Previous Studies:

Three experiments have been carried out at beamline ID17. In the first study, C57 BL mice bearing the B16 murine melanoma on footpad, and to whom InTMPyP(4) had and had not been administered, were irradiated with photon energies above and below the K absorption edge of In with a total dose of 20 Gy to the tumor. Irradiations in the group that received both the drug and above the In K edge radiation showed a slightly longer delay in tumor growth than tumors irradiated below the edge as can be seen in Table 1 and Figure 1. The difference in the delay was greater between both groups shortly after the irradiation, but, upon entering the exponential growth phase, the delay became less remarkable.

Table 1. Indium Auger Electron Emission Tumor Therapy using Synchrotron X-Rays at ESRF

Tumor Volume as a Function of Time (days), post-irradiation
Irradiation, day 20

Treatment	Days $100\ mm^3$	Days $200\ mm^3$	Days $300\ mm^3$
Control	26.3 ± 4.3	30.6 ± 4.1	33.1 ± 3.6
InTMPyP Only	26.9 ± 4.0	30.4 ± 3.7	32.9 ± 2.5
Saline and Irradiation (above K-edge)	23.4 ± 4.5	31.1 ± 2.6	34.2 ± 2.1
Saline and Irradiation (below K-edge)	30.8 ± 9.3	38.4 ± 8.1	44.3 ± 7.3
InTMPyP and Irradiation (above K-edge)	42.1 ± 4.2	49.5 ± 5.0	54.5 ± 6.5
InTMPyP and Irradiation (below K-edge)	37.4 ± 7.5	45.8 ± 8.9	53.8 ± 12.7

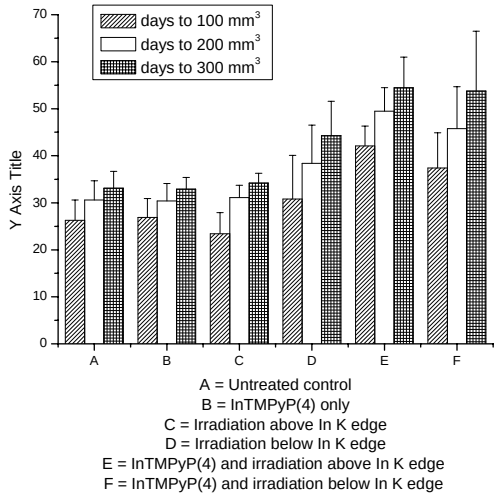


Figure 1.

The second study carried out at beamline ID 17 was carried out in an attempt to delay the exponential growth phase that was evident in the first experiment and possibly achieve a cure. The radiation dose was increased to 40 Gy. The B16 melanoma borne on the footpads of C57 BL mice was the tumor model used. InTMPyP(4) was administered at a concentration of 40mg/kg. The growth of all footpad tumors in all groups, with the exception of the 'sham' saline above the K edge, that served as controls for the InTMPyP(4) above the K edge group, were controlled. However, 60% of the tumors reappeared in the leg joint region (tibia-femoral junction) within several days, up to two weeks after irradiation. These secondary tumors grew very rapidly and seemed to be painful to the mice; consequently, the mice were euthanized as soon as these tumors began to appear. Autopsy revealed no other metastatic sites. This anomalous result was very discouraging, and remains unexplained. Results are shown in Fig. 2. below.

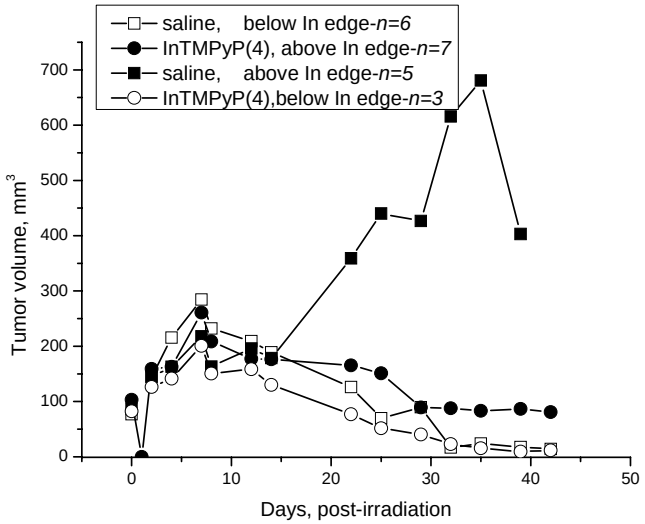


Figure 2.

A third study was carried out at the same time. In this study, PtTMPyP(4) was administered at a concentration of 40 mg/kg to BALB/c mice bearing the KHJJ murine melanoma on thigh. A radiation dose of 40 Gy was delivered to the thigh tumors. In Fig. 3 below, it is apparent that a significant reduction in the tumor burden was observed in all groups. It had been anticipated that irradiations of tumors above the Pt K edge would result in a slower growth pattern, showing a delay in tumor growth. Several of the irradiated mice in all groups died on different days from unknown causes within 2 weeks after irradiation. It was postulated that these mice might have received too large whole-body dose, perhaps from back-scatter of photons due to the difficulty in positioning the thigh tumor as an isolated structure. Unlike the footpad tumors where only the extremity was exposed, it is possible that a segment of the lower pelvis was pulled through the opening of the holder in order to irradiate the entire tumor.

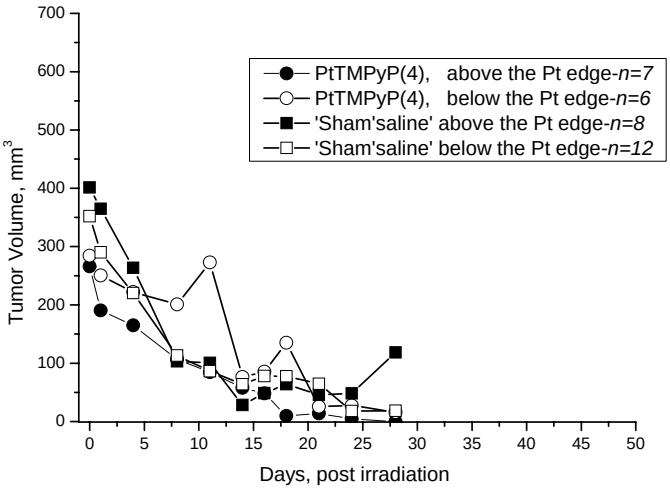
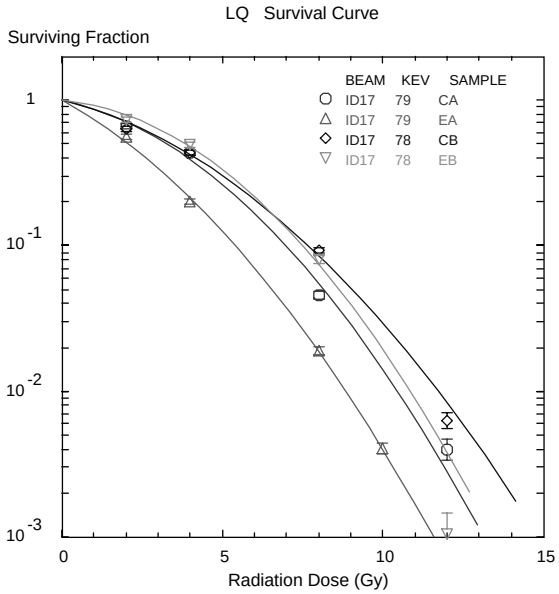


Figure 3.

The last study carried out under the previous long term proposal provided very fruitful scientific information about the lack of repair in V-79 Chinese hamster cells irradiated in a split dose regimen. Cells, in medium with (E) and without (A) PtTMPyP(4), were irradiated above and below the Pt K edge. Data were plotted using the cell survival model developed by Albight.

Figure 4.



Data fit the single hit multi target and linear quadratic models equally well.

In this study, the RBE for irradiations above and below the Pt K edge after cells were incubated with PtTMPyP(4) [0.1 mg/ml] was ~1.4 at the 10% survival level (data points, EA and EB). The ratio of D_0 values between these same two points was 1.2. Extra cells that were not taken for irradiation, but were from the same dishes as those irradiated, were sent for ICP-MS analysis of the results. Measurements of Pt uptake in cells are not yet available.

A second more intensive study was also carried out at ID17. The study was designed to determine if there were any differences in the repair capacity between cells irradiated above and below the Pt K edge. It was anticipated that repair in cells irradiated above the Pt K edge would be inhibited due to the induction of more DNA double strand breaks by Auger electrons emitted from the Pt atom. The manner in which the experiment was carried out is given below.

Cell preparation.

$1.5 \cdot 10^6$ V-79 Chinese hamster cells were seeded into 6, 100 mm Petri dishes in complete DMEM medium (Bet HaEmek) containing 10% FBS (Hyclone), 2% L-glutamine (Bet HaEmek), and 2% antibiotic/antimycotic (Bet HaEmek). After 18 hours, the seeding medium was aspirated and replaced with either complete medium (control-C), or complete medium containing PtTMPyP(4) (experimental –E) at a concentration of 0.1 mg/ml. Medium was again aspirated after 18 hours, the dishes washed vigorously three times with PBS, the cells trypsinized without EDTA, harvested and counted. Cells were diluted to a population density of 10^5 cells per ml, and 1 ml of the cell suspension was placed in each of 40, 1.5 ml microfuge tubes. The tubes were placed on ice and taken to the ID17 beamline for irradiation. All procedures were carried out under amber light conditions. Excess harvested cells were retained for ICP-MS analysis of Pt uptake.

Irradiation:

The experiment comprised 8 groups, all in the exponential growth phase, with different treatments, all given a total radiation dose of 8 Gy. These were: a) single acute dose, no PtTMPyP(4) and irradiated above the K absorption edge of Pt-CSA; b) no PtTMPyP(4) and irradiated below the K absorption edge of Pt-CSB; c) no PtTMPyP(4), irradiated above the K absorption edge of Pt in 2 fractions CFA; d) single acute dose, no PtTMPyP(4) and irradiated below the K absorption edge of Pt-CFB; e) single acute dose, PtTMPyP(4) and irradiated above the K absorption edge of Pt-ESA; f) single acute dose, PtTMPyP(4) and irradiated below the K absorption edge of Pt-ESB; g) PtTMPyP(4), irradiated above the K absorption edge of Pt in 2 fractions-EFA; h) PtTMPyP(4) and irradiated below the K absorption edge of Pt in 2 fractions-EFB. All treatment groups consisted of 5 samples each, an untreated control (0), and four samples representing the interval (0.5, 1, 2, 3) allotted to the cells for recovery of radiation damage.

Method:

All untreated control samples (0) were kept on ice and plated for colony growth at the end of the radiation experiment. All samples were maintained on ice during transport to and from the laboratory, or while awaiting plating for colony growth. Liquid nitrogen was released into the radiation chamber to maintain a cool environment (~4˚C) for the cells during the irradiation. Samples with and without PtTMPyP(4) were irradiated simultaneously by placing two microfuge tubes into a horizontal and hollow rod, with caps at either end. Tubes containing the cells that received a fractionated dose (F) of 4 Gy were incubated until the time point was reached (0.5, 1, 2, and 3 hours), whereupon they were removed from the incubator and taken to the beamline for the second 4 Gy fraction. Once the second fraction was delivered, those cells that received a single acute radiation dose of 8 Gy and were kept on ice, were plated at the same time as their matching fractionated sample. Cell samples were irradiated either above (79 keV) or below (78.1 keV) the Pt K absorption edge (78.4 keV).

Survival analysis:

Results in Figure 5. were obtained according to the following formula:

$$\frac{Number\ of\ colonies\ obtained}{Number\ of\ cells\ seeded}\ X\ PE\ (Plating\ efficiency)\ X\ 100$$

Preliminary results of this experiment are shown in Fig. 5 below. The individual recovery pattern for each treatment group is shown. In the figure, all below edge samples seem to show a greater capacity for repair after the first hour, than those irradiated above the edge as indicated by the open symbols (CSB, ESB, CFB, and EFB). The exception to this rule is the fractionated control, at 3 hours (CFA), whose results are anomalous. Treatments in the legend are given above in section on Irradiation.

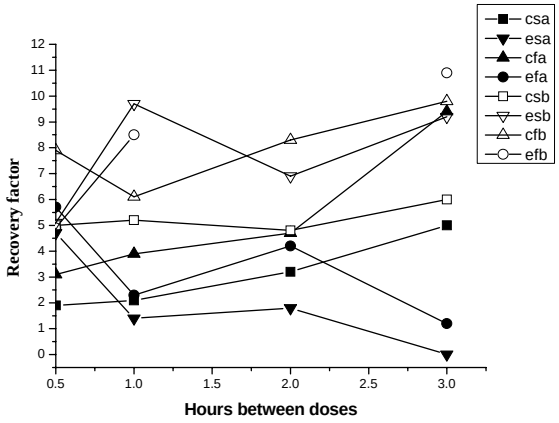


Figure 5.

In Figure 5, no remarkable increase in survival was apparent in cells that received PtTMPyP(4) in the single or fractionated regimen, at 3 hours (ESA and EFA). In fact, there seems to be a decrease in survival after the first half hour of incubation. What was expected in this experiment was an increase in survival for all treatment groups, with the exception of the PtTMPyP(4), above edge. It was postulated that if damage from Auger electrons emitted from the Pt atom emulated high-LET type damage due to the dense deposition of ionizing energy directly in the DNA, then repair of the cells would be minimal. The results obtained and analyzed as described above support the hypothesis that damage, after inducing a photoelectric process in DNA, is significantly less repairable.

Safety:

There are no safety issues associated with the cell experiments. The drugs and reagents represent no biological hazards. Approval from our Institutional Animal Care and Use Committee will have to be obtained prior to carrying out animal experiments under this proposal.

Discussion:

According to Dr. Foray (personal communication), the V-79 cell line is not only radioresistant, but also proficient in repair, with the effect normally demonstrable at a dose of 2 Gy. His assessments of DNA damage and repair phenomena are well-recognized (23). As can be seen in Figure 4, at a dose of 4 Gy, survival of PAT cells (EA) is 20% compared to 55% for all other samples. Taking these points into consideration, a significant effect on the capacity of the EA cells to repair DNA double strand breakage is suggested. When comparing the different treatments at the same level of biological effect (10% survival), the ~1.4 ratio obtained is quite similar to that obtained in previous experiments carried out on ID17 (7).

Dr. Foray suggests that for V-79 Chinese hamster cells, the induction rate of double strand breaks (DSB) is 20 DSB/Gy. He suggests that the number of DSB might differ among the different treatment groups as shown below:

Dose	Time interval between fractions	PtTMPyP(4) cells (split dose), irradiated above the K edge (PAT effect) No. of DSB anticipated	Control cells No. of DSB anticipated (after 24 hours)
4 Gy	1	55	15
"	2	48	10
"	3	44	8
"	10	40	0
8 Gy	10	64	0

Relevant to the studies shown in Figure 5, he concludes that there is good agreement with the survival data shown in Fig.4. Irradiations above the K edge of Pt show an excess of ~40 unrepaired DSB for the fractionated samples compared to the controls

that would correspond to the effect of an additional 2 Gy. For the single acute dose samples (irradiated above the Pt K edge), there are an excess of 60 unrepaired DSB, compared to controls which corresponds to an additional 3 Gy.