, the actual presence of tumor was not systematically verified by imaging.

Taken together, our data demonstrate the efficiency of an innovative anticancer treatment involving both CDDP and synchrotron radiation. To emphasize the actual feasibility of the proposed therapeutic protocol and its transfer to clinical trials, it is noteworthy that several intracerebral drug injections have been performed in patients suffering from brain tumors (54, 55). Further investigations are in progress at ESRF, notably to introduce high concentrations of drugs containing platinum atoms into tumors. As an example, carboplatin may be an interesting alternative to cisplatin because of its low nephro- and neuro-toxicity (56). Hence, our approach, validated by molecular analysis and preclinical trials, demonstrates the possibility of powerful monochromatic X-ray sources as new tools against solid tumors.

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**References.**


Cure of Fisher Rats Bearing Radioresistant F98 Glioma Treated with *cis*-Platinum and Irradiated with Monochromatic Synchrotron X-Rays

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